Review

Idiopathic pulmonary fibrosis: Are any of the morphological-molecular markers useful in clinical management?

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Summary. Idiopathic pulmonary fibrosis (IPF), the most common form of chronic interstitial lung disease, is a severe progressive fibrotic disorder of unknown aetiology. The disease has a heterogeneous clinical course, with frequent poor prognosis, similar to malignant disease. Correctly diagnosing IPF has become particularly important in view of the availability of more precise therapeutic indications, thus avoiding steroid treatment and allowing new approaches with novel drugs.

To date we have limited information about biomarkers predictive of progressive disease and associated complications. Efforts should be made in the future to more appropriately study lung tissue and then to extrapolate the most clinically fitting biomarkers.

This approach is already used in routine management of many cancers and provides a potential road map for more appropriate clinical care of IPF.

This review will mainly focus on histology and etiopathogenesis highlighting some morphological and molecular features that may influence the overall management of IPF.

Key words: IPF, Morphology, Markers, Management

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Introduction

Idiopathic pulmonary fibrosis (IPF), the most common form of chronic interstitial lung disease, is a chronic, devastating disease, and lethal fibrotic disorder. The American Thoracic Society and European Respiratory Society (ATS/ERS) published international consensus statements in 2000 and in 2011 on the diagnosis and management of IPF (American Thoracic Society, 2000; Raghu et al., 2011). In both statements the importance of recognizing IPF as a distinct clinical entity was highlighted. Specific recommendations for a more correct diagnosis and management of the disease were provided. According to the current guidelines, in about two thirds of cases a confident diagnosis of IPF can be achieved on an appropriate clinical history and a typical high-resolution computed tomography (HRCT). Surgical lung biopsy is recommended when clinical and HRCT data are not diagnostic. Correctly diagnosing IPF has become particularly important in view of the availability of some novel therapies and overall the demonstration that a previously widely used immunosuppressive treatment regimen (prednisone, azathioprine and NAC) increases the risk of death and hospitalization (Idiopathic Pulmonary Fibrosis Clinical Research Network et al., 2012). IPF has a heterogeneous clinical course, with a median survival after diagnosis of only 2.5-3.5 years (King et al., 2011a), similar to malignant disease. Equally variable is the development of important complications such as acute exacerbation (Ley and Collard, 2012), pulmonary hypertension and lung cancer (King and Nathan, 2016). To date we have

limited information of predictors on progressive disease and associated complications. This review, after a brief overview of the disease concerning epidemiology and clinical presentation, will mainly focus on histology and etiopathogenesis, highlighting some morphological and molecular features which may impact on the overall management of IPF.

Epidemiology

IPF is known for being the most common among the interstitial idiopathic pneumonias, but its prevalence and incidence still remain unclear, with large variability across reports due to several reasons. The sources of uncertainty include the complexities of establishing a diagnosis, lack of validity of the reported and/or noted diagnosis in medical records, variation in physician diagnostic approaches over time and variations across geographic regions and countries (Raghu et al., 2011).

It is still unknown whether the incidence and prevalence of IPF are influenced by geographic, ethnic, cultural, or racial factors (Raghu et al., 2011). Worldwide, the incidence of IPF is estimated to be 10.7 cases per 100,000 person-years for males and 7.4 cases per 100,000 person-years for females (Kim et al., 2006a,b), with different incidence rates reported in various countries (Hodgson et al., 2002; Ohno et al., 2008; Karakatsani et al., 2009; Fernández Pérez et al., 2010; Navaratnam et al., 2011). It is unclear how much of this variation among studies is due to geographic or demographic differences in the risk of IPF.

Data on IPF-related mortality are typically obtained from national registries compiled from information on death certificates. These studies are often limited by disease under-recognition and diagnostic mis-classification. In studies that used the revised diagnostic criteria for IPF, only 20 to 30% of subjects were alive 5 years after diagnosis with an estimated mean survival of 2-5 years from the time of diagnosis (Raghu et al., 2011). Whether or not the increasing mortality from IPF is due to an actual rise in mortality or is a consequence of recognition or reporting bias is unclear. In public health terms, IPF-related mortality is similar in depth to that of many malignancies (eg, non-Hodgkin's lymphoma, renal cancer, esophageal cancer). Respiratory failure from IPF accounted for 60% of IPF-related deaths in a US-based study (most commonly after an acute exacerbation), followed by cardiovascular diseases (8.5%), lung cancer (2.9%), infection, and pulmonary embolism (Kim et al., 2006a,b).

Clinical features

The signs and symptoms of IPF develop over time, and may not even begin to appear until the disease has done serious damage to the lungs. The most common signs and symptoms are: shortness of breath (at first, only during exercise, over time even at rest), a dry, hacking cough, bibasilar inspiratory crackles and finger clubbing. The disease involves older subjects typically in the sixth and seventh decades. Patients with IPF aged less than 50 years are rare (Raghu et al., 2011).

The natural history of IPF is highly variable among patients. Some patients with IPF experience rapid decline, others progress much more slowly, and some patients show periods of relative stability interspersed with acute deteriorations in respiratory function. However, it is impossible at present to predict at the time of the diagnosis, how the disease will behave in the single individual (Selman et al., 2007). Some clinical variables have been reported to be associated with shortened survival time and these include: older age, smoking history, lower body mass index, more severe physiologic impairment, greater radiologic extent of disease, and the development of other complications or conditions, in particular, pulmonary hypertension, emphysema, and lung cancer (King et al., 2001b; Collard et al., 2003; Nadrous et al., 2005; Alakhras et al., 2007).

Several years ago a clinical prediction model named CRP score model (including Clinical, Radiological and Physiological determinants) was developed (King et al., 2001).

It incorporates age, smoking status, clubbing, profusion of fibrosis and pulmonary hypertension on chest radiography, total lung capacity, and partial pressure of arterial oxygen at maximal exercise. CRP scores were highly predictive of survival in the cohort who were studied. However this model has not been widely adopted because it lacks formal external validation and uses some variables that are not routinely measured in current clinical practice (Ley et al., 2011). Moreover, they provide no insights into underlying pathobiology, thus failing to identify distinct molecular phenotypes of the disease.

According to the recent evidence-based guidelines there are unfortunately no definitive treatments for IPF: novel agents such pirfenidone and nintedanib potentially slow disease progression (King et al., 2014; Richeldi et al., 2014) and surgical therapy with lung transplantation is available to only a small minority of patients (Raghu et al., 2011, 2015). A better knowledge of specific biomarkers detected in the lung tissue and then confirmed in less invasive tools such as broncoalveolar lavage or in the peripheral circulation are worthy of future investigation.

Morphological findings

As described in the first 2002 joint consensus statement of the American Thoracic Society (ATS) and the European Respiratory Society (ERS), and confirmed in the subsequent large consensus statement, the pathological manifestation of IPF is usual interstitial pneumonia (UIP) (American Thoracic Society/European Respiratory Society, 2002; Travis et al., 2013). UIP, a process that basically involves the periphery of the lung lobules and primarily the lower lobes, is characterized by "patchy distribution" of the lesion with a

heterogeneous appearance with involved and uninvolved parenchymal areas. Hereinafter histological features will be described in detail, highlighting in the section titled "*key morphological-molecular biomarkers*" those that are now the most widely discussed as possible biomarkers.

The disease develops stepwise producing the characteristic spatial heterogeneous appearance characterized by the pathological processes at different stages of development: presence of subacute injury (fibroblastic foci), dense scarring and normal lung. Fibroblastic foci, characterized by fibroblasts and myofibroblasts arranged in a linear fashion with a pale staining matrix, are usually detected in the transitional area from dense scarring to normal lung and appear a quite distinct IPF lesion. The fibroblastic foci are localized immediately beneath the alveolar lining epithelium obscuring the epithelial basement membrane. Fibroblastic proliferation bulges towards the airspace but does not make a polypoid structure such as a Masson body seen in organizing pneumonia. Fibroblastic foci may be detected in other non IPF interstitial pneumonia such as non-specific interstitial pneumonia and chronic hypersensitivity pneumonia, albeit less profuse (Nicholson and Wells, 2001; Travis et al., 2008; Churg et al., 2009; Chiba et al., 2016). Several studies have highlighted the importance of the fibroblastic focus as a manifestation of active lung injury and in the setting of IPF as a poor prognostic marker (see below *key morphological-molecular biomarkers*).

Fibroblast/myofibroblasts derive from resident cells, circulating fibrocytes or from epithelial cells through epithelial mesenchymal transition (EMT). According to lineage tracing in murine models of lung fibrosis, it is estimated that up to one-third of fibroblasts may be of epithelial origin (Tanjore et al., 2009). EMT is a process well known in normal development, wound healing and cancer metastasis (Savagner, 2010; Nakamura and Tokura, 2011; Kerosuo and Bronner-Fraser, 2012). Several works have demonstrated that in the context of fibroblastic foci from IPF lung tissue the epithelial cells lose their distinct marker expression and gain a mesenchymal expression profile and morphology (Chilosi et al., 2002; Willis et al., 2005; Kim et al., 2006a; Seibold et al., 2013; Jonsdottir et al., 2015). The expressional switch includes an increase in the expression of various mesenchymal markers, such as vimentin, alphaSMA, N-cadherin, calponin 1 and fibronectin (Kim et al., 2006a,b; Marmai et al., 2011). The immunostaining colocalization of cytockeratin markers and mesenchymal proteins is not present in normal lungs. Although the contribution of these mesenchymal changes to the fibrotic process remains unclear, the identification of the signalling pathways that lead to the activation of the EMT program is recently providing new insights into the potential biomarkers (see below *key morphological-molecular biomarkers*).

Inflammation is not extensively detected in the disease and is not considered to date a crucial contributing factor to the pathogenesis of IPF. Although some forms of pulmonary fibrosis may result from inflammation, IPF is currently thought to result from cell death primarily and inflammation secondarily. This assumption is also supported by the ineffectiveness of anti–inflammatory therapies, particularly corticosteroids, in improving IPF patient survival. According to the new statement the presence of inflammation, particularly if aggregate in follicular patterns and/or significantly present around the airway, should take into consideration the diagnosis of non IPF UIP patterns such as immunological disease (Raghu et al., 2011).

In IPF, inflammation is rarely found close to fibroblastic foci or EMT while it is more often detected close to honeycomb lesions or in IPF with acute exacerbation or more recently in forms of IPF with rapid functional decline called "rapidly progressive IPF" (or accelerated variant) (Balestro et al., 2016). This last form includes a subgroup of patients who had a rapidly progressive disease and a different gene expression profile than those with slower progression and longer survival (Boon et al., 2009). While previous studies did not show any different histological findings with slowly progressive IPF (Selman et al., 2007; Brett et al., 2011), our group detected more evident inflammation, both adaptive and innate, in both lung biopsies and explant tissues from rapidly progressive IPF. Some authors have reported that inflammation has been detected more frequently in IPF explanted lungs compared to lung biopsies, but they did not specify the IPF phenotype (Todd et al., 2013). Future studies on larger case series and continued efforts to understand the precise role of inflammation and possible mechanisms of corti-costeroid-resistant inflammation are warranted in IPF patients.

Extracellular matrix deposition (EMD) with dense collagen and honeycomb areas are other typical histological changes which are part of the heterogeneous aspect of the disease. Vascularity is decreased in the areas of more pronounced fibrosis and in the context of fibroblastic foci, while increased vascular density has been noted in areas of mild fibrosis. In more pathological lung areas, vascular remodelling characterized by changes in collagen and elastic fibres with different degrees of occlusion are frequently detected. Vascular remodelling may be, at least in part, responsible for pulmonary hypertension, an important complication with detrimental impact on disease progression.

Several studies have focused on precise analyses of EMD and vascular changes highlighting some specific markers for IPF (see below *key morphological-molecular biomarkers*).

Dense fibrosis causes remodelling of the lung architecture resulting in collapse of alveolar walls and formation of cystic spaces or honeycombing. The honeycomb lesion was originally defined by radiologists as a single or multicystic lesion within a fibrotic lung area (Schmidt et al., 2009). Given a different resolution between HRCT and histology, a substantial difference in

size is seen between the two tools. At histology a so called honeycomb lesion is a cystic lung lesion involving a secondary lobule. Microscopic honeycomb in the range of 1-3 mm characterizes one of the early findings of the gross honeycomb cysts seen in end stage UIP. Inspissated mucus and inflammatory cell infiltration both inside the cyst and in the pericystic area can frequently be detected. As recently emphasized by several authors (Popper, 2013; Kradin, 2015), a deeper understanding of these changes could significantly improve the final diagnosis and the investigation of its predictive value. The spaces are lined by cuboidal or ciliated columnar cells and at least some of them constitute the in-growth of bronchiolar epithelium along alveolar ducts or directly into alveoli via the bronchoalveolar canals of Lambert (lambertosis). Metaplastic cuboidal, bronchiolar and squamous cells lining cystic spaces can frequently show different degrees of dysplastic changes. In 1965, Meyer and Liebow provided the first description of atypical epithelial lesions in honeycombing areas, raising the possibility that these atypical epithelial lesions in UIP might be precancerous lesions (Meyer and Liebow, 1965). Our group demonstrated high-grade dysplasia foci, mainly squamous type, in 15% of end-stage IPF lungs from patients who underwent lung transplantation (Calabrese et al., 2012). Squamous cell carcinoma was first reported as the most frequent histotype (Aubry et al., 2002) even if other forms have been subsequently described. The impact of epithelial dysplasia/lung cancer on IPF outcome has been investigated by several authors (see below *key morphological-molecular biomarkers*).

Etiopathogenesis

The paradigm about the disease has currently shifted to the idea that it results from aberrant activation of alveolar epithelial cells damaged by sequential injuries with a consequent deregulated wound healing response. It is becoming increasingly clear that the heterogeneous IPF phenotype is likely the result of complex interactions between genetic and environmental factors. This multiple-pathway model could explain the disappointing results of therapies targeting single receptors or pathways.

Evidence for a genetic basis of pulmonary fibrosis is substantial, with familial aggregation confirmed through a variety of studies in twins, siblings raised apart, and multigenerational families. Several genetic alterations have been reported and now genetic counselling and screening is strongly suggested in familial cases and/or young people (see below *key molecular biomarkers*).

Although in theory genetic mutations could lead directly to lung fibrosis, there are no data to support this possibility. For example, telomerase-deficient mice have not been reported to develop lung fibrosis (Rudolph et al., 1999; Lee et al., 2009). Similarly, when the L188Q mutated form of the surfactant protein C (SPC) is overexpressed in mouse alveolar type II cells, the mice do not develop lung fibrosis despite activation of the unfolded protein response and increased apoptosis of alveolar type II cells (Lawson et al., 2011). However, aged mice develop more severe lung fibrosis after exposure to bleomycin or murine gamma-herpesvirus infection (Redente et al., 2011; Sueblinvong et al., 2012; Torres-González et al., 2012). In the case of gamma-herpesvirus infection, the predisposition to more severe fibrosis in aged mice is associated with greater activation of the unfolded protein response and increased apoptosis (Miki et al., 2000). Similarly, mice expressing the L188Q mutated form of SPC develop lung fibrosis after administration of bleomycin in doses that do not cause fibrosis in control animals (Lawson et al., 2011). These observations suggest that genetic mutations may not be sufficient to cause lung fibrosis; rather, a second event such as environmental exposure which may act in concert with a genetically predisposed epithelium.

Several environmental factors have been shown to increase the risk of developing the disease, such as cigarette smoke, dust exposure, gastroesophageal reflux (GER) and microbial agents; below GER and infections are discussed in depth (see below *key morphological molecular biomarkers*). When and what environ-mental agents activate profibrotic molecular programming in predisposed individuals need further investigation.

Key morphological-molecular biomarkers

They have been summarized in the Table 1.

Fibroblastic foci

Numerous studies have carefully analysed fibroblastic foci focusing on several aspects concerning the specific genotype/phenotype, spatial distribution and abundance. Although some authors have reported interesting data concerning a different genotype/phenotype (Miki et al., 2000; Nagao et al., 2001) as well as a peculiar spatial distribution (Cool et al., 2006; Jones et al., 2016) of fibroblastic foci in patients with IPF, surely the most important one, at least to date, is that related to profusion. High numbers of fibroblastic foci may predict physiologic decline and mortality (King et al., 2001a,b; Nicholson et al., 2002).

Fibroblasts derived from fibrotic tissue have been shown to have both high and low proliferative capacity and a high variability in DNA methylation (Huang et al., 2014). While it has long been known that IPF fibroblasts are polyclonal and do not possess the classical characteristics of malignant transformation (Mio et al., 1992), newer methods have facilitated the finding that these cells contain genome-wide abnormalities in translational control similar to changes found in cancer cells (Larsson et al., 2008).

EMT

EMT can be induced or regulated by various growth

and differentiation factors, which act through receptor tyrosine kinases, such as transforming growth factor-β (TGF- β), fibroblast growth factor, hepatic growth factor platelet derived growth factor (PDGF), Wnt and Notch proteins (Moustakas and Heldin, 2007). Among these mediators, the TGF-β signaling system is considered a master inducer of EMT, another crucial cytokine is PDGF which promotes fibroblast proliferation (Hetzel et al., 2005). Several therapeutic interventions that interfere with the tyrosine kinase pathway have been developed to slow the fibrotic process.

Multi-kinase inhibitors have effectively antagonized the TGF-β1-mediated EMT both *in vitro* and *in vivo* studies (Lamouille and Derynck, 2014).

This possibility was succesfully tested in a clinical trial examining whether a tyrosine kinase inhibitor that targets several receptors, including PDGF receptor, slows the progression of IPF (Richeldi et al., 2011, 2014).

EMD and vascular remodelling

Collagen I and other minor isoforms (type III, V) are the primary components of the new abnormal matrix, with changes during disease progression. Previous studies have found that collagen III is characteristic of early IPF, whereas collagen I dominates in late-stage disease (Bateman et al., 1983; Kirk et al., 1984; Raghu et al., 1985). Changes in elastin also contribute to ECM remodelling with different patterns in early and late IPF (Rozin et al., 2005; Enomoto et al., 2013). In early IPF, collagen and elastic fibers are degraded by several types of metalloproteinases (MMPs) and elastases. As IPF progresses and elastin is degraded, fibroblasts respond through synthesis of both collagen and elastin; however, the new elastin is highly disordered yielding poor mechanical properties of the new lung matrix. MMPs, a large family of proteinases, play a major role in the degradation and remodeling of the ECM. The contribution of MMPs to extracellular matrix remodeling is complex. They are not only responsible for matrix degradation but may also contribute to processing a variety of bioactive mediators such as growth factors, cytokines, chemokines, and cell-surface-receptors, thus modulating other local biopathological processes such as apoptosis, migration, proliferation and angiogenesis (Pardo and Selman, 2006). It has been reported that several MMPs have been overexpressed in IPF lungs and also in serum (MMP-1, -2, and -7) and in broncho-alveolar lavage fluid (MMP-3, -8, and -9) (McKeown et al., 2009; Dancer et al., 2011). Among MMPs, MMP-7 is one of the most extensively investigated MMPs in IPF. A recent work reported a fairly sensitive value of the combined index of three biomarkers: MMP-7, SP-D and osteopontin for the diagnosis of IPF versus non-IPF ILDs. Even if the combined biomarker index was not different in RA-ILD and was not comparatively investigated in all fibrosing ILDs (e.g. hypersensitivity pneumonitis, or pneuconiosis) it may be of help in the diagnosis of IPF, particularly for those patients who can not undergo surgical lung biopsy and where the HRCT scan is not diagnostic (White et al., 2016). A better knowledge of the complexity of MMP proteolysis and the in-vivo substrate repertoires should be defined for a greater understanding of the fibrosis-specific effects of MMPs in IPF.

Table 1. Key morphological/molecular markers in Idiopathic Pulmonary Fibrosis.

	Marker	Diagnosis	Prognosis	Therapy
Histology				
Fibroblastic foci	Profusion	-	+	-
EMT	TGF-β, PDGF	-	+	+
EMD/Vascular remodeling	MMP (particularly MMP7)	-	+	-
	Osteopontin	-	+/-	-
	Periostin	-	+	-
	VEGF	-	+	+
Epithelial dysfunction/ regeneration/lung cancer	Presence of lung cancer	-	+	-
	KL-6 (MUC1)	-/+	+/-	-
	Serpins	-/+	+	-
	K-RAS, P53, guanine nitration	-	-	-
Genetic factors				
	SPA, SPC (familial IPF)	+	+/-	-
	MUC5B	- /+	+	-
	TERT/TERC (familial IPF)	+	+/-	-
Environmental factors				
	Infections	-	+	-
	GERD	-	-	+

EMT: epithelial-mesenchymal transition; TGF: transforming growth factor; PDGF: platelet-derived growth factor; EMD: extracellular matrix deposition; MMP: metalloproteases; VEGF: vascular endothelial growth factor; KL: Krebs von den Lungen; SP: surfactant protein; TERT: telomerase reverse transcriptase; TERC: telomerase RNA component; GERD: gastroesophageal reflux disease.

MMP7 has also been demonstrated to be an interesting prognostic biomarker. Several studies have shown that serum MMP-7 correlates with both FVC and DLCO and is associated with survival in patients with IPF (Richards et al., 2012).

High serum levels of MMP-7 combined with SP-A and Krebs von den Lungen-6 antigen (KL-6) have been more significantly associated with shorter survival in patients with IPF (Rosas et al., 2008; Song et al., 2013). The exact mechanism of increased plasma MMP-7, SP-A and KL6 is uncertain; injured/activated epithelial cells in IPF lungs are the likely source. However, most of these studies have been performed with small numbers of subjects and therefore their use as a bio-marker for prognosis or response to therapy in ILD still needs to be validated with an unbiased and representative study population. A prerequisite for the use of biomarkers in clinical practice is validation in large, well-phenotyped cohorts with longitudinal follow up of both clinical and molecular parameters. In the PROFILE study concentrations of protein fragments (six neoepitopes: BGM, C1M, C3A, C3M, C6M and CRPM) generated by MMP activity have been found increased in the serum of individuals with IPF compared with healthy controls (Jenkins et al., 2015). Increased neoepitope concentrations were associated with disease progression, and the rate of this increase predicted survival. Although these data require testing in an appropriately designed, prospective, therapeutic trial, the predictive prognostic value of the these markers is additional important information supporting the importance of ECM turnover in the pathobiology of IPF.

Periostin is another crucial ECM protein demonstrated to promote ECM deposition, mesenchymal cell proliferation and parenchymal fibrosis. The protein has been found to be highly expressed in lung tissues and blood of patients with IPF (Okamoto et al., 2011; Naik et al., 2012; Hambly et al., 2015). It localizes in areas of active fibrosis, fibroblastic foci, subepithelial and subendothelial regions in IPF lungs, and plasma levels of periostin at baseline predict clinical progression at 48 weeks in patients with IPF (Naik et al., 2012).

Vascular endothelial growth factor (VEGF) signalling has been demonstrated as an important pathway involved not only in vascular but also in fibrotic remodelling, indeed, it acts as a trigger of PDGFR activation, thereby regulating mesenchymal cell migration and proliferation (Ball et al., 2007). In patients with IPF, baseline plasma VEGF concentration was reported to be positively related to the fibrosis score on high-resolution computed tomography (Simler et al., 2004). Moreover, patients with IPF who developed progressive disease (≥10% decrease in FVC from baseline to month 6) had significantly higher plasma VEGF concentrations at baseline than non-progressors (Simler et al., 2004). However, VEGF levels in the bronchoalveolar lavage fluid are reported to be depressed in patients with IPF (Meyer et al., 2000; Koyama et al., 2002).

In a rat model of pulmonary fibrosis, VEGF expression was almost absent in fibrotic lesions, but strong in epithelial and endothelial cells (Farkas et al., 2009). Similarly, in patients with IPF, VEGF expression was low in fibroblasts and leukocytes in fibrotic lesions, but increased in capillary endothelial cells and alveolar type II epithelial cells in highly vascularised alveolar septa (Ebina et al., 2004). Experimental models in rats suggest that inhibition of VEGFR can reduce fibrosis (Hamada et al., 2005) while the administration of VEGF aggravates the fibrogenic process (Farkas et al., 2009). The new drug nintedanib has been demonstrated to be a potent inhibitor of several receptor tyrosine kinases, including PDGFR, FGFR and also VEGFR, thus playing a key role not only in EMT but also EMD and vascular remodeling (Wollin et al., 2015).

Honeycomb lesions and epithelial dysplasia/lung cancer

Several studies have shown a clinical impact of honeycomb lesions on follow-up computed tomography (traction bronchiectasis, progression of fibrosis, and honeycomb associated with poorer outcomes) (Hwang et al., 2011; Oda et al., 2014). Few papers have specifically focused on honeycomb cysts and fibrosis semiquantitative/quantitative evaluations reporting contradictory data in terms of clinical outcomes (King et al., 2001b; Calabrese et al., 2008, 2012). While no studies are to date available about the predictive significance of epithelial dysplasia in IPF, several works have recently focused on the impact of lung cancer on survival of patients with IPF. Patients with IPF and lung cancer showed a worse prognosis than those with IPF without lung cancer (Tomassetti et al., 2015). Moreover, other studies have demonstrated a significantly poor impact of lung cancer in surgery, particularly after lung transplantation (Kumar et al., 2003; Calabrese et al., 2009). Few tissue markers for epithelial instability have been described as being overexpressed in lung tissue from patients with IPF/UIP, including mutations in K-ras and p53, the occurrence of guanine nitration, and overexpression of SCCA in proliferating metaplastic and neoplastic cells of IPF (Kato, 1996; Calabrese et al., 2008, 2012). Given the significant impact of this complication on IPF management, larger studies are mandatory for the identification of early and sensitive biomarkers linked to neoplastic transformation in the disease.

Genetic alterations

It has been estimated that up to to 20% of cases of idiopathic interstitial pneumonia are located in families. The inheritance of familial pulmonary fibrosis (FPF) is not clear. Complex inheritance (including multifactorial inheritance and autosomal dominant inheritance with reduced penetrance) appears likely, though autosomal recessive inheritance remains a possibility (Steele et al., 2005; Lawson and Loyd, 2006).

FPF has been associated with private mutations in SPC and SPA and genes that maintain telomere length (TERT and TERC) (Nogee et al., 2001; Diaz de Leon et al., 2010; Garcia, 2011; Ono et al., 2011). Although mutations in TERT or TERC have been identified in a small minority of sporadic IPF patients, a significantly higher proportion of affected patients have short telomeres when measured in either their peripheral blood or alveolar epithelial cells (Alder et al., 2008; Cronkhite et al., 2008). Collectively, these studies report mutations in the aging-related genes TERT and TERC in 18% of familial cases, while these are rare in sporadic IPF cases (about 3%).

Other genetic variants have been described in IPF, including genes encoding ELMOD2, IL-1, CR-1, IL12p40 and IFN-gamma, NOD2/CARD15, MMP-1, ENA-78, IP-10 and VEGF, CD16b, IL-8 and HER2, but the majority of these results have not been replicated. More recently, Seibold et al. identified a SNP in the putative promoter of MUC5B (rs35705950) that was associated with familial interstitial pneumonia (Zhang and Kaminski, 2012). This SNP has been reported in sporadic IPF cases. The analysis found SNP in 38% of individuals in a cohort of sporadic IPF patients and 9% of healthy controls. The rs35705950 allele was associated with lung expression of MUC5B that was 37.4 times higher than in unaffected subjects (Zhang et al., 2011). Although these data provide a strong association for a linkage of MUC5B polymorphisms to IPF, the molecular consequences of SNP and how increased MUC5B protein expression may contribute to the development of lung fibrosis require further explanation. The estimated frequencies of genetic mutations predisposes to IPF is shown in Fig. 1.

Infections

There is evidence of a potential role of herpesviruses as co-factors in the initiation and progression of IPF. The reported frequency of herpesviruses in IPF lungs ranges from 30 to 100% (Calabrese et al., 2013). These differences may be related to technical sensitivity, disease heterogeneity or selection of patients (Egan et al., 1995; Kuwano et al., 1997; Stewart et al., 1999; Kelly et al., 2002; Tang et al., 2003; Arase et al., 2008; Pulkkinen et al., 2012). There are few studies that demonstrate a bad impact of viral infections on IPF patient outcome, both in terms of respiratory dysfunction (Egan et al., 1995) and the development of exacerbations and comorbidities (such as pulmonary hypertension) (Calabrese et al., 2013; Moore and Moore, 2015). There is recent evidence that a more complex influence of infective agents including bacterial burden (the so-called respiratory microbiome) in the lungs of patients with IPF may predict a poor prognosis (Molyneaux and Maher, 2014).

GERD

The prevalence of abnormal GERD is higher in patients with IPF than in matched control subject, and retrospective, anecdotal data suggest a beneficial role of proton-pump inhibitors in IPF, including stabilization of lung function, reduction in episodes of acute exacerbation, and enhanced longevity. It has not yet been clarified if the beneficial effect of this drug is related to GERD or to its anti-fibrotic and anti-inflammatory effect (Ghebre and Raghu, 2016).

Conclusion

In the last few years great efforts have been made in investigating morphological and pathogenetic substrates of IPF which led to the identification of some candidate biomarkers. The recent approval of new drugs and the identification of other potential targets have highlighted the need for theragnostic markers able to assess early onset of the disease and therapeutic response to new drugs. The significant diagnostic improvement of other non invasive tools (HRCT, PFT), lead to less use of
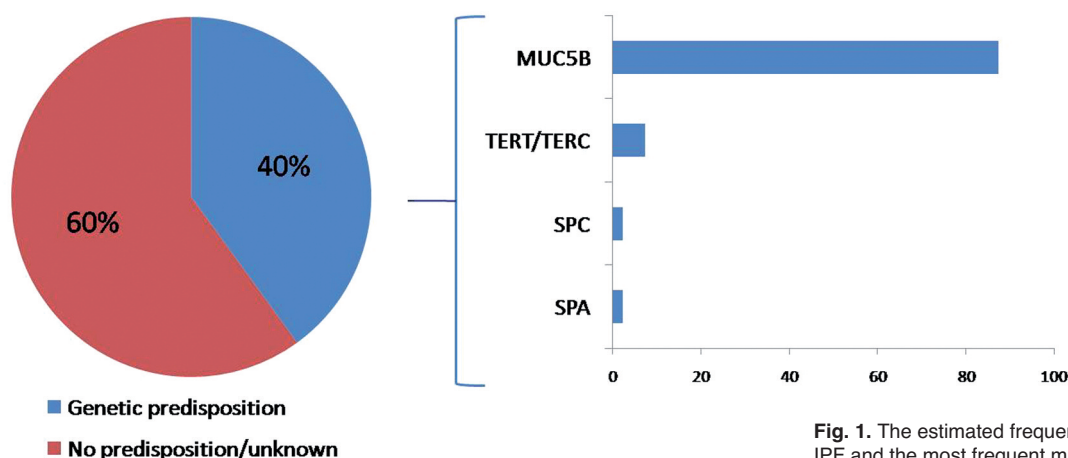

Fig. 1. The estimated frequency of genetic predisposition to IPF and the most frequent mutated genes are shown.

surgical biopsies and consequently less tissue availability. However, lung tissue evaluation should still be considered the bedrock to study specific markers and subsequently extrapolate the most clinically fitting biomarkers. As occurs in the oncological field, in the future it would be desirable to apply more specific tissue analysis using high throughput omic technologies for a wide screening of molecules rather than using a cherry-picking approach.

The development and validation of crucial biomarkers in less invasive tools (such as blood) will consequently lead to an earlier and more precise diagnosis of IPF and a better prediction of future disease behaviour.

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