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Comorbidity in idiopathic pulmonary fibrosis - what can biomarkers tell us?

Tiago M. Alfaro and Carlos Robalo Cordeiro

***Abstract*:** Idiopathic pulmonary fibrosis (IPF) is characterized by progressive parenchymal scarring, leading to dyspnoea, respiratory failure and premature death. Although IPF is confined to the lungs, the importance of IPF comorbidities such as pulmonary hypertension and ischaemic heart disease, lung cancer, emphysema/chronic obstructive pulmonary disease, gastroesophageal reflux, sleep apnoea and depression has been increasingly recognized. These comorbidities may be associated with increased mortality and significant loss of quality of life, so their identification and management are vital. The development of good-quality biomarkers could lead to numerous gains in the management of these patients. Biomarkers can be used for the identification of predisposed individuals, early diagnosis, assessment of prognosis, selection of best treatment and assessment of response to treatment. However, the role of biomarkers for IPF comorbidities is still quite limited, and mostly based on evidence coming from populations without IPF. The future development of new biomarker studies could be informed by those that have been studied independently for each of these conditions.

For now, clinicians should be mostly attentive to clinical manifestations of IPF comorbidities, and use validated diagnostic methods for diagnosis. As research on biomarkers of most common diseases continues, it is expected that useful biomarkers are developed for these diseases and then validated for IPF populations.

The reviews of this paper are available via the supplemental material section.

Keywords: biomarkers, comorbidity, idiopathic pulmonary fibrosis, diagnosis, therapeutics, hypertension, pulmonary, lung neoplasms, gastroesophageal reflux, depression

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Idiopathic pulmonary fibrosis and its comorbidities

Idiopathic pulmonary fibrosis (IPF) is a specific lung disorder characterized by progressive parenchymal scarring, leading to dyspnoea, respiratory failure and premature death. As implied by the name, there is no identified cause for the disease, but patients with IPF are generally older males with a history of smoking with or without occupational exposure to agriculture or dusts from wood or metal.[1]

The diagnosis requires the identification of the histological or radiological pattern of usual interstitial pneumonia and exclusion of known causes for pulmonary fibrosis, such as hypersensitivity pneumonitis, autoimmune disease and drugs. This tends to be rather complex, so international guidelines recommend a multidisciplinary approach.[2,3]

The treatment is based on the use of antifibrotics, nintedanib or pirfenidone, which reduce the progression of the disease, improving prognosis. Younger patients should be referred for lung transplant, as this is the only cure for the disease.[3]

Although IPF as a disease is limited to the lungs, middle aged and older subjects, such as the typical IPF patient, are at risk for comorbidities. Indeed, the importance of IPF comorbidities has been increasingly recognized. A 2016 study showed that 88% of 272 IPF patients have at least one

Correspondence to:
Carlos Robalo Cordeiro
Pneumology Unit, Centro Hospital e Universitário de Coimbra, Praceta Mota Pinto, Coimbra 3000-085, Portugal
Centre of Pneumology, Faculty of Medicine, University of Coimbra, Coimbra, Portugal
Carlos.crobalo@gmail.com

Tiago M. Alfaro
Pneumology Unit, Centro Hospital e Universitário de Coimbra, Coimbra, Portugal
Centre of Pneumology, Faculty of Medicine, University of Coimbra, Coimbra, Portugal

comorbidity, with 70% having two or more.[4] The most frequent and relevant ones are pulmonary hypertension and ischaemic heart disease, lung cancer, emphysema/chronic obstructive pulmonary disease (COPD), gastroesophageal reflux, sleep apnoea and depression.[5]

These comorbidities can have a profound impact on the prognosis of IPF. Arteriosclerosis, cardiovascular disease and cancer were all associated with increased mortality in IPF populations. Additionally, they may lead to significant loss of quality of life and to further difficulties or delays in diagnosing and treating IPF.[6]

It is clear that the identification and management of comorbidities are vital for the optimal management of this severe disease, but the diagnosis and evaluation of these conditions may be complex and delayed. The development of improved methods for the assessment of IPF comorbidities could have profound impacts on the quality of life and possibly the survival of IPF patients.[7]

Biomarkers

Biomarkers have been 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'.[8]

The diagnosis of IPF and other interstitial lung diseases is frequently complex and delayed, as clinical manifestations tend to be protracted and nonspecific. The same problem is frequently found for assessment of severity and progression, and even response to therapy.[9]

The development of good-quality biomarkers for this application could lead to numerous gains in the management of these patients. Biomarkers can be used for the identification of predisposed individuals, early diagnosis of those affected, assessment of prognosis, selection of best treatment and assessment of response to treatment. This last use would be particularly beneficial for selection of best candidates or as a surrogate endpoint in clinical trials. Understandably, there has been a significant research effort on the development of new biomarkers for IPF.[10]

The ideal biomarker should be reproducible, have high sensitivity and specificity, accuracy, acceptable costs, added value and should be validated in multicentric studies in heterogenous populations. As availability and ease of assessment are critical for the success of any new biomarker, the best sources of biomarkers for IPF are probably the peripheral blood, with exhaled breath condensate as another possible option. Importantly, biomarkers can be molecular, histological, radiographic and physiological, but the development of biomarkers for interstitial lung disease (ILD) has focused mostly on molecular biomarkers.[7]

Biomarkers for IPF

Molecular biomarkers for IPF can be developed using two main approaches. An unbiased approach uses methods from systems biology, such as genomics or proteomics, to screen a wide range of candidate markers. This increases discovery efficiency, but also the probability of false discovery. On the hypothesis-driven method, candidate biomarkers are selected *a priori* from previous knowledge about the mechanisms of the disease, and then studied and validated on patient populations. Most of existing research followed this second path.[7]

Ideally, molecular biomarkers should reflect the presence and activity levels of relevant pathogenic mechanisms. The pathogenesis of IPF is not yet fully known, but probably includes an initial injury, such as smoking, chronic viral infection or occupational exposure, followed by abnormal repair, leading to excess extracellular matrix deposition. Some additional characteristics include immune system activation, and vascular and epithelial damage.[1]

Consequently, the most promising biomarkers for IPF are those associated with epithelial cell damage or dysfunction, fibrogenesis and matrix remodelling, immune dysregulation and oxidative stress.[11]

Biomarkers for comorbidities

Cardiovascular

Pulmonary hypertension (PH) is characterized by loss and obstructive remodelling of the pulmonary vascular bed, leading to a rise in pulmonary arterial pressure and pulmonary vascular resistance (PVR). This results in progressive right-heart failure, functional decline and increased risk for mortality.[12]

PH is, in fact, a group of diseases, as the condition may be associated with different pathophysiological mechanisms, clinical presentations, haemodynamic characteristics and response to therapy. The most recent clinical classification of PH, from the 6th World Symposium on Pulmonary Hypertension maintains a five-group classification: group 1: pulmonary arterial hypertension; group 2: PH due to left heart disease; group 3: PH due to lung diseases or hypoxia; group 4: PH due to pulmonary artery obstructions; and group 5: PH with unclear or multifactorial mechanisms. The same task force also proposed a new haemodynamic definition of PH: precapillary PH, found in groups 1, 3, 4 and 5 is defined by the concomitant presence of mean pulmonary arterial pressure (mPAP) $>$ 20 mmHg, pulmonary arterial wedge pressure (PAWP) $\leqslant$ 15 mmHg and PVR = (mPAP–PAWP) / cardiac output $\geqslant$ 3 Wood units.[13]

Patients with IPF are at risk for group 2 PH, but the reported prevalence ranges from 3% to 86%, with most estimates varying between 30% and 50%. This wide range results from differences in the definition of PH and the characteristics of the IPF population that was studied. Several studies have estimated the prevalence of PH based on the use of echocardiography, which is useful as a non-invasive screening tool, but cannot establish diagnosis, as diagnosis must be confirmed by right-heart catheterization.[14]

The prevalence of PH increases with the severity of IPF, with reports of a prevalence under 10% at diagnosis and over 32% in more advanced patients on a transplant list.[15]

Several studies have found a higher risk for PH in patients with coexistent emphysema, which is referred to as 'combined pulmonary fibrosis and emphysema' (CPFE). Importantly, it has been shown that patients with IPF and PH have increased risk for death, despite adjustment for lung function.[16]

The pathogenesis of PH in patients with IPF is complex and only partially known. An important consideration is that the development of PH is probably more dependent on the mechanisms for fibrosis than on the chronic hypoxemia or lung function reduction. Some of the molecular pathways involved on the development of PH include endothelin-1, transforming growth factor beta, platelet-derived growth factor, adenosine signalling and bone morphogenetic protein receptor type 2.[15,17]

The development of biomarkers for PH in IPF can benefit from current understanding of the pathogenesis, but none of the candidate biomarkers are currently recommended for routine clinical use. Some of the biomarkers being studied for PH include molecular targets associated with endothelial dysfunction, inflammation, oxidative stress, cardiac function, metabolism and extracellular matrix. Some of these biomarkers may also be useful for the diagnosis and prognosis of IPF-associated PH.[18]

A Danish study assessed 212 patients with ILD (29 with PH), and found that serum N-terminal probrain natriuretic peptide $<$ 95 ng/l can be useful as a rule-out test for PH. There was also an association between higher values of this marker and mortality. Increased uric acid was associated with the presence of PH, and uric acid, troponin T and fibrin d-dimer levels were associated with a worse prognosis.[19]

Some other biomarkers shown to predict PH in IPF include CXCL13, a B-cell homing chemokine. A 2014 study found higher plasma concentrations for this protein in those IPF patients with PH, as well as those with more severe disease.[20]

Another molecule involved in immune and oxidative stress mechanisms, S100A12 (Calgranulin C, EN-RAGE), was found in higher levels in peripheral blood mononuclear cells from patients with pulmonary-fibrosis-associated PH.[21]

Patients with IPF are at higher risk for arrythmia, mainly atrial fibrillation, in those referred for lung transplant. Several mechanisms may be involved, including hypoxia, pulmonary haemodynamic changes, coronary artery disease (CAD) and chronic inflammation.[22]

The classes of possible biomarkers for arrythmia that have been studied include markers of inflammation, oxidative stress, renin–angiotensin–aldosterone system, mechanical stress, myocardial remodelling, thrombosis and renal function. The most promising markers for the risk of atrial fibrillation (AF) are C-reactive protein as a marker of inflammation and B-type natriuretic peptide. Galectin-3, matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs), are also

promising as biomarkers for the risk of incident or relapsing AF.[23]

CAD shares major risk factors with IPF, such as smoking and ageing. Additionally, IPF may contribute for the development of CAD through hypoxia and increased levels of cytokines and other inflammatory mediators.[22] A 2009 study found a 28.6% prevalence of coronary disease in patients with IPF compared with 9.8% in those with emphysema.[24]

Importantly, the presence of IPF may lead to delays in the identification and treatment of CAD, as new complaints may be attributed to IPF and not comorbid CAD. Patients with IPF are less likely to receive statins and beta-blockers than those without IPF.[25]

The biomarker classes being researched for CAD are similar to those for arrythmia and include markers for myocardial stretch, myocardial injury, inflammation and oxidative stress. One general concern is that patients with severe pulmonary disease can have elevation of these markers even in the absence of CAD. Additionally, many studies for biomarkers exclude those patients with pulmonary diseases.[24]

Gastrointestinal disease

Gastro-oesophageal reflux disease (GORD) is a common comorbidity of IPF, with studies reporting a prevalence of about 60%, although a large proportion of patients are asymptomatic.[26] IPF and GORD seem to be associated, although a significant confounding from smoking is likely.[27] The persistent micro-aspiration of gastric acid to the respiratory system may lead to chronic inflammation and possibly fibrosis. Conversely, pulmonary fibrosis leads to reduced lung compliance, which increases thoracic pressure and may lead to further reflux.[27]

The effects of GORD on the prognosis of IPF are less clear. A retrospective study on pretransplant IPF subjects showed more severe disease in those with GORD, while others even showed improved survival in those with comorbid GORD.[4,28]

A long-time hypothesis has been that the treatment of GORD could reduce the progression of IPF, but this has been difficult to study. Several retrospective studies showed that the use of antiacid drugs led to improved outcomes in IPF populations, but these results may have been biased.[29] A *post hoc* analysis of the placebo group of three randomized control trials for antifibrotics on IPF showed no effect of antiacid treatment but found a higher risk for infections on those with more severe fibrosis.[30] International guidelines for the treatment of IPF, make a weak recommendation for regular antiacid treatment of patients with IPF, but most experts agree that the benefits of medical treatment for GORD in IPF are uncertain.[3,27]

The diagnosis of GORD is usually based on the use of 24-h-long oesophageal pH and pressure measuring, but the cost and availability of these tests limit their usefulness. The development of diagnosis biomarkers is necessary.[27]

Pepsin, a gastric proteolytic enzyme, has been studied as a biomarker for reflux. Salivary pepsin levels are easy to collect and assess and are currently being validated as a diagnostic biomarker.[31] The levels of this protein on bronchoalveolar lavage (BAL) seem to predict aspiration with good sensitivity and specificity and this may be more valuable. In addition, BAL pepsin levels are higher during IPF exacerbations, and are associated with the prognosis of chronic fibrosing lung disease. Another possible biomarker is the assessment of bile acids, as not all reflux episodes are acidic. BAL bile acids may be assessed in conjunction with pepsin, but it is not clear if this would be useful. An important consideration for the use of BAL pepsin is that BAL is frequently not performed in patients with a final diagnosis of IPF, and it is too invasive for repeat testing.[32] The use of exhaled-breath condensate could overcome these limitations, but EBC pepsin levels did not predict GORD, while bile acids were below the detection limit in one study in transplanted subjects.[33,34]

Lung cancer

Patients with IPF are recognized as having a high risk for lung cancer, with one study showing a cumulative incidence of 40% after 1 year of follow up and 82% after 3 years. IPF has been recognized as risk factor for cancer, independent from smoking and other environmental exposures.[35]

Most patients with this association do not display symptoms and are diagnosed incidentally. Lung cancer has an important effect on IPF mortality,

with an adjusted hazard ratio of 7.0 (95% confidence interval, 3.81–12.90; $p < 0.001$) compared with those with only IPF.[35] Most diagnoses are on older male smokers, and those with CPFE.[36]

Some other specificities of lung cancer on subjects with IPF are a basal location, near areas of fibrosis and a higher frequency of squamous cell carcinoma than adenocarcinoma. The increased mortality in patients with LC-IPF was also associated with a higher risk for adverse events from cancer diagnosis and treatment. This makes clinical decisions about the diagnosis and treatment of lung cancer more complex. However, despite a high risk for acute exacerbation and death from surgery and other treatments in this population, some patients may experience long-term survival after surgery, and this should be taken into consideration.[36,37]

The mechanisms underlying the association between lung cancer and IPF have been the subject of several reviews.[38,39] Several common pathways between these two disorders have been reported, and include genetic and epigenetic changes, imbalances in cellular/tissue growth and migration, as well as changes in extracellular mediators.[40]

Concerning biomarkers, lung cancer has been one of the most successful areas of biomarker development, but most are used for treatment selection. International recommendations for their use are available and frequently updated.[41]

It would be useful, however, to have biomarkers for early detection of lung cancer in IPF, but these are less well established. The only procedure that has shown an improvement on the early diagnosis and treatment for non-small cell lung cancer is annual low-dose computed tomography, but this has not yet been validated in IPF patients.[42,43] Several serum/plasma biomarkers have been studied, and are available, but their clinical value is also not established. Some of the most promising serum markers include the carcino-embryonic antigen, cytokeratin 19-fragments (CYFRA 21-1) and squamous cancer-cell antigen for non-small cell lung cancer, as well as progastrin-releasing peptide and neuron-specific enolase (NSE) for small-cell lung cancer.[44] Another promising area for biomarkers is microribonucleic acids, which can be found in serum and used as a marker for diagnosis. The most promising are miR-25, 141, 155, 223, 629 and 1254.[45]

In addition to blood, sputum and exhaled breath may be a less-invasive way for collection of biomarkers, and several studies have pointed to possible utility of these systems. One study integrated breath analysis with artificial intelligence in order to assess pulmonary nodules for malignancy with good results.[46,47]

Psychiatric disease

Anxiety and depression are common in those with chronic respiratory disorders. The prevalence of depression in IPF is about 25%, which is similar to what is found on COPD.[48,49] The causes for this association include both psychological and physiological mechanisms. One hypothesis for the physiological effects is that hypoxia leads to central nervous system changes that may lead to depression.[50] In fact, studies have shown that people living at higher altitudes and subject to hypobaric hypoxia are at higher risk for depression and suicide.[51]

Psychiatric disease was not associated with survival in IPF but had a major impact on patient quality of life, so active identification and treatment is recommended. The best screening method for depression in IPF has not been validated, so a diagnosis biomarker could lead to better outcomes, at least on quality of life.[52]

The most promising biomarkers for the diagnosis of depression include those associated with inflammation, neuroendocrine function, neurotropic growth factors, neurotransmitters and metabolism. However, several of these systems may be changed in IPF patients, so specific validation for this population is important, but has not been done yet.[53]

Sleep disorders

The prevalence of respiratory sleep disturbances on IPF is higher than in the general population, with studies reporting a prevalence of 50% to about 90%. The pathogenic relationship between these two conditions has not been proven, but current hypotheses include reduced lung volumes predisposing to upper airway closure. Other contributing factors may include treatment with steroids and ventilatory control instability caused by higher chemo-responsiveness to hypoxia. On the other hand, sleep apnoea may lead to enhanced oxidative stress and gastroesophageal reflux, which

can worsen IPF.[54] One study found an association between obstructive sleep apnoea (OSA) and coronary heart disease in IPF patients.[55]

The identification and treatment of OSA in IPF patients is recommended, as continuous positive airway pressure leads to improvement in quality of life.[56] However, OSA complaints tend to be nonspecific. The main complaint is usually daytime fatigue. Questionnaires are not accurate for the diagnosis of OSA and performing sleep studies in all patients is too expensive. A search for biomarkers for OSA on IPF is warranted, but so far, no marker has been validated in this specific population.[54,57]

The most promising markers for the diagnosis of OSA on the general population include inflammatory markers, such as interleukin 6 (IL-6) and IL-10. A combination of biomarkers has also been studied as a screening method for OSA.[58,59]

Emphysema

Pulmonary emphysema is usually a consequence of smoking and as such shares a major risk factor with IPF. In addition to smoking, COPD and IPF are both related to ageing and share some pathogenic mechanisms.[60] The rates of emphysema in IPF vary from 8% to 51%.[5] CPFE is considered a specific IPF phenotype and is characterized by preserved lung volumes, reduced diffusion capacity, and higher risk for pulmonary hypertension, lung cancer and mortality. The identification of emphysema has important prognostic implications, but the consequences of this comorbidity on treatment strategies are less known. Antifibrotic treatments are generally recommended for this population, as patients with emphysema were recruited to major trials for these drugs. There is no evidence on other treatments, such as inhaled bronchodilators or steroids.[61]

Considering diagnosis, emphysema is usually identified and quantified by high-resolution chest tomography, which is always performed in patients with IPF.[62] Nevertheless, biomarkers can be useful for predicting prognosis and predict/assess response to therapy. One study assessed the tissue levels of 34 proteins and found a similar inflammatory signature in CPFE and IPF.[63] Other studies found lower levels of Krebs von den lungen-6 and CYFRA21-1 in peripheral blood of those with CPFE compared with IPF.[64,65]

Conclusion

There is wide consensus on the importance of identifying and treating IPF comorbidities to improve the quality of life and prognosis of this population.

Table 1. Biomarkers used for diagnosis or as a treatment guide for comorbidities in IPF.

Comorbidity	Function	Group	Examples
Cardiovascular: pulmonary hypertension	Diagnosis	Endothelial cell	Caveolin-1[66]
		Blood-vessel components	Desmosine, isodesmosine[67]
		Angiogenic markers	Angiogenin, tumour necrosis factor-alpha[68]
		Genetic	miR-23a[69]
	Prognosis	Heart function	B-type natriuretic peptide[70]
		Inflammation	Interleukin-6,[71]CXCL13, osteopontin[72]
		Neuroendocrine activation	Midregional pro-adrenomedullin[73]
		Vascular remodelling	N-terminal propeptide (type III procollagen)[74]
	Treatment	Heart function	B-type natriuretic peptide[70]
		Therapeutic pathway components	FENO,[75]cGMP[76]

(Continued)

Table 1. (Continued)

Comorbidity	Function	Group	Examples
Gastrointestinal: gastro-oesophageal reflux disease	Diagnosis	Gastric enzymes	Pepsin,[77] bile acids
		Inflammation	IL-8, substance P[32]
		Oxidative stress	8-isoprostane[78]
Lung cancer	Diagnosis	Tumour markers	NSE, CEA, CYFRA, ProGRP[44]
Psychiatric: depression	Diagnosis/ treatment	Inflammation	IL-6, CRP[79]
		HPA	Cortisol[80]
		Growth factors	BDNF[81]
		Metabolism	Adipokines[82]
Sleep apnoea	Diagnosis	Metabolism	HbA1c[59]
		Inflammation	CRP[59]
Pulmonary: emphysema	Diagnosis	Tumour markers	CYFRA21-1[65]
		Alveolar cells	KL-6[65]

Noncomprehensive list of biomarkers used for diagnosis or as a treatment guide for comorbidities in IPF. Importantly, no biomarker has been validated for clinical use in IPF.
BDNF, brain-derived neurotrophic factor; CEA, carcinoembryonic antigen; CRP, C-reactive protein; CXCL13, chemokine (C-X-C motif) ligand 13; CYFRA 21-1, cytokeratin-19 soluble fragment; FENO, fractional exhaled nitric oxide; cGMP, cyclic guanosine monophosphate; HbA1c, glycated haemoglobin; HPA, hypothalamic–pituitary axis; IL, interleukin; IPF, idiopathic pulmonary fibrosis; KL-6, Krebs von den lungen-6; miR, microribonucleic acid; NSE, neuron-specific enolase; ProGRP, progastrin-releasing peptide.

The role of biomarkers for IPF comorbidities is still quite limited, and mostly based on evidence coming from populations without IPF. A non-comprehensive list of biomarkers for some of the IPF comorbidities is presented in Table 1.

Biomarkers have a promising role for the screening, early diagnosis and selection of best treatment for many of these associated conditions, but the number of studies on this specific population is quite limited. However, future development of studies could be informed by those biomarkers studied independently for each of these conditions.

For now, clinicians should be mostly attentive to clinical manifestations of IPF comorbidities, and use validated diagnostic methods for diagnosis. As research on biomarkers for most common diseases continues, it is expected that useful biomarkers are developed and then validated for IPF populations.

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Conflict of interest statement
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ORCID iD
Carlos Robalo Cordeiro https://orcid.org/0000-0002-8264-3856

Supplemental material
The reviews of this paper are available via the supplemental material section.

References
1. Martinez FJ, Collard HR, Pardo A, *et al.* Idiopathic pulmonary fibrosis. *Nat Rev Dis Primers* 2017; 3: 17074.

2. Raghu G, Remy-Jardin M, Myers JL, *et al.* Diagnosis of idiopathic pulmonary fibrosis. An official ATS/ERS/JRS/ALAT clinical practice guideline. *Am J Respir Crit Care Med* 2018; 198: e44–e68.
3. Raghu G, Rochwerg B, Zhang Y, *et al.* An official ATS/ERS/JRS/ALAT clinical practice guideline: treatment of idiopathic pulmonary fibrosis. An update of the 2011 clinical practice guideline. *Am J Respir Crit Care Med* 2015; 192: e3–e19.
4. Kreuter M, Ehlers-Tenenbaum S, Palmowski K, *et al.* Impact of comorbidities on mortality in patients with idiopathic pulmonary fibrosis. *PLoS One* 2016; 11: e0151425.
5. Torrisi SE, Vancheri A, Pavone M, *et al.* Comorbidities of IPF: how do they impact on prognosis. *Pulm Pharmacol Ther* 2018; 53: 6–11.
6. Margaritopoulos GA, Kokosi MA and Wells AU. Diagnosing complications and co-morbidities of fibrotic interstitial lung disease. *Expert Rev Respir Med* 2019; 13: 645–658.
7. Ley B, Brown KK and Collard HR. Molecular biomarkers in idiopathic pulmonary fibrosis. *Am J Physiol Lung Cell Mol Physiol* 2014; 307: L681–L691.
8. Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin Pharmacol Ther* 2001; 69: 89–95.
9. Barratt SL, Creamer A, Hayton C, *et al.* Idiopathic pulmonary fibrosis (IPF): an overview. *J Clin Med* 2018; 7.
10. Drakopanagiotakis F, Wujak L, Wygrecka M, *et al.* Biomarkers in idiopathic pulmonary fibrosis. *Matrix Biol* 2018; 68-69: 404–421.
11. Guiot J, Moermans C, Henket M, *et al.* Blood biomarkers in idiopathic pulmonary fibrosis. *Lung* 2017; 195: 273–280.
12. Humbert M, Guignabert C, Bonnet S, *et al.* Pathology and pathobiology of pulmonary hypertension: state of the art and research perspectives. *Eur Respir J* 2019; 53: 1801887.
13. Simonneau G, Montani D, Celermajer DS, *et al.* Haemodynamic definitions and updated clinical classification of pulmonary hypertension. *Eur Respir J* 2019; 53: 1801913.
14. Raghu G, Amatto VC, Behr J, *et al.* Comorbidities in idiopathic pulmonary fibrosis patients: a systematic literature review. *Eur Respir J* 2015; 46: 1113–1130.
15. Collum SD, Amione-Guerra J, Cruz-Solbes AS, *et al.* Pulmonary hypertension associated with idiopathic pulmonary fibrosis: current and future perspectives. *Can Respir J* 2017; 2017: 1430350.
16. Caminati A, Cassandro R and Harari S. Pulmonary hypertension in chronic interstitial lung diseases. *Eur Respir Rev* 2013; 22: 292–301.
17. Singh I, Ma KC and Berlin DA. Pathophysiology of pulmonary hypertension in chronic parenchymal lung disease. *Am J Med* 2016; 129: 366–371.
18. Frost A, Badesch D, Gibbs JSR, *et al.* Diagnosis of pulmonary hypertension. *Eur Respir J* 2019; 53: 1801904.
19. Andersen CU, Mellemkjaer S, Nielsen-Kudsk JE, *et al.* Diagnostic and prognostic role of biomarkers for pulmonary hypertension in interstitial lung disease. *Respir Med* 2012; 106: 1749–1755.
20. Vuga LJ, Tedrow JR, Pandit KV, *et al.* C-X-C motif chemokine 13 (CXCL13) is a prognostic biomarker of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2014; 189: 966–974.
21. Tzouvelekis A, Herazo-Maya JD, Ryu C, *et al.* S100A12 as a marker of worse cardiac output and mortality in pulmonary hypertension. *Respirology* 2018; 23: 771–779.
22. Agrawal A, Verma I, Shah V, *et al.* Cardiac manifestations of idiopathic pulmonary fibrosis. *Intractable Rare Dis Res* 2016; 5: 70–75.
23. Krummen DE and Daniels LB. Biomarkers in arrhythmias, sudden death, and device therapy. In: Maisel AS and Jaffe AS (eds) *Cardiac biomarkers: case studies and clinical correlations*. Cham: Springer International Publishing, 2016, pp.329–343.
24. Izbicki G, Ben-Dor I, Shitrit D, *et al.* The prevalence of coronary artery disease in end-stage pulmonary disease: is pulmonary fibrosis a risk factor? *Respir Med* 2009; 103: 1346–1349.
25. Hubbard RB, Smith C, Le Jeune I, *et al.* The association between idiopathic pulmonary fibrosis and vascular disease: a population-based study. *Am J Respir Crit Care Med* 2008; 178: 1257–1261.
26. Raghu G, Freudenberger TD, Yang S, *et al.* High prevalence of abnormal acid gastro-oesophageal reflux in idiopathic pulmonary fibrosis. *Eur Respir J* 2006; 27: 136-142.
27. Wang Z, Bonella F, Li W, *et al.* Gastroesophageal reflux disease in idiopathic pulmonary fibrosis: uncertainties and controversies. *Respiration* 2018; 96: 571–587.
28. Gavini S, Borges LF, Finn RT, *et al.* Lung disease severity in idiopathic pulmonary fibrosis is more strongly associated with impedance measures of bolus reflux than pH parameters of acid reflux alone. *Neurogastroenterol Motil* 2017; 29: e13001.

29. Kreuter M and Raghu G. Gastro-oesophageal reflux and idiopathic pulmonary fibrosis: the heart burn in patients with IPF can no longer be silent. *Eur Respir J* 2018; 51: 1800921.
30. Kreuter M, Wuyts W, Renzoni E, *et al.* Antacid therapy and disease outcomes in idiopathic pulmonary fibrosis: a pooled analysis. *Lancet Respir Med* 2016; 4: 381–389.
31. Guo Z, Wu H, Jiang J, *et al.* Pepsin in saliva as a diagnostic marker for gastroesophageal reflux disease: a meta-analysis. *Med Sci Monit* 2018; 24: 9509–9516.
32. Meyer KC. Gastroesophageal reflux and lung disease. *Expert Rev Respir Med* 2015; 9: 383–385.
33. Lee AL, Button BM, Denehy L, *et al.* Exhaled breath condensate pepsin: potential noninvasive test for gastroesophageal reflux in COPD and bronchiectasis. *Respir Care* 2015; 60: 244–250.
34. Reder NP, Davis CS, Kovacs EJ, *et al.* The diagnostic value of gastroesophageal reflux disease (GERD) symptoms and detection of pepsin and bile acids in bronchoalveolar lavage fluid and exhaled breath condensate for identifying lung transplantation patients with GERD-induced aspiration. *Surg Endosc* 2014; 28: 1794–1800.
35. Tomassetti S, Gurioli C, Ryu JH, *et al.* The impact of lung cancer on survival of idiopathic pulmonary fibrosis. *Chest* 2015; 147: 157–164.
36. Antoniou KM, Tomassetti S, Tsitoura E, *et al.* Idiopathic pulmonary fibrosis and lung cancer: a clinical and pathogenesis update. *Curr Opin Pulm Med* 2015; 21: 626–633.
37. Tzouvelekis A, Spagnolo P, Bonella F, *et al.* Patients with IPF and lung cancer: diagnosis and management. *Lancet Respir Med* 2018; 6: 86–88.
38. Vancheri C. Idiopathic pulmonary fibrosis and cancer: do they really look similar? *BMC Med* 2015; 13: 220.
39. Ballester B, Milara J and Cortijo J. Idiopathic pulmonary fibrosis and lung cancer: mechanisms and molecular targets. *Int J Mol Sci* 2019; 20.
40. Vancheri C. Common pathways in idiopathic pulmonary fibrosis and cancer. *Eur Respir Rev* 2013; 22: 265–272.
41. Planchard D, Popat S, Kerr K, *et al.* Metastatic non-small cell lung cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2018; 29: iv192–iv237.
42. Seijo LM, Peled N, Ajona D, *et al.* Biomarkers in lung cancer screening: achievements, promises, and challenges. *J Thorac Oncol* 2019; 14: 343–357.
43. Fulton BG and Ryerson CJ. Managing comorbidities in idiopathic pulmonary fibrosis. *Int J Gen Med* 2015; 8: 309–318.
44. Holdenrieder S. Biomarkers along the continuum of care in lung cancer. *Scand J Clin Lab Invest Suppl* 2016; 245: S40–S45.
45. Qin X, Xu H, Gong W, *et al.* The tumor cytosol miRNAs, fluid miRNAs, and exosome miRNAs in lung cancer. *Front Oncol* 2014; 4: 357.
46. Nakhleh MK, Amal H, Jeries R, *et al.* Diagnosis and classification of 17 diseases from 1404 subjects via pattern analysis of exhaled molecules. *ACS Nano* 2017; 11: 112–125.
47. Peled N, Hakim M, Bunn PA Jr., *et al.* Non-invasive breath analysis of pulmonary nodules. *J Thorac Oncol* 2012; 7: 1528–1533.
48. Lee YJ, Choi SM, Lee YJ, *et al.* Clinical impact of depression and anxiety in patients with idiopathic pulmonary fibrosis. *PLoS One* 2017; 12: e0184300.
49. Matte DL, Pizzichini MM, Hoepers AT, *et al.* Prevalence of depression in COPD: a systematic review and meta-analysis of controlled studies. *Respir Med* 2016; 117: 154–161.
50. Kumar GK. Hypoxia. 3. Hypoxia and neurotransmitter synthesis. *Am J Physiol Cell Physiol* 2011; 300: C743–C751.
51. Kious BM, Kondo DG and Renshaw PF. Living high and feeling low: altitude, suicide, and depression. *Harv Rev Psychiatry* 2018; 26: 43–56.
52. Oldham JM and Collard HR. Comorbid conditions in idiopathic pulmonary fibrosis: recognition and management. *Front Med (Lausanne)* 2017; 4: 123.
53. Strawbridge R, Young AH and Cleare AJ. Biomarkers for depression: recent insights, current challenges and future prospects. *Neuropsychiatr Dis Treat* 2017; 13: 1245–1262.
54. Troy LK and Corte TJ. Sleep disordered breathing in interstitial lung disease: a review. *World J Clin Cases* 2014; 2: 828–834.
55. Gille T, Didier M, Boubaya M, *et al.* Obstructive sleep apnoea and related comorbidities in incident idiopathic pulmonary fibrosis. *Eur Respir J* 2017; 49.
56. Mermigkis C, Bouloukaki I, Antoniou K, *et al.* Obstructive sleep apnea should be treated in patients with idiopathic pulmonary fibrosis. *Sleep Breath* 2015; 19: 385–391.
57. Lancaster LH, Mason WR, Parnell JA, *et al.* Obstructive sleep apnea is common in idiopathic pulmonary fibrosis. *Chest* 2009; 136: 772–778.

58. Canto Gde L, Pacheco-Pereira C, Aydinoz S, *et al.* Biomarkers associated with obstructive sleep apnea: a scoping review. *Sleep Med Rev* 2015; 23: 28–45.

59. Fleming WE, Holty JC, Bogan RK, *et al.* Use of blood biomarkers to screen for obstructive sleep apnea. *Nat Sci Sleep* 2018; 10: 159–167.

60. Chilosi M, Carloni A, Rossi A, *et al.* Premature lung aging and cellular senescence in the pathogenesis of idiopathic pulmonary fibrosis and COPD/emphysema. *Transl Res* 2013; 162: 156–173.

61. Millan-Billi P, Serra C, Alonso Leon A, *et al.* Comorbidities, complications and non-pharmacologic treatment in idiopathic pulmonary fibrosis. *Med Sci (Basel)* 2018; 6.

62. Cottin V. The impact of emphysema in pulmonary fibrosis. *Eur Respir Rev* 2013; 22: 153–157.

63. Cornwell WD, Kim C, Lastra AC, *et al.* Inflammatory signature in lung tissues in patients with combined pulmonary fibrosis and emphysema. *Biomarkers* 2019; 24: 232–239.

64. Kokuho N, Ishii T, Kamio K, *et al.* Diagnostic values for club cell secretory protein (CC16) in serum of patients of combined pulmonary fibrosis and emphysema. *COPD* 2015; 12: 347–354.

65. Xu L, Bian W, Gu XH, *et al.* Differing expression of cytokines and tumor markers in combined pulmonary fibrosis and emphysema compared to emphysema and pulmonary fibrosis. *COPD* 2017; 14: 245–250.

66. Wang KY, Lee MF, Ho HC, *et al.* Serum caveolin-1 as a novel biomarker in idiopathic pulmonary artery hypertension. *Biomed Res Int* 2015; 2015: 173970.

67. Minkin R, Sandhu G, Grosu H, *et al.* Desmosine and isodesmosine as a novel biomarker for pulmonary arterial hypertension: a pilot study. *Am J Ther* 2017; 24: e399–e404.

68. Rameh V and Kossaify A. Role of biomarkers in the diagnosis, risk assessment, and management of pulmonary hypertension. *Biomark Insights* 2016; 11: 85–89.

69. Sarrion I, Milian L, Juan G, *et al.* Role of circulating miRNAs as biomarkers in idiopathic pulmonary arterial hypertension: possible relevance of miR-23a. *Oxid Med Cell Longev* 2015; 2015: 792846.

70. Nagaya N, Nishikimi T, Uematsu M, *et al.* Plasma brain natriuretic peptide as a prognostic indicator in patients with primary pulmonary hypertension. *Circulation* 2000; 102: 865–870.

71. Heresi GA, Aytekin M, Hammel JP, *et al.* Plasma interleukin-6 adds prognostic information in pulmonary arterial hypertension. *Eur Respir J* 2014; 43: 912–914.

72. Rosenberg M, Meyer FJ, Gruenig E, *et al.* Osteopontin (OPN) improves risk stratification in pulmonary hypertension (PH). *Int J Cardiol* 2012; 155: 504–505.

73. Kolditz M, Seyfarth HJ, Wilkens H, *et al.* MR-proADM predicts exercise capacity and survival superior to other biomarkers in PH. *Lung* 2015; 193: 901–910.

74. Safdar Z, Tamez E, Chan W, *et al.* Circulating collagen biomarkers as indicators of disease severity in pulmonary arterial hypertension. *JACC Heart Fail* 2014; 2: 412–421.

75. Machado RF, Londhe Nerkar MV, Dweik RA, *et al.* Nitric oxide and pulmonary arterial pressures in pulmonary hypertension. *Free Radic Biol Med* 2004; 37: 1010–1017.

76. Michelakis E, Tymchak W, Lien D, *et al.* Oral sildenafil is an effective and specific pulmonary vasodilator in patients with pulmonary arterial hypertension: comparison with inhaled nitric oxide. *Circulation* 2002; 105: 2398–2403.

77. Johnston N, Dettmar PW, Ondrey FG, *et al.* Pepsin: biomarker, mediator, and therapeutic target for reflux and aspiration. *Ann N Y Acad Sci* 2018; 1434: 282–289.

78. Emilsson OI, Benediktsdottir B, Olafsson I, *et al.* Respiratory symptoms, sleep-disordered breathing and biomarkers in nocturnal gastroesophageal reflux. *Respir Res* 2016; 17: 115.

79. Haapakoski R, Mathieu J, Ebmeier KP, *et al.* Cumulative meta-analysis of interleukins 6 and 1 beta, tumour necrosis factor alpha and C-reactive protein in patients with major depressive disorder. *Brain Behav Immun* 2015; 49: 206-215.

80. Fischer S, Strawbridge R, Vives AH, *et al.* Cortisol as a predictor of psychological therapy response in depressive disorders: systematic review and meta-analysis. *Br J Psychiatry* 2017; 210: 105–109.

81. Molendijk ML, Spinhoven P, Polak M, *et al.* Serum BDNF concentrations as peripheral manifestations of depression: evidence from a systematic review and meta-analyses on 179 associations (N=9484). *Mol Psychiatry* 2014; 19: 791–800.

82. Carvalho AF, Rocha DQ, McIntyre RS, *et al.* Adipokines as emerging depression biomarkers: a systematic review and meta-analysis. *J Psychiatr Res* 2014; 59: 28–37.