Chapter 4

Pulmonary arterial hypertension in connective tissue diseases

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SUMMARY: Pulmonary arterial hypertension (PAH) is a common complication of connective tissue diseases (CTD) and a leading cause of death in this population. Despite significant advances in therapy for PAH, particularly for idiopathic PAH (IPAH), the response to treatment in patients with CTD associated with PAH, mainly in scleroderma-associated PAH (SSc-PAH), has been quite disappointing. This chapter reviews the epidemiology, clinical manifestations, pathophysiology and currently available therapies for CTD-associated PAH with a particular focus on SSc-PAH, animal models of disease, and newly identified potential targets for therapy for this devastating syndrome.

KEYWORDS: Connective tissue disease, pulmonary arterial hypertension, systemic sclerosis, therapy

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Pulmonary arterial hypertension (PAH) is a relentlessly progressive disease, consisting of structural remodelling and obliteration of the pulmonary distal vessels, with significant morbidity leading eventually to death through right ventricular (RV) failure [1, 2]. PAH, or Group 1 of the classification of pulmonary hypertension (PH), includes several clinical entities sharing similar pathological alterations, among them idiopathic PAH (IPAH) and heritable PAH (associated with known or presumed gene defects), and PAH associated with diseases or conditions such as connective tissue diseases (CTD), porto-pulmonary hypertension, infectious conditions (*e.g.* HIV) and drugs and toxins [3].

While the histological changes of PAH have been well characterised [4], the cellular and molecular mechanisms underlying this syndrome remain poorly understood [5]. However, there has been a growing interest in autoimmunity and inflammatory processes as putative driving forces in the development of pulmonary vascular changes [6] based on several clinical observations, including the presence of circulating autoantibodies [7] and pro-inflammatory cytokines (*e.g.* interleukin (IL)-1 and IL-6) [8], in patients with IPAH and the common association of PAH with autoimmune diseases such as systemic sclerosis (SSc) and systemic lupus erythematosus (SLE). In this chapter, we will review the characteristics of PAH associated with CTD, with special emphasis

on SSc-PAH since it is the most common but also most severe syndrome among all forms of CTD including SLE, mixed connective tissue disease (MCTD), rheumatoid arthritis, dermatomyositis, and Sjögren's syndrome.

Pathology of vascular remodelling in SSc-PAH

Two recent studies [9, 10] support the hypothesis of SSc-PAH as a distinct clinical entity and outline histopathological differences of vascular remodelling in IPAH compared with SSc-PAH. Briefly, the main characteristic features of SSc-PAH compared with IPAH are: 1) a higher degree of intimal fibrosis, especially of small vessels; 2) the absence of plexiform lesions; 3) a more pronounced perivascular and interstitial inflammation/fibrosis; and 4) in particular the presence of pulmonary veno-occlusive disease (PVOD)-like changes. Although preliminary, these findings are intriguing and would need to be confirmed in larger studies, preferably with samples that do not only reflect late-stage disease.

One of the most interesting observations of these studies was the high proportion of PVOD in patients with SSc-PAH. PVOD is a rare subtype of PAH [11] with a prevalence of 0.1–0.2 per million persons and per year [12]. It is characterised by extensive and diffuse occlusion of pulmonary veins and venules. Capillary angiectasia and capillary angioproliferation with concomitant postcapillary congestion [11] represent additional features. The estimated proportion of PVOD in PAH is about 5–10% [13]. Interestingly, in the study by DORFMÜLLER *et al.* [9], 85% of patients with CTD-PAH (50% of which had SSc) showed PVOD-like features compared with 17.2% of patients with IPAH. In the study by OVERBEEK *et al.* [10], PVOD occurred in 50% of the patients with SSc-PAH whereas it was absent in patients with IPAH.

These histological characteristics, as well as other differences detailed below, may partly explain the fact that the two diseases have quite divergent clinical courses and response to therapy. Indeed, survival is significantly worse in SSc-PAH compared with IPAH patients treated with modern medical therapy [14–18].

Inflammation and fibrosis

Current concepts of the development of vasculopathy in both IPAH and SSc-PAH support the role of pro-inflammatory cytokines such as IL-1 or IL-6 [8, 19–21], chemokines such as CX3CL1, CC chemokine (C-C motif) ligand 5 (CCL5), monocyte chemotactic protein (MCP)-1 [6] and growth factors such as vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) [6, 9, 10, 22]. Besides inflammatory processes, the presence of both intimal fibrosis and interstitial lung disease (ILD) might also account for the worse clinical performance and the observed differences in the diffusing capacity of the lung for carbon monoxide ($D_{L,CO}$) of patients with SSc-PAH compared with patients with IPAH [15, 23].

Autoimmunity

Dysregulation of B-cells might play a role in the pathogenesis of SSc linking autoimmunity and fibrosis [19]. In SSc, levels of circulating CD19+ can be observed, whereas memory B-cells, although reduced in numbers, show markers of activation. In the skin and lungs of SSc patients, increased numbers of infiltrating B-cells and a dermal B-cell gene expression signature were reported [24]. Experimental mouse models of skin and lung fibrosis support a role of B-cells in autoimmunity and fibrosis [25]. Whether autoimmunity plays a preponderant role in (pulmonary) vasculopathy has yet to be addressed.

Autoantibodies are often associated with certain phenotypes in SSc and, thus, anti-fibrillarin [26, 27], anti-U1RNP [28], and anti-centromere antibodies [29] are commonly found in patients with SSc-PAH. So far, no functional properties have been found for these autoantibodies. However, there is increasing data on novel, potentially pathogenic autoantibodies in patients with IPAH and SSc-PAH.

Injury and apoptosis of endothelial cells are considered initial events in the pathogenesis of SSc [30, 31]. Anti-endothelial cell antibodies are found in patients with IPAH and SSc-PAH [32] and are suggested to play a role in the pathogenesis of PAH by displaying distinct reactivity against micro- and macrovascular antigens [33] and inducing the activation of EC and the expression of adhesion molecules leading finally to apoptosis [34, 35]. Recently, anti-β(2)-glycoprotein I antibodies were reported to be associated with PAH in SSc, although it has yet to be investigated whether these autoantibodies are pathogenic or simply represent a marker of endothelial cell injury [26, 36].

Key cellular players of vascular remodelling include vascular smooth muscle cells (VSMC) and activated fibroblasts. Immunoglobulin (Ig)G antibodies targeting stress-induced phosphoprotein 1 and α-enolase on the surface of VSMC have been identified in the serum of patients with SSc with/without PAH and IPAH and have been suggested to modulate vascular contraction, thus potentially contributing to the vascular remodelling [37]. Anti-fibroblast antibodies are present in the sera of patients with IPAH and SSc-PAH [38] and have been shown to induce pro-adhesive and pro-inflammatory responses in normal fibroblasts *in vitro* [39]. Furthermore, identified target antigens include proteins involved in cytoskeletal function, cell contraction and other key cellular pathways [40]. Therefore, it is possible that these anti-fibroblast antibodies might mediate the secretion of cytokines and growth factors involved in the vascular remodelling in PAH.

Recently, functional autoantibodies against vascular receptors were identified in the sera of patients with SSc [41, 42]. Antibodies against angiotensin II type 1 receptor and endothelin (ET)-1 type A receptor on endothelial cells were associated with more severe disease including complications such as PH, lung fibrosis and digital ulcers. Of note, both antibodies exerted biological effects by inducing the phosphorylation of extracellular signal-related kinase 1 or 2 and by increasing the expression of transforming growth factor (TGF)-β in endothelial cells which could be blocked by specific receptor antagonists [41].

Additionally, stimulatory antibodies targeting the PDGF receptor in SSc patients have been detected [43]. Given the key role of PDGF in the pathogenesis of IPAH and SSc-PAH, addressing the pathogenic role of these antibodies in the pulmonary vascular remodelling in SSc will be important.

Epidemiology and clinical features of CTD-PAH

SSc-PAH

SSc is a complex multi-system disorder characterised essentially by endothelial dysfunction, fibroblast dysregulation resulting in excess production of collagen, and profound alterations of the immune system [44]. In combination, such alterations lead to progressive fibrosis of the skin and internal organs resulting in premature organ failure and death. Genetic and environmental factors may contribute to host susceptibility [45, 46], however, the aetiology of the disease remains largely elusive. Whether in its limited or diffuse form, SSc has the potential for multiple organ involvement; foremost among them are the gastro-intestinal tract, heart, kidneys and lungs [44]. However, SSc-PAH has now emerged as one of the two leading causes of mortality [47, 48]. In this context, it is quite vexing that despite remarkable advances in disease-targeted therapies for other forms of PAH (such as IPAH), response to therapy remains largely suboptimal and survival rather dismal in SSc-PAH [14–17, 49].

Prevalence and incidence

Using strict right heart catheterisation (RHC) for diagnosis of PAH, the prevalence of SSc-PAH in prospective studies is between 7.8 and 12% [50–52], with variations probably dependent on referral biases. Given significant geographic variations in the prevalence of SSc, and with an estimated US prevalence of SSc of about 240 cases per million, the overall prevalence of SSc-PAH (in the USA) may be as high as 24 individuals per million, a figure significantly higher than that of

the estimated prevalence of IPAH [12]. However, in two large registries of PAH, CTD-PAH (mainly represented by SSc) accounts for only 15.3% [12] and 25% (including 17% of SSc-PAH patients in the USA registry) of all PAH cases, suggesting a clear under recognition of the syndrome [53]. In a large single US centre registry, the proportion of SSc-PAH is about 30% of all PAH patients [54]. The overall higher prevalence of SSc in the USA [55] probably accounts for a somewhat higher prevalence of SSc-PAH in the USA compared with France. A recent prospective study estimates the incidence of PAH among patients with SSc at 0.61 cases per 100 patient-years [49].

Risk factors for the development of PAH in SSc

An increased risk of developing PAH in the setting of SSc disease includes limited (as opposed to diffuse) skin involvement [23, 56, 57]; SSc disease duration greater than 10 years [57], although PAH can develop at any time throughout the course of SSc [49]; older age at the onset of SSc [52, 56]; and severity [23] or length of duration [58] of Raynaud phenomenon. Other clinical parameters, such as reduced nailfold capillary density [57, 59] or an isolated reduction in $D_{L,CO}$ or a progressive decline of $D_{L,CO}$ [23, 56], also appear to be independent risk factors for the subsequent development of PAH in these patients. While the decrease in $D_{L,CO}$ is probably the result of progressive pulmonary vascular remodelling over time, it is interesting to note that this alteration is significantly more pronounced in SSc-PAH compared with IPAH patients [15], perhaps suggesting more profound small vessel remodelling in the former compared with the latter patients, although this may also represent associated subclinical lung fibrosis in these patients.

Clinical features

SSc-PAH patients are typically predominantly females (reflecting the sex predilection for SSc) with limited sclerosis; they are older and have a more favourable baseline haemodynamic profile compared with IPAH patients, although this is clearly misleading since their survival is worse [15, 16]. As in IPAH, presenting clinical symptoms are nonspecific and include mainly dyspnoea and functional limitation, the latter often being worse than in IPAH due to older age and frequent involvement of the musculoskeletal system. SSc-PAH patients also tend to have other organ involvement such as renal dysfunction and intrinsic heart disease. Even in the absence of PAH, SSc patients tend to have depressed RV function [60, 61] and left ventricular (LV) systolic as well as diastolic dysfunction [62]. At presentation, SSc-PAH patients have severe RV dysfunction similar to IPAH patients; however, they display more severely depressed RV contractility [63]. In addition, they are more likely to have LV diastolic dysfunction and evidence of pericardial effusion (34% compared with 13% for IPAH) [15]. The latter portends a particularly poor prognosis [15]. In addition, SSc-PAH patients often present with more severe metabolic abnormalities such as high levels of N-terminal pro-brain natriuretic peptide (NT-proBNP) [64, 65] and hyponatraemia [66]. Both NT-proBNP and hyponatraemia, at baseline (or with serial changes (for NT-proBNP [64])) correlate with survival in PAH [64, 66].

Early diagnosis

Because of a relatively high prevalence of PAH in SSc, there is an opportunity for early diagnosis in this population at risk, which can lead to therapeutic intervention at an earlier stage and potentially improved outcome. An algorithm for early detection of PAH based on a combination of symptoms and screening echocardiography was recently tested in a large French study encompassing a total of 21 referral centres. SSc patients with tricuspid regurgitation velocity (TRV) jet by transthoracic echocardiography greater than 3 $m \cdot s^{-1}$, or TRV between 2.5 and 3 $m \cdot s^{-1}$ if patients had unexplained dyspnoea, were systematically referred for RHC. Using this approach, incident cases of SSc-PAH with less haemodynamic impairment (compared with the ''prevalent'' patients, *i.e.* patients with known disease) were identified [51]. Whether this approach leads to improved outcomes [67, 68] or merely reflects a so-called ''lead bias'' remains to be determined in larger prospective studies. At any rate, it seems reasonable to recommend a routine diagnostic workup in those SSc patients with unexplained dyspnoea in the setting of low or declining single-breath $D_{L,CO}$ measurements,

echocardiographic findings suggestive of pulmonary hypertension (elevated TRV jet or dilated RV or atrium) and/or elevated levels of NT-proBNP [51].

Prognosis of SSc-PAH

SSc-PAH patients are almost four times more likely to die from their disease than IPAH patients [15], and their outcome in general is significantly worse compared with patients with any other CTD complicated by PAH [14, 17]. Despite substantial improvements in other PAH categories, the figures remain sobering for these patients with a 3-year survival well below 60% [14, 15, 17, 49, 50, 69]. Markers of poor survival include male sex [17], late age at diagnosis [17], pericardial effusion [15], functional severity based on New York Heart Association (NYHA) functional class [17, 70], right heart dysfunction [15, 50, 70], hyponatraemia [66] and renal impairment [70]. In addition, measures of RV afterload, both proximal and distal vascular resistance (pulmonary arterial capacitance estimated by stroke volume divided by pulmonary artery pulse pressure and pulmonary vascular resistance (PVR), respectively) independently predict survival in SSc-PAH [70]. Finally, in patients with SSc-PAH admitted to the hospital for treatment of RV failure, hyponatraemia and hypotension upon admission are the main prognostic factors for in-hospital mortality. Furthermore, short-term outcomes after discharge are poor and remarkably worse in patients with underlying CTD, with mortality close to 50% within the following year after discharge [71].

Systemic lupus erythematosus

Similar to SSc, endothelial dysfunction in SLE may be prominent in this disease, resulting in a potential imbalance between vasodilators and vasoconstrictors. Other causes of pulmonary vascular disease and PH in SLE include recurrent thromboembolic disease, particularly in patients with anti-phospholipid antibodies (such as anti-cardiolipin antibody present in up to 10% of patients with SLE) [72], pulmonary vasculitis and parenchymal disease (including ILD and the shrinking lung syndrome from myositis of the diaphragm). Combined vasculitis and chronic hypoxia are frequent contributing offenders in these syndromes. In addition, pulmonary venous hypertension can be a consequence of LV dysfunction and myocarditis.

The exact prevalence of PAH in SLE remains unclear but is in the order of about 0.5–14% patients (most certainly less than in SSc) based on a review of the literature encompassing over 100 patients [73]. The patients are predominantly female (90%), young (average age of 33 years at the time of diagnosis), and often suffer from Raynaud's phenomenon (underscoring a generalised endothelial dysfunction in these patients with SLE and PH). The pathological lesions are often indistinguishable from IPAH or SSc-PAH lesions, with intimal hyperplasia, smooth muscle cell hypertrophy and medial thickening. Survival, initially thought to be quite poor (25–50% at 2 years) in studies preceding specific PAH treatment, is now estimated at 75% at 3 years [17], clearly significantly better than SSc-PAH.

Mixed connective tissue disease

MCTD is a separate entity among the CTDs linked to anti-U1RNP antibodies. The main clinical features are Raynauds phenomenon, myositis/myopathy, polyarthritis and puffy fingers. The exact prevalence of PAH in MCTD is unknown and has been reported to be as high as 50% [74]. The syndrome may occasionally respond to immunosuppressive drugs [75, 76] and, therefore, these patients appear to have a better prognosis overall compared with SSc-PAH patients with an estimated 2- and 3-year survival of 89 and 63%, respectively [17].

Rheumatoid arthritis

PAH is a rare complication of rheumatoid arthritis and both the prevalence and impact of PH in these patients is not well known. The 2- and 3-year estimated survival in these patients is 83 and 66%, respectively [17].

Primary Sjögren's syndrome

Although primary Sjögren's syndrome is a relatively common autoimmune disease with glandular and extraglandular manifestations, it is very rarely complicated by PAH. In a recent review by LAUNAY *et al.* [77] of 28 well characterised patients with primary Sjögren's syndrome and PAH, the mean age at diagnosis of PAH of these almost exclusively female patients (27 out of 28 patients) was 50 years. Patients had severe functional class (III and IV) and haemodynamic impairment. Standard therapy (with endothelin receptor antagonists (ERAs), phosphodiesterase inhibitors or prostanoids) was typically ineffective despite an initial improvement. Some patients were reported to respond to immunosuppressive treatment. However, conclusion regarding treatment is limited by the small size of this case report. Survival rate was low (66% at 3 years) [77].

Therapies for CTD-PAH

General measures

General guidelines for the treatment of PAH include the use of supplemental oxygen in patients who are hypoxic at rest or with exercise and in patients who have evidence of oxygen desaturation at night. Loop diuretics are used in most patients at variable dosages for the management of volume overload and in acute situations of right heart failure. Digitalis may be used as adjunct therapy for the management of symptomatic right heart failure and sometimes for control of atrioventricular conduction [78].

Calcium channel blockers

Treatment for PAH over the past 20 years has largely resulted from the recognition of a profoundly impaired pulmonary vascular endothelial function [79–81] with consequent alterations in both vascular tone and remodelling [82]. Early on, vasodilator therapy with high dose calcium channel blockers (CCB) was found to be an effective long-term therapy [83], but only for a minority of patients (~6% of IPAH patients [84]) who demonstrated acute vasodilation upon challenge (*e.g.* to nitric oxide (NO) or adenosine) during cardiac haemodynamic testing. However, high dose calcium channel therapy is not indicated for patients with CTD-PAH such as SSc-PAH since there are only about 2.6% responders in that group in one large study [12], and long-term response to these drugs remains very rare in these patients [85]. CCB remain the norm for treatment of Raynaud phenomenon.

Anti-inflammatory drugs

It is now increasingly recognised that inflammation may play a significant role in various types of PH, including IPAH and CTD-PAH. However, only rare patients with CTD-PAH (mainly patients with SLE, primary Sjögren syndrome, and MCTD) have had anecdotal dramatic improvement of their pulmonary vascular disease with corticosteroids and/or conventional immunosuppressive therapy [75, 76]. However, clearly SSc-PAH patients have been recalcitrant to such therapy [75].

Prostaglandin analogues

Prostacyclin (epoprostenol), which has potent pulmonary vasodilator activity but also anti-platelet aggregating and antiproliferative properties [86], given by continuous infusion has proven effective in improving the exercise capacity, cardiopulmonary haemodynamics, NYHA functional class, symptoms and survival in IPAH patients [87–89]. In SSc-PAH, continuous intravenous epoprostenol marginally improves exercise capacity and haemodynamics [90], compared with conventional therapy, and may have long-term beneficial effects [91]; however, an effect on survival in these patients has yet to be demonstrated.

Treprostinil, an analogue of epoprostenol suitable for continuous subcutaneous administration, has modest effects on symptoms and haemodynamics in PAH [92]. In a small study of 16 patients (including six CTD-PAH patients), *i.v.* treprostinil improved haemodynamics, 6-minute walking distance (6MWD) and functional class [93]. Although the safety profile of this drug is similar to *i.v.* epoprostenol, required maintenance doses are usually twice as much as for epoprostenol. However, for patients with SSc-PAH, the lack of requirement of ice packing and less frequent mixing of the drug offer obvious advantages.

The use of prostacyclin analogues in SSc-PAH patients has been limited by occasional reports of pulmonary oedema, raising the suspicion of increased prevalence of PVOD in these patients [94, 95]. However, this remains a therapeutic option for patients with SSc-PAH with NYHA class IV with the caveat that frequent digital problems and disabilities that these patients often experience, and the number of serious adverse effects associated with this form of therapy (*e.g.*, infection and possibility of pump failure [96]), may render treatment quite challenging.

Endothelin receptor antagonists

Randomised, placebo-controlled trials have now established the beneficial effect of bosentan therapy on functional class, 6MWD, time to clinical worsening and haemodynamics in PAH [97, 98]. In these trials, roughly one-fifth of the population consisted of SSc-PAH patients while a large majority had a diagnosis of IPAH. In subgroup analysis, a nonsignificant trend towards a positive treatment effect on 6MWD was shown for SSc-PAH patients treated with bosentan compared with placebo [98]. At most, bosentan therapy prevented deterioration in these patients (as assessed by a stable 6MWD in the treated group compared with a substantial decrease in the placebo group). However, in another analysis of patients with CTD-PAH (*e.g.* SLE, overlap syndrome and other rheumatological disorders) included in several randomised clinical trials of bosentan, there was a trend toward improvement in 6MWD and improved survival compared with historical cohorts [99]. Single-centre experience suggests that long-term outcome of first-line bosentan monotherapy is inferior in SSc-PAH compared with IPAH patients, with no change in functional class and worse survival in the former group [69]. Considering that ET-1 may be an important player in the development of vascular lesions in SSc-PAH, perhaps contributing to vascular damage and fibrosis, inhibiting ET-1 remains a rational strategy. In a small study of SSc patients (10 of whom had SSc-PAH), bosentan treatment appeared to reduce endothelial cells (as determined by endothelial soluble serum factors such as intercellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1, P-selectin and platelet/endothelial cell adhesion molecule (PECAM)-1) and T-cell subset (assessed by expression of lymphocyte function-associated antigen-1, very late antigen-4, and L-selectin on CD3 T-cells) activation [100]. An additional beneficial effect of bosentan therapy is a significant reduction in the occurrence of new digital ulcerations without, however, evidence of healing of pre-existing ulcers [101].

The selective ETA receptor antagonist sitaxentan improved exercise capacity (*i.e.* change in peak oxygen uptake ($V'O_2$) at week 12, the main end-point of the study) [102] and, in patients with CTD-PAH (representing less than one-quarter of the study group), improved exercise capacity, quality of life and haemodynamics, although elevated liver enzymes were reported [103]. This drug has since been removed from the market due to significant hepatotoxicity and death in a few patients. A large placebo-controlled, randomised trial of ambrisentan, the only currently Food and Drug Administration (FDA)-approved selective ERA, improved 6MWD in PAH patients at week 12 of treatment, however, the effect was clearly larger in IPAH compared with CTD-PAH patients (range of 50–60 m *versus* 15–23 m, respectively) [104]. Ambrisentan is generally well tolerated although peripheral oedema (in up to 20% of patients [104]) and fluid overload are quite common side-effects often requiring an increase in diuretic dosage.

Phosphodiesterase inhibitors

Sildenafil, a phosphodiesterase type-5 inhibitor (PDE-5 I) that reduces the catabolism of cyclic guanosine monophosphate (cGMP), thereby enhancing the cellular effects mediated by NO, has become a widely used and highly efficacious therapy for PAH. A large clinical trial showed that sildenafil therapy led to an improvement in the 6MWD in patients with IPAH and associated PAH (mainly CTD-PAH or repaired congenital heart disease (CHD)), who were predominantly functional class II or III, at all three doses tested (20, 40, and 80 mg, given three times daily) [105]. Based on the lack of significant differences in clinical effects and time to clinical worsening at week 12 between doses, the recommended dosage is now 20 mg three times daily. In a *post hoc* subgroup analysis of 84 patients with CTD-PAH (45% of whom had SSc-PAH), data from the Sildenafil Use in Pulmonary Arterial Hypertension (SUPER) study suggest that sildenafil at a dose of 20 mg improved exercise capacity (6MWD), haemodynamic measures and functional class after 12 weeks of therapy [106]. However, for reasons that remain unclear (but in part related to study limitations such as *post hoc* subgroup analysis), there was no significant effect for 80 mg three times daily on haemodynamics in this CTD-PAH subgroup of patients [106]. For this reason and because of the potential of increased side-effects (such as bleeding from arterio-venous malformations) at high doses, a sildenafil dosage of 20 mg three times daily is recommended for SSc-PAH patients (and perhaps for patients with other forms of CTD-PAH) as standard initial therapy. Higher doses may occasionally be attempted in case of limited response. The impact of long-term sildenafil therapy on survival in these patients remains to be determined. Finally, tadalafil, another phosphodiesterase inhibitor, is also effective for PAH [107], although subgroup analysis has not been performed yet and thus its effects on CTD-PAH remain unclear. Tadalafil has the advantage over sildenafil of a single daily dosage.

Combination therapy

Adding PAH drugs when patients fail to improve on monotherapy has now become routine. Adding inhaled iloprost to patients receiving bosentan has been shown to be beneficial in a small, randomised trial. Combining inhaled iloprost with sildenafil is mechanistically appealing and anecdotally efficacious [108, 109] as these drugs target separate, potentially synergistic pathways. Several multicentre trials are now exploring the efficacy of various combinations of oral drugs and inhaled drugs. The results of the Pulmonary Arterial Hypertension Combination study of Epoprostenol and Sildenafil (PACES) trial demonstrated that adding sildenafil (80 mg three times daily) to *i.v.* epoprostenol improves exercise capacity, haemodynamic measurements, time to clinical worsening and quality of life [110]. About 21% of these patients had CTD-PAH, including 11% with SSc-PAH. Although no specific subgroup analysis is provided, improvement was apparently mainly in patients with IPAH. In a smaller single centre clinical trial, adding sildenafil to patients with IPAH or SSc-PAH after they failed initial monotherapy with bosentan, demonstrated that combination therapy improved the 6MWD and functional class in IPAH patients. The outcome in patients with SSc-PAH was less favourable, although combination therapy may have halted clinical deterioration. Importantly, there were more side-effects reported in the SSc-PAH compared with the IPAH patients, including hepatotoxicity that developed after addition of sildenafil to bosentan monotherapy [111].

Anticoagulation

The rationale and recommendation for the use of anticoagulation in severe PAH is based on pathologic evidence of pulmonary thromboembolic arterial disease and thrombosis *in situ* in patients with IPAH [112], and a few non-randomised or retrospective clinical studies [83, 113] demonstrating a significant beneficial effect of anticoagulation on survival in IPAH. The rationale for anticoagulation in other forms of PAH, in particular in SSc-PAH or other forms of CTD is even less sound. Theoretically, there is potential for increased bleeding in patients with CTD, particularly with SSc in whom gastric antral vascular ectasia may be common.

An unpublished review of our experience with anticoagulation in over 100 patients with SSc-PAH suggests that less than 50% of these patients remain on long-term anticoagulation therapy, mainly because of the occurrence of occult bleeding in the gastro-intestinal tract, the source of which is often very difficult to diagnose (unpublished data).

Tyrosine kinase inhibitors

A pathological hallmark of PAH is aberrant proliferation of endothelial and smooth muscle cells and fibroblasts which, combined with an increased expression of secreted growth factors such as VEGF and basic fibroblast growth factor (bFGF), has caused a paradigm shift in treatment strategies. Some investigators have even linked this condition to a neoplastic process reminiscent of advanced solid tumours [114]. As a result, anti-neoplastic drugs have been tested over the past few years in experimental models [115, 116] and in some patients [117, 118], with two essential strategies: disruption of the PDGF or the VEGF signalling pathway. A proof of concept, phase II, multicentre trial to evaluate the safety, tolerability and efficacy of imatinib in patients with PAH recently demonstrated that imatinib is well tolerated. However, there was no significant change in 6MWD (primary end-point) although there was a significant decrease in PVR and an increase in cardiac output in imatinib-treated patients compared with placebo [119]. Whether these new anti-neoplastic drugs with anti-tyrosine kinase activity will have a role in CTD-PAH, particularly SSc-PAH, remains to be determined. It is noteworthy that RV function improved significantly in response to imatinib treatment in a single case report of SSc-PAH [120]. In a very small open label phase I/IIa pilot trial of imatinib treatment for SSc-related ILD, there were significant side-effects of the drug without evidence of significant improvement in pulmonary function tests (PFTs) [121]. Other tyrosine kinase inhibitors (TKIs) (targeting VEGF, PDGF, or EGF receptors) may perhaps be effective for SSc-related complications; however, these should be tested in carefully controlled trials [122].

Lung transplantation

Lung transplantation is offered as a last resort to patients with PAH who fail medical therapy. However, CTD-PAH patients often have associated morbidity and organ dysfunction other than the lung that place them at a specifically high risk for this procedure. As an example, the post-operative potential of aspiration due to gastro-oesophageal reflux combined with intrinsic oesophageal dysmotility may place the transplanted organ at increased risk. For these reasons, patients with SSc-PAH are sometimes denied the lung transplant option in some centres, although the results of two studies in SSc patients [123, 124] suggest that lung transplantation, in carefully selected patients, is a viable therapeutic option for these patients. Lung transplant experts now suggest that candidates for transplantation should be evaluated on an individual basis.

Animal models of SSc-PAH

Animal models are of great utility to identify molecular key players and potential therapeutic targets and for preclinical proof of concept studies in SSc-PAH. Unfortunately, there is a lack of well characterised and validated animal models of SSc that mimic complications of SSc such as ILD and/or pulmonary vasculopathy [125].

The UCD200/206 chicken model [30] was the first SSc animal of vasculopathy, however, to date, due to the drawbacks of chicken housing and hatching, rodent models are preferred. So far, there are three SSc animal models with an additional vascular phenotype. The caveolin-1 knockout mice develop both pulmonary vasculopathy and fibrosis in the context of increased TGF-β signalling [126]. The transgenic (tg) mouse strain Tbeta RIIDeltak-fib, characterised by balanced ligand-dependent upregulation of TGF-β signalling, provides insights into the altered biomechanical properties of large elastic arteries in human SSc in the context of perturbed TGF-β and ET activity [127]. However, in both models, pulmonary vasculopathy has not been evaluated in detail.

The Fra-2 (Fos-related antigen-2) tg mouse model combines vasculopathy with fibrosis of the skin and internal organs, and Fra-2 protein is overexpressed in the skin and lungs of patients with SSc [31, 128]. In these animals, most of the characteristic pathologic features of SSc-PAH could be observed, except for PVOD-like lesions which were absent. Additionally, interstitial inflammation and fibrosis occurred, closely resembling human nonspecific interstitial pneumonia (NSIP) as the most common form of ILD in SSc patients [129]. When assessing potential molecular key players of vascular remodelling, the PDGF signalling axis was found to be activated which is consistent with human data on IPAH [130] and SSc-PAH [22]. To test the model's response to treatment, a subgroup of Fra-2 tg mice was treated with the TKI nilotinib which inhibits both TGF-β and PDGF signalling pathways. Nilotinib is an orally administered second-generation TKI with a superior toxicity profile that was developed for patients with treatment refractory CML or with intolerance to imatinib. Similar to imatinib, nilotinib inhibited the development of fibrosis in preclinical models of SSc [131]. In Fra-2 tg mice, treatment with nilotinib had striking effects since it completely prevented vascular remodelling and remarkably reduced the development of interstitial lung fibrosis. Thus, Fra-2 tg mice might represent the first preclinical model of SSc-PAH [132].

Novel targets for therapy of SSc-PAH and SSc-PH

Since SSc-PAH differs from IPAH, further studies are needed to define whether the key molecules identified in IPAH might also play a role in the pathogenesis of SSc-PAH, especially since at least at subclinical level tissue fibrosis and inflammation might be more prevalent. Thus, the ideal drug should simultaneously target both pulmonary vascular and fibrotic changes which might also apply to SSc-PH due to ILD.

TGF-β and PDGF are key players in the development of fibrosis and vasculopathy in SSc, and since both molecules signal through tyrosine kinases, TKIs have become of major interest as potential anti-fibrotic agents. In SSc, larger prospective and partially controlled trials on imatinib have been initiated [121, 133], and so far, moderate but significant effects on skin and lung fibrosis have been reported [133], whereas in a randomised, placebo-controlled, double-blind trial in patients with mild-to-moderate idiopathic pulmonary fibrosis (IPF), no effect on survival or clinical outcome measures such as lung function could be observed [134]. However, another TKI, BIBF 1120, which targets PDGF, VEGF, and fibroblast growth factor (FGF) receptors, was recently tested in a phase II trial in patients with IPF, and compared with placebo treatment, a trend toward a reduction in the decline in lung function could be observed [135]. In IPAH, the PDGF, and in particular the PDGF-BB pathways are activated, especially in vascular cells and perivascular inflammatory cells [130]. Interestingly, PDGF receptor (PDGFR)-β immunoreactivity is more common in the pulmonary vessels of patients with SSc-PAH compared with IPAH [22]. The recent findings from the Fra-2 tg mouse model, where PDGF-BB signalling is activated and treatment with nilotinib prevents vascular remodelling and development of lung fibrosis [132], additionally support a potential role for TKIs in patients with SSc-PAH.

Furthermore, since Fra-2 has been found to be overexpressed in the skin and lungs of SSc patients [31, 128], Fra-2 as part of the activator protein (AP)-1 complex might itself represent a potential future therapeutic target in SSc. Based on previous data on microvasculopathy and dermal fibrosis in SSc patients [31, 80, 81], a recent study demonstrated substantial anti-fibrotic effects of AP-1 inhibition in different animal models of SSc [136]. Thus, Fra-2/AP-1 might represent an additional relevant molecular target for future SSc-specific therapies.

The role of 5-hydroxytryptamine (5-HT) signalling in the pathogenesis of IPAH has long been established [137, 138], and in animal models of IPAH substantial effects on vascular remodelling were observed [139, 140]. Recent data additionally suggested an important role in the development of IPF [141, 142]. In SSc, the pathogenic implication of 5-HT signalling pathways has only recently been studied in detail [143]. 5-HT is released upon activation of platelets due to microvascular injury. Since blood levels are elevated in SSc patients, it is hypothesised that 5-HT

might link vascular injury and fibrosis in SSc. *In vitro*, 5-HT strongly induces the synthesis of extracellular matrix proteins in dermal fibroblasts *via* activation of 5-HT2B receptors (5-HTR2B) in a TGF-β-dependent manner. *In vivo*, 5-HT deficiency or inhibition by 5-HT2 inhibitors reduced dermal fibrosis both in inducible and genetic models of fibrosis. Thus, these recent findings in SSc and the previous data on IPAH and IPF suggest 5-HT/5-HT2B signalling as a potential molecular target to simultaneously treat vascular remodelling and fibrosis in SSc. Terguride, a potent 5-HT2A and 5-HT2B inhibitor already approved for the treatment of ovulation disorders caused by hyperprolactinemia and hyperprolactemic pituitary adenoma, seems a promising drug to test in SSc-PAH. Since terguride acts as a non-surmountable 5-HT2B antagonist, there are few concerns regarding potential negative side effects such as those seen in chronic treatment with ergoline derivatives (*e.g.* pergolide, cabergoline) or with anorexic drugs (*e.g.* fenfluramine) which have caused retroperitoneal, pleural and pericardial fibrosis as well as valvular heart disease due to their 5-HT2B agonist activity.

Finally, given the emerging role of IL-6 in SSc and PH [8, 19, 144, 145], the clinically approved IL-6 antagonist, tocilizumab, might also be considered a future treatment option, although further preclinical and clinical studies are needed to address the role of IL-6 in SSc-PAH. So far, there are only single case reports on the use of tocilizumab in a patient with MCTD-PAH [146] and PAH in Castleman's disease [147].

Conclusion

PH is a common complication of CTD, particularly SSc where outcome is significantly worse compared with other diseases (such as IPAH). In addition, modern therapy for PAH appears to be of limited value in SSc-PAH. Similarly, currently available markers of disease onset (in a population at risk), disease severity or response to therapy in SSc-PAH and other CTD are either limited or lacking. Care of these complex patients calls for a multidisciplinary approach in order to ensure comprehensive therapy and monitoring [18]. Whether early diagnosis and treatment of SSc-PAH patients improves outcomes is still uncertain and needs to be confirmed in properly designed studies. Finally, there is a pressing need to identify potential genetic causes and establish novel physiologic, molecular, and imaging biomarkers that will improve the understanding of the pathogenesis of this disease and potentially serve as reliable tools to monitor therapy in this devastating syndrome.

Statement of Interest

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