VIII

Summary and aims for further research

Summary

Systemic sclerosis-associated pulmonary arterial hypertension (SScPAH) is the focus of this thesis, with emphasis on the characteristics of the pulmonary vasculopathy and the right ventricle (RV). First, the histopathological characteristics of the diseased pulmonary vasculature of patients with SScPAH were studied. Second, the adaptive behaviour of the RV of SScPAH patients to the increased resistance of the pulmonary vasculature was examined. Findings were compared with the idiopathic form of PAH (IPAH) as a reference group, to investigate the presence of distinguishing features, supporting further arguments for the need of an individualised approach to the SScPAH patient group in therapy and research. Furthermore, the partitioned transfer factor of the lung for canbonmonoxide (TLCO) was examined for its potential benefit as a screening tool in the diagnostic work-up for PAH in SSc.

Chapter 1 introduces the focus and rationale of this thesis. It presents an overview, introducing SSc pathology and clinic in general and SSc complicated by PAH. Epidemiology, prognosis, difficulties in diagnostic procedures and therapy of SScPAH are outlined in this section. Moreover, reference is made to the differences in clinical behaviour between SScPAH and IPAH are outlined.

Chapter 2 explores the histopathologic characteristics of lesions of pulmonary vessels in SScPAH and compares these with the well-documented plexogenic arteriopathy in IPAH. Parameters of vasculopathy were assessed of lung tissue of PAH patients with limited cutaneous SSc (n=8) and with IPAH (n=11). Intimal fibrosis of the small vessels (*i.e.* arterioles or venules that cannot be distinguished by their anatomical localisation, and as such collectively designated as "small vessels") of the pulmonary vessels was identified in all SScPAH patients, a significant difference as compared with the IPAH patient group. Fibrosis of pulmonary veins and/or venules was also significantly more frequently observed in SScPAH as compared with IPAH. In half of the SScPAH patients, fibrosis of veins and/or venules was focal and associated with capillary congestion as in pulmonary veno-occlusive disease (PVOD). The majority of the IPAH group, ten out of 11 IPAH patients, had evidence of plexogenic arteriopathy, as compared with none of the SScPAH patients.

This study demonstrates that pulmonary vasculopathy in SScPAH is different from that observed in IPAH. It can be characterised by a heterogeneic picture, including small vessel intimal fibrosis and PVOD-like features in some cases, and the absence of plexiform lesions.

The findings support the notion that different pathogenic mechanisms may account for the development of PAH. It could be speculated that differences in vasculopathy also account for differences in clinical behaviour such as the lower TLCO values and the worse response to vasodilative therapy, which emphasises the need for further study into specific therapeutic strategies in SScPAH. The PVOD-like changes observed in part of the SScPAH patients provide incentives for further research of the presence of a histopathological and/or clinical subset within this disease group, potentially relevant for tailored treatment.

Chapter 3 further characterises the SScPAH-pulmonary vascular bed by examining its immunohistochemical reactivity of the growth factor receptors plateled-derived growth factor receptor β (PDGFR-β) and epidermal growth factor receptor (EGFR). These growth factor receptors are implicated in the pathogenesis of SSc. Moreover, they have shown to play a role in the pathogenesis of animal models of pulmonary hypertension and, anecdotically, inhibition of these growth factor receptors by blocking agents demonstrated some effect in IPAH and PVOD. Therefore, they are potential targets for tyrosine kinase inhibitors and/or monoclonal antibodies. In this chapter we compare immunoreactivity of PDGFR-β and EGFR in SScPAH to IPAH and PVOD. Lung tissue specimens from 5 SScPAH, 9 IPAH, 7 PVOD patients and 5 controls were stained with antibodies directed against PDGFR-β and EGFR. Immunoreactivity was scored for presence, distribution and intensity. All SScPAH patients showed PDGFR-β-immunoreactivity in small vessels. This was significantly different from the IPAH group, which demonstrated small vessel-PDGFR-β staining only in 3 of 9 patients. SScPAH patients also displayed significantly higher prevalence of venous staining when compared to IPAH. The intensity of PDGFR-β immunoreactivity was significantly stronger in SScPAH in the pooled arterioles and small vessels, when compared with IPAH. No differences were found between SScPAH and PVOD. One of 5 controls demonstrated a focally, mild PDGFR-β-staining. In IPAH, plexiform lesions showed PDGFR-β-expression. EGFR-staining was weak in pre-capillary media/intima, without differences between groups. Plexiform lesions showed weak EGFR-staining, mostly in stromal cells. No expression was observed in control vasculature. No EGFR staining was observed in control vasculature. It can be concluded from these results that PDGFR-β- and EGFR-staining of pulmonary vessels distinguishes PAH patients from controls. Moreover, PDGFR-β-expression in SScPAH is more common and intense in small- and post-capillary pulmonary vessels than in IPAH and does not differ from PVOD. This fits in with the histomorphological distribution of vascular lesions as described in Chapter 2.

These findings not only provide further support to the presence of different pathogenetic mechanisms underlying SScPAH, but also suggest that PDGFR-β inhibiting therapy may be effective in the treatment of PAH and of SScPAH in particular. Moreover, it is tantalising to speculate that the different pattern of immunoreactivity indicates a different functional outcome of targeted therapy. Furthermore, the mild EGFR staining in PAH may suggest its pathogeneity. Therefore, the search for tailored therapy in (SSc)PAH should include the consideration of multikinase inhibitors as an option in future treatment strategies.

The remodelled pulmonary vasculature causes increased RV afterload. There is no knowledge of the RV systolic and diastolic performance of SScPAH under these conditions. However, there are indications that RV adapts worse to increased afterload than IPAH. The next chapters describe aspects of the systolic and diastolic RV function in SScPAH and compare these with IPAH. Moreover, it is examined whether SSc-disease specific cardiac pathology might explain these differences.

In **chapter 4** we characterise the RV pump function in SScPAH. In 13 limited cutaneous SScPAH patients and 17 IPAH patients, the RV pump function was described by the pump function graph, which relates mean RV pressure (mPrv) and stroke volume index (SVI). Differences in pump function result in shift or rotation of the pump function graph. Mean Prv and SVI were measured by standard catheterisation. The mean of the hypothetical RV isovolumic pressure ($mPrv_{iso}$) was estimated using a single-beat method. The pump function graph was approximated by a parabola: $mPrv = mPrv_{iso}[1 - (SVI/SVI_{max})^2]$, enabling calculation of SVI_{max}, the hypothetical maximal SVI at zero mPrv. There were no differences in SVI and SVI_{max} between SScPAH and IPAH. Both mPrv and $mPrv_{iso}$ were significantly lower in SScPAH than in IPAH. Since higher pressures were found at similar SVI, the pump function graph of IPAH moves in a clockwise rotation around the SVImax point as compared with SScPAH. This indicates that RV pump function in SScPAH is lower than in IPAH. This means that for any given mPrv, SV is lower in SScPAH, suggesting a decreased contractility. As SV and exercise capacity are closely related, this finding provides an explanation for the poor exercise capacity of SScPAH patients despite similar pulmonary arterial pressure (Ppa). Moreover, these results show that a similar increase in Ppa affects SV more in SScPAH as compared with IPAH, which might, in part, explain the premature death in this patient group. These differences in RV function also underpin the need for more research of the underlying mechanisms: the first steps to unravel such mechanisms are described in Chapter 6. The lower RV pump function also raises questions concerning the diastolic RV function of SScPAH. RV diastole is the focus of the Chapter 5.

Chapter 5 describes the RV filling pattern in SScPAH patients and assesses differences with IPAH patients. Moreover, to investigate whether involvement of SSc in the RV myocardium of SScPAH patients may explain possible differences, we assessed differences in RV filling patterns while afterload between the SScPAH and IPAH groups was similar. Ten SScPAH, 14 IPAH and 10 healthy subjects were studied. SScPAH was age-matched with controls. SScPAH and IPAH were matched for afterload, *i.e.*, similar pulmonary vascular resistance and compliance. RV mass index (RVMI) and diastolic function, described by early peak filling rate (E), atrium-induced peak filling rate (A) and E/A ratio, were measured with MRI. E was significantly lower in SScPAH than in IPAH and than in controls. A was not different between SScPAH and IPAH. However, A was significantly higher in SScPAH than in controls. E/A ratio in SScPAH was significantly lower than in IPAH. RVMI was significantly higher in SScPAH than in controls, but did not differ from IPAH.

These results indicate that RV filling in SScPAH is more impaired than IPAH with similar afterload and that this difference might be explained by intramyocardial pathology related to SSc, directing further study towards the SScPAH myocardium per se.

Underlying explanations for altered RV function and adaptation in SScPAH have not been unraveled yet. It might be hypothesized that SSc pathology occurring in the myocardium of SScPAH RV's plays a role. Therefore, we investigated three important features of SSc disease, *i.e.* fibrosis, vasculopathy and inflammation at histopathological level in cardiac tissue from SScPAH patients, and compared these with IPAH patients and controls. The results of this study are described in **Chapter 6**. Tissue samples of RV and left ventricle (LV) from SScPAH and IPAH patients and controls were picrosirius red stained for interstitial fibrosis, which was quantified semi-automatically. Interstitial granulocytes (MPO), macrophages (CD68), and lymphocytes (CD45) were counted. Presence of epi- or endocardial inflammation, and of perivascular- or intimal fibrosis of coronary arteries was assessed semi-quantitatively. RV's of SScPAH showed significantly more interstitial inflammatory cells than RV's of IPAH and than controls, but did not show more inflammatory cells in the LV as compared with IPAH. RV fibrosis was similar in SScPAH and IPAH, there was no difference in comparison with controls either. In SScPAH and IPAH RV's, foci of replacement fibrosis were found. No differences were found on epi- or endocardial inflammation or perivascular- or intimal fibrosis of coronary arteries.

These data show that inflammatory status, but not fibrosis displays differences in SScPAH as compared to IPAH. This suggests that this results from mechanical stress on the RV as interstitial inflammation of the LV was not different between

the groups. Whether this increased inflammatory status in SScPAH influences RV function is speculative and should be investigated in further research.

As therapeutic intervention implemented at an earlier phase might modify the disease course in SScPAH, new tools that assess PAH in patients with SSc are warranted. **Chapter 7** examines the usefulness of the partitioned TLCO, i.e. the diffusion of the alveolar capillary membrane (Dm) and the capillary blood volume (Vc), as screening tools of PAH in SSc patients. SSc patients with PAH (SScPAH+), SSc patients without PAH (SScPAH-) and healthy control subjects were included. Pulmonary function testing took place at diagnosis of PAH. TLCO was partitioned according to Roughton and Forster. As pulmonary fibrosis in SSc influences values of the (partitioned) TLCO, these were adjusted for fibrosis score as assessed on HRCT. TLCO as percentage of predicted (%) was significantly lower in SScPAH+ than in SScPAH-. Dm% in SScPAH+ was significantly decreased as compared with SScPAH-, also after adjustment for total fibrosis score. No difference was found in Vc%. There were no correlations between pulmonary haemodynamic parameters and Dm% in the PAH groups. Moreover, there were no indications that Dm was a superior discriminator than DLCO between the groups.

These findings do not support further study of the role of partitioning TLCO in the diagnostic work- up for PAH in SSc.

Future research directions

In summary, this thesis demonstrates that in SScPAH, the pulmonary vasculature and the right ventricle display unique characteristics at histopathological and functional level. This implicates that SScPAH deserves to be set apart from other forms of PAH in pathobiological and pathophysiological research, as well as in clinical trials and treatment algorithms. More insight in the specific features of SScPAH pathobiology, -physiology, and clinical behaviour may eventually lead to improved therapeutic regimens. This is important as SScPAH has a high mortality and morbidity.

The distinct pulmonary vasculopathy in SScPAH points to a different pathogenesis than other forms of PAH. This is supported by the different pattern of pulmonary vascular immunoreactivity of the examined growth factor receptors. Therefore, research is warranted of the specific pathogenic mechanisms in SScPAH resulting in the pulmonary vasculopathic lesions. Early vascular changes, dysregulated angiogenesis and inflammatory processes such as cell recruitment and presence auto-antibodies seem to be predominant features in SSc and offer a few of many starting points to focus on. Moreover, lessons from pathologic processes in SScPAH vasculopathy could lead to a better understanding of the inflammatory pathologic mechanisms known to play a role in IPAH. Ideally, these issues should be investigated in prospective studies of histology specimens from open lung biopsies in SSc and SScPAH patients which does not get along with ethical standards. Alternative research methods include the assessment of more accessible tissue such as (affected) skin, construction of a biobank to detect pathogenetic/predictive agents of PAH in SSc and the study of preclinical models. The development of such models for a heterogeneous disease as SSc is no less than a challenge. However, a recently developed mice model seems promising for the study of SSc microvasculopathy[1].

More insight in clinical behaviour in SScPAH is warranted, especially concerning the prediction of development of PAH in SSc and concerning the initiation of therapy. There are indications, as described in the discussion in Chapter 2, that pulmonary vascular lesions are already present without clinically manifest PAH. An unresolved issue is if and when vasculopathy results in clinical manifestation of PAH, and which parameters determine and/or predict this transition. Again, animal models as well as study on the follow up of (new) biomarkers and/or haemodynamic parameters may be helpful. Furthermore, of interest are possible relations between specific SScPAH vasculopathic lesions and clinical characteristics such as the lower TLCO values and the worse response to vasodilative therapy compared with IPAH. The observation of a PVOD-like pattern in some of the SScPAH patients may indicate the presence of a subset within SScPAH. This demands the study of larger series of

histologic vessel morphology and of the study on biomarkers and radiologic markers to set such subsets apart, as the occurrence of PVOD-like features has important clinical and therapeutic consequences.

In SScPAH, the RV is a yet unexplored area of research, while the RV is a close interactor with the pulmonary circulation, and in addition determines mortality and morbidity in PAH. This thesis shows that RV contractility and filling in SScPAH are disturbed. Research should focus on prognostic and diagnostic determinants that influence the transition from an adapted to a failing RV in SScPAH. In this context, more knowledge is necessary concerning for example the value of the cardiac hormone NT-proBNP, released due to myocardial stress, in SScPAH. Whether the increased inflammation in the RV myocardial interstitium, demonstrated in Chapter 6, influences the RV function in SScPAH patients, is another question to be answered, either in larger functional studies on autopsy material, on RV endomyocardial biopsies or pre-clinical models. Interstitial fibrosis in RV myocardium in SScPAH might display different qualitative characteristics than IPAH and as such contribute to different RV adaptation and/or earlier failure. Studies that evaluate these characteristics should include the evaluation of differences in cross-linking of collagen between these groups.

The enhancement of knowledge concerning determinants influencing the transition from an adapted to a failing RV in SScPAH may add to the improvement of therapy, consisting of components not only directed at pathologic mechanisms of pulmonary vascular remodeling, but also at pathogenic mechanisms of altered RV adaptation in SScPAH.

Reference List

1. Maurer B, Busch N, Jungel A, Pileckyte M, Gay RE, Michel BA *et al*.: Transcription factor fos-related antigen-2 induces progressive peripheral vasculopathy in mice closely resembling human systemic sclerosis. *Circulation* 2009, 120: 2367-2376.