Chapter 8

Conclusions & future directions

P. Trip

Conclusions

Pulmonary arterial hypertension is a disease affecting the pulmonary vasculature leading to an increase in pulmonary vascular resistance and consequently pulmonary artery pressure. Despite of the disease being in the lungs, clinical outcome in PAH is mainly determined by RV function.[1] While with the introduction of therapies pulmonary vascular resistance can be reduced, patients still progress towards right heart failure[2], which is the main cause of death in PAH.[3] Therefore, enhanced insight into RV function and adaptation is essential in PAH patients and may provide new tools to identify, at an early stage, patients at risk for developing right heart failure.

Recently, load-independent assessment of RV systolic and diastolic function, by means of pressure-volume analysis, has become possible in PAH patients due to the development of single-beat methods.[4–6] Pressure-volume analysis not only allows load-independent assessment of RV function, but also the characterization of the arterial load.[7–9] A more detailed evaluation of RV function is therefore available in PAH patients, allowing the separation of the different factors (RV preload, afterload, RV contractile function, RV diastolic function) that may be additive to the presently used RV function parameters, such as RV ejection fraction, cardiac output or mean right atrial pressure that are both RV and load dependent. As such, in the first part of the present thesis, we aimed to describe load-independent RV systolic and diastolic function and its clinical relevance in a large cohort of PAH patients using single-beat pressure-volume analysis and revealed the following:

- For an accurate assessment of load-independent RV systolic function (by means of end-systolic elastance) RV pressures and volumes are required. Volume alone gives inadequate information (**CHAPTER 3**).
- A reduced RV-arterial coupling ratio, reflecting a reduced RV systolic adaptation to arterial load, is discriminative at baseline and in treated patients for disease progression (**CHAPTER 4**).
- RV diastolic stiffness is related to clinical progression both at baseline and in treated patients. In patients with a long survival, RV diastolic stiffness is explained by increased wall thickness, while in patients with a short survival RV diastolic stiffness is not explainable by wall thickness alone, suggesting that intrinsic myocardial processes such as fibrosis and sarcomeric stiffening play a distinctive role in different disease stages (**CHAPTER 5**).

In the second part of this thesis we aimed to get more insight into the group of IPAH patients that is characterized by advanced age, lower diffusion capacity for carbon monoxide (DLCO) and worse prognosis. These patients are increasingly seen in pulmonary hypertension referral centers[10,11], and a more detailed description of this subgroup is needed. By using DLCO as a tool to identify patients as a subgroup within IPAH, we were able to show that:

- A severely reduced DLCO is related to advanced age and greater tobacco exposure. This suggests that a severe reduction in DLCO identifies a subtype of IPAH in which the disease is likely related to smoking. Furthermore, we confirmed that patients with a severely reduced DLCO have a worse survival that is related to age, gender and the presence of coronary disease (**CHAPTER 6**).

As a continuation of this study, we studied in greater detail at the difference in DLCO between non *BMPR2*- and *BMPR2*-mutation carriers, as it has been suggested that these patients have different vascular processes underlying their pulmonary hypertension. By using DLCO as a tool to describe the alveolar-capillary structure, we were able to show that:

- In non-smoking patients the DLCO is more preserved in patients with a *BMPR2* mutation when compared to patients without the mutation, suggesting a distinct vascular process in *BMPR2* mutation carriers (**CHAPTER 7**).

Future directions - Part I

Physiology of Right Ventricular Function & Adaptation in Pulmonary Arterial Hypertension

Prognostic value & clinical applicability of pressure-volume analysis in PAH

There are several questions that remain to be answered regarding load-independent assessment of RV function by means of pressure-volume analysis in PAH. First of all, in the present thesis we show that RV-arterial coupling and RV diastolic stiffness relate to survival. Future studies are needed to compare RV-arterial coupling and RV diastolic stiffness with known prognostic parameters such as right atrial pressure, cardiac output and RV ejection fraction. Studies with large patient populations and a greater number of events will allow a multivariate statistical analysis in order to assess whether

RV-arterial coupling and/or RV diastolic stiffness at baseline, or during follow-up, plays an additive role in predicting clinical outcome in PAH patients.

Larger and longitudinal studies are warranted to address the additive role of pressure-volume analysis. Yet, due to the invasiveness of pressure-volume analysis, this type of analysis is of limited clinical value when it comes to applicability. For that reason, noninvasive measures that reflect RV systolic and diastolic function in a load-independent matter are needed to make the assessment of load-independent RV systolic and diastolic function in a longitudinal way, and in larger patient populations, possible. Ideally, these non-invasive methods are largely load-independent and should be applicable not only in PAH patients, but also in healthy controls. Possible noninvasive methods that need to be validated for the RV and assessed for its load-independency are: myocardial acceleration during isovolumic contraction by tissue Doppler velocity imaging (systolic function)[12], and RV diastolic wall strain.[13,14] RV-arterial coupling is noninvasively assessed in literature by dividing end-systolic volume by stroke volume.[15] Although used frequently, this method has not been validated in PAH and has been shown to be based on the assumption that cannot hold in PAH patients, leading to an underestimation of RV end-systolic elastance and thus RV-arterial coupling, as is shown in **CHAPTER 3** of this thesis.

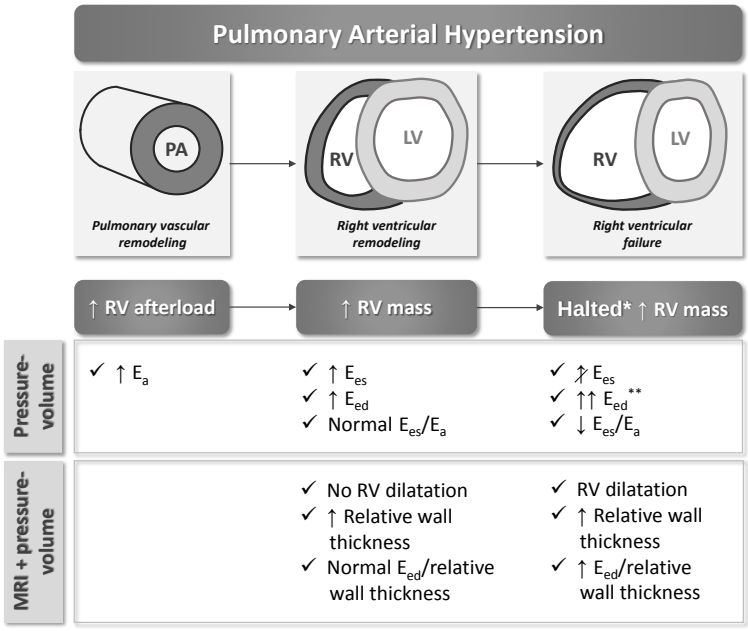

FIGURE 8.1. Schematic overview of our findings and hypotheses on changes in RV function over time. An increase in afterload (E_a) stimulates the hypertrophic process. An increased RV mass then results in enhanced RV systolic function (E_{es}) and RV diastolic stiffness (E_{ed}). In this stage of adaptive remodeling, RV-arterial coupling is maintained (E_{es}/E_a) and no dilatation has occurred yet. E_{ed} is increased proportional to the observed increase in

relative wall thickness. In patients with a maladapted or failing RV, RV-arterial coupling is reduced, possibly because RV E_{es} cannot further increase due to a halted hypertrophic response* or RV diastolic stiffening. The RV dilates and there a signs of fibrosis and/or sarcomeric stiffening as E_{ed} is further and disproportionally increased to relative wall thickness**. PA: pulmonary arteriole; LV: left ventricle.

Mechanisms of RV diastolic stiffness & the effect of reducing it

We observed in **CHAPTER 5** that RV diastolic stiffness is disproportionately increased in progressive PAH patients, while in stable patients, the ratio between RV diastolic stiffness and relative wall thickness is maintained at a normal level. We therefore hypothesize that RV diastolic stiffening as a result of hypertrophy alone is a good sign, while RV diastolic stiffening due to intrinsic RV wall changes, such as fibrosis or sarcomeric stiffening, is not a beneficial sign and occurs mainly in patients who are transitioning from adaptative remodeling to RV failure. Therefore, it could be of great importance to assess the presence of fibrosis and sarcomeric stiffening in PAH patients. However, the relation between hypertrophy and RV diastolic stiffening has never been described in literature, as taking RV tissue from PAH patients during disease progression is contraindicated due to the high risk of bleeding or rupture of the RV myocardium.[16] Consequently, the presence of fibrosis and/or sarcomeric stiffening cannot be assessed in the human and our hypothesis not investigated. We therefore propose to establish a method to distinguish hypertrophy, fibrosis and sarcomeric stiffening.

There are two directions that can be taken to establish such a method. First, in a preclinical setting the relation between the different tissue characteristics and RV diastolic stiffness can be assessed by measuring hypertrophy, the extent of fibrosis and sarcomeric stiffening in animal models with stable and progressive PAH. In cardiomyocytes taken from these different PAH models, total passive-tension relations, titin-based (sarcomeric stiffening) and collagen-based (fibrosis) passive-tension relations can be derived using extraction and degradation techniques.[17] As such, the contribution of the different tissue characteristics to RV diastolic stiffness can be determined.

The other direction is set in patients. In patients, cardiac MRI could be used to assess the different tissue characteristics. Hypertrophy can be assessed with cardiac MRI by measuring RV mass. Fibrosis can be assessed by means of T_1 and T_2 relaxation times. These times vary significantly from one type of tissue to another, but also within the same tissue depending on the presence of fibrosis.[18] Gadolinium contrast agents can further increase the visibility of fibrosis.[18] Whether these methods are sensitive enough to detect relevant differences in the amount of fibrosis is unclear and should be investigated. For sarcomeric stiffening there is no method currently available, as the resolution of

currently used imaging techniques doesn't allow visualization of sarcomeres within the cardiomyocyte for being able to measure its function.

In our recently published study on RV diastolic function in PAH and in the present thesis we demonstrate the clinical relevance of RV diastolic stiffness in PAH.[6] Targeting RV diastolic stiffness may therefore be beneficial and may improve survival. In animal models of PAH, it has been shown that a reduction in RV diastolic stiffening can be achieved by β-blocker therapy.[19] However, β-blocker therapy is currently not recommended in patients with PAH due to its side effects.[19] Recently, a report of an open-label pilot study of β-blocker therapy in six patients with PAH showed that treatment was safe.[20] Results of randomized, placebo-controlled trials are on the way and should answer the question whether β-blocker therapy improves important clinical parameters that are related to outcome.

Evaluation of changes in RV function over time

How the RV shifts from adaptive remodeling to right heart failure is currently not well understood. Several cellular/molecular process have been postulated to play a role in this transition.[16] However, how at the ventricular level RV function changes over time has never been revealed. Based on the results presented in the present thesis, we can conclude that in stable disease, a preserved RV-arterial coupling relates with normal RV volumes. However, it remains unknown whether this preserved RV-arterial coupling in stable disease is followed first by a reduction in RV-arterial coupling before RV dilatation occurs, a hypothesis postulated in literature.[21]

We hypothesize that the following sequence of events occurs (see also **FIGURE 8.1**): first, in adaptive remodeling the RV is hypertrophied in response to the increased pressure resulting from the increased resistance expressed as arterial elastance. Diastolic wall stress is normalized and no dilatation has occurred. Due to hypertrophy, both RV end-systolic elastance and RV diastolic stiffness are increased. Consequently, RV-arterial coupling is preserved. On the way to maladaptive remodeling and right heart failure, the process of hypertrophy is halted by limitations in capillarization and possibly other mechanisms currently unknown.[16] In addition, long-term neurohormonal activation may lead to an additional increase in RV diastolic stiffness attributable to sarcomeric stiffening. This may result in a reduced contractile function.[16] The combination of both halted hypertrophy and impaired contractile function of the cardiomyocytes will then lead to a reduction in RV end-systolic elastance and thus worse RV-arterial coupling. RV dilatation will then occur and will lead to an enhancement of RV

myocyte contractile force through the Frank-Starling mechanism.[22] However, excessive dilatation will stretch the cardiomyocytes too much, leading to a decrease in contractile force. In maladaptive remodeling, an increase in RV end-diastolic wall stress, RV fibrosis, further RV diastolic stiffening and impaired contractility will put the RV in a vicious circle of worsening of function leading to right heart failure and death.

Pressure-volume analysis and cardiac MRI performed on a regular basis (for example once a year) in PAH patients, from time of diagnosis until clinical deterioration and death, will give more insight into the sequence of RV functional changes that occur when the RV shifts from adaptive remodeling to right heart failure.

Future directions - Part II

Pulmonary diffusion capacity in patients with pulmonary arterial hypertension

To enhance insight into IPAH patients with a low diffusion capacity

Over the past decades knowledge on pulmonary hypertension has considerably increased. Several epidemiological studies have revealed the clinical characteristics of different groups of PAH and their survival.[23,24] Based on these studies the image of the IPAH patient is presented as being young, female, and without comorbidities. As a consequence of these earlier registries, IPAH is usually considered a homogenous disease. However, recent registries give a different image of the IPAH patient today.[10,11] Patients with IPAH are now older and have more comorbidities, posing the idea that a different phenotype is emerging. Nevertheless, currently there are no good tools to assess these different phenotypes. By using DLCO in IPAH patients in the present thesis, we provided a tool to portray a subgroup of patients with a severely reduced DLCO that is characterized by a higher age, a higher tobacco exposure, and more comorbidities (CHAPTER 6), suggesting that the pulmonary hypertension in these patients is possibly related to smoking or to the comorbidities described. To obtain more insight into the different phenotypes of IPAH patients, additional assessments are required. These assessments, as described below, may lead to a better clinical classification of patients and consequently a better treatment strategy suited for the phenotype presented.

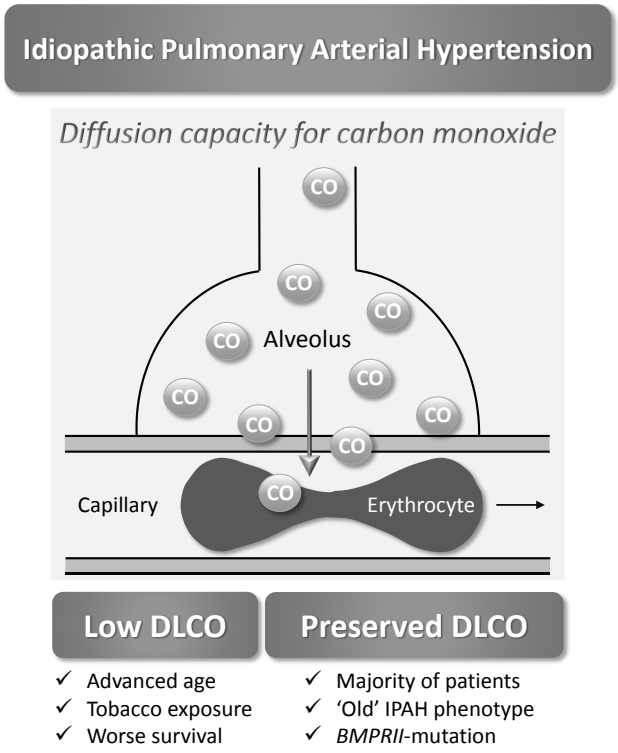

FIGURE 8.2 Schematic overview of the findings on DLCO. Idiopathic PAH patients that present with a severely reduced DLCO are more likely to be of advanced age and have a high tobacco exposure. They have worse survival that is related to age, sex and coronary disease (**Chapter 6**). A *BMPRII*-mutation is only found in patients with a preserved DLCO.

Assessing occult left ventricular diastolic dysfunction

Coronary artery disease was more frequently present in patients with a severely reduced DLCO. As such, occult left ventricular (LV) diastolic dysfunction could be present in this group of patients. In the present thesis, we were not able to assess the presence of occult LV diastolic dysfunction in this group of patients. Therefore, future studies are needed to do so. This could include the measurement of pulmonary capillary wedge pressure (PCWP) during fluid administration or exercise in all IPAH patients or in for example IPAH patients who present with a resting PCWP >10 mmHg.[25] All patients should then be treated like IPAH patients, with careful follow-up registrations of pulmonary hemodynamics, including PCWP, RV and LV function. This to assess whether there are differences in PAH-treatment responses between IPAH patients with a normal PCWP after fluid administration and IPAH patients with an increase in PCWP >15 mmHg after fluid administration. This could give information on whether IPAH patients with occult LV diastolic dysfunction (defined by a PCWP >15 mmHg after fluid

administration) require a different therapeutic strategy and should possibly be classified within group II of the Nice classification.[26]

Assessing mild interstitial or emphysematous lung disease

In addition to the additional analysis of patients with a severely reduced DLCO described above, a more detailed evaluation of high resolution CT scans in this group could give more information on the presence of mild interstitial abnormalities or emphysematous changes. High resolution CT scans should not only be analyzed at baseline, but also during follow-up to assess possible progression of mild abnormalities or the emergence of new abnormalities during the disease of PH. Also, as in the assessment of occult LV diastolic dysfunction described above, treatment response should be carefully documented to determine whether patients with lung abnormalities exhibit a different response compared to patients without abnormalities.

Assessing pulmonary vasculopathy

Pathological analysis is further needed to assess the type of vasculopathy present and to determine whether this is different from IPAH patients with a more preserved DLCO. This should include the assessment of plexiform lesions, intima, media and adventitia thickening, interstitial inflammation, fibrotic changes, and the presence of venous lesions.[27] Pathological analysis may further reveal why DLCO is severely reduced in these patients. By studying the alveolar-capillary structures, by for example electron microscopy, one could reveal thickening of the alveolar-capillary membrane not detectable by current high-resolution CT scans. Pathological analysis may also show differences in alveolar-capillary structures between *BMPR2*-mutation carriers and non-carriers, as is hypothesized in **CHAPTER 8** of this thesis.

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