Right ventricular remodeling and function in pulmonary arterial hypertension

Mariëlle C. van de Veerdonk

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Voor mijn ouders

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General introduction and thesis outline

Mariëlle C. van de Veerdonk

General introduction

The normal right ventricle and pulmonary circulation

In his ‘Exercitatio Anatomica de Motu Cordis et Sanquinis in Animalibus’, the English physician William Harvey described in 1628 for the first time the importance of the right ventricle (RV) and its interaction with the pulmonary circulation. However, for four centuries the RV remained largely understudied and it is only during the last decades that the RV is finally receiving increased attention.

The RV receives deoxygenated blood from the right atrium and conducts it into the pulmonary artery and the lungs where the blood becomes oxygenated. Subsequently the oxygenated blood flows from the lungs into the left atrium and left ventricle (LV). The LV pumps the blood into the aorta and systemic circulation that provides the tissues of the body with oxygenated blood. Finally, the deoxygenated blood from the body flows via the vena cava into the right atrium and the cycle will start over again (Figure 1A).

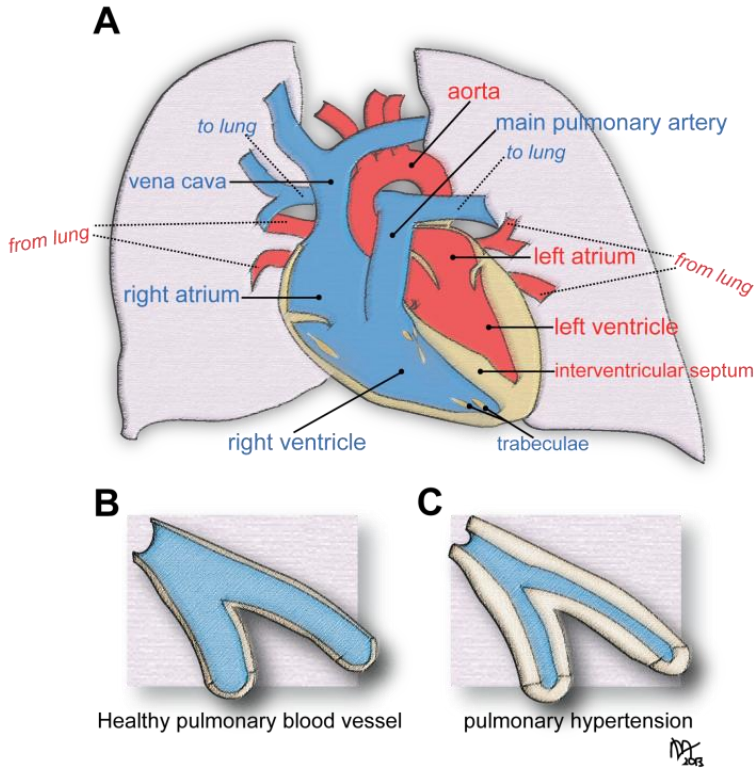

Figure 1. Pulmonary circulation in health and pulmonary hypertension.

(A) Schematic presentation of the pulmonary circulation. The right atrium receives deoxygenated blood from the body. The right ventricle pumps the blood into the main pulmonary artery and the lungs. Subsequently, the left atrium and left ventricle receive oxygenated blood from the lungs and pump it into the aorta towards the body. **(B)** Normal pulmonary arteries. **(C)** In patients with pulmonary arterial hypertension (PAH) the pulmonary arteries are remodeled and narrowed.

The normal RV has a complex crescent shape, a thin wall with prominent trabeculae (*i.e.* muscle bundles that support the RV wall) and is separated from the LV by the interventricular septum (IVS). The RV is primarily build to conduct blood volume and to function in a low-resistance, low-pressure system in relation with the pulmonary circulation. The normal blood pressure in the lungs of an healthy adult is on average 14 mmHg. When the pulmonary vascular resistance (PVR) and the pulmonary artery pressure (PAP) are elevated, the RV has to adapt in order to maintain adequate blood flow. Unfortunately, the RV is built as a volume pump and is poorly able to cope with rising pulmonary pressures[1].

Pulmonary arterial hypertension

Pulmonary hypertension (PH) is defined by an elevated mean PAP ≥25 mmHg at rest, invasively measured during a right heart catheterization. PH can be classified into five groups (Table 1).

Table 1. Clinical classification of pulmonary hypertension.

Group 1	Pulmonary arterial hypertension (PAH) Idiopathic Heritable Drugs and toxins induced Associated pulmonary arterial hypertension (APAH) Connective tissue diseases HIV infection Portal hypertension Congenital heart disease Schistosomiasis Chronic haemolytic anemia Persistent pulmonary hypertension of the new born (Group 1') Pulmonary veno-occlusive disease and/or pulmonary capillary hemangiomatosis
Group 2	Pulmonary hypertension due to left heart disease
Group 3	Pulmonary hypertension due to lung diseases and/or hypoxia
Group 4	Chronic thromboembolic pulmonary hypertension (CTEPH)
Group 5	Pulmonary hypertension with unclear or multifactorial mechanism

PH related to left heart disease (Group 2) and PH related to lung diseases (Group 3) are the most common forms of PH. Group 1, pulmonary arterial hypertension (PAH) is a progressive disease of the small pulmonary arteries that results in increased PVR and PAP[2]. The pulmonary vessels

show vasoconstriction, obstruction and remodeling (Figure 1B,C). Histopathologic examinations revealed intima hyperplasia, media thickening, fibrosis of the adventitia, thrombotic lesions and the presence of plexiform lesions in the small pulmonary arteries which are thought to be major contributors to the elevated PVR[3].
PAH is a rare disease with an estimated prevalence of 15 cases per million in the general adult population. The incidence was estimated at 2.4 cases / million adult people / year. Most PAH patients are female (ratio female:male: 1.9:1) with a peak prevalence at the age of 50 years[4]. Most frequently, the cause is idiopathic (unknown) but PAH can also be heritable or associated with other conditions including congenital heart disease, collagen vascular disease, portal hypertension, HIV infection and exposure to drugs or toxins such as appetite suppressants.

The RV determines the symptoms and survival in patients with PAH
PAH patients present with symptoms of a limited exercise capacity and dyspnea on exertion. Due to the non-specific nature of its symptoms and its insidious development, PAH is often ignored by the patient and mistreated by the medical doctor, contributing to a delay in the establishment of the diagnosis. The symptoms are related to the presence of RV dysfunction. Chronic elevated pressure overload initially results in a temporarily adapted RV and preserved RV function, ultimately followed by RV maladaptation and dysfunction (Figure 2). RV failure is the primary cause of death[5,6]. Without treatment, the natural median survival of PAH is 2.8 years[7].

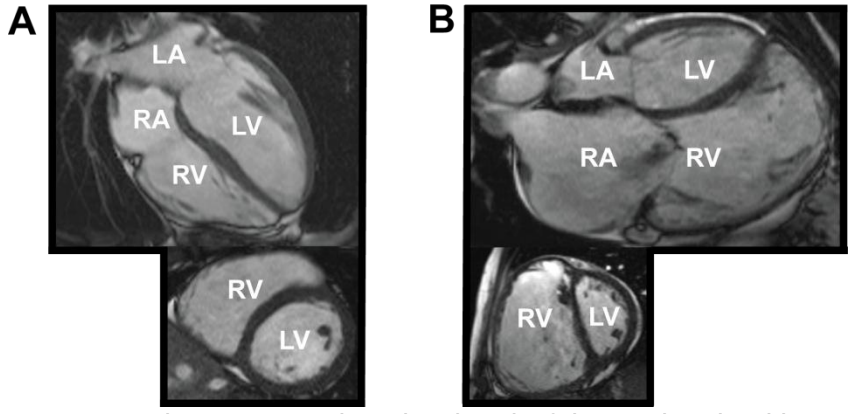

Figure 2. Magnetic resonance imaging (MRI) of the RV in a healthy individual and in a patient with PAH.
(A) The normal RV has a thin wall, crescent shape and numerous trabeculae. **(B)** The RV of a PAH patient has developed a spherical shape, hypertrophy, pronounced trabecularisation, severe dilatation, apical ballooning, bulging of the (IVS) into the LV, LV underfilling, an enlarged right atrium (RA), tricuspid insufficiency and pericardial effusion. LA = left atrium.

Pathophysiology of RV remodeling and dysfunction

The heart functions as an 'on-demand' pump in order to fulfill the needs of sufficient oxygen delivery to the body[8]. During chronic pressure overload, the RV has to increase its work in order to sustain cardiac output (CO). According to the Law of Laplace (*i.e.* wall stress = intraluminal pressure times chamber internal radius, divided by wall thickness), wall stress in PAH is elevated and the RV has to compensate by increasing its wall mass. Hypertrophy is initially considered a favorable adaptation mechanism by reducing wall stress and improving pumping effectiveness to maintain adequate blood flow. An additional adaptive mechanism is RV dilatation which aids in sustaining CO via the Frank-Starling mechanism, but at the same time contributes to increased RV wall stress. The elevated RV mass and wall stress are associated with an increased myocardial oxygen demand and simultaneous impairment of myocardial blood flow[9,10]. The balance between oxygen demand and supply is further disturbed by a decreased myocardial mechanical efficiency which increases the amount of oxygen needed for a given amount of work[9]. Whether or not associated with an impaired oxygen balance, metabolite use in the hypertrophic RV of PAH patients switches from predominantly fatty acid towards glucose in order maintain the supply of adenosine triphosphate (ATP) for force generation[11-14].

A disturbed balance between RV pressure overload, hypertrophy and progressive dilatation result in a persistent increase of wall stress. In addition to concurrent ischemia, changes in metabolism and potential other factors such as altered RV contractility and activation of the neurohormonal system[15-17], are all detrimental for the RV in the long run and contribute to progressive RV dysfunction and failure[18,19].

Most studies have focused on remodeling of the RV free wall. The pressure-overloaded RV is heavily trabecularised but it is unknown whether hypertrophic responses are similar in the RV free wall and in trabeculae. In addition, the IVS significantly contributes to normal RV function[20,21]. In patients with PAH, the IVS cannot function properly and bows into the LV, which is associated with LV underfilling, decreased stroke volume and poor survival[22-25]. Leftward IVS bowing is considered a consequence of interventricular dyssynchrony[23,25-27]. It is unknown whether underlying remodeling processes occur in the IVS as well and if so, whether these processes are comparable to the changes in the RV free wall. If the IVS reflects the changes of the RV free wall, than taking biopsies from the IVS could allow clinical assessment of RV failure mechanism and treatment response monitoring, because taking biopsies directly from the RV free wall is considered unsafe[28].

Advanced assessment of the RV by cardiac MRI and- PET imaging

Cardiac magnetic resonance imaging (MRI) provides high-resolution imaging, does not require geometric assumptions, lacks ionizing radiation and is considered the gold-standard to assess RV mass, volumes and function[29]. Global RV function is usually quantified by RV ejection fraction (RVEF)[1], which is determined as the amount of blood ejected per heart beat relative to the amount of RV filling (*i.e.* RV end-diastolic volume (RVEDV)). Although the diagnostic role of MRI in PAH is limited, it is valuable in the monitoring of RV therapeutic effects[30,31]. Furthermore, it has been demonstrated that increased RVEDV, low RVEF and stroke volume on MRI are strong predictors of a poor survival[32]. Disadvantages of MRI are that the technique is relative expensive, requires operator expertise and relative long scan times. Positron emission tomography (PET), despite its high costs and labor intensity, is a rapidly evolving technique that allows assessment of RV molecular and pathophysiological tissue processes.

Therapeutic approach to improve RV function

The current therapeutic strategy in PAH is aimed at improving RV function by reducing RV load. This approach is based on the results obtained after lung transplantation and pulmonary endarterectomy in chronic thromboembolic PH patients in which normalization or a strong decrease in PVR guarantees restoration of the function of the RV[33,34].

During the last decade, multiple medical therapies (*i.e.* prostacyclins, endothelin receptor antagonists, phosphodiesterase five inhibitors) have been developed that have mainly pulmonary vasodilator effects resulting in reduced PVR and improved CO[35,36]. These therapies have contributed to a more stable clinical condition and better survival of PAH patients[35,37,38]. However, PAH cannot be cured with these therapies and long-term survival remains unsatisfactory[38,39]. The contrast between hemodynamic success and a poor prognosis after medical treatment, raises the question whether a reduced, but non-normalized PVR is automatically followed by an improved RV function. The relationship between changes in PVR and the effects on RV function after medical therapies have been scarcely assessed however. Little is known about the influence of additional factors contributing to a successful response to medical therapies. Of interest, females have a better survival than male patients despite optimal medical treatment[39,40] but the cause for this gender difference is currently unknown.

An increasing clinical problem is that even after years of a stable clinical condition under medical treatment, patients may experience an unexpected, rapid clinical deterioration which is associated with progressive RV dysfunction and is related to poor survival[41-43]. In order to prevent this worsening, it is of great importance to identify the transition from adaptive RV remodeling to refractory RV dysfunction and failure as early as possible.

Rationale and outline of this thesis

In 1997, the VU University Medical Center was the first hospital in the Netherlands to introduce a program for diagnosis, assessment and treatment of patients with PAH. During more than 15 years, the RV has been studied extensively using MRI as part of a standard clinical protocol. More recently, PET imaging has also been applied. The data gathered into a large, growing PAH database and formed the basis of the research performed in this thesis.

The aims of the studies described in this thesis were twofold. The first aim was to improve knowledge of RV structural adaptation to chronic pressure overload. In **Chapter 2**, the structural remodeling of the RV free wall and trabeculae are studied. Using MRI to assess RV structural remodeling and right heart catheterization (RHC) to measure hemodynamics, we assessed whether trabeculare and papillary muscles showed a similar remodeling process as the RV free wall in relationship to pressure overload. In C**hapter 3**, we focused on the role of the IVS and did not only study the morphological changes by MRI but also assessed the changes in glucose metabolism using PET imaging. In addition, alterations on a cellular level of the IVS were studied in a rat model with PH. The aim of this study was to assess whether the changes in the IVS resembled the changes in the RV free wall.
The second aim of this thesis was to assess the changes in RV adaptation and function over time in relationship to the changes in RV afterload after medical therapies. In **Chapter 4** of this thesis, the relationship between the changes in PVR and RV function and survival was investigated. We assessed whether a decrease in PVR after medical treatment was automatically followed by improved RV function and better survival. In **Chapter 5** we compared the PVR-RV function relationship between female and male PAH patients under medical treatment. We investigated whether worse survival of male patients is caused by more severe pulmonary vascular disease, more impaired RV function or a different therapeutic response. In **Chapter 6** of this thesis, the hypothesis is tested that accomplishment of a large decrease in PVR after medical treatment is associated with a guaranteed improvement in RV function and survival. In C**hapter 7**, we studied PAH patients with a longstanding stable response to medical treatment. Some of these patients can show an ultimate unexpected clinical deterioration and it was hypothesized that clinical deterioration in these patients, was preceded by progressive adverse RV remodeling during the initial seemingly stable disease period. Assessment of this adverse RV remodeling would enable early detection of an ultimate clinical worsening.

In **Chapter 8** we provide a general overview of current and future applications of MRI and PET imaging in the assessment of chronic RV failure. We review the current clinical relevance of MRI and PET applications and explain how advanced imaging techniques could improve our knowledge in the mechanisms of RV failure under chronic pressure overload. Finally, in **Chapter 9**, we provide conclusions of this thesis and present future perspectives.

References

1. Haddad F, Doyle R, Murphy DJ, et al. Right ventricular function in cardiovascular disease, part II: pathophysiology, clinical importance, and management of right ventricular failure. Circulation. 2008; 117:1717-1731.
2. Galie N, Hoeper MM, Humbert M, et al. Guidelines for the diagnosis and treatment of pulmonary hypertension: the Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS), endorsed by the International Society of Heart and Lung Transplantation (ISHLT). Eur Heart J. 2009; 30:2493-2537.
3. Pietra GG, Capron F, Stewart S, et al. Pathologic assessment of vasculopathies in pulmonary hypertension. J Am Coll Cardiol. 2004; 43:25S-32S.
4. Humbert M, Sitbon O, Chaouat A, et al. Pulmonary arterial hypertension in France: results from a national registry. Am J Respir Crit Care Med. 2006; 173:1023-1030.
5. Voelkel NF, Quaife RA, Leinwand LA, et al. Right ventricular function and failure: report of a National Heart, Lung, and Blood Institute working group on cellular and molecular mechanisms of right heart failure. Circulation. 2006; 114:1883-1891.
6. McLaughlin VV, McGoon MD. Pulmonary arterial hypertension. Circulation. 2006; 114:1417-1431.
7. D'Alonzo GE, Barst RJ, Ayres SM, B, et al. Survival in patients with primary pulmonary hypertension. Results from a national prospective registry. Ann Intern Med. 1991; 115:343-349.
8. Hall J. Guyton and Hall Textbook of Medical Physiology. Elsevier Health Sciences. 2010 (12th ed).
9. Wong YY, Ruiter G, Lubberink M, et al. Right ventricular failure in idiopathic pulmonary arterial hypertension is associated with inefficient myocardial oxygen utilization. Circ Heart Fail. 2011; 4:700-706.
10. van Wolferen SA, Marcus JT, Westerhof N, et al. Right coronary artery flow impairment in patients with pulmonary hypertension. Eur Heart J. 2008; 29:120-127.
11. Bokhari S, Raina A, Rosenweig EB, et al. PET imaging may provide a novel biomarker and understanding of right ventricular dysfunction in patients with idiopathic pulmonary arterial hypertension. Circ Cardiovasc Imaging. 2011; 4:641-647.
12. Can MM, Kaymaz C, Tanboga IH, et al. Increased right ventricular glucose metabolism in patients with pulmonary arterial hypertension. Clin Nucl Med. 2011; 36:743-748.
13. Oikawa M, Kagaya Y, Otani H, et al. Increased [18F]fluorodeoxyglucose accumulation in right ventricular free wall in patients with pulmonary hypertension and the effect of epoprostenol. J Am Coll Cardiol. 2005; 45:1849-1855.
14. Nagaya N, Goto Y, Satoh T, et al. Impaired regional fatty acid uptake and systolic dysfunction in hypertrophied right ventricle. J Nucl Med. 1998; 39:1676-1680.

15. Rain S, Handoko ML, Trip P, et al. Right ventricular diastolic impairment in patients with pulmonary arterial hypertension. Circulation. 2013; 128:2016-2025.
16. Tedford RJ, Mudd JO, Girgis RE, et al. Right ventricular dysfunction in systemic sclerosis-associated pulmonary arterial hypertension. Circ Heart Fail. 2013; 6:953-963.
17. de Man FS, Handoko ML, Guignabert C, et al. Neurohormonal axis in patients with pulmonary arterial hypertension: friend or foe? Am J Respir Crit Care Med. 2013; 187:14-19.
18. Handoko ML, de Man FS, Allaart CP, et al. Perspectives on novel therapeutic strategies for right heart failure in pulmonary arterial hypertension: lessons from the left heart. Eur Respir Rev. 2010; 19:72-82.
19. Bogaard HJ, Abe K, Vonk Noordegraaf A, et al. The right ventricle under pressure: cellular and molecular mechanisms of right-heart failure in pulmonary hypertension. Chest. 2009; 135:794-804.
20. Buckberg GD. The ventricular septum: the lion of right ventricular function, and its impact on right ventricular restoration. Eur J Cardiothorac Surg. 2006; 29:S272-278.
21. Banka VS, Agarwal JB, Bodenheimer MM, et al. Interventricular septal motion: biventricular angiographic assessment of its relative contribution to left and right ventricular contraction. Circulation. 1981;64:992-996.
22. Puwanant S, Park M, Popovic ZB, et al. Ventricular geometry, strain, and rotational mechanics in pulmonary hypertension. Circulation. 2010; 121:259-266.
23. Marcus JT, Gan CT, Zwanenburg JJ, et al. Interventricular mechanical asynchrony in pulmonary arterial hypertension: left-to-right delay in peak shortening is related to right ventricular overload and left ventricular underfilling. J Am Coll Cardiol. 2008; 51:750-757.
24. Gan C, Lankhaar JW, Marcus JT, et al. Impaired left ventricular filling due to right-to-left ventricular interaction in patients with pulmonary arterial hypertension. Am J Physiol Heart Circ Physiol. 2006; 290:1528-1533.
25. Roeleveld RJ, Marcus JT, Faes TJ, et al. Interventricular septal configuration at mr imaging and pulmonary arterial pressure in pulmonary hypertension. Radiology. 2005; 234:710-717.
26. Lopez-Candales A, Dohi K, Bazaz R, et al. Relation of right ventricular free wall mechanical delay to right ventricular dysfunction as determined by tissue Doppler imaging. Am J Cardiol. 2005; 96:602-606.
27. Tanaka H, Tei C, Nakao S, et al. Diastolic bulging of the interventricular septum toward the left ventricle. An echocardiographic manifestation of negative interventricular pressure gradient between left and right ventricles during diastole. Circulation. 1980; 62:558-563.
28. Brooksby IA, Jenkins BS, Coltart DJ, et al. Left-ventricular endomyocardial biopsy. Lancet. 1974; 2:1222-1225.
29. Pennell DJ, Sechtem UP, Higgins CB, et al. Clinical indications for cardiovascular magnetic resonance (CMR): Consensus Panel report. Eur Heart J. 2004; 25:1940-1965.
30. Peacock AJ, Crawley S, McLure L, et al. Changes in Right Ventricular Function Measured by Cardiac Magnetic Resonance Imaging in Patients Receiving Pulmonary Arterial Hypertension-targeted Therapy: The EURO-MR Study. Circ Cardiovasc Imaging. 2014; 7:107-114.
31. McLaughlin VV, Archer SL, Badesch DB, et al. ACCF/AHA 2009 expert consensus document on pulmonary hypertension a report of the American College of Cardiology Foundation Task Force on Expert Consensus Documents and the American Heart Association developed in collaboration with the American College of Chest Physicians; American Thoracic Society, Inc.; and the Pulmonary Hypertension Association. J Am Coll Cardiol. 2009; 53:1573-1619.

32. van Wolferen SA, Marcus JT, Boonstra A, et al. Prognostic value of right ventricular mass, volume, and function in idiopathic pulmonary arterial hypertension. Eur Heart J. 2007; 28:1250-1257.
33. Reesink HJ, Marcus JT, Tulevski II, et al. Reverse right ventricular remodeling after pulmonary endarterectomy in patients with chronic thromboembolic pulmonary hypertension: utility of magnetic resonance imaging to demonstrate restoration of the right ventricle. J Thorac Cardiovasc Surg. 2007;133:58-64.
34. Pasque MK, Trulock EP, Cooper JD, et al. Single lung transplantation for pulmonary hypertension. Single institution experience in 34 patients. Circulation. 1995; 92:2252-2258.
35. Galie N, Manes A, Negro L, et al. A meta-analysis of randomized controlled trials in pulmonary arterial hypertension. Eur Heart J. 2009; 30:394-403.
36. Humbert M, Sitbon O, Simonneau G. Treatment of pulmonary arterial hypertension. N Engl J Med. 2004; 351:1425-1436.
37. Savarese G, Paolillo S, Costanzo P, et al. Do changes of 6-minute walk distance predict clinical events in patients with pulmonary arterial hypertension? A meta-analysis of 22 randomized trials. J Am Coll Cardiol. 2012; 60:1192-1201.
38. Benza RL, Miller DP, Barst RJ, et al. An evaluation of long-term survival from time of diagnosis in pulmonary arterial hypertension from the REVEAL Registry. Chest. 2012; 142:448-456.
39. Humbert M, Sitbon O, Chaouat A, et al. Survival in patients with idiopathic, familial, and anorexigen-associated pulmonary arterial hypertension in the modern management era. Circulation. 2010; 122:156-163.
40. Benza RL, Miller DP, Gomberg-Maitland M, et al. Predicting survival in pulmonary arterial hypertension: insights from the Registry to Evaluate Early and Long-Term Pulmonary Arterial Hypertension Disease Management (REVEAL). Circulation. 2010; 122:164-172.
41. Haddad F, Peterson T, Fuh E, et al. Characteristics and outcome after hospitalization for acute right heart failure in patients with pulmonary arterial hypertension. Circ Heart Fail. 2011; 4:692-699.
42. Sztrymf B, Souza R, Bertoletti L, et al. Prognostic factors of acute heart failure in patients with pulmonary arterial hypertension. Eur Respir J. 2010; 35:1286-1293.
43. Greyson CR. Pathophysiology of right ventricular failure. Crit Care Med. 2008; 36:S57-65.

Chapter 2

The importance of trabecular hypertrophy in right ventricular adaptation to chronic pressure overload

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Abstract

Introduction. To assess the contribution of right ventricular (RV) trabeculae and papillary muscles (TPM) to RV mass and volumes in controls and patients with pulmonary arterial hypertension (PAH). Furthermore, to evaluate whether TPM shows a similar response as the RV free wall (RVFW) to changes in pulmonary artery pressure (PAP) during follow-up.
Methods. 50 patients underwent cardiac magnetic resonance imaging (CMR) and right heart catheterization at baseline and after one-year follow-up. Furthermore 20 controls underwent CMR. RV masses were assessed with and without TPM.
Results. TPM constituted a larger proportion of total RV mass and RV end-diastolic volume (RVEDV) in PAH than in controls (Mass: 35 ± 7% vs. 25 ± 5%, p<0.001, RVEDV: 17 ± 6% vs. 12 ± 6%, p = 0.003). TPM mass was related to the RVFW mass in patients (baseline: R = 0.65, p <0.001, follow-up: R = 0.80, p <0.001) and controls (R = 0.76, p <0.001). In PAH and controls, exclusion of TPM from the assessment resulted in altered RV mass, volumes and function than when included (all p <0.01). Changes in RV TPM mass (β = 0.44, p = 0.004) but not the changes in RVFW mass (p = 0.095) were independently related to changes in PAP during follow-up.
Conclusions. RV TPM showed a larger contribution to total RV mass in PAH (~35%) compared to controls (~25%). Inclusion of TPM in the analyses significantly influenced the magnitude of the RV volumes and mass. Furthermore, TPM mass was stronger related to changes in PAP than RVFW mass. Our results implicate that TPM are important contributors to RV adaptation during pressure overload and cannot be neglected from the RV assessment.

Introduction

An increased right ventricular (RV) load in patients with pulmonary arterial hypertension (PAH) results in marked RV hypertrophy[1,2]. Generally studies have focused on hypertrophy of the compact layer of the RV free wall. However the RV is highly trabecularised in PAH and even in the normal RV, trabeculae and papillary muscles (TPM) are prominent components. It is unknown whether in PAH during the process of hypertrophy, all RV mass components increase in size to the same extent.
Cardiac magnetic resonance imaging (CMR) provides high resolution imaging that enables visual contrast between the cardiac free wall, trabeculae and blood pool[3]. CMR RV parameters are of strong diagnostic[4-6], therapeutic[7-11] and prognostic[12] importance in PAH. Yet the issue of trabeculations has not received much attention, while previous studies have variably included[5,7,10,13-15] or excluded[16-19] TPM from the cardiac assessment of control subjects and PAH patients. However it is unknown whether TPM contributions to RV measures are constant over time in individual subjects and across patient populations. It therefore remains unascertained if it is possible to neglect TPM during RV assessment. Studies on the left ventricle (LV) of normal subjects and patients with LV cardiomyopathies have shown that LV assessments are greatly affected by the in- or exclusion of TPM[20-26].
The aim of the present study was to assess the contribution of RV TPM to RV mass and volumes using CMR in patients with PAH and in normal RV subjects. Furthermore to evaluate in PAH patients whether TPM shows a similar response as the RV compact free wall mass to changes in RV load during follow-up.

Methods

Study population

The present study is a retrospective data-analysis partly obtained from an ongoing prospective research program to assess the value of CMR in patients with pulmonary hypertension. Since several years CMR has become part of our clinical protocol and the other part of the CMR data included in this study were initially obtained for clinical purposes. The study was approved by The Medical Ethics Review Committee of the VU University Medical Center. Due to the fact that the study does not fall within the scope of the Medical Research Involving Human Subjects Act (WMO), the study was approved without requirement of a consent statement.
PAH was diagnosed according to World Health Organization guidelines, including a right heart catheterization (RHC)[27]. From an imaging database

consisting of 103 PAH patients who underwent CMR at baseline and after one-year of follow-up at our institution between 2003 and 2012, 50 patients were randomly selected. All included patients underwent CMR and RHC at baseline and after a median time period of 13 months (interquartile range: 12 - 17 months). CMR and RHC were obtained within a median time period of one day. Patients received optimal PAH targeted therapies. Patients with a positive response to the acute vasodilator challenge[27] were treated with calcium channel antagonists. NYHA II and III patients received oral medical therapy consisting of endothelin receptor antagonsists (ERA) and/or phophodiesterase 5 inhibitors (PDE-5I) and NYHA IV patients received intravenous prostacyclins with or without additional oral medical treatment. During follow-up, many patients went through one or multiple treatment regimens. All patients received oral anticoagulation.
In addition, 20 control subjects with no known risk factors for or history of pulmonary and cardiovascular diseases were selected from a database consisting of 30 subjects and included in the present study. Control subjects and patients were matched for age and gender.

Right heart catheterization
Hemodynamic assessment was performed with a 7F balloon tipped, flow directed Swan-Ganz catheter (131HF7, Baxter, Healthcare Corp Irvine, California). During continuous electrocardiographic monitoring, the following variables were recorded: mean pulmonary artery pressure (mPAP), mean right atrial pressure (RAP), cardiac output (CO), pulmonary arterial wedge pressure (PAWP). Pulmonary vascular resistance (PVR) is calculated as: 80*(mPAP – PAWP)/CO.

CMR image acquisition
Image acquisition was performed on a Siemens 1.5T Sonata scanner (Siemens Medical Solutions, Erlangen, Germany), equipped with a 6-element phased-array receiver coil. Electrocardiographic-gated cine imaging was performed using a balanced steady-state precession pulse sequence during repeated breath-holds. Short-axis images from base to apex of the ventricles were obtained with a typical slice thickness of 5 mm and an interslice gap of 5 mm. MR parameters were: temporal resolution between 35 and 45 ms, typical voxel size 1.8 x 1.3 x 5.0 mm^3, flip angle 60°, receiver bandwith 930 Hz/pixel, field of view 280 x 340 mm^2, repetition time/echo time 3.2/1.6 ms, and matrix 156 x 256.

CMR image analysis
During post-processing, two independent observers analyzed the CMR-images by the MASS software package (MEDIS Medical Imaging Systems, Leiden, the Netherlands). On end-diastolic (ED) images (first cine after the R-wave trigger) and end-systolic (ES) short-axis cine images (cine with

visually the smallest cavity), endocardial and epicardial contours of the RV were obtained by manual tracing. Short-axis images of the ventricles were cross-referenced with the four-chamber cine to determine whether basal slices should be included in the analysis. For each patient and image section, the contrast and brightness settings were optimized to achieve the best possible contrast between myocardium, TPM and blood pool.
RV volumes and masses were calculated using Simpson's rule. Stroke volume (SV) was calculated as (RVEDV - RVESV) and RV ejection fraction was calculated as SV/RVEDV multiplied by 100%, where RVEDV is RV end-diastolic volume and RVESV is RV end-systolic volume. For mass calculation, the myocardial volume was multiplied by the specific density of the heart (1.05 g/cm^3)[28]. RV mass was reported as the average of RV ED and ES mass. Masses and volumes were corrected for body surface area (BSA).
Analysis time and observer variability were recorded in a subset of patients. To assess intraobserver variability, observer 1 analyzed a random subset of 20 baseline and 20 follow-up CMR-scans of the PAH patients and 10 control scans twice with a three month interval between repeat measurements. Observer 2 assessed the same scans to analyze interobserver variability. The observers did not know whether a scan was a baseline or follow-up measurement. Contours from each dataset were saved in separate databases and both observers were blinded to the initial results.

Determination of RV mass components
The compact myocardium of the RV free wall was defined as a myocardial layer of homogeneous medium signal intensity on the image without inclusion of blood of brighter signal intensity. TPM was defined as a meshwork of medium signal intensities interspersed with blood of bright signal intensity.
All contours were traced twice, using two different methods. First, RV TPM were included in the compact myocardium of the RV free wall (Figure 1A, C, RV total wall mass = RV compact free wall mass + TPM). Second, TPM were excluded from the RV total wall mass and included in the blood volume (Figure 1B, D, RV compact free wall mass). The difference between the RV total mass and RV compact free wall mass represents the RV TPM mass. The difference between the RVEDV with and without TPM represents the RV TPM volume.

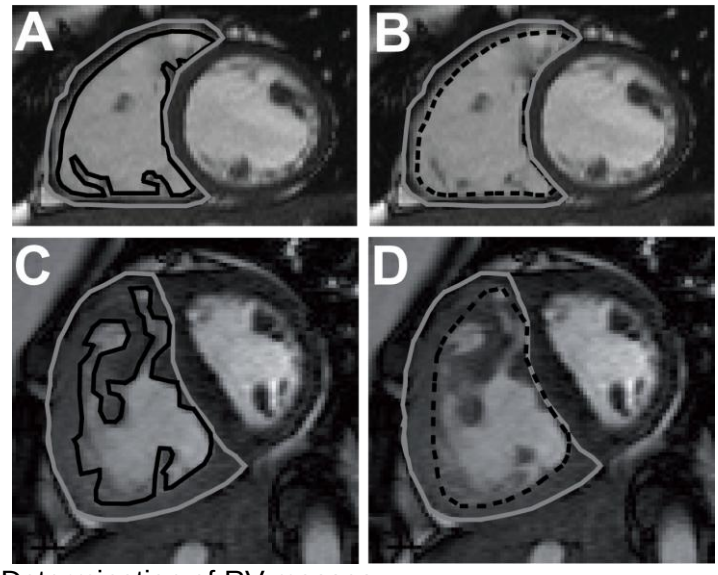

Figure 1. Determination of RV masses
The volume between the grey continuous line and black continuous line illustrates the right ventricular (RV) total mass: RV compact free wall mass with inclusion of trabeculae and papillary muscles (TPM) in controls **(A)** and PAH patients **(C)**. The volume between the black dotted line and the grey continuous line represents the RV compact free wall mass without TPM in controls **(B)** and PAH patients **(D)**.

Statistical analysis

Continuous data are presented as mean ± SD for continuous variables and absolute for categorical variables. Differences between the PAH patient-group and control-group were calculated using the unpaired Student *t* test. The absolute difference between RV volumes and mass with and without TPM and between ED and ES were tested using the paired Student *t* test. Pearson correlation analysis was applied to test the relationship between RV total mass, RV compact free wall mass, TPM mass and RV load (*i.e*. mPAP and PVR). Multivariate linear regression analysis was applied to determine the independent relationships between RV load, RV compact free wall mass and RV TPM mass after correction for potential confounding by age, gender and type of PAH diagnosis. Regression analysis was repeated for the second set of analyses from observer 1 and the analyses from observer 2. The intra and- interobserver variability were tested using Bland-Altman analysis. In addition, the intra and- interobserver coefficient of variability (*i.e.* the SD of the difference between the two measurements divided by the mean of the two measurements, expresses as a percentage) was calculated. All statistical analyses were performed using SPSS (version 19.0, SPSS, inc, Chicago, Illinois). P-values <0.05 were considered statistically significant.

Results

Study population

Table 1 summarizes the demographic and hemodynamic data of the study population. After baseline measurements, 28 patients were initiated on ERA, 5 patients received PDE5I, 8 patients were treated with prostacyclins, 2 patients received calcium channel antagonists and 7 patients received upfront medical combination therapies.
PAH patients showed greater RV mass, higher RV volumes and diminished RV function compared to controls (Table 2) (p-value between groups for all RV parameters: p-inter <0.001).

Table 1. Demographics and hemodynamics of the study population.

Variable	PAH patients (n = 50)	Control subjects (n = 20)	P-value
Age, years	46 ± 13	48 ± 18	0.465
Female, n (%)	43 (86)	16 (80)	0.717
BSA, m^2	1.8 ± 0.2	1.8 ± 0.2	0.862
Diagnosis, n (%)			
Idiopathic PAH	37 (74)		
Familial PAH	4 (8)		
Associated PAH			
Connective tissue	5 (10)		
disease	2 (4)		
Portal hypertension	2 (4)		
Pulmonary veno occlusive disease			
Hemodynamics			
mPAP, mmHg	51 ± 15		
PVR, dyne.s.cm^{-5}	833 ± 416		
CO, L/min	4.7 ± 1.4		
RAP, mmHg	8 ± 5		
PAWP, mmHg	8 ± 4		

BSA = body surface area, CO = cardiac output, mPAP = mean pulmonary artery pressure, PAWP = pulmonary arterial wedge pressure, PVR = pulmonary vascular resistance, RAP = right atrial pressure.

Table 2. RV mass and volumes at baseline in PAH patients and control subjects.

	PAH patients (n = 50)			Control subjects* (n = 20)		
Variable	**TPM in RV mass**	**TPM ex RV mass**	**P-intra**	**TPM in RV mass**	**TPM ex RV mass**	**P-intra**
RV mass, g/m^2	50 ± 15	32 ± 9	<0.001	19 ± 4	14 ± 3	<0.001
RVEDV, ml/m^2	79 ± 23	95 ± 28	<0.001	66 ± 14	70 ± 15	0.002
RVESV, ml/m^2	52 ± 22	68 ± 27	<0.001	26 ± 9	31 ± 10	<0.001
RVEF, %	36 ± 13	29 ± 12	<0.001	61 ± 8	57 ± 8	<0.001
SV, ml/m^2	27 ± 11	27 ± 11	0.215	40 ± 9	40 ± 9	0.268

RV = right ventricular, RVEDV = right ventricular end-diastolic volume, RVESV = right ventricular end-systolic volume, RVEF = right ventricular ejection fraction, SV = stroke volume, TPM = trabeculae and papillary muscles.
TPM in RV mass = TPM included in the RV mass assessment. TPM ex RV mass = TPM excluded from the RV mass assessment and included in the blood pool.
*Difference in TPM in RV mass and TPM ex RV mass between PAH patients and control subjects, P-inter <0.001 for all parameters.

Contributions of the RV compact free wall mass and TPM mass in a normal and hypertrophic RV

TPM constituted a larger proportion of RV mass in PAH patients than in control subjects (PAH: 35 ± 7% vs. controls: 25 ± 5%, p <0.001) (Figure 2A). In addition, the TPM mass was linear but non-proportionally related to the RV compact free wall mass (Figure 2B) (PAH: total RV mass = 1.93 * RV TPM mass + 16.6). RV TPM volume constituted 17 ± 6% of RV EDV in PAH and 12 ± 6% in controls (p for difference: 0.003).

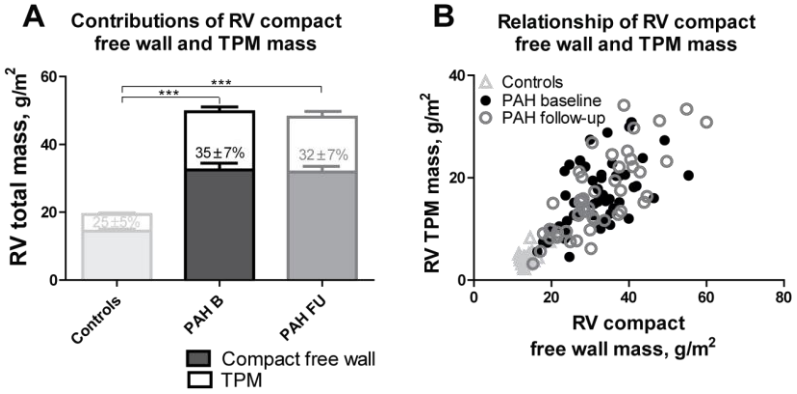

Figure 2 Relationship between TPM mass and RV compact free wall mass.
(A) Relative contributions of RV compact free wall mass (filled bars) and TPM mass (open bars) in controls (light grey), PAH patients at baseline (B) (black) and at follow-up (FU) (dark grey). **(B)** RV TPM mass was related to the RV compact free wall mass in PAH patients at baseline (R = 0.65, p <0.001); at follow-up (R = 0.80, p <0.001) and in controls (R = 0.76, p <0.001).

At baseline RV compact free wall mass and TPM mass were both related to mPAP and PVR (Table 3). Linear multivariate regression analysis consisting of RV TPM mass, RV free wall mass and mPAP revealed that RV TPM mass was an independent predictor of baseline mPAP (β = 0.040, p = 0.043) whereas RV compact free wall mass was not (p = 0.630). Multivariate analysis for PVR did not reveal an independent RV mass predictor.
After one year of medical treatment, PAH patients as a group showed no change in RV compact free wall mass (-0.1 ± 7.1 g/m^2, p = 0.924) or TPM mass (-0.7 ± 4.9 g/m^2, p = 0.305) whereas mPAP and PVR showed small average improvements (-6 ± 9 mmHg and -177 ± 273 dyne.s.cm^{-5} resp., both: p <0.001). The changes in TPM mass were moderately related to the changes in RV compact free wall mass (r = 0.530, p = 0.001). The changes in TPM mass showed a stronger correlation to the changes in mPAP and PVR than he changes in RV compact free wall mass (Table 3). The changes in mPAP during follow-up were independently predicted by the changes in RV TPM mass (β = 0.444, p = 0.004) and not by the changes in RV compact free wall mass (β = 0.246, p = 0.095). Similar results were found for the other analyses of observers 1 and 2. In addition comparable results were obtained for the relationship with the changes in PVR. After correction for possible confounding by age, gender and type of PAH diagnosis, the results remained unchanged.

Table 3. Correlations between RV masses and hemodynamics in PAH patients.

	mPAP		**PVR**	
Baseline	***r***	**P-value**	***r***	**P-value**
RV total mass, g/m^2	0.447	0.014	0.254	0.075
RV compact free wall mass, g/m^2	0.478	0.007	0.215	0.138
RV TPM mass, g/m^2	0.586	<0.001	0.433	0.019
Percentage RV TPM mass, %	0.396	0.039	0.378	0.049
	ΔmPAP		**ΔPVR**	
Changes during follow-up	***r***	**P-value**	***r***	**P-value**
ΔRV total mass, g/m^2	0.427	0.003	0.223	0.136
ΔRV compact free wall mass, g/m^2	0.472	0.001	0.183	0.228
ΔRV TPM mass, g/m^2	0.580	<0.001	0.502	0.001
ΔPercentage RV TPM mass, %	0.288	0.043	0.394	0.007

Influence of RV TPM assessment on the magnitude and reproducibility of RV volumes and mass

Measurements of RV mass in PAH did not differ between ED and ES (RV compact free wall mass + TPM: PAH: ED: 49 ± 15 g/m^2, ES: 50 ± 15 g/m^2, p = 0.330, RV compact free wall mass: ED: 32 ± 9 g/m^2, ES: 33 ± 10 g/m^2, p = 0.396). Both in controls and in PAH, measurements of RVEDV and RVESV were significantly larger when TPM were excluded from the RV

mass (Table 2) (p values within groups for all RV parameters: p-intra: <0.01). In the overall study population no differences in SV were observed between both methods. Consequently, a similar SV and larger RVEDV resulted in lower values of RVEF.
Bland-Altman analysis of the intra and- inter observer differences between RV compact free wall mass with and without inclusion of TPM for PAH patients and control subjects are presented in Figure 3. The intra-observer coefficient of variation was better when RV TPM were included in the RV mass assessment than when excluded (8.5% vs. 12.9%). Similarly, inclusion of TPM in the RV mass assessment showed a smaller inter-observer coefficient of variation than exclusion of TPM (12.5% vs. 17.7%).
Analysis time was significantly shorter when TPM were not included in the analysis in comparison with the inclusion of TPM (mean of 14 vs. 21 minutes).

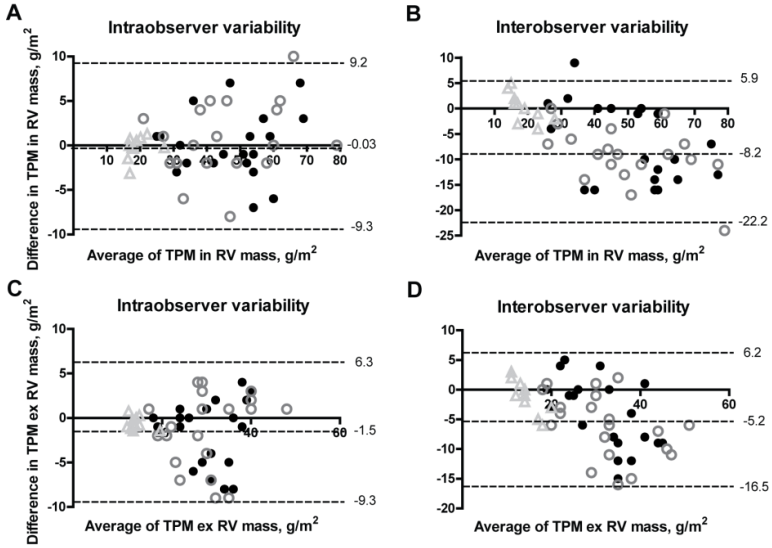

Figure 3. Bland Altman analysis for the RV mass determination.
(A) Intra-observer agreement of RV total mass assessment with inclusion of TPM (TPM in RV mass) and **(C)** without TPM (TPM ex RV mass). **(B)** Inter-observer agreement of RV mass assessment with inclusion of TPM and **(D)** without TPM of PAH patients at baseline (black), at follow-up (dark-grey) and in controls (triangle, light-grey). On each plot, the dotted lines represent the bias and limits of agreement of the PAH patients.

Discussion

In the present study, we showed that TPM mass comprised a larger proportion of total RV wall mass in PAH patients (~35%) than in controls (~25%). Furthermore in PAH, changes in RV TPM were independently related to changes in loading conditions whereas such a relationship does

not exist for changes in the RV compact free wall mass. Because the RV TPM constitutes a large part of total RV mass and volume in both PAH patients and controls, exclusion of TPM measurements significantly affected the magnitude of CMR RV measures.

RV hypertrophy is more prominent in TPM than in the RV compact free wall

We found a significant linear but non-proportional relationship between TPM and RV compact free wall mass in PAH and controls. In addition we showed that TPM constituted a larger proportion of total RV mass in PAH patients (~35%) compared to controls (~25%). In line with our findings, in the autopsy study of Fulton et al. it was observed that RV trabeculae were hypertrophied in a hypertrophic RV but the relative contribution of trabeculae to total RV weight was not studied[29]. In addition, Vogel-Claussen et al. observed a moderate relationship between septomarginal trabeculae and RV free wall mass in controls and PAH patients. Although not statistically tested it appeared that septomarginal trabeculae constituted a larger proportion of total RV free wall mass in PAH than in controls[30]. An echocardiographic study on the LV showed that LV papillary muscles form a larger fraction of LV volumes in the hypertrophied human heart than in the normal LV[31]. In addition, Janik et al. found by CMR assessment a twofold larger impact of TPM to total LV mass in the hypertrophic LV compared to controls[32].

The significant correlation between TPM and the RV compact free wall mass might be explained by a similar embryological origin implicating that RV trabeculae are an integral part of RV structural remodeling[33]. However we found that RV TPM have a relative larger contribution in the hypertrophic RV and showed a stronger relation to changes in pulmonary pressures than the RV compact free wall which might suggest that TPM are more responsive to changes in loading conditions. A possible physiological explanation is a greater sensitivity of RV TPM to changes in wall stress. Damon et al. found greater strain and more stretch in trabeculae than in the compact layer of the embryonic animal heart[34]. Similarly, a CMR study demonstrated that high myocardial strain was related to TPM in the human LV[35]. Another explanation could be that TPM respond differently to changes in oxygen delivery. RV papillary muscles are more susceptible to ischemia since blood is required to course uphill to perfuse the papillary muscle by coronary blood flow[36]. RV trabeculae receive blood from both the coronary circulation and surrounding ventricular blood but it has been found that the capillary density of trabeculae in the normal animal RV is about the half of that of the ventricular walls[37]. In autopsy tissues from the RV compact free wall of PAH patients, a reduced capillary density has been found[38] but it is unknown what happens to the oxygenation of RV TPM in PAH.

Influence of TPM assessment on the magnitude and reproducibility of CMR RV measures

In- or exclusion of TPM in CMR measures of RV mass, volumes and function significantly affected the magnitude of the measure. Our results correspond to the 1.5 times higher measured RV mass in PAH studies that included TPM[7-12] compared to studies that excluded TPM[14,16]. When TPM were excluded from the RV mass and included into the blood pool, it resulted in significantly higher measured RVEDV, RVESV and lower RVEF in PAH patients. In theory the amount of TPM is similar in diastole and systole and therefore does not influence SV measurements. Previously Winter et al. observed similar variations in RV volume measurements with or without inclusion of TPM in patients with a systemic RV[39]. In line with our findings, Sievers et al. observed systemic differences between the method with or without inclusion of TPM in control subjects[40].

Our reproducibility results are consistent with previous studies in PAH[28,41] and control subjects[15,18]. Although analysis time was reduced by ~35% when TPM were excluded from the RV mass analyses, observer variability was larger than when TPM were included in the RV mass of PAH patients. These findings correspond to the study of Bradlow et al.[41]. For complete assessment of RV mass, volumes and function, the RV has to be assessed both in ED and ES. If only RV mass assessment is required than it simply needs to be assessed during ED saving 50% of time due to the fact that RV mass measures were similar in ED and ES.

Clinical implications

In the present study, we found that the relative contributions of TPM and compact free wall to total RV mass alter during the RV hypertrophic process which implicates that RV TPM and the compact free wall should not be considered as strictly homogeneous. TPM are important contributors to RV adaptation and might be sensitive components to detect changes in RV afterload. Although future studies are required to assess the physiological importance of TPM, it might be that hypertrophy of TPM significantly contributes to a favorable RV adaptation pattern in order to lower the wall stress in the direction orthogonal to the RV wall.

In addition, TPM constitute a large proportion of RV mass and volumes in controls and PAH patients and thus cannot be neglected from the RV assessment. Observer variability was better when TPM were included but was still relatively modest for RV mass measurements. Therefore measuring RV mass is reliable when measured in a group but might not be sensitive enough to accurately detect changes in the individual patient.

Limitations

TPM might be relatively small in controls and therefore more difficult to visualize by CMR leading to potential underestimation of the TPM mass.

Although we cannot completely exclude measurement errors, previous studies have demonstrated that CMR is highly accurate to determine RV mass in a healthy subject[28,32,42,43]. Therefore it is unlikely that the absolute difference in TPM mass between controls and PAH patients of 18 g/m^2 can be explained by underestimation of RV TPM mass in controls.
In the image analysis of ventricular masses window/level display settings influence ventricular measurements. Although, the settings were optimized during each analysis, in future studies standardized settings should be sought. In the present study, inter-study reproducibility was not addressed. However, in a previous study the repeatability of image acquisition in PAH patients has been assessed and a coefficient of variation of 8% was found for RV mass with inclusion of TPM and of 12% when TPM were excluded[41].

Conclusions

RV TPM showed a larger contribution to total RV mass in the hypertrophic RV of PAH patients (~35%) compared to controls (~25%). Furthermore TPM mass was stronger related to changes in loading conditions than mass of the RV compact free wall. Inclusion of TPM in the RV analyses significantly altered the magnitude of the RV parameters and resulted in better observer variability. Our results implicate that TPM are important contributors to RV adaptation during pressure overload and cannot be neglected from RV volumetric and mass assessments.

References

1. McLaughlin VV, McGoon MD. Pulmonary arterial hypertension. Circulation. 2006; 114:1417-1431.
2. Voelkel NF, Quaife RA, Leinwand LA, et al. Right ventricular function and failure: report of a National Heart, Lung, and Blood Institute working group on cellular and molecular mechanisms of right heart failure. Circulation. 2006; 114:1883-1891.
3. Pennell DJ, Sechtem UP, Higgins CB, et al. Clinical indications for cardiovascular magnetic resonance (CMR): Consensus Panel report. Eur Heart J. 2004; 25:1940-1965.
4. Hagger D, Condliffe R, Woodhouse N, et al. Ventricular mass index correlates with pulmonary artery pressure and predicts survival in suspected systemic sclerosis-associated pulmonary arterial hypertension. Rheumatology. 2009; 48:1137-1142.
5. Roeleveld RJ, Marcus JT, Boonstra A, et al. A comparison of noninvasive MRI-based methods of estimating pulmonary artery pressure in pulmonary hypertension. J Magn Reson Imaging. 2005; 22:67-72.
6. Saba TS, Foster J, Cockburn M, et al. Ventricular mass index using magnetic resonance imaging accurately estimates pulmonary artery pressure. Eur Respir J. 2002; 20:1519-1524.
7. Wilkins MR, Ali O, Bradlow W, et al. Simvastatin as a treatment for pulmonary hypertension trial. Am J Respir Crit Care Med. 2010; 181:1106-1113.

8. Reesink HJ, Marcus JT, Tulevski II, et al. Reverse right ventricular remodeling after pulmonary endarterectomy in patients with chronic thromboembolic pulmonary hypertension: utility of magnetic resonance imaging to demonstrate restoration of the right ventricle. J Thor Cardiovasc Surg. 2007; 133:58-64.
9. D'Armini AM, Zanotti G, Ghio S, et al. Reverse right ventricular remodeling after pulmonary endarterectomy. J Thor Cardiovasc Surg. 2007; 133:162-168.
10. Wilkins MR, Paul GA, Strange JW, et al. Sildenafil versus Endothelin Receptor Antagonist for Pulmonary Hypertension (SERAPH) study. Am J Respir Crit Care Med. 2005; 171:1292-1297.
11. Frist WH, Lorenz CH, Walker ES, et al. MRI complements standard assessment of right ventricular function after lung transplantation. Ann Thor Surg. 1995; 60:268-271.
12. van Wolferen SA, Marcus JT, Boonstra A, et al. Prognostic value of right ventricular mass, volume, and function in idiopathic pulmonary arterial hypertension. Eur Heart J. 2007; 28:1250-1257.
13. Hudsmith LE, Petersen SE, Francis JM, et al. Normal human left and right ventricular and left atrial dimensions using steady state free precession magnetic resonance imaging. J Cardiovasc Magn Reson. 2005; 7:775-782.
14. Grothues F, Moon JC, Bellenger NG, et al. Interstudy reproducibility of right ventricular volumes, function, and mass with cardiovascular magnetic resonance. Am Heart J. 2004; 147:218-223.
15. Lorenz CH, Walker ES, Morgan VL, et al. Normal human right and left ventricular mass, systolic function, and gender differences by cine magnetic resonance imaging. J Cardiovasc Magn Reson. 1999; 1:7-21.
16. Hardziyenka M, Campian ME, Reesink HJ, et al. Right ventricular failure following chronic pressure overload is associated with reduction in left ventricular mass evidence for atrophic remodeling. J Am Coll Cardiol 2011; 57:921-928.
17. Dibble CT, Lima JA, Bluemke DA, et al. Regional left ventricular systolic function and the right ventricle: the multi-ethnic study of atherosclerosis right ventricle study. Chest. 2011; 140:310-316.
18. Sandstede J, Lipke C, Beer M, et al. Age- and gender-specific differences in left and right ventricular cardiac function and mass determined by cine magnetic resonance imaging. Eur Radiol. 2000; 10:438-442.
19. Doherty NE, Fujita N, Caputo GR, et al. Measurement of right ventricular mass in normal and dilated cardiomyopathic ventricles using cine magnetic resonance imaging. Am J Cardiol. 1992; 69:1223-1228.
20. Chuang ML, Gona P, Hautvast GL, et al. Correlation of trabeculae and papillary muscles with clinical and cardiac characteristics and impact on CMR measures of LV anatomy and function. JACC Cardiovasc Imaging. 2012; 5:1115-1123.
21. Jacquier A, Thuny F, Jop B, et al. Measurement of trabeculated left ventricular mass using cardiac magnetic resonance imaging in the diagnosis of left ventricular non-compaction. Eur Heart J. 2010; 31:1098-1104.
22. Janik M, Cham MD, Ross MI, et al. Effects of papillary muscles and trabeculae on left ventricular quantification: increased impact of methodological variability in patients with left ventricular hypertrophy. J Hypertens. 2008; 26:1677-1685.
23. Weinsaft JW, Cham MD, Janik M, et al. Left ventricular papillary muscles and trabeculae are significant determinants of cardiac MRI volumetric measurements: effects on clinical standards in patients with advanced systolic dysfunction. Int J Cardiol. 2008; 126:359-365.
24. Han Y, Osborn EA, Maron MS, et al. Impact of papillary and trabecular muscles on quantitative analyses of cardiac function in hypertrophic cardiomyopathy. J Magn Reson Imaging. 2009; 30:1197-1202.

25. Vogel-Claussen J, Finn JP, Gomes AS, et al. Left ventricular papillary muscle mass: relationship to left ventricular mass and volumes by magnetic resonance imaging. J Comput Assist Tomogr. 2006; 30:426-432.
26. Papavassiliu T, Kuhl HP, Schroder M, et al. Effect of endocardial trabeculae on left ventricular measurements and measurement reproducibility at cardiovascular MR imaging. Radiology. 2005; 236:57-64.
27. Galie N, Torbicki A, Barst R, et al. Guidelines on diagnosis and treatment of pulmonary arterial hypertension. The Task Force on Diagnosis and Treatment of Pulmonary Arterial Hypertension of the European Society of Cardiology. Eur Heart J. 2004; 25:2243-2278.
28. Katz J, Whang J, Boxt LM, et al. Estimation of right ventricular mass in normal subjects and in patients with primary pulmonary hypertension by nuclear magnetic resonance imaging. J Am Coll Cardiol. 1993; 21:1475-1481.
29. Fulton RM, Hutchinson EC, Jones AM. Ventricular weight in cardiac hypertrophy. Br Heart J. 1952; 14:413-420.
30. Vogel-Claussen J, Shehata ML, Lossnitzer D, et al. Increased right ventricular Septomarginal trabeculation mass is a novel marker for pulmonary hypertension: comparison with ventricular mass index and right ventricular mass. Invest Radiol. 2011; 46:567-575.
31. Madu EC, Baugh DS, Johns C, et al. Papillary muscle contribution to ventricular ejection in normal and hypertrophic ventricles: a transesophageal echocardiographic study. Echocardiography. 2001; 18:633-638.
32. Shors SM, Fung CW, Francois CJ, et al. Accurate quantification of right ventricular mass at MR imaging by using cine true fast imaging with steady-state precession: study in dogs. Radiology. 2004 Feb;230(2):383-8.
33. Wenink AC, Gittenberger-de Groot AC. Left and right ventricular trabecular patterns. Consequence of ventricular septation and valve development. Br Heart J. 1982; 48:462-468.
34. Damon BJ, Remond MC, Bigelow MR, et al. Patterns of muscular strain in the embryonic heart wall. Dev Dyn. 2009; 238:1535-1546.
35. Fransson P, Merboldt KD, Petersson KM, et al. On the effects of spatial filtering--a comparative fMRI study of episodic memory encoding at high and low resolution. Neuroimage. 2002; 16:977-984.
36. Roberts WC, Cohen LS. Left ventricular papillary muscles. Description of the normal and a survey of conditions causing them to be abnormal. Circulation. 1972; 46:138-154.
37. Goo S, Joshi P, Sands G, et al. Trabeculae carneae as models of the ventricular walls: implications for the delivery of oxygen. J Gen Physiol. 2009; 134:339-350.
38. Ruiter G, Ying Wong Y, de Man FS, et al. Right ventricular oxygen supply parameters are decreased in human and experimental pulmonary hypertension. J Heart Lung Transplant. 2013; 32:231-240.
39. Winter MM, Bernink FJ, Groenink M, et al. Evaluating the systemic right ventricle by CMR: the importance of consistent and reproducible delineation of the cavity. J Cardiovasc Magn Reson. 2008; 10:40.
40. Sievers B, Kirchberg S, Bakan A, et al. Impact of papillary muscles in ventricular volume and ejection fraction assessment by cardiovascular magnetic resonance. J Cardiovasc Magn Reson. 2004; 6:9-16.
41. Bradlow WM, Hughes ML, Keenan NG, et al. Measuring the heart in pulmonary arterial hypertension (PAH): implications for trial study size. J Magn Reson Imaging. 2010; 31:117-124.
42. Bloomgarden DC, Fayad ZA, Ferrari VA, et al. Global cardiac function using fast breath-hold MRI: validation of new acquisition and analysis techniques. Magn Reson Med. 1997; 37:683-692.

43. McDonald KM, Parrish T, Wennberg P, et al. Rapid, accurate and simultaneous noninvasive assessment of right and left ventricular mass with nuclear magnetic resonance imaging using the snapshot gradient method. J Am Coll Cardiol. 1992; 19:1601-1607.

Chapter 3

The interventricular septum in pulmonary hypertension does not show features of right ventricular failure

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To the Editor:

Chronic pressure overload in patients with pulmonary hypertension (PH) results in hypertrophy, dilatation, metabolic alterations[1-3] and disturbed oxygen and calcium handling of the right ventricular (RV) free wall, leading to RV failure[4,5]. Being structurally and functionally related to the left ventricle (LV), the interventricular septum (IVS) might adapt differently to high RV pressures compared with the RV free wall.
The IVS plays a major role in the maintenance of RV function[6-8]. In PH, its function is impaired and associated with poor prognosis[9,10]. A comparison of remodeling processes of the IVS and the RV free wall may contribute to better understand the development of RV dysfunction in PH. Moreover if IVS remodeling does not reflect global processes of right heart remodeling, this would imply that IVS biopsies which have been advocated for the clinical management of PH patients because of a higher complication risk of RV wall biopsies[11], might have limited potential.
The aim of the current study was to assess whether the IVS shows similar changes in morphology and glucose metabolism as the RV free wall in PH patients *in vivo*. In addition, we studied whether cellular alterations of the IVS were comparable to the RV free wall in a monocrotaline PH rat model.

Seventeen prevalent patients with a clinical stable condition of idiopathic pulmonary arterial hypertension (IPAH) were included. They underwent right heart catheterization and exercise testing[12]. The IVS, RV and LV free wall mass and myocardial glucose uptake rate (MRglu) were studied using magnetic resonance imaging (MRI) and [^{18}F]-2-fluoro-2-deoxy-D-glucose (FDG) positron emission tomography (PET) respectively as described previously[12-15]. A priori, the study was approved by the local Medical Ethics Committee and each patient gave written informed consent (approval number: 2007/259). In addition, seventeen age and gender matched control subjects without history of cardiopulmonary diseases underwent MRI.
A male Wistar rat PH model was used to assess cardiac cellular changes[16]. The study was approved by the local Animal Ethics Committee (approval number: FYS11-03). Severe PH was induced in eight rats with subcutaneous injection of 60 mg/kg monocrotaline. Eight animals were used as healthy controls. After three weeks follow-up, *in vivo* cardiac function was measured using echocardiography before the rats were sacrificed. Cardiomyocyte cross sectional area (CSA), capillary density, fibrosis and leukocyte infiltration were measured[16-19].

Cardiac segments were compared by two-way repeated measurements analysis of variance with Bonferroni post-hoc testing. Differences between groups were assessed using unpaired t-tests. SPSS 20.0 (SPSS Inc.,

Chicago) was used for statistical analysis. Data are presented as mean ± SD, unless stated otherwise. A p-value <0.05 was considered significant.

Table 1 shows the characteristics and MRI-parameters of IPAH patients and controls.

Table 1. Clinical characteristics and MRI measurements of patients with idiopathic pulmonary arterial hypertension (IPAH) and control subjects.

Variable	IPAH patients (n = 17)	Control subjects (n = 17)	P-value
Age, years	46 ± 13	46 ± 16	0.89
Female / male, n	16 / 1	16 / 1	1.00
BSA, g/m^2	1.83 ± 0.19	1.79 ± 0.15	0.53
NYHA class, II / III, n	9 / 8		
NT-proBNP, ng/L	1383 ± 1790		
6MWT, distance, m	453 ± 140		
Hemodynamics			
mPAP, mmHg	52 ± 15		
RAP, mmHg	8 ± 7		
PVR, dyne·s·cm^{-5}	693 ± 369		
PAWP, mmHg	10 ± 4		
CI, L/min/m^2	3.1 ± 1.2		
SvO_2, %	65 ± 8		
MRI parameters			
RV free wall mass, g/m^2	56 ± 12	18 ± 3	<0.001
RVEDV, ml/m^2	82 ± 15	65 ± 14	<0.01
RVEF, %	39 ± 15	61 ± 8	<0.001
SV, ml/m^2	33 ± 11	44 ± 10	0.001
LVEDV, ml/m^2	52 ± 16	67 ± 15	<0.01
LVEF, %	64 ± 9	66 ± 6	0.41
LV free wall mass, g/m^2	41 ± 10	41 ± 9	0.81
IVS mass, g/m^2	17 ± 4	14 ± 2	0.05

6MWT = six-minute walking test, BSA = body surface area, CI = cardiac index, IVS = interventricular septum, LV = left ventricular, LVEDV = left ventricular end-diastolic volume, LVEF = left ventricular ejection fraction, mPAP = mean pulmonary artery pressure, NT-proBNP = N-terminal pro-brain natriuretic peptide, NYHA = New York Heart Association, PAWP = pulmonary arterial wedge pressure, PVR = pulmonary vascular resistance, RAP = right atrial pressure, RV = right ventricular, RVEDV = right ventricular end-diastolic volume, RVEF = right ventricular ejection fraction, SV = stroke volume, SvO_2 = mixed venous oxygen saturation.

Figure 1 demonstrates a higher RV free wall mass (p <0.001) but comparable IVS mass (p >0.05) in IPAH compared to controls. PET in IPAH patients showed that MRglu in the IVS was higher than in the RV (0.34 ± 0.09 vs. 0.25 ± 0.08 µmol/g/min, p <0.01) and comparable to the LV (0.33 ± 0.09 µmol/g/min, p = 0.38).

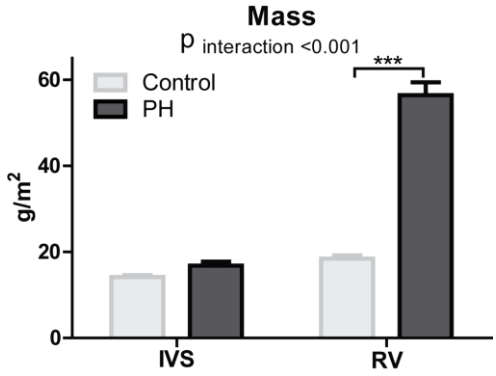

Figure 1. Interventricular septum (IVS) and right ventricular (RV) free wall mass measured by cardiac magnetic resonance imaging (MRI).
Control subjects (grey) and patients with idiopathic pulmonary arterial hypertension (IPAH) (black). RV free wall mass index was significantly increased in IPAH compared to controls. In contrast, IVS mass was similar in both groups. Data are presented as mean ± SEM. ***p <0.001.

Table 2 shows echocardiography results of the PH rats. Although RV free wall thickness was increased in PH compared with controls (p <0.001), IVS wall thickness was not (p = 0.24) (p-interaction <0.001).

Table 2. Rat echocardiographic parameters.

Variable	PH rats (n = 8)	Control rats (n = 8)	P-value
eRVSP, mmHg	81.4 ± 5.5	24.4 ± 5.1	<0.001
PVR, mmHg/ml/min/mg	13.46 ± 3.37	0.76 ± 0.20	<0.001
SV, ml	0.07 ± 0.01	0.21 ± 0.04	<0.001
Heart rate, bpm	264 ± 28	432 ± 22	<0.001
CI, ml/min/g	0.08 ± 0.02	0.34 ± 0.07	<0.001
TAPSE, mm	1.4 ± 0.4	3.1 ± 0.3	<0.001
RVWT, mm	1.3 ± 0.1	1.1 ± 0.1	<0.001
IVSWT, mm	1.5 ± 0.1	1.6 ± 0.1	0.24
LVWT, mm	1.7 ± 0.1	1.7 ± 0.1	0.74
RVEDD, mm	7.2 ± 0.4	3.5 ± 0.7	<0.001
LVEDD, mm	5.2 ± 0.6	8.4 ± 0.7	<0.001
RVWT / RVEDD	0.18 ± 0.01	0.32 ± 0.08	<0.001
LVWT / LVEDD	0.34 ± 0.06	0.20 ± 0.02	<0.001

eRVSP = estimated right ventricular systolic pressure, IVSWT = interventricular septum wall thickness, LVEDD = left ventricular end diastolic diameter, LVWT = left ventricular wall thickness, PH = pulmonary hypertension, PVR = pulmonary vascular resistance, RVEDD = right ventricular end diastolic diameter, RVWT = right ventricular wall thickness, TAPSE = tricuspid annular plane systolic excursion.

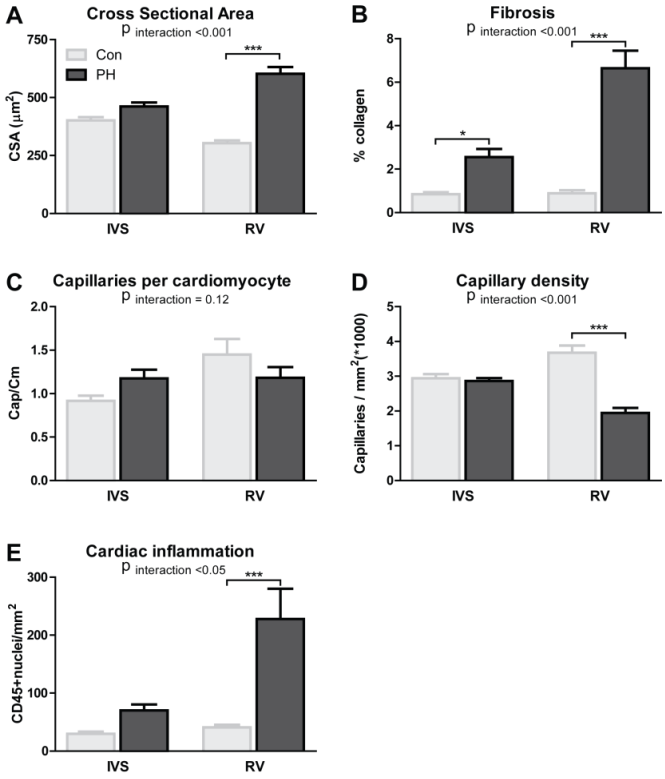

Figure 2. Cellular changes in the IVS in control (grey) and pulmonary hypertension (PH) rats (black).
(A) Although the RV cross-sectional area (CSA) of the cardiomyocytes was significantly increased in PH compared to controls, the IVS CSA was comparable in controls and PH rats. **(B)** The number of capillaries remained unchanged in both the IVS and RV of PH rats compared to controls. **(C)** The capillary density was decreased in the RV but remained preserved in the IVS. **(D)** Leukocyte infiltration is highly present in the RV in PH, but is absent in the PH IVS. **(E)** In both the IVS and the RV, fibrosis was increased in PH compared to controls, but the magnitude of increase was significantly smaller in the IVS. Data are presented as mean ± SEM. ***p <0.001.

Figure 2 demonstrates a higher cardiomyocyte CSA in the RV free wall of PH rats than of controls (p <0.001). In PH but not in controls, we found larger cardiomyocytes on the right side of the IVS similar to RV free wall and smaller cardiomyocytes on the left side of the IVS, comparable to the LV free wall (Figure 3).

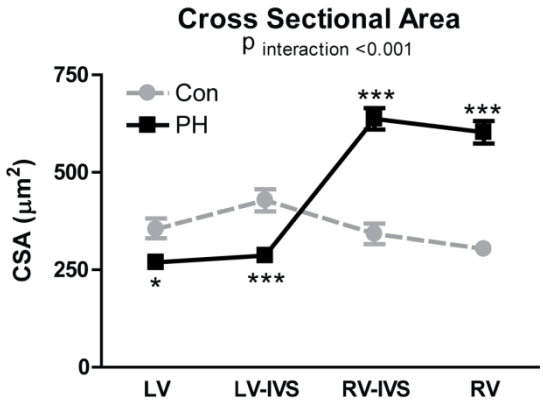

Figure 3. Cross-sectional area (CSA) of cardiomyocytes in rats with pulmonary hypertension (PH) and controls (Con).
Cardiomyocytes are significantly larger at the RV cavity side of the IVS (RV-IVS) and smaller at the LV cavity side of the IVS (LV-IVS) in PH rats compared to controls. Data are presented as mean ± SEM. *p <0.05, ***p <0.001.

Although the number of capillaries was similar in both groups, the capillary density in PH was decreased in the RV free wall due to increased CSA but preserved in the IVS (p-interaction <0.001). The amount of fibrosis was increased in both the RV free wall and IVS of PH rats compared with controls but the magnitude of increase was smaller in the IVS (p-interaction <0.001). Leukocyte infiltration was present in the RV free wall but absent in the IVS of PH rats. We did not observe regional heterogeneity in the IVS with respect to capillary density, fibrosis and leukocyte infiltration (not shown).

In the transition from RV adaptation towards failure in PH, several RV free wall changes have been reported: hypertrophy, metabolic changes, altered cardiomyocyte contractile properties, disturbed calcium and oxygen handling, fibrosis and inflammation[4,5]. The absence of such changes in the IVS in the present study indicates a different biological response to pressure overload than the RV free wall. Both IVS mass and MRglu were unrelated to the RV free wall in IPAH patients. In addition, we found in PH rats that the CSA of RV free wall cardiomyocytes was increased whereas the average CSA in the IVS remained unaltered. Although the RV free wall showed capillary rarefaction, IVS capillary density was preserved. A reduced capillary density seems to be a heart-failure specific symptom[20] and therefore our results may imply that the IVS is not clearly involved in the process of RV failure in PH. Although not observed in the IVS, in the failing RV free wall high inflammatory activity results in oxidative stress, cell damage and apoptosis[5].
The absence of failure signs in the IVS might suggest that IVS cardiomyocytes are capable to sustain higher pressures/wall stress than

RV free wall cardiomyocytes. Another possible explanation could be that, with the exception of leftward IVS bulging during the RV post-systolic contraction period[10], the IVS is not stretched/dilated during most part of the cardiac contraction cycle due to counterbalancing LV pressures, which translates into a relatively lower wall stress.
To conclude, we showed that despite similar pressure overload in IPAH patients, right heart remodeling is different in the IVS and RV free wall. The RV free wall shows hypertrophic and metabolic changes that do not occur in the IVS. In PH rats, in contrast to the RV free wall, the IVS demonstrates preserved cardiomyocyte size and capillary density, no fibrosis and leukocyte infiltration. Our results imply that the IVS is an inadequate substitute to study remodeling processes in the right heart. Therefore, the future role of IVS biopsies in PH might be limited.

References

1. Bokhari S, Raina A, Rosenweig EB, et al. PET imaging may provide a novel biomarker and understanding of right ventricular dysfunction in patients with idiopathic pulmonary arterial hypertension. Circulation. 2011; 4:641-647.
2. Can MM, Kaymaz C, Tanboga IH, et al. Increased right ventricular glucose metabolism in patients with pulmonary arterial hypertension. Clin Nucl Med. 2011; 36:743-748.
3. Oikawa M, Kagaya Y, Otani H, et al. Increased [18F]fluorodeoxyglucose accumulation in right ventricular free wall in patients with pulmonary hypertension and the effect of epoprostenol. J Am Coll Cardiol. 2005; 45:1849-55.
4. Voelkel NF, Gomez-Arroyo J, Abbate A, et al. Pathobiology of pulmonary arterial hypertension and right ventricular failure. Eur Respir J. 2012; 40:1555-1565.
5. Bogaard HJ, Abe K, Vonk Noordegraaf A, et al. The right ventricle under pressure: cellular and molecular mechanisms of right-heart failure in pulmonary hypertension. Chest. 2009; 135:794-804.
6. Buckberg GD. The ventricular septum: the lion of right ventricular function, and its impact on right ventricular restoration. Eur J Cardiothorac Surg. 2006; 29:S272-278.
7. Banka VS, Agarwal JB, Bodenheimer MM, et al. Interventricular septal motion: biventricular angiographic assessment of its relative contribution to left and right ventricular contraction. Circulation. 1981; 64:992-996.
8. Fixler DE, Monroe GA, Wheeler JM. Hemodynamic alterations during septal or right ventricular ischemia in dogs. Am Heart J. 1977; 93:210-215.
9. Puwanant S, Park M, Popovic ZB, et al. Ventricular geometry, strain, and rotational mechanics in pulmonary hypertension. Circulation. 2010; 121:259-266.
10. Marcus JT, Gan CT, Zwanenburg JJ, et al. Interventricular mechanical asynchrony in pulmonary arterial hypertension: left-to-right delay in peak shortening is related to right ventricular overload and left ventricular underfilling. J Am Coll Cardiol. 2008; 51:750-757.
11. Brooksby IA, Jenkins BS, Coltart DJ, et al. Left-ventricular endomyocardial biopsy. Lancet. 1974; 2:1222-1225.
12. Wong YY, Ruiter G, Lubberink M, et al. Right ventricular failure in idiopathic pulmonary arterial hypertension is associated with inefficient myocardial oxygen utilization. Circ Heart Fail. 2011; 4:700-706.

13. van Wolferen SA, Marcus JT, Boonstra A, et al. Prognostic value of right ventricular mass, volume, and function in idiopathic pulmonary arterial hypertension. Eur Heart J. 2007; 28:1250-1257.
14. Rijzewijk LJ, van der Meer RW, Lamb HJ, et al. Altered myocardial substrate metabolism and decreased diastolic function in nonischemic human diabetic cardiomyopathy: studies with cardiac positron emission tomography and magnetic resonance imaging. J Am Coll Cardiol. 2009; 54:1524-1532.
15. Patlak CS, Blasberg RG. Graphical evaluation of blood-to-brain transfer constants from multiple-time uptake data. Generalizations. J Cereb Blood Flow Metab. 1985; 5:584-590.
16. Handoko ML, de Man FS, Happe CM, et al. Opposite effects of training in rats with stable and progressive pulmonary hypertension. Circulation. 2009; 120:42-49.
17. Hadi AM, Mouchaers KT, Schalij I, et al. Rapid quantification of myocardial fibrosis: A new macro-based automated analysis. Anal Cell Pathol. 2010; 33:257-269.
18. Lee-de Groot MB, Tombe AL, van der Laarse WJ. Calibrated histochemistry of myoglobin concentration in cardiomyocytes. J Histochem Cytochem. 1988; 46:1077-1084.
19. de Man FS, Handoko ML, Groepenhoff H, et al. Effects of exercise training in patients with idiopathic pulmonary arterial hypertension. Eur Respir J. 2009; 34:669-75.
20. Ruiter G, Ying Wong Y, de Man FS, et al. Right ventricular oxygen supply parameters are decreased in human and experimental pulmonary hypertension. J Heart Lung Transplant. 2013; 32:231-240.

Chapter 4

Progressive right ventricular dysfunction in pulmonary arterial hypertension patients responding to therapy

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Abstract

Introduction. Despite the fact that medical therapies reduce pulmonary vascular resistance (PVR), the prognosis of patients with pulmonary arterial hypertension (PAH) is still poor. The primary cause of death is right ventricular (RV) failure. One possible explanation for this apparent paradox is the fact that a reduction in PVR is not automatically followed by an improvement in RV function. The purpose of this study was to examine the relationship between changes in PVR, RV ejection fraction (RVEF) and survival in PAH patients under PAH targeted therapies.
Methods. A cohort of 110 incident PAH patients underwent baseline right heart catheterization, cardiac magnetic resonance imaging and six-minute walk testing. These measurements were repeated in 76 patients after 12 months of therapy.
Results. Two patients underwent lung transplantation, 13 patients died during the first year, and 17 patients died in the subsequent follow-up of 47 months. Baseline RVEF (hazard ratio (HR): 0.938, p = 0.001) and PVR (HR: 1.001, p = 0.031) were predictors of mortality. During the first 12 months, changes in PVR were moderately correlated to changes in RVEF (R = 0.330, p = 0.005). Changes in RVEF (HR: 0.929, p = 0.014) were associated with survival but changes in PVR (HR: 1.001, p = 0.920) were not. In 68% of patients, PVR decreased after medical therapy. Twenty-five percent of those patients with decreased PVR showed a deterioration of RV function and had a poor prognosis.
Conclusions. After PAH-targeted therapy, RV function can deteriorate despite a reduction in PVR. Loss of RV function is associated with a poor outcome, irrespective of any changes in PVR.

Introduction

Pulmonary arterial hypertension (PAH) is a progressive disease of the pulmonary vasculature, leading to increased pulmonary vascular resistance (PVR), an elevated pulmonary artery pressure (PAP), right ventricular (RV) dysfunction, and ultimately, RV failure and death[1,2]. Prognosis is strongly associated with RV parameters, such as cardiac index and right atrial pressure[3-5]. Guided by the premise that RV failure follows an increased load, the current strategy to preserve RV function is by attempting to reduce the PVR. This strategy is effective when loading conditions can be normalized, which is the case in PAH patients after lung transplantation and in patients with chronic thrombo-embolic pulmonary hypertension after pulmonary endarterectomy[6-8]. Although PVR can be reduced by means of PAH-specific medication, PVR remains elevated in the vast majority of patients and the prognosis remains unsatisfactory[3,9]. This apparent contrast between hemodynamic success and poor prognosis raises the question whether RV dysfunction can progress even when the PVR is lowered but not normalized by current medical therapies.
Therefore, the aim of the present study is to investigate the relationship between changes in PVR, right ventricular ejection fraction (RVEF) and survival as assessed by means of right heart catheterization (RHC) and cardiac magnetic resonance imaging (CMR) in a cohort of PAH patients receiving PAH-targeted medical therapy.

Methods

Patients

This study is part of a prospective on-going research program to assess the value of CMR in patients with pulmonary hypertension. Between March 2002 and March 2007, 657 patients were referred to the VU University Medical Center because of a suspected diagnosis of pulmonary hypertension. Based on world health organisation (WHO) guidelines[10], 179 patients were diagnosed as having PAH.
Inclusion criteria were: (1) patients diagnosed with PAH; (2) RHC, CMR and six-minute walking test (6MWT) completed within 2 weeks of diagnosis and before the initiation of therapy. Exclusion criteria were: (1) congenital systemic-to-pulmonary shunts (n = 32), (2) contraindications for CMR (e.g., implanted devices, claustrophobia) (n = 28).
In total, 119 PAH patients met the criteria and were enrolled. Nine patients were excluded because of incomplete data. Baseline measurements were completed in 110 patients. Thirteen patients died during the first year of follow-up. Seven patients did not undergo a second RHC and were

excluded from the follow-up analysis. Ninety out of the 110 patients underwent follow-up measurements consisting of a second RHC, CMR and 6MWT, after 12 months of PAH-targeted medical treatment. Six patients were excluded from the final analysis because the time between the second RHC and CMR was >1 month. Five patients were excluded due to incomplete CMR data and three patients were excluded due to insufficient CMR quality. Seventy-six patients completed follow-up measurements (Figure 1). All 110 patients were followed clinically on a regular basis by outpatient visits and telephone contacts during a period up to May 1, 2010.
Medical treatment comprised of prostacyclins, endothelin receptor antagonists and phosphodiesterase 5 inhibitors, either alone or in various combinations. Patients with a positive response to an acute vasodilator challenge were treated with calcium antagonists[10]. All patients received oral anticoagulants. During follow-up, many patients went through one or more treatment regimens.
This study was approved by the institutional "Review Board on Research Involving Human Projects" of the VU University Medical Centre. All participants gave written informed consent.

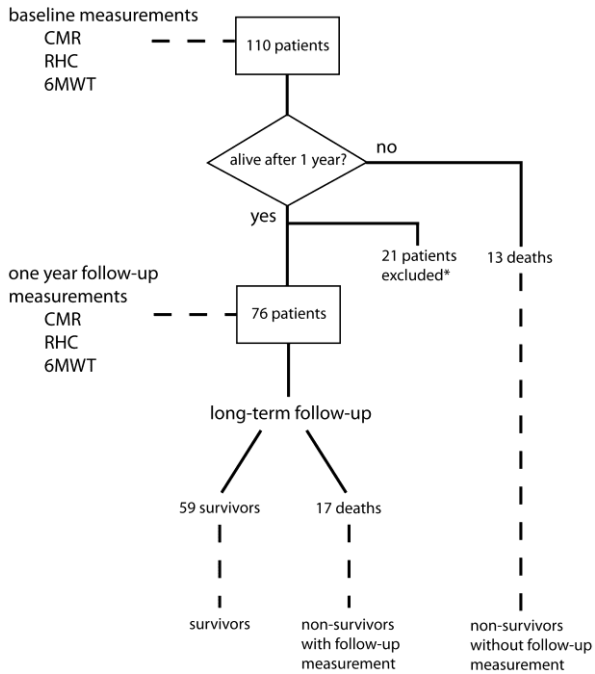

Figure 1. Study profile.
*Excluded due to: a missing second right heart catheterization (RHC) (n = 7), interval between the second RHC and cardiac magnetic resonance imaging (CMR) >1 month (n = 6), incomplete CMR cines (n = 5), and insufficient CMR image quality (n = 3).

Right heart catheterization
Hemodynamic assessment was performed with a 7F balloon tipped, flow directed Swan-Ganz catheter (131HF7; Baxter Healthcare Corp; Irvine, CA) during continuous electrocardiography monitoring. PVR was calculated as: 80*(mPAP - PCWP)/CO (mPAP is mean pulmonary artery pressure, PCWP is pulmonary capillary wedge pressure and CO is cardiac output).

Six-minute walking test
The 6MWT was performed according to American Thoracic Society guidelines[11].

Cardiac magnetic resonance imaging
CMR was performed on a Siemens 1.5-T Sonato scanner (Siemens Medical Solutions, Siemens, Erlangen, Germany), equipped with a 6-element phased-array receiver coil. Electrocardiographic gated cine imaging was performed using a balanced steady-state precession pulse sequence, during repeated breath-holds. Short-axis images from base to apex of the ventricles were obtained with a typical slice thickness of 5 mm and an interslice gap of 5 mm. MR parameters were: temporal resolution between 35 and 45 ms, typical voxel size 1.5 x 1.8 x 5.0 mm^3, flip angle 60^o, receiver bandwidth 930 Hz/pixel, field of view 280 x 340 mm^2; TR/TE 3.2/1.6 ms, matrix 256 x 156.
During post-processing, a blinded observer analysed the short-axis images with the MASS software package (MEDIS Medical Imaging Systems, Leiden, the Netherlands). On end-diastolic images (first cine after the R-wave trigger) and end-systolic images (cine with visually the smallest cavity area), endocardial contours of the left ventricle (LV) and RV were obtained by manually tracing. Papillary muscles and trabeculae were excluded from the cavity. Ventricular volumes were estimated using the Simpsons rule. Ejection fraction was calculated as: (EDV – ESV)/EDV (EDV is end-diastolic volume and ESV is end-systolic volume). Ventricular volumes were indexed by correcting for body surface area.

Statistical analysis
Data were expressed as mean ± SD for continuous variables and absolute for categorical variables. $P < 0.05$ was considered significant. Comparisons between and within groups were calculated using unpaired and paired Student t-tests. Correlation coefficients were calculated by the Pearson method. Univariate Cox proportional hazards analyses were applied to test the relationship between survival and selected demographic, New York Heart Association functional class (NYHA), distance at 6MWT, hemodynamic and CMR variables measured at baseline. Kaplan-Meier survival estimates were stratified by the optimal cut-off values of PVR and RVEF and compared by log-rank tests. The optimal cut-off values were

identified from receiver operating characteristic (ROC) curve analyses by taking the sum of the highest specificity and sensitivity. Bivariate Cox regression analysis was used to test the relationship between baseline RVEF, PVR and mortality. Survival was estimated from time of enrolment with cardiopulmonary death and lung transplantation as the endpoints (median period 59 months (interquartile range: (IQR): 30 to 74 months)). Other causes of death were censored. We performed a sensitivity analysis to test whether missing values influenced the results.
The follow-up analysis was performed in 76 patients after 12 months of follow-up. Univariate Cox-proportional hazard analyses were performed to analyse the relationship between survival and the changes in 6MWT, selected hemodynamic and CMR variables during 1 year of follow-up. Multivariable Cox survival analyses were used to examine the independent effect of RVEF and PVR on survival after correction for potential confounders. These analyses take into account the number of events and the number of non-events to achieve sufficient power of the test. Based on baseline RVEF and PVR and the changes in RVEF and PVR during follow-up, a backward multivariable survival analysis was applied to compare the prognostic values of baseline parameters with those of follow-up parameters.
Patients were considered to have a decreased PVR after a decrease in PVR of at least 15 dyne·s·cm^{-5}. In addition, according to the results of Bradlow et al.[12], a change of +3% defined an increased RVEF and a value of -3% defined a decreased RVEF. Patients with a decreased PVR were dichotomised: decreased PVR + stable/increased RVEF and decreased PVR + decreased RVEF. A landmark analysis (landmark at month 12) was applied to compare survival rates of both subgroups.
All statistical analyses were carried out with SPSS (Version 15.0, SPSS, Inc, Chicago, Illanois).

Results

Patient characteristics

Table 1 summarizes the demographics of the study population and Table 2 shows the hemodynamics and volume measurements. All baseline measures were obtained in treatment naïve patients with PAH. The mean age of the study population was 53 ± 15 years, 76% were female, and most patients (66%) were diagnosed as having idiopathic PAH. The time between baseline measurements and the end of the study represents a long-term median follow-up period of 59 months (IQR: 30 to 74 months. During that period, 30 patients died from cardiopulmonary causes and two patients underwent lung transplantation. Thirteen patients died during the first year, and 17 patients died during the median subsequent follow-up of

47 months. One patient who died during follow-up was treated as a censored case: the cause of death was given as lung cancer.

Table 1. Patient demographics.

Variable	Total study population (n = 110)	Population without follow-up (n = 34)	Follow-up population (n = 76)	P-value
Age, years	53 ± 15	57 ± 17	50 ± 14	0.023
Female, n (%)	84 (76)	21 (62)	63 (83)	0.139
Diagnosis, n (%)				
Idiopathic PAH	73 (66)	19 (56)	54 (71)	0.445
Familial PAH	7 (6)	2 (6)	5 (7)	0.834
Associated PAH				
CTD	20 (18)	9 (26)	11 (14)	0.419
PHT	5 (5)	3 (9)	2 (3)	0.326
HIV infection	2 (2)	1 (3)	1 (1)	0.542
Drugs/toxins	3 (3)	0	3 (4)	0.550
Body surface area, m^2	1.8 ± 0.2	1.8 ± 0.2	1.9 ± 0.2	0.170
NYHA, n (%)				
I/II	53 (48)	16 (47)	37 (49)	0.222
III	51 (46)	17 (50)	34 (45)	0.217
IV	6 (6)	1 (3)	5 (7)	0.628
6MWT				
Distance, m	414 ± 135	405 ± 170	421 ± 117	0.675
Therapy, n (%)*				
None	2 (2)	2 (6)	0	0.011
Ca^{2+} antagonists	3 (3)	2 (6)	1 (1)	0.966
ERA	39 (35)	13 (38)	26 (34)	0.387
PDE5I	17 (15)	7 (21)	10 (13)	0.291
Prostacyclins	15 (14)	7 (21)	8 (11)	0.251
Combination	34 (31)	3 (9)	31 (41)	<0.001

*Refers to the period after baseline measurements. 6MWT = six-minute walk test, CA^{2+} = calcium, CTD connective tissue disease, ERA = endothelin receptor antagonists, n = number, NYHA = New York Heart Association functional class, PAH = pulmonary arterial hypertension, PDE5I = phosphodiesterase 5 inhibitors, PHT = portal hypertension.

Table 2. Baseline hemodynamics and volume measurements.

Variable	Baseline population (n = 110)	Population without follow-up (n = 34)	Follow-up population (n = 76)	P-value
RHC				
mPAP, mmHg	49 ± 16	47 ± 17	50 ± 16	0.474
mRAP, mmHg	7 ± 5	6 ± 5	7 ± 4	0.623
PCWP, mmHg	7 ± 4	8 ± 4	7 ± 4	0.220
PVR, dyne·s·cm^{-5}	745 ± 432	720 ± 513	772 ± 384	0.463
CO, L/min	5.1 ± 1.9	5.2 ± 2.4	4.9 ± 1.3	0.444
CI, L/min/m^2	2.8 ± 1.0	2.8 ± 1.3	2.7 ± 0.7	0.325
Heart rate, bpm	82 ± 14	80 ± 16	85 ± 16	0.313
SvO_2, %	66 ± 9	65 ± 10	66 ± 8	0.727
CMR				
RVEDVI, ml/m^2	71 ± 23	69 ± 22	72 ± 24	0.611
RVESV, ml/m^2	47 ± 21	45 ± 18	48 ± 22	0.709
RVEF, %	36 ± 11	38 ± 12	35 ± 10	0.160
LVEDVI, ml/m^2	42 ± 14	45 ± 18	41 ± 13	0.268
LVESVI, ml/m^2	15 ± 9	16 ± 12	14 ± 7	0.364
LVEF, %	67 ± 10	68 ± 10	66 ± 10	0.323
SVI, ml/m^2	28 ± 9	30 ± 11	27 ± 8	0.133

CI = cardiac index, CMR = cardiac magnetic resonance imaging, CO = cardiac output, LVEDVI = left ventricular end-diastolic volume index, LVEF = left ventricular ejection fraction, LVESV = left ventricular end-systolic volume index, mPAP = mean pulmonary artery pressure, mRAP = mean right atrial pressure, PCWP = pulmonary capillary wedge pressure, PVR = pulmonary vascular resistance, RHC = right heart catheterization, RVEDVI = right ventricular end-diastolic volume index, RVEF = right ventricular ejection fraction RVESVI = right ventricular end-systolic volume index, SVI = stroke volume index, SvO_2 = mixed venous oxygen saturation.

Baseline survival analyses

Table 3 shows univariate Cox regression analyses. It was found that both RVEF (Hazard ratio (HR): 0.938, 95% confidence interval (CI): 0.902 to 0.975, p = 0.001) and PVR (HR: 1.001, 95% CI: 1.001 to 1.002, p = 0.031) were associated with survival. In addition, age and connective-tissue-disease PAH were associated with outcome. Multivariable analyses showed that RVEF and PVR remained significantly associated with survival after correction for age and type of underlying diagnosis (RVEF: HR: 0.921, 95% CI: 0.884 to 0.959, p <0.001; PVR: HR: 1.001, 95% CI: 1.001 to 1.002, p = 0.002).

Table 3. Univariate Cox-regression analyses of baseline variables.

	Baseline population (n = 110)		
Variable	**Hazard ratio**	**95% CI**	**P-value**
Age, years	1.027	1.001 – 1.052	0.040
Gender			
Male	1		
Female	0.789	0.323 - 1.926	0.603
Diagnosis			
Idiopathic PAH	2.064	0.866 - 4.238	0.163
Familial PAH	0.982	0.862 - 1.119	0.784
Associated PAH			
CTD	0.306	0.143 - 0.654	0.002
PHT	0.364	0.086 - 1.544	0.170
HIV infection	0.876	0.545 - 1.408	0.585
Drugs/toxins	0.726	0.099 - 5.342	0.753
6MWT			
Distance, m	0.993	0.990 - 0.997	<0.001
RHC			
mPAP, mmHg	0.998	0.976 - 1.020	0.850
mRAP, mmHg	1.048	0.981 - 1.120	0.167
PCWP, mmHg	0.986	0.898 - 1.082	0.761
PVR, dyne·s·cm^{-5}	1.001	1.001 - 1.002	0.031
CO, L/min	0.669	0.483 - 0.928	0.016
CI, L/min/m^2	0.560	0.323 - 0.970	0.039
Heart rate, bpm	1.014	0.989 - 1.039	0.274
$SvO_{2,}$ %	0.936	0.900 - 0.972	0.001
CMR			
RVEDVI, ml/m^2	1.011	0.996 - 1.024	0.121
RVESVI, ml/m^2	1.014	1.001 - 1.027	0.048
RVEF, %	0.938	0.902 - 0.975	0.001
LVEDVI, ml/m^2	0.962	0.931 - 0.994	0.019
LVESVI, ml/m^2	0.942	0.888 - 0.998	0.045
LVEF, %	0.998	0.960 - 1.036	0.900
SVI, ml/m^2	0.945	0.899 - 0.993	0.025

95% CI = 95% confidence interval.

ROC-curve analysis revealed that RVEF and PVR at a cut-off of 35% and 650 dyne·s·cm^{-5} respectively were indicators of survival (RVEF: area under the ROC curve (AUC): 0.749, p = 0.007, PVR: AUC: 0.628, p = 0.035). Univariate Cox-regression analyses based on cut-off values showed that a low RVEF (HR: 0.237, 95% CI: 0.102 to 0.551, p = 0.001) and a high PVR (HR: 2.296, 95% CI: 1.016 to 5.184, p = 0.046) were associated with mortality. Bivariate analysis showed that a low RVEF was independently associated with a poor survival (HR: 0.260, 95% CI: 0.101 to 0.670, p = 0.005). Figure 2 shows Kaplan-Meier survival analyses based on the cut-off values of PVR and RVEF. Patients with low RVEF (groups 3 and 4) had a

significantly poorer prognosis compared with patients with high RVEF (groups 1 and 2), regardless of their PVR (Figure 2C). Bivariate Cox regression analysis applied to the combination of the binary values of RVEF and PVR showed that the patients with high RVEF/high PVR (group 2) did not have a different prognosis compared to the patients with high RVEF/low PVR (group 1) (p = 0.579). Patients with low RVEF/low PVR (group 3) had a similar prognosis compared to patients with low RVEF/high PVR (group 4) (p = 0.830). In addition, patients of group 3 and patients of group 4 had 5.2 times greater HRs compared to high RVEF/low PVR patients (group 1) (p <0.01).

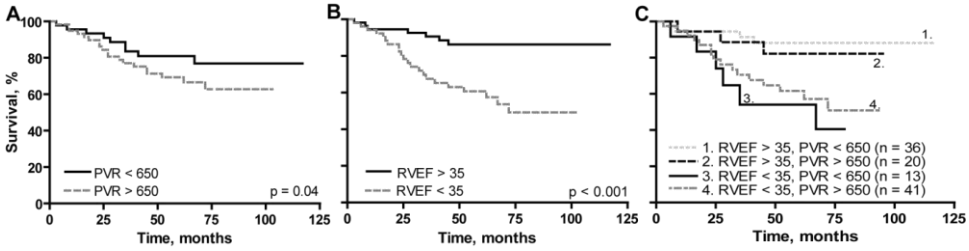

Figure 2. Survival rates of PAH patients stratified according to PVR and RVEF at baseline.
(A) Patients with pulmonary vascular resistance (PVR) <650 dyne·s·cm^{-5} showed better survival rates than patients with PVR >650 dyne·s·cm^{-5} (p = 0.04). **(B)** Patients with right ventricular ejection fraction (RVEF) >35% showed better survival rates compared to patients with RVEF <35% (p <0.001). **(C)** Survival rates based on the coupling of PVR and RVEF. PAH = pulmonary arterial hypertension.

Changes with follow-up

After a median period of 12 months (IQR: 10 to 16 months) of PAH-specific medical treatment, pulmonary pressures remained almost unaltered while PVR was significantly decreased. In addition, CI was improved and the 6MWT was stable. No other changes in cardiac functional parameters were observed (Table 4). Furthermore, with respect to the effects of the different classes of drugs, we found no significant differences between groups (Table 5).

Table 4. Differences between characteristics at baseline and at 12 months follow-up.

	(n = 76)		
Variable	**Baseline**	**Follow-up**	**P-value**
6MWT			
Distance, m	421 ± 117	425 ± 139	0.727
RHC			
mPAP, mmHg	50 ± 16	47 ± 16	0.176
mRAP, mmHg	7 ± 4	7 ± 5	0.557
PCWP, mmHg	7 ± 4	7 ± 4	0.966
PVR, dyne·s·cm^{-5}	772 ± 384	660 ± 378	0.003
CO, L/min	4.9 ± 1.3	5.4 ± 2.4	0.032
CI, L/min/m^2	2.7 ± 0.7	3.0 ± 1.2	0.026
Heart rate, bpm	85 ± 16	83 ± 12	0.182
$SvO_{2,}$ %	66 ± 8	65 ± 10	0.641
CMR			
RVEDVI, ml/m^2	72 ± 24	76 ± 32	0.099
RVESVI, ml/m^2	48 ± 22	51 ± 30	0.167
RVEF, %	35 ± 10	36 ± 13	0.413
LVEDVI, ml/m^2	41 ± 13	43 ± 14	0.374
LVESVI, ml/m^2	14 ± 7	14 ± 8	0.965
LVEF, %	66 ± 10	67 ± 10	0.267
SVI, ml/m^2	27 ± 8	29 ± 8	0.224

Table 5. Differences between different classes of medical therapies (n = 76).

Variable*	**ERA** (n = 26)	**PDE5I** (n = 10)	**Prosta** (n = 8)	**Combi** (n = 31)	**P-value**
Changes in PVR, dyne·s·cm^{-5}	-133 ± 315	-33 ± 271	95 ± 201	-180 ± 271	0.311
Changes in RVEF, %	1 ± 8	-1 ± 6	-3 ± 10	2 ± 9	0.360

*One patients was treated with calciumantagonists and not included in the analysis. Combi = combination therapy, prosta = prostacyclins.

Follow-up survival analyses

Changes in PVR correlated moderately to changes in RVEF (R = 0.330; p = 0.005) (Figure 3). PVR decreased in both survivors (-121 ± 297 dyne·s·cm^{-5}) and non-survivors (-132 ± 432 dyne·s·cm^{-5}) (p = 0.927). Changes in RVEF differed significantly between survivors (+3 ± 9%) and non-survivors (-5 ± 6%) (p = 0.001) (Figure 4). Similar results were found for the relative changes in PVR (survivors: -13%, non-survivors: –11%, p = 0.765) and relative changes in RVEF (survivors: +10%, non-survivors: -20%, p <0.001). Changes in PVR were not associated with outcome (HR: 1.000, 95% CI: 0.998 to 1.001, p = 0.820), whereas changes in RVEF were

independently related to mortality (HR: 0.929, 95% CI: 0.875 to 0.985, p = 0.014).

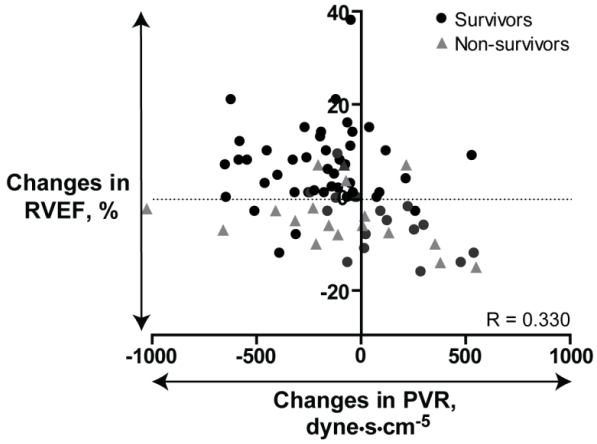

Figure 3. Relation between changes in PVR and changes in RVEF.
Changes in PVR were moderately correlated to changes in RVEF (R = 0.330, p = 0.005).

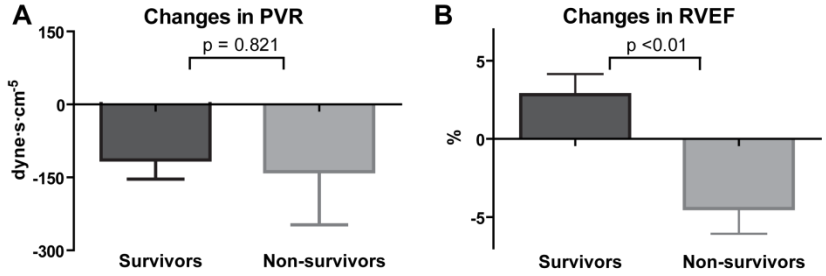

Figure 4. Changes in PVR and RVEF after 12 months follow-up according to survival.
(A) Changes in PVR did not differ between survivors (black) and non-survivors (grey). **(B)** Survivors showed an increased RVEF whereas non-survivors showed a decreased RVEF during follow-up. Data are presented as mean ± SEM.

Table 6 shows univariate analyses of changes in hemodynamic and imgaging variables during follow-up. After correction for age and connective tissue disease PAH, changes in RVEF remained significantly associated with survival (HR: 0.928, 95% CI: 0.870 to 0.991, p = 0.026). A backward multivariable survival analyses based on baseline RVEF and PVR and the changes in RVEF and PVR showed that baseline RVEF and the changes in RVEF during follow-up had similar prognostic value (baseline RVEF: HR: 0.926, 95% CI: 0.876 to 0.978, p = 0.006; changes in RVEF: HR: 0.909, 95% CI: 0.846 to 0.976, p = 0.009).

Table 6. Univariate survival analyses of changes in follow-up variables.

	(n = 76)		
Variable	**Hazard ratio**	**95% CI**	**P-value**
Changes in 6MWT			
Distance, m	0.996	0.989 - 1.003	0.239
Changes in RHC			
mPAP, mmHg	1.013	0.975 - 1.053	0.504
mRAP, mmHg	1.050	0.945 -1.167	0.360
PCWP, mmHg	1.027	0.944 - 1.118	0.530
PVR, dyne·s·cm^{-5}	1.000	0.998 - 1.001	0.820
CO, L/min	0.811	0.619 - 1.062	0.128
CI, L/min/m^2	0.705	0.443 - 1.123	0.141
Heart rate, bpm	0.986	0.950 - 1.024	0.475
$SvO_{2,}$ %	0.965	0.911 - 1.096	0.314
Changes in CMR			
RVEDVI, ml/m^2	1.029	1.013 - 1.045	<0.001
RVESVI, ml/m^2	1.036	1.018 - 1.053	<0.001
RVEF, %	0.929	0.875 - 0.985	0.014
LVEDVI, ml/m^2	0.928	0.937 - 1.014	0.179
LVESVI, ml/m^2	0.971	0.909 - 1.037	0.377
LVEF, %	0.988	0.947 - 1.031	0.576
SVI, ml/m^2	0.928	0.843 - 1.015	0.110

In total, 52 patients (68%) showed a significant decrease of PVR after therapy and were included in the landmark analysis. In this group, patients with a decreased RVEF had a significantly poorer survival than patients with stable/increased RVEF (p <0.001) (Figure 5). Both groups had a similar decrease in PVR (mean: -284 ± 248 dyne·s·cm^{-5}, difference in PVR: p = 0.437). We observed no differences in the baseline characteristics that could account for a different RV response to a similar decrease in PVR (Supplement, Table A1). Supplement, Table A2 shows the characteristics of both groups after follow-up.

Thirteen patients did not survive the first year and therefore did not undergo follow-up measurements. The non-survivors without follow-up measurements showed similar characteristics as the seventeen non-survivors with follow-up measurements (Supplement, Table A3).

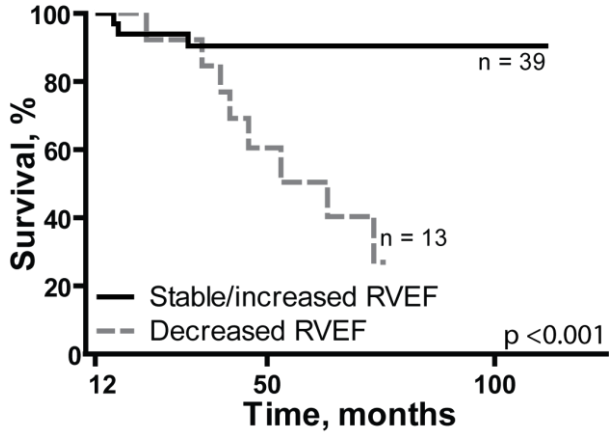

Figure 5. Landmark analysis.
Landmark at month 12 of 52 patients with decreased PVR after therapy. Patients with stable/increased RVEF (n = 39) had better survival rates than patients with decreased RVEF (n = 13) (p <0.001).

Discussion

Our study shows that in a large group of World Health Organisation group 1 patients with PAH on PAH-targeted therapies, RVEF measured at baseline is a better predictor of mortality than PVR. Changes in RVEF after 12 months predict long-term outcome, whereas changes in PVR do not. In addition, we found that changes in PVR were moderately related to changes in RVEF and that after medical therapy, RV dysfunction can progress despite a decrease in PVR.

Significance of baseline parameters

In accordance with previous studies, we showed that RVEF as assessed by CMR had a strong prognostic value[13,14]. Kawut et al. showed that RVEF is an independent predictor of long-term outcome[15]. In correspondence with earlier studies, we found that baseline PVR was a prognostic predictor[3,4]. However, we showed that although a high PVR at baseline was associated with outcome, the prognosis was primarily determined by RVEF. Previously, Ghio et al. found similar results in patients with pulmonary hypertension secondary to left heart disease[16].

Effects of medical therapies

Thus far, only few studies have studied the therapeutic effects on changes in PVR and RV function. It was previously shown by Roeleveld et al. that epoprostenol therapy lowered PVR but did not affect RV dilation and hypertrophy[17]. Chin et al. reported that while bosentan reduced PVR, it did not affect either RVEF or RVEDV[18]. Wilkins et al. showed that RV mass

decreased after sildenafil treatment and remained stable after bosentan therapy[19]. A randomised clinical trial by Galie et al. showed that bosentan treatment was associated with improvement of RV systolic function as assessed by the RV Doppler index[20]. The last two studies cited did not include hemodynamic measures in the analyses. Although the former studies analysed treatment effects in PAH-patients, the relationship between changes in load and RV function was not been quantified.

The majority of patients in our cohort (68%) had a reduced PVR after medical treatment. However, the reduction in PVR was modest (-12%) and mPAP remained almost unaltered (-5%). Despite the small patient groups, we found non-heterogeneity in the effects between different classes of medical treatment. These results are in correspondence with meta-analyses of randomised controlled trials showing moderate reductions in PVR and only small reductions in mPAP over an average study duration of 14 weeks[9]. Furthermore, our results agree with the findings of previous studies reporting a lowered PVR (ranging from –11% to –39%) after a long-term treatment period[21-26].

Significance of follow-up parameters

Our results on PVR show that although this parameter, when measured at baseline was of prognostic significance, a change over time of this parameter was not. However, we cannot conclude from this result that a change in PVR is not important. It has been found in an earlier study that PVR reduction only will lead to an improvement in survival only if reduced to more than 30%[25]. Because this was the case in a minority of our patients, no conclusions can be made whether a larger reduction in PVR will lead to an improved survival in our study.

In the present study, we showed that the changes in RVEF during follow-up had similar prognostic value in comparison with baseline RVEF. In addition, a previous study of our group found that the changes in stroke volume index, RV and LV volumes were associated with mortality[13]. The results of both studies suggest that follow-up parameters may provide important prognostic insights.

The paradox of progressive RV dysfunction despite decreased PVR

We found that the changes in RVEF were moderately correlated to the changes in PVR. The most important finding of this study is that in 25% of the patients with a reduced PVR, RV function deteriorated further after follow-up. We showed that the group with a deteriorating RV function had a poor outcome. This deterioration was not explained by the PVR since the reduction in PVR occurred to a similar extent in patients with improving and deteriorating RVEF.

RV load consists of PVR, compliance and impedance of the proximal pulmonary artery. In previous studies of our group[27,28], it was shown that PVR and compliance are inversely related (PVR = Constant * 1/compliance). As a consequence, compliance is strongly correlated to PVR (= peripheral resistance + characteristic impedance), and therefore we do not think that compliance can explain additional variance in relation with RVEF. Recently, we showed that RV total power (associated with total load, *i.e.* compliance and PVR) and mean power (associated with non-pulsatile load, *i.e.* PVR) are proportional[29]. These findings emphasize that PVR is a valid reflection of the load on the RV.
The moderate correlation between PVR and RVEF indicated that RV function does not fully adapt to changes in vascular properties, as is expected in healthy subjects due to "coupling" of the heart and arterial functions. Therefore, we expected that others factors play an important role in the changes in RVEF over time. Kawut et al. showed that older age, male gender and higher von Willebrand factor were associated with lower RVEF[30]. We speculate that genetic differences in RV adaptation to pressure overload[2] and possible direct effects of current PAH treatments on the heart are responsible for different RV responses. In addition, we hypothesize that the deterioration in RV function might possibly be explained by an important physiological principle: ventricular wall tension. The current results show that despite a reduction in PVR, pulmonary pressures were unaltered after medical treatment and consequently, ventricular wall tension will remain unchanged[31]. In case that wall tension is the driving force for the RV to fail, therapies will not prevent the RV from failure if failing conditions were already present at baseline.

Implications
Here we showed that changes in PVR as accomplished by currently available therapies do not prevent RV deterioration in 25% of the patients. Therefore, as RV function is the primary determinant of prognosis, it is important to analyse the factors that predict RV dysfunction.
It has been shown that larger reductions in PVR and mPAP (e.g. after lung transplantation or endarterectomy) can result in improved RV function. We therefore consider that a medical treatment strategy that is more effective at onset could have a more pronounced effect on patient outcome. Furthermore, understanding the pathways that underlie RV failure could lead to the development of strategies that are directly targeted at improving RV function.

Limitations
A limitation of this study is that RHC and CMR measurements could not be obtained simultaneously which may have potentially resulted in measurements in different hemodynamic states. However, the median time

between CMR imaging and RHC was one day; therefore, it was unlikely that the delay affected our conclusions.
In addition, since our study required follow-up measurements, patients who died between baseline and follow-up measurements could not be included in the follow-up analyses (immortal time bias)[32]. However, we observed no differences in baseline characteristics between the non-survivors without follow-up measurements and the non-survivors with follow-up measurements.

Conclusions

In PAH, baseline RVEF was a stronger prognostic predictor than baseline PVR. Changes in PVR after follow-up were moderately correlated with changes in RVEF. Moreover, this study showed that in the presence of PAH, right heart dysfunction may progress despite a reduced PVR by PAH-targeted medical therapies. A deterioration of RV function was associated with a poor outcome, irrespective of any changes in PVR.

References

1. McLaughlin VV, McGoon MD. Pulmonary arterial hypertension. Circulation. 2006; 114:1417-1431.
2. Voelkel NF, Quaife RA, Leinwand LA, et al. Right ventricular function and failure: report of a National Heart, Lung, and Blood Institute working group on cellular and molecular mechanisms of right heart failure. Circulation. 2006; 114:1883-891.
3. Humbert M, Sitbon O, Chaouat A, et al. Survival in patients with idiopathic, familial, and anorexigen-associated pulmonary arterial hypertension in the modern management era. Circulation. 2010;122:156-63.
4. Benza RL, Miller DP, Gomberg-Maitland M, et al. Predicting survival in pulmonary arterial hypertension: insights from the Registry to Evaluate Early and Long-Term Pulmonary Arterial Hypertension Disease Management (REVEAL). Circulation. 2010; 122:164-172.
5. D'Alonzo GE, Barst RJ, Ayres SM, et al. Survival in patients with primary pulmonary hypertension. Results from a national prospective registry. Ann Intern Med. 1991; 115:343-349.
6. Reesink HJ, Marcus JT, Tulevski, II, et al. Reverse right ventricular remodeling after pulmonary endarterectomy in patients with chronic thromboembolic pulmonary hypertension: utility of magnetic resonance imaging to demonstrate restoration of the right ventricle. J Thorac Cardiovasc Surg. 2007; 133:58-64.
7. Mayer E, Dahm M, Hake U, et al. Mid-term results of pulmonary thromboendarterectomy for chronic thromboembolic pulmonary hypertension. Ann Thorac Surg. 1996; 61:1788-1792.
8. Pasque MK, Trulock EP, Cooper JD, et al. Single lung transplantation for pulmonary hypertension. Single institution experience in 34 patients. Circulation. 1995; 92:2252-2258.

9. Galie N, Manes A, Negro L, Palazzini M, Bacchi-Reggiani ML, Branzi A. A meta-analysis of randomized controlled trials in pulmonary arterial hypertension. Eur Heart J. 2009; 30:394-403.
10. Galie N, Torbicki A, Barst R, et al. Guidelines on diagnosis and treatment of pulmonary arterial hypertension. The Task Force on Diagnosis and Treatment of Pulmonary Arterial Hypertension of the European Society of Cardiology. Eur Heart J. 2004; 25:2243-2278.
11. ATS statement: guidelines for the six-minute walk test. Am J Respir Crit Care Med. 2002; 166:111-117.
12. Bradlow WM, Hughes ML, Keenan NG, et al. Measuring the heart in pulmonary arterial hypertension (PAH): implications for trial study size. J Magn Reson Imaging. 2010; 31:117-124.
13. van Wolferen SA, Marcus JT, Boonstra A, et al. Prognostic value of right ventricular mass, volume, and function in idiopathic pulmonary arterial hypertension. Eur Heart J. 2007;28:1250-7.
14. Zafrir N, Zingerman B, Solodky A, et al. Use of noninvasive tools in primary pulmonary hypertension to assess the correlation of right ventricular function with functional capacity and to predict outcome. Int J Cardiovasc Imaging. 2007; 23:209-215.
15. Kawut SM, Horn EM, Berekashvili KK, et al. New predictors of outcome in idiopathic pulmonary arterial hypertension. Am J Cardiol. 2005; 95:199-203.
16. Ghio S, Gavazzi A, Campana C, et al. Independent and additive prognostic value of right ventricular systolic function and pulmonary artery pressure in patients with chronic heart failure. J Am Coll Cardiol. 2001; 37:183-188.
17. Roeleveld RJ, Vonk-Noordegraaf A, Marcus JT, et al. Effects of epoprostenol on right ventricular hypertrophy and dilatation in pulmonary hypertension. Chest. 2004; 125:572-579.
18. Chin KM, Kingman M, de Lemos JA, et al. Changes in right ventricular structure and function assessed using cardiac magnetic resonance imaging in bosentan-treated patients with pulmonary arterial hypertension. Am J Cardiol. 2008; 101:1669-1672.
19. Wilkins MR, Paul GA, Strange JW, et al. Sildenafil versus Endothelin Receptor Antagonist for Pulmonary Hypertension (SERAPH) study. Am J Respir Crit Care Med. 2005; 171:1292-1297.
20. Galie N, Hinderliter AL, Torbicki A, et al. Effects of the oral endothelin-receptor antagonist bosentan on echocardiographic and doppler measures in patients with pulmonary arterial hypertension. J Am Coll Cardiol. 2003; 41:1380138-6.
21. Hoeper MM, Gall H, Seyfarth HJ, et al. Long-term outcome with intravenous iloprost in pulmonary arterial hypertension. Eur Respir J. 2009; 34:132-137.
22. Benza RL, Rayburn BK, Tallaj JA, Pamboukian SV, Bourge RC. Treprostinil-based therapy in the treatment of moderate-to-severe pulmonary arterial hypertension: long-term efficacy and combination with bosentan. Chest. 2008; 134:139-145.
23. Provencher S, Sitbon O, Humbert M, Cabrol S, Jais X, Simonneau G. Long-term outcome with first-line bosentan therapy in idiopathic pulmonary arterial hypertension. Eur Heart J. 2006; 27:589-595.
24. Ghofrani HA, Rose F, Schermuly RT, et al. Oral sildenafil as long-term adjunct therapy to inhaled iloprost in severe pulmonary arterial hypertension. J Am Coll Cardiol. 2003; 42:158-164.
25. Sitbon O, Humbert M, Nunes H, et al. Long-term intravenous epoprostenol infusion in primary pulmonary hypertension: prognostic factors and survival. J Am Coll Cardiol. 2002; 40:780-788.
26. McLaughlin VV, Shillington A, Rich S. Survival in primary pulmonary hypertension: the impact of epoprostenol therapy. Circulation. 2002; 106:1477-1482.

27. Lankhaar JW, Westerhof N, Faes TJ, et al. Pulmonary vascular resistance and compliance stay inversely related during treatment of pulmonary hypertension. Eur Heart J. 2008; 29:1688-1695.
28. Lankhaar JW, Westerhof N, Faes TJ, et al. Quantification of right ventricular afterload in patients with and without pulmonary hypertension. Am J Physiol Heart Circ Physiol. 2006; 291:H1731-1737.
29. Saouti N, Westerhof N, Helderman F, et al. Right Ventricular Oscillatory Power is a Constant Fraction of Total Power Irrespective of Pulmonary Artery Pressure. Am J Respir Crit Care Med. 2010; 182:1315-1320.
30. Kawut SM, Al-Naamani N, Agerstrand C, et al. Determinants of right ventricular ejection fraction in pulmonary arterial hypertension. Chest. 2009; 135:752-759.
31. Sniderman AD, Fitchett DH. Vasodilators and pulmonary arterial hypertension: the paradox of therapeutic success and clinical failure. Int J Cardiol 1988; 20:173-181.
32. Suissa S. Immortal time bias in pharmaco-epidemiology. Am J Epidemiol. 2008; 167:492-499.

Data supplement

Table A1. Subanalysis of 52 patients with decreased PVR after medical treatment.
Baseline characteristics of patients with stable/increased RVEF (PVR ↓, RVEF = / ↑) were compared to patients with decreased RVEF (PVR ↓, RVEF ↓).

Variable	PVR ↓, RVEF = / ↑ (n = 39)	PVR ↓, RVEF ↓ (n = 13)	P-value
Age, years	52 ± 14	47 ± 13	0.265
Diagnosis, n (%)			
Idiopathic PAH	33 (89)	10 (77)	0.780
Associated PAH			
CTD	4 (11)	3 (23)	0.357
Female, n (%)	32 (82)	9 (69)	0.420
Survivors, n (%)	36 (92)	5 (38)	<0.001
RHC			
mPAP, mmHg	53 ± 14	51 ± 17	0.712
mRAP, mmHg	6 ± 4	9 ± 6	0.158
PCWP, mmHg	8 ± 4	6 ± 4	0.108
PVR, dyne·s·cm^{-5}	789 ± 326	969 ± 622	0.335
CI, L/min/m^2	2.7 ± 0.8	2.5 ± 0.7	0.347
Heart rate, bpm	84 ± 14	85 ± 16	0.820
$SvO_{2,}$ %	67 ± 6	67 ± 11	0.270
CMR			
RVEDVI, ml/m^2	70 ± 27	77 ± 24	0.430
RVESVI, ml/m^2	46 ± 25	52 ± 23	0.492
RVEF, %	35 ± 10	36 ± 10	0.973
LVEDVI, ml/m^2	41 ± 12	44 ± 17	0.632
LVESVI, ml/m^2	14 ± 5	16 ± 10	0.541
LVEF, %	67 ± 9	67 ± 13	0.897
SVI, ml/m^2	28 ± 8	28 ± 9	0.793

CI = cardiac index, CMR = cardiac magnetic resonance imaging, CO = cardiac output, CTD = connective tissue disease, LVEDVI = left ventricular end-diastolic volume index, LVEF = left ventricular ejection fraction, LVESVI = left ventricular end-systolic volume index, mPAP = mean pulmonary artery pressure, mRAP = mean right atrial pressure, PCWP = pulmonary capillary wedge pressure, PVR = pulmonary vascular resistance, RHC = right heart catheterization, RVEDVI = right ventricular end-diastolic volume index, RVEF = right ventricular ejection fraction, RVESVI = right ventricular end-systolic volume index, SVI = stroke volume index, SvO_2 = mixed venous oxygen saturation.

Table A2. Subanalysis of 52 patients with decreased PVR after therapy. Changes in characteristics during follow-up of patients with stable/increased RVEF (PVR ↓, RVEF = / ↑) were compared to patients with decreased RVEF (PVR ↓, RVEF ↓).

Variable	PVR ↓, RVEF = / ↑ (n = 39)	PVR ↓, RVEF ↓ (n = 13)	P-value
Changes in 6MWT			
Distance, m	24 ± 68	58 ± 115	0.349
Changes in RHC			
mPAP, mmHg	-10 ± 10	-4 ± 16	0.112
mRAP, mmHg	-1 ± 5	-2 ± 4	0.433
PCWP, mmHg	7 ± 4	5 ± 4	0.247
PVR, dyne·s·cm^{-5}	-268 ± 212	-331 ± 308	0.437
CI, L/min/m^2	0.7 ± 1.3	0.5 ± 0.6	0.650
Heart rate, bpm	-2 ± 13	-7 ± 23	0.392
Changes in CMR			
RVEDVI, ml/m^2	2 ± 21	1 ± 32	0.909
RVESVI, ml/m^2	-2 ± 18	5 ± 26	0.377
RVEF, %	7 ± 8	-6 ± 3	<0.001
LVEDVI, ml/m^2	6 ± 10	-1 ± 13	0.092
LVESVI, ml/m^2	1 ± 7	-1 ± 10	0.619
LVEF, %	3 ± 9	-3 ± 12	0.077
SVI, ml/m^2	5 ± 6	-2 ± 5	0.001

6MWT = six-minute walking test, n = number, PAH = pulmonary arterial hypertension.

Table A3. Differences in baseline characteristics between survivors, non-survivors without follow-up measurements and non-survivors with follow-up measurements.

Variable	Survivors (n = 78)	Non-survivors without follow-up measurements (n = 14)	Non-survivors with follow-up measurements (n = 18)
Age, years	52 ± 15	57 ± 18	56 ± 15
Female, n (%)	60 (77)	11 (79)	13 (72)
Diagnosis, n (%)			
Idiopathic PAH	57 (73)	7 (50)	9 (50)
CTD	10 (13)	5 (36)	5 (28)
Other	11 (14)	2 (14)	6 (33)
6MWT			
Distance at 6WT, m	443 ± 128	247 ± 132	388 ± 102[†,‖]
RHC			
mPAP, mmHg	50 ± 16	49 ± 15	49 ± 16
mRAP, mmHg	6 ± 4	7 ± 8	8 ± 5
PCWP, mmHg	7 ± 4	8 ± 4	6 ± 4
PVR, dyne·s·cm^{-5}	731 ± 372	871 ± 631	915 ± 508
CO, L/min	5.2 ± 1.8	4.2 ± 2.0	4.4 ± 1.0
CI, L/min/m^2	2.9 ± 1.1	2.5 ± 1.2	2.5 ± 0.6
Heart rate, bpm	82 ± 14	80 ± 16	86 ± 17
$SvO_{2,}$ %	66 ± 9	62 ± 11[*]	61 ± 10[§]
CMR			
RVEDVI, ml/m^2	69 ± 23	70 ± 20	79 ± 37
RVESVI, ml/m^2	45 ± 16	48 ± 21	56 ± 33
LVEDVI, ml/m^2	44 ± 15	35 ± 8[*]	38 ± 11
LVESVI, ml/m^2	16 ± 9	10 ± 5[*]	14 ± 7
RVEF, %	38 ± 11	33 ± 10	30 ± 8[§]
LVEF, %	67 ± 10	70 ± 8	65 ± 11
SVI, ml/m^2	30 ± 9	25 ± 6	25 ± 5[‡]

[*]p <0.05 survivors vs. non-survivors <12months, [†]p <0.001 survivors vs. non-survivors <12 months, [‡]p <0.05 survivors vs. non-survivors >12 months, [§]p <0.01 survivors vs. non-survivors >12 months, [‖]p <0.05 non-survivors <12 months vs. non-survivors >12 months.

Chapter 5

The right ventricle explains sex differences in survival in idiopathic pulmonary arterial hypertension

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Abstract

Introduction. Male sex is an independent predictor of worse survival in pulmonary arterial hypertension (PAH). This finding might be explained by more severe pulmonary vascular disease, worse right ventricular (RV) function or different response to therapy. The aim of this study was to investigate the underlying cause of sex differences in survival in treated PAH patients.
Methods. This was a retrospective cohort study of 101 patients with PAH (82 idiopathic, 15 heritable, 4 anorexigen associated) who were diagnosed at our institute between February 1999 and January 2011 and underwent right heart catheterization and cardiac magnetic resonance imaging to assess RV function. Change in pulmonary vascular resistance (PVR) was taken as a measure of treatment response on the pulmonary vasculature, whereas change in right ventricular ejection fraction (RVEF) was used to assess right ventricular (RV) response to therapy.
Results. PVR and RVEF were comparable between men and women at baseline, however male patients had a worse transplant-free survival compared to female patients ($p = 0.002$). While male and female patients showed a similar reduction in PVR after one year, RVEF improved in female patients whereas it deteriorated in male patients. In a mediator analysis, after correcting for confounders, 39.0% of the difference in transplant-free survival between men and women was mediated through changes in RVEF after initiating PAH medical therapies.
Conclusions. This study suggests that differences in RVEF response with initiation of medical therapy in PAH explain a significant portion of the worse survival seen in males.

Introduction

Pulmonary arterial hypertension (PAH) is a rare disease characterized by obstructive lesions of the small pulmonary vessels, leading to increased pulmonary artery pressure (PAP), right-sided heart failure and death within several years[1-2]. Despite the advent of improved therapies outcome remains poor[3,4]. Prognosis correlates with severity of right ventricular (RV) structure and function[2,5]. More recently, male sex was identified as an independent predictor of mortality[6-10]. Men treated with endothelin receptor antagonists had less six minute walk improvement[11]. The cause of these sex differences is unknown, however a distinct vascular and/or RV response to medical therapies is one possibility. Considering the need for improved treatments and "personalized therapy", a better understanding of these sex differences would be important. The aim of our study was to investigate the role of the pulmonary vasculature and the right ventricle in explaining sex differences in survival of treated IPAH.

Methods

Patients

All idiopathic (IPAH), anorexigen associated (APAH) and heritable PAH (HPAH) treated at the VU University Medical Center (VUMC) between February1999 and January 2011 were eligible. Diagnosis was according to the guidelines and included right heart catheterization (RHC). Medical treatment comprised prostacyclin analogues, endothelin receptor antagonists (ERA) and phosphodiesterase type-5 inhibitors (PDE5-I) either alone or in various combinations. Patients with a positive vasodilator challenge were treated with calcium antagonists[1]. This was a retrospective cohort study of patients enrolled in an ongoing prospective study to assess the clinical value of cardiac magnetic resonance imaging (CMR) in PAH. All patients who had RHC and CMR performed prior to initiation of medical therapy (n = 101 out of n = 186 patients evaluated during this period) were included.

Right heart catheterization

Hemodynamic assessment was performed with a 7-F balloon tipped flow directed Swan-Ganz catheter (131HF7, Baxter Healthcare Corp., Irvine, California). Baseline and follow-up RHC measurements of pulmonary artery pressure (PAP), right atrial pressure (RAP), pulmonary capillary wedge pressure (PCWP), cardiac output (CO) were obtained. Pulmonary vascular resistance (PVR) was calculated as 80*(meanPAP-PCWP)/CO. Vasoreactivity testing was with inhaled nitric oxide (20 ppm). Acute

vasoreactivity defined as a mean PAP reduction ≥10 mmHg to reach an absolute value ≤40 mmHg with increased or unchanged CO.
Venous blood sampling was performed to measure glomerular filtration rate (GFR) (Cockgroft), creatinine and N-terminal pro-brain natriuretic peptide (NT-proBNP).

Six-minute walking test
The six-minute walking test (6MWT) was performed according to ATS-guidelines.

Cardiac magnetic resonance imaging
CMR was performed on a Siemens Avanto 1.5 T and 1.5 T Sonata scanner (Siemens Medical Solutions, Erlangen, Germany), equipped with a 6-element phased-array coil. ECG-gated cine imaging was performed using a balanced steady, free precession pulse sequence, during repeated breath-holds. Short-axis slices were obtained with, slice thickness 5 mm and interslice gap 5 mm, fully covering both ventricles from base to apex. Temporal slice resolution between 35 and 45 ms, voxel size 1.8 x 1.3 x 5.0 mm^3, flip angle 60^o, receiver bandwidth 930 Hz/pixel, TR/TE 3.2/1.6 ms, matrix 256 x 156.
End-diastolic and end-systolic endocardial and epicardial contours were delineated manually by an observer blinded to other clinical information and processed using MASS software (MEDIS Medical Imaging Systems, Leiden, the Netherlands) to obtain RV end-diastolic and end-systolic volumes (RVEDV and RVESV respectively) and RV mass. Papillary muscles and trabeculae were excluded from the cavity, and included in RV mass. RV stroke volume (RVSV) and ejection fraction (RVEF) were calculated: RVSV = RVEDV – RVESV and RVEF = RVSV/RVEDV[12]. RV mass / RVEDV was used as a measure of relative RV wall thickness[13,14].

Statistical analysis
Measurements are reported as mean ± standard deviation and median (interquartile range) where appropriate. Continuous variables were compared using student t-tests or Mann-Whitney U, where not normally distributed. Categorical variables compared using Pearson Chi-square tests and Fisher's exact tests, as needed.
Follow-up was until September 2011. Transplant-free sex survival differences were confirmed using Kaplan Meier curves and log-rank test. Confounders accounted for by Cox regression. Variables leading to a ≥10% change in the coefficient for sex were included in the final survival prediction model. Variables screened for confounding included: age, height, weight, World Health Organization functional class (WHO FC), number of comorbidities (1, 2 and ≥3), RVEF, RV wall thickness, GFR, PVR and type

of medical therapy used (prostacyclins yes/no, endothelin receptor antagonist yes/no and phosphodiesterase type 5 inhibitor yes/no).
Sex differences in secondary treatment outcomes, NT-proBNP, 6-minute walking distance, renal function, RHC hemodynamics and CMR were confirmed using linear regression with the follow-up measurement as the dependent variable and the baseline measurement and sex as independent variables. WHO FC change differences were confirmed by ordinal regression. Multiple imputation was used for missing follow-up CMR variables. We multiply imputed 100 datasets. Linear regression models were estimated in each dataset and regression coefficients and standard errors pooled and the p-value of each coefficient in the model determined. To correct for confounders a similar approach was used as discussed above for the survival analysis.
An exploratory mediator analysis was done to confirm that transplant-free sex survival differences were mediated through differences in RVEF change. Analysis was done according to Baron and Kenny[15] and consists of 3 steps. In step 1, sex was confirmed as an independent predictor of transplant-free survival by Cox regression. Step 2 was to confirm that sex was an independent predictor of the proposed mediator by linear regression. Step 3 employs a Cox regression model for transplant-free survival including sex and the potential mediator as independent variables and its purpose is to confirm the proposed mediator is a significant predictor of survival, while controlling for sex. RVEF and PVR changes were both examined as potential mediators. This was done by adding follow-up measurements of respectively RVEF and PVR to a Cox regression equation containing gender and the baseline value. A greater than 10% change in the coefficient of sex after adding the follow-up value of the proposed mediator was accepted as evidence of significant mediation. The magnitude of the indirect (mediated) effect was calculated according to the following formula:

$$\text{Indirect effect} = 1 - (c' / c)$$

In the formula, c is the coefficient for sex in the Cox regression formula predicting survival, corrected for baseline RVEF; c' is the coefficient for sex in the Cox regression formula predicting survival corrected for RVEF baseline value and RVEF change by adding the follow-up RVEF value to the equation. In addition a mediator analysis corrected for all potential confounders mentioned earlier was performed[16].
Analysis were performed using IBM SPSS statistics 19.0 software. This study was approved by the VUMC Research and Ethics Review boards (METC) (approval number 2012288).

Results

Patient characteristics and treatments

One-hundred-eighty-six patients (155 IPAH, 25 HPAH and 6 APAH) were treated at the VUMC between February 1999 and January 2011. Eight-five patients were excluded. Reasons for exclusion were: no CMR due to logistical reasons (n = 44), first-line treatment elsewhere (n = 25), contraindications for CMR (n = 11) and no PAH medication initiated (n = 5). Apart from age, Table A1 (Supplement) indicates similar characteristics compared to those included for further analysis (n = 101). The six-minute walking distance tended to be greater in those included, however the % predicted distance was similar.

The remaining 101 patients had CMR and RHC at baseline before starting PAH specific medical therapies (Table 1). In these patients men had larger RVEDV and RVESV, but had similar invasively measured hemodynamics and similar RVSV and RVEF compared to women. Median (IQR) time between baseline CMR and RHC was 0.2 months (0.0 - 1.95 months).

Table A2 (Supplement) depicts prescribed medications between baseline and follow-up assessment. Follow-up CMR and RHC were performed after respectively 1.1 years (0.9 - 1.7 years) and 1.1 years (0.9 - 2.2 years). Time on PAH specific medication was 5.4 years (2.1 - 7.7 years). Time to addition of other PAH specific therapy was 5.0 months (2.3 - 6.0 months) for those patients who had PAH specific drugs added before follow-up measurements.

Table 1. Baseline patient characteristics, RHC and CMR measurements in male and female pulmonary arterial hypertension (PAH).

Variable	Male (n = 26)	Female (n = 75)	P-value
Age, years	50 ± 19	47 ± 15	0.31
Idiopathic, n (%)	23 (88)	59 (79)	0.55
Heritable, n (%)	3 (12)	12 (16)	0.75
Anorexigen, n (%)	0 (0)	4 (5)	0.57
BMI, kg/m^2	27 ± 3	26 ± 6	0.34
WHO FC, n (%)			0.09
Class I	1 (4)	0 (0)	
Class II	7 (27)	12 (16)	
Class III	13 (50)	40 (53)	
Class IV	5 (19)	23 (31)	
Comorbidities, n (%)			0.82
0	9 (35)	25 (33)	
1	6 (23)	26 (35)	
2	8 (31)	12 (16)	
≥ 3	3 (12)	12 (16)	
6MWD, m	388 ± 189	353 ± 150	0.40
6MWD, % predicted	62 ± 27	61 ± 23	0.82
Creatinine, mmol/L	110 ± 27	94 ± 17	0.001
GFR, ml/min	88 ± 31	75 ± 19	0.01
NT-proBNP, ng/L[*]	1414 ± 1668	1887 ± 1913	0.34
RHC			
RAP, mmHg	10 ± 6	9 ± 5	0.11
mPAP, mmHg	53 ± 15	57 ± 13	0.29
PCWP, mmHg	8 ± 4	8 ± 5	0.65
CO, L/min	4.73 ± 1.63	4.55 ± 1.63	0.61
PVR, dyn·s·cm^{-5}	903 ± 545	963 ± 473	0.61
Acute vasoreactivity [#]	3/23 (13%)	7/66 (11%)	0.71
CMR			
RVEDV, ml	177 ± 68	137 ± 41	0.001
RVEDVI, ml/m^2	89 ± 36	76 ± 21	0.03
RVESV, ml	124 ± 54	93 ± 35	0.001
RVESVI, ml/m^2	62 ± 28	52 ± 19	0.04
RVEF, %	31 ± 13	33 ± 11	0.44
RVSV, ml	53 ± 30	44 ± 19	0.38
RVSVI, ml/m^2	27 ± 17	25 ± 10	0.38
RV mass, g	104 ± 41	81 ± 28	0.009

RV mass / RVEDV, g/ml	0.64 ± 0.31	0.62 ± 0.23	0.75

Data are presented as mean ± SD. 6MWD = six-minute walk distance, BMI = body mass index, CMR = cardiac magnetic resonance imaging, CO = cardiac output, GFR = glomerular filtration rate, mPAP = mean pulmonary artery pressure, NT-proBNP = N-terminal pro-brain natriuretic peptide, PCWP = pulmonary capillary wedge pressure, PVR = pulmonary vascular resistance, RAP = right atrial pressure, RHC = right heart catheterization, RVEDV = right ventricular end-diastolic volume, RVEF = right ventricular ejection fraction, RVESV = right ventricular end-systolic volume, RVSV = right ventricular stroke volume, WHO FC = world health organization functional class. CMR volumes are also provided indexed (I) for body surface area. *NT-proBNP was measured in a subgroup of n = 20 males and n = 52 females. #Acute vasoreactivity was measured in a subgroup of n = 66 females and n = 23 males. RV mass / RVEDV is a measure of relative RV wall thickness.

Survival and secondary treatment outcomes

In the 101 patients included median (IQR) follow-up time was 5.7 years (2.5 to 8.1 years), and there were 26 deaths and 5 lung transplantations. In males, cumulative transplant-free survival was 84% at 1 year and 57% at 5 years. In females, survival was 100% at 1 year and 85% at 5 years (log-rank, p = 0.002, HR: 3.04, 95% CI: 1.45-6.41, Figure 1). The association between sex and survival after adjustment for confounders in multivariate analysis remained (HR: 7.21, 95% CI: 4.18-12.43, p<0.001). The confounders retained in the final model were height, GFR and WHO FC.
Male patients had higher NT-proBNP, lower 6-minute walking distance and more severe WHO FC at follow-up in basic (Table 2) and covariate-adjusted models (Table 3).

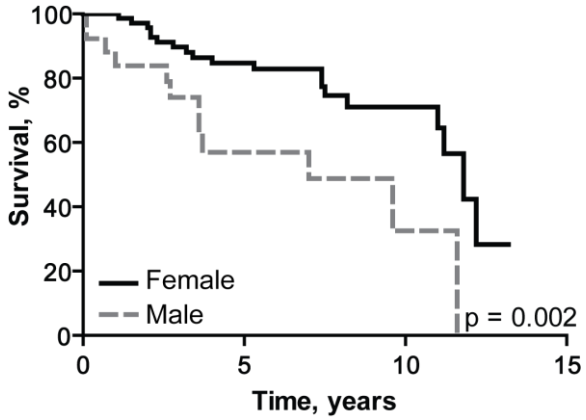

Figure 1. Kaplan-Meier survival analyses of female (black) and male (grey) patients with pulmonary arterial hypertension (PAH) starting first-line PAH-specific therapies.

Table 2. Results of linear regression of sex differences in hemodynamics, CMR measurements and other secondary treatment outcome parameters corrected for the baseline value.

Variable	Difference for men vs. women in follow-up measure after adjustment for baseline	95% CI	P-value
WHO FC	+1.4	+0.4 to +2.3	<0.01
6MWD, m	-71	-123 to -19	<0.01
Creatinine, mmol/L	+17	+6 to +29	<0.01
GFR, ml/min	-5	-11 to +1	0.12
NT-proBNP, ng/L	+1385	+482 to +2288	<0.01
RHC			
Heart rate, bpm	+3	-7 to +13	0.56
RAP, mmHg	+2	-1 to +6	0.17
mPAP, mmHg	+1	-7 to +9	0.81
CO, L/min	+0.2	-1 to +1	0.78
SV, ml	-4	-19 to +11	0.59
PVR, $dyn \cdot s \cdot cm^{-5}$	-60	-301 to +182	0.63
CMR			
RVEF, %	-8.1	-14 to -2	<0.01
RVEDV, ml	+11.9	-5 to +29	0.18
RVESV, ml	+13.8	-2 to +30	0.09
RVSV, ml	-5.5	-14 to +3	0.19
RV mass, g	+2.9	-12 to +18	0.70
RV mass / RVEDV, g/ml	+0.04	-0.09 to +0.16	0.57

b = coefficient for sex (male = 1, female = 0).

Table 3. Results of multivariate analysis* of sex specific RHC, CMR measurements and other treatment outcome parameter changes compared to baseline.

Variable	Difference for men vs. women in follow-up measure after adjustment for baseline and confounders	95% CI	P-value
WHO FC	+1.9	+0.9 to +3.0	<0.001
6MWD, m	-70	-127 to -12	0.02
Creatinine, mmol/L	+14	+3 to +25	0.01
GFR, ml/min	-6	-13 to 0	0.05
NT-proBNP, ng/L	+1385	+482 to +2288	<0.01
RHC			
Heart rate, bpm	+5	-7 to +17	0.42
RAP, mmHg	+2	-1 to +6	0.25
mPAP, mmHg	+2	-8 to +11	0.73
CO, L/min	+0.0	-1 to +1	0.99
SV, ml	-7	-24 to +11	0.45
PVR, dyn·s·cm^{-5}	-35	-337 to +267	0.82
CMR			
RVEF, %	-7.2	-13 to -1	0.02
RVEDV, ml	-0.4	-19 to +18	0.97
RVESV, ml	+5.2	-13 to +23	0.58
RVSV, ml	-9.5	-19 to 0	0.04
RV mass, g	+3.8	-13 to +21	0.67
RV mass / RVEDV, g/ml	+0.09	-0.05 to +0.24	0.22

*Multivariate analysis results showing the coefficient b for sex (male = 1; female = 0) corrected for potential confounding by age, weight, height, number of comorbidities, baseline RVEF, GFR, PVR, WHO FC and type of PAH specific medical therapy initiated.

RHC and CMR

RHC showed no significant differences in treatment response associated with sex (Tables 2 and 3). Median PVR changes (IQR) were -78 dyn·s·cm^{-5} (-523 to +10 dyn·s·cm^{-5}) in males and -165 dyn·s·cm^{-5} (-436 to +92 dyn·s·cm^{-5}) in females.

Eighty patients had baseline and follow-up CMR performed. Reasons for not performing follow-up measurements in males were: patient deceased (n

= 3), patient follow-up <1 year (n = 3), patient too disabled to undergo CMR (n = 3), unknown (n = 1). In females these were patient refusal (n = 4), patient follow-up <1 year (n = 3), patient too disabled (n = 2), psychiatric disorder (n = 1) and technical CMR problems (n = 1). Corrections for missing follow-up measurements were made by multiple imputation.
After the baseline assessment, RVEF decreased in males (median, IQR) -1.0 % (-11.9 to +6.9 %) and increased in females +3.6 % (-3.0 to +13.0 %). Tables 2 and 3 depict results of univariate and multivariate analysis of sex difference in CMR changes. Calculated RVEF change corrected for confounders was -1.8 ± 6.5 % in males and +5.3 ± 5.4 % in females (p <0.001).

Mediator analysis

Step 1 and step 2 of the mediator analysis were reported above. In step 1 sex was confirmed as an independent predictor of survival. In step 2 sex was confirmed as an independent predictor of RVEF change. Results of step 3 are reported in Table 4, which shows the results of cox regression for transplant-free survival with sex and the baseline value of the potential mediator. The B coefficient of sex changed substantially after RVEF follow-up measurements were added to the equation and significance of sex as predictor of transplant-free survival was lost, thus showing evidence that the impact of sex on survival was mediated through RVEF at follow-up. There is no evidence for mediation through PVR changes as the B coefficient for sex remains similar in the cox regression formula with sex and baseline PVR compared to the formula with sex, baseline PVR and follow-up PVR. The amount of change in B for sex after adding follow-up values of RVEF or PVR to the Cox regression equation gives a sense of how much of the variance in outcome associated with sex is explained by changes of each hemodynamic parameter. In the basic model, 42.8 % of the effect of sex on survival was mediated through RVEF. After adjustment for confounders this was 39.0 %.

Table 4. Results from cox-regression for transplant-free survival with respectively sex and the baseline measurement and subsequently sex, the baseline measurement and the follow-up measurement for respectively RVEF and PVR. Crude analysis (A) and analysis including corrections for confounders (B) is reported.

	B	Exp (B)	95% CI of Exp (B)	P-value
A				
Gender (male vs. female)	1.029	2.80	1.33 - 5.91	0.007
Baseline RVEF	-0.05	0.95	0.92 - 0.99	0.007
Gender	0.589	1.80	0.81 - 4.01	0.15
Baseline RVEF	-0.01	0.99	0.95 - 1.04	0.81
Follow-up RVEF	-0.07	0.94	0.89 - 0.98	0.006
B				
Gender	1.397	4.04	2.50 - 6.54	0.004
Baseline RVEF	-0.05	0.95	0.93 - 0.97	0.009
Gender	0.852	2.34	0.93 - 5.92	0.07
Baseline RVEF	-0.01	0.99	0.95 - 1.04	0.76
Follow-up RVEF	-0.07	0.94	0.89 - 0.98	0.006
A				
Gender	1.11	3.04	2.08 - 4.45	0.003
Baseline PVR	0.00	1.00	1.00 - 1.00	0.95
Gender	1.20	3.31	1.53 - 7.16	0.002
Baseline PVR	0.00	1.00	1.00 - 1.00	0.53
Follow-up PVR	0.00	1.00	1.00 - 1.00	0.12
B				
Gender	1.51	4.52	1.83 - 11.18	0.001
Baseline PVR	0.00	1.00	1.00 - 1.00	0.84
Gender	1.476	4.38	1.77 - 10.84	0.001
Baseline PVR	0.00	1.00	1.00 - 1.00	0.48
Follow-up PVR	0.00	1.00	1.00 - 1.00	0.19

Discussion

Our data confirmed previous findings of worse outcome in males[6]. This survival difference was not associated with either baseline characteristics

or differences in responsiveness of the pulmonary vascular bed to therapy, but rather differences in RVEF after starting medical therapies. NT-proBNP changes are correlated with RV strain and RVEF measured by CMR and the NT-proBNP differences found in our study further support our CMR findings[17-19]. In an earlier study RVEF change difference between survivors and non-survivors in PAH was 8%, further illustrating that the difference found in our study is clinically meaningful[20].

Sex differences have been well documented in diseases of the left ventricle. In the Framingham study, worse survival was observed in male heart failure patients[21]. Systolic heart failure is predominantly found in men whereas women rather present with heart failure with preserved ejection fraction[22]. In analogy female pressure loaded hearts showed more preserved ejection fractions in aortic stenosis[23]. In a recent study of hypertensive patients left ventricular mass variance explained by arterial blood pressure was much higher in females. This could be interpreted as further evidence of better cardiac adaptation in females[24].
Little is known about sex differences in disease of the right ventricle. Healthy women have lower RV mass, smaller RV volumes, and higher RVEF than men[25]. Ventetuolo et al. showed an association between higher estradiol levels and improved RVEF in women and an association between increased androgen levels and increased RV mass and RV volumes[26]. In a rodent model testosterone and estradiol both caused pulmonary vasodilation[27]. In male mice testosterone affected RV hypertrophic stress response after pulmonary artery banding through increased myocyte size and increased fibrosis. Testosterone deprivation through castration improved survival in these mice[28]. In addition estrogen and estrogen receptor agonist therapy restored RV structure and function in a rodent model of monocrotaline induced PH[29].

Our study found no differences in pulmonary vascular responses to PAH specific medications. Hitherto no other studies in humans reported on sex differences in pulmonary vascular response. We found no sex differences in cardiac output, and this further points out the problems with only looking at resting CO, rather than at RV structure and RV systolic function (RVEF). During disease progression resting CO can be maintained through an increased heart rate. In addition stroke volume can be relatively preserved through the Starling mechanism. However, in progressive RV dilation RVEF will decrease and RVEF may be a more sensitive parameter for disease progression[2]. It cannot be ruled out that CO differences do occur upon exercise.

Study limitations

Our study has some limitations. Not all patients evaluated at our center were included. While those included appeared similar to those excluded, selection bias could still be possible. We attempted to account for a variety of confounders, however we cannot exclude residual or unmeasured confounding. There were some missing data; we used multiple imputation to allow inclusion of all subjects in the study sample in all analyses. Finally, this is an observational study, preventing us from confirming causality, however the use of sex as our exposure and prospective reassessments of RV function support causal inferences. We only studied the idiopathic, heritable and anorexigen associated form of PAH, so these findings may not be generalizable to other forms of PAH. However sex differences in survival are also reported in connective tissue disease associated PAH[30], although in associated PAH the survival difference was limited to elderly patients[9]. Since RVEF could explain 40% of the observed survival difference, other factors must contribute. However, these factors cannot be identified through our study, as the small patient number prohibits further exploratory analysis.

Conclusions

Our study suggests a sex difference in cardiac adaptation to treatment with long-term improvements in RVEF in women, but not in men. Mediator analysis suggests this different cardiac adaptation may cause decreased survival in males. To further improve treatments, the pathophysiology of sex differences in cardiac response to medical therapies should further be elucidated. Evidence for differences in cardiac responses in associated forms of PAH should be studied. Furthermore, the role of sex hormones, and the potential of substances targeting sex-specific pathways, such as estrogen receptor agonists should be further evaluated[29].

References

1. Galiè N, Hoeper MM, Humbert M, et al. Guidelines for the diagnosis and treatment of pulmonary hypertension. Eur Heart J. 2009; 30:2493-2537.
2. van Wolferen SA, Marcus JT, Boonstra A, et al. Prognostic value of right ventricular mass, volume, and function in idiopathic pulmonary arterial hypertension. Eur Heart J. 2007; 28:1250-1257.
3. Thenappan T, Shah SJ, Rich S, et al. A USA-based registry for pulmonary arterial hypertension: 1982-2006. Eur Respir J. 2007; 30:1103-1110.
4. Gomberg-Maitland M, Dufton C, Oudiz RJ, et al. Compelling evidence of long-term outcome in pulmonary arterial hypertension? A clinical perspective. J Am Coll Cardiol. 2011; 57:1053-1061.

5. Kawut SM, Horn EM, Berekashvili KK, et al. New predictors in outcome in idiopathic pulmonary arterial hypertension. Am J Cardiol. 2005; 95:199-203.
6. Humbert M, Sitbon O, Chaouat A, et al. Survival in patients with idiopathic, familial and anorexigen-associated pulmonary arterial hypertension in the modern management era. Circulation. 2010; 122:156-153.
7. Humbert M, Sitbon O, Yaïci A, et al. Survival in incident and prevalent cohorts of patients with pulmonary arterial hypertension. Eur Respir J. 2010; 36:549-555.
8. Kane GC, Maradit-Kremers H, Slusser JP, et al. Integration of clinical and hemodynamic parameters in the prediction of long-term survival in patients with pulmonary arterial hypertension. Chest. 2011; 139:1285-1293.
9. Shapiro S, Traiger GL, Turner M, et al. Sex differences in the diagnosis, treatment, and outcome of patients with pulmonary arterial hypertension enrolled in the registry to evaluate early and long-term pulmonary arterial hypertension disease management. Chest. 2012; 141:363-373.
10. Thenappan T, Glassner C, Gomberg-Maitland M. Validation of the pulmonary hypertension connection equation for survival prediction in pulmonary arterial hypertension. Chest. 2012; 141:642-650.
11. Gabler NB, French B, Strom BL, et al. Race and sex differences in response to endothelin receptor antagonists for pulmonary arterial hypertension. Chest. 2012; 141:20-26.
12. Vonk Noordegraaf A, Galiè N. The role of the right ventricle in pulmonary arterial hypertension. Eur Respir Rev. 2011; 20:243-253.
13. Gaash WH, Zile MR. Left ventricular structural remodelling in health and disease. J Am Coll Cardiol. 2011; 58:1733-1740.
14. Lorenz CH, Walker ES, Graham TP, et al. Right ventricular performance and mass by use of cine MRI late after atrial repair of transposition of the great arteries. Circulation. 1995; 92:233-239
15. Baron RM, Kenny DA. The moderator-mediator variable distinction in social psychological research: conceptual, strategic and statistical considerations. J Pers Soc Psychol. 1986;51:1173-1182.
16. Kenny DA, Kashy DA, Bolger N. Data analysis in social psychology. In D Gilbert, S Fiske, G lLindzey (Eds.), The handbook of social psychology, New York. 1998; 1:115-139.
17. Oyama-Manabe N, Sato T, Tsujino I, et al. The strain encoded (SENC) MR imaging for detection of global right ventricular dysfunction in pulmonary hypertension. Int J Cardiovasc Imaging. 2013; 29:371-378.
18. Blyth KG, Groening BA, Mark PB, et al. NT-proBNP can be used to detect right ventricular systolic dysfunction in pulmonary hypertension. Eur Respir J. 2007; 29:737-744.
19. Vonk Noordegraaf A, Westerhof N. Right ventricular ejection fraction and NT-proBNP are both indicators of wall stress in pulmonary hypertension. Eur Respir J. 2007; 29:622-623.
20. van de Veerdonk MC, Kind T, Marcus JT, et al. Progressive right ventricular dysfunction in patients with pulmonary arterial hypertension responding to therapy. J Am Coll Cardiol. 2011; 58:2511-2519.
21. Ho KKL, Anderson KM, Kannel WB, et al. Survival after the onset of congestive heart failure in the Framingham heart study subjects. Circulation. 1993; 88:107-115.
22. Cleland JGF, Swedberg K, Follath F, et al. The euroheart failure survey programme – a survey on the quality of care among patients with heart failure in Europe. Part 1: patient characteristics and diagnosis. Eur Heart J. 2003; 24:442-463.
23. Carroll JD, Carroll EP, Feldman T, et al. Sex-associated differences in left ventricular function in aortic stenosis of the elderly. Circulation. 1992; 86:1099-1107.

24. Cipolline F, Arcangeli E, Greco E, et al. Gender difference in the relation blood pressure-left ventricular mass and geometry in newly diagnosed arterial hypertension. Blood Press. 2012; 21:255-264.
25. Kawut SM, Lima JA, Barr RG, et al. Sex and race differences in right ventricular structure and function: the Multi-ethnic study of atherosclerosis-right ventricle study. Circulation. 2011; 123:2542-2551.
26. Ventetuolo CE, Ouyang P, Bluemke DA, et al. Sex hormones are associated with right ventricular structure and function. The MESA-Right ventricle study. Am J Respir Crit Care Med. 2011; 183:659-667.
27. English KN, Jones RD, Jones TH, et al. Gender differences in the vasomotor effects of different steroid hormones in rat pulmonary and coronary arteries. Horm Metab Res. 2001; 33:645-652.
28. Hemnes AR, Maynard KN, Champion HC, et al. Testosterone negatively regulates right ventricular load stress responses in mice. Pulm Circ. 2012; 2:352-358.
29. Umar S, Lorga A, Matori H, et al. Estrogen rescues preexisting severe pulmonary hypertension in rats. Am J Respir Crit Care Med. 2011; 184:715-723.
30. Condliffe R, Kiely DG, Peacock AJ, et al. Connective tissue disease-associated pulmonary arterial hypertension in the modern treatment era. Am J Respir Crit Care Med. 2009; 179:151-157.

Data supplement

Table A1. Patient characteristics and hemodynamics in patients with pulmonary arterial hypertension (PAH) included in study compared to those excluded.

Variable	Included (n = 101)	Excluded (n = 85)
Age, years	48 ± 16	57 ± 18
Gender, m/f	26/75	28/57
BMI, kg/m^2	26 ± 5	28 ± 6
WHO FC, n		
Class I	3	1
Class II	14	19
Class III	41	53
Class IV	27	28
Comorbidities, n		
0	34	30
1	32	22
2	20	19
≥ 3	15	14
6MWD, m	362 ± 162	307 ± 126
6MWD, % predicted	61 ± 24	58 ± 21
Creatinine, mmol/L	98 ± 21	100 ± 23
GFR, ml/min	78 ± 24	76 ± 31
NT-proBNP, ng/L*	1765 ± 1865	1824 ± 2486
RHC		
RAP, mmHg	9 ± 5	9 ± 6
mPAP, mmHg	56 ± 14	49 ± 12
PCWP, mmHg	8 ± 5	10 ± 7
CO, L/min	4.60 ± 1.63	4.65 ± 1.75
CI, L/min/m^2	2.50 ± 0.93	2.51 ± 0.96
PVR, dyn·s·cm^{-5}	957 ± 493	802 ± 462
PVRI, dyn·s·cm^{-5}·m^2	1760 ± 919	1505 ± 835

6MWD = six-minute walk distance, BMI = body mass index, BSA = body surface area, CI = cardiac index, CO = cardiac output, GFR = glomerular filtration rate (Cochroft), mPAP = mean pulmonary artery pressure, NT-proBNP = N-terminal pro-brain natriuretic peptide, PCWP = pulmonary capillary wedge pressure, PVR = pulmonary vascular resistance, PVRI = pulmonary vascular resistance index, RAP = right atrial pressure, RHC = right heart catheterization, WHO FC = world health organization functional class. *NT-proBNP was measured in a subgroup of respectively n = 72 and n = 40.

Table A2. PAH medical treatment regimens in respectively men and women.

Variable	Male (n = 26)	Female (n = 75)	P-value
First-line therapy, n (%)			
Prostacyclins	4 (15%)	23 (31%)	0.20
ERA	13 (50%)	30 (40%)	0.74
PDIE5	4 (15%)	9 (12%)	0.74
ERA + PDIE5	2 (8%)	3 (4%)	0.60
ERA + Prostacyclins	0 (0%)	3 (4%)	0.57
Ca^{2+} blockers	3 (12%)	7 (9%)	0.71
Add-on therapy, n (%)			
ERA + PDIE5	4 (15%)	17 (23%)	0.58
ERA + Prostacyclins	1 (4%)	1 (1%)	0.45
Prostacyclins + PDIE5	0 (0%)	2 (3%)	1.00
PDIE5 + Prostacyclins	1 (4%)	0 (0%)	0.26
Ca^{2+} blockers + PDIE5	0 (0%)	1 (1%)	1.00
Ca^{2+} blockers + Prostacyclins	0 (0%)	2 (3%)	1.00
Switch, n (%)			
From ERA to PDIE5	1 (4%)	3 (4%)	1.00
From Ca^{2+} blockers to Prostacyclins	0 (0%)	2 (3%)	1.00

Data are presented as number of patients n (% within sex). CA^{2+} blockers = calcium channel blockers, ERA = endothelin receptor antagonists, PDIE5 = phosphodiesterase type 5 inhibitors.

Chapter 6

Improved right ventricular function and survival after substantial afterload reduction in patients with pulmonary arterial hypertension

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In progress

Abstract

Introduction. In patients with pulmonary arterial hypertension (PAH), the mean reduction in pulmonary vascular resistance (PVR) after medical treatment is modest but heterogeneous responses with respect to changes in load and right ventricular (RV) function exist. The aim of this study was to compare the effects of varying degrees of PVR reduction after medical treatment on hemodynamics, RV function and survival in patients with PAH.
Methods. 123 patients underwent right heart catheterization and a subset of 81 patients underwent cardiac magnetic resonance imaging at baseline and after 1 year of treatment. The study population was divided into three equal groups based on tertiles of relative changes in PVR: (1) unchanged/small decrease <-12%, (2) modest decrease -12 to -42%, (3) large decrease >-42%. In these groups, hemodynamics, RV function and survival were compared.
Results. Compared to groups 1 and 2, group 3 showed the strongest improvements in pulmonary pressures and normalisation of cardiac output (CO). RV fraction remained unchanged in groups 1 and 2 but improved in group 3. During a follow-up of 47 months, 38 patients died/underwent lung transplantation. Survival was similar in groups 1 and 2 (54% and 64% resp.) but significantly better in group 3 (survival: 90%, sensitivity: 90%, specificity: 42%, $p = 0.01$).
Conclusions. Compared to a small and modest PVR reduction, only a large PVR decrease >-42% after medical treatment is associated with normalisation of CO, improvement of RV function and is a sensitive predictor of long-term survival in patients with PAH.

Introduction

In patients with pulmonary arterial hypertension (PAH) a progressive increase in pulmonary vascular resistance (PVR) and pulmonary artery pressure result in right ventricular (RV) dysfunction and ultimately RV failure and death[1,2]. It has been shown that normalisation of PVR as achieved after lung transplantation, result in restoration of RV function[3]. Furthermore in patients with chronic thromboembolic pulmonary hypertension (CTEPH), a strong decrease in PVR after pulmonary endarterectomy lead to significant RV improvements[4,5]. Multiple PAH-targeted medical therapies have been developed in the past 20 years and are proven to lower the PVR[6,7]. Although the mean PVR decrease is modest and has relatively small effects on RV function and survival, previous studies have reported a broad range of PVR changes after medical therapy[7-12]. Furthermore, it has been observed that some patients responded favourably with improved RV function while others showed progressive RV deterioration despite a PVR reduction after therapy and was associated with poor survival[8]. Improved insights in the heterogeneous medical therapeutic responses are essential. Therefore the aim of the present study was to compare the effects of varying degrees of PVR reduction after medical treatment on hemodynamics, RV function and survival in patients with PAH.

Methods

Study design and study population

We performed a retrospective cohort study. Cardiac magnetic resonance imaging (CMR) data of this study were partly obtained from an on-going prospective research program. The other part of our CMR data and all right heart catheterization (RHC) data were obtained for clinical purposes. The study was approved by the Medical Ethics Review Committee of the VU University Medical Center. Due to the fact that the study does not fall within the scope of the Medical Research Involving Human Subjects (WMO), the study was approved without requirement of informed consent.

Between January 2000 and January 2012, 1565 patients were referred to the VU University Medical Center, Amsterdam, the Netherlands because of a suspected diagnosis of pulmonary hypertension (PH). According to the World Health Organisation guidelines[13], PAH group 1 was diagnosed in 453 patients. Inclusion criteria for the study were: (1) idiopathic, hereditary, anorexigen-related PAH patients or patients with pulmonary veno-occlusive disease; (2) incident PAH patients with optimal PAH-targeted medical therapies directly initiated after diagnostic baseline measurements; (3) RHC

at baseline and after 1 year of follow-up. Exclusion criteria were: PAH patients with associated conditions (*i.e.* congenital systemic to pulmonary shunts, connective tissue disease, portal hypertension or HIV infection). 133 patients met the inclusion criteria but ten patients were excluded due to incomplete/insufficient PVR measures. In the present study, a total of 123 PAH patients were included. 81 out of the 133 patients underwent CMR both at baseline and after 1 year of follow-up. CMR and RHC were performed within a median time interval of 1 day. Some patients did not undergo complete CMR assessment due to logistical reasons (n = 22) or contraindications for CMR (n = 12). In addition, eight patients had incomplete/insufficient CMR data.
All patients were followed clinically on a regular basis by outpatient visits and telephone contacts until January 1, 2013. Patients with a positive response to the acute vasodilator challenge[13] were treated with calcium channel antagonists. Before 2002, all unresponsive NYHA functional class III and IV patients were initiated on prostacyclins. After 2002, NYHA II and III patients received oral medical therapy consisting of endothelin receptor antagonsists (ERA) and/or phophodiesterase 5 inhibitors (PDE 5I) and NYHA IV patients received intravenous prostacyclin with/without additional oral medical treatment. All patients received oral anticoagulation. During follow-up, many patients went through one or multiple treatment regimens.

Right heart catheterization
Hemodynamic assessment was performed with a 7F balloon tipped, flow directed Swan-Ganz catheter (131HF7, Baxter, Healthcare Corp Irvine, California) as previously described[14].

Six-minute walking testing
The six-minute walking test (6MWT) was performed according to American Thoracic Society guidelines[15].

Blood sampling
Since November 2002, N-terminal pro-brain natriuretic peptide (NT-proBNP) measurements have become part of our clinical assessment. NT-proBNP plasma levels were analysed using the Elecsys 1010 electrochemiluminescense immunoassay (Roche Diagnostics, the Netherlands) as outlined before[16].

Cardiac magnetic resonance imaging
Before April 2008 CMR was performed on a Siemens 1.5T Sonata scanner thereafter CMR was performed on a Siemens 1.5T Avanto scanner (Siemens, Medical Solutions, Erlangen, Germany). CMR data acquisition was obtained according to our routine protocol[14].

During post-processing, a blinded observer assessed the ventricular volumes, mass and function using the MASS-software package (MEDIS, Medical Imaging Systems, Leiden, The Netherlands) as described previously[14]. Briefly, stroke volume (SV) was calculated as end-diastolic volume (EDV) – end-systolic volume (ESV). RV ejection fraction (RVEF) was calculated as SV divided by EDV and multiplied by 100%. Volume measurements were indexed to body surface area (BSA). RV systolic wall stress was calculated according to the Law of Laplace as explained before[17].

Statistical analysis

Data are expressed as mean ± SD for continuous variables and absolute for categorical variables. P <0.05 was considered significant. Comparisons between and within groups were calculated by unpaired and paired Student *t*-tests respectively. The study population was divided into three equal groups, each consisting of 41 patients based on tertiles of the relative changes in PVR: (1) unchanged/increased PVR: PVR decline smaller than -12% (mean: 20 ± 34%), (2) modest PVR decrease: PVR decrease of -12 to -42% (mean: -28 ± 9%), (3) large PVR decrease: PVR decrease of more than -42% (mean: -59 ± 9%). In each group, 27 patients had underwent CMR measurements. Within these three groups, baseline characteristics were compared to follow-up measurements by means of a two-way repeated measures ANOVA with Bonferroni post-hoc test. In addition, survival rates were compared using a Kaplan-Meier landmark analysis (landmark at 1 year of follow-up). Cardiopulmonary death and lung transplantation were used as endpoint, other causes of death were censored. Receiver-operating characteristic (ROC) curve analysis was applied to determine the sensitivity and specificity of different threshold changes in PVR as indicators of survival. All statistical analyses were carried out using SPSS (version 19.0, SPSS inc. Chicago, Illinois).

Results

Patient demographics

Table 1 summarizes the demographics of the study population with and without CMR measurements. The mean age of the total study population was 48 ± 17 years, 78% was female. Most patients received upfront monotherapy consisting of ERA or combination treatment. The median time between baseline and follow-up measurements was 12 months (IQR: 10-16 months). During a subsequent long term median follow-up period of 37 months (IQR: 13-84 months), 38 patients died due to cardiopulmonary causes or underwent lung transplantation.

Table 1. Patient demographics.

Variable	Total study population (n = 123)	Patients with CMR (n = 81)	Patients without CMR (n = 42)	P-value
Age, yrs	48 ± 17	46 ± 16	52 ± 17	0.083
Female, n (%)	96 (78)	69 (85)	27 (64)	0.036
Diagnosis, n (%)				0.368
Idiopathic	103 (84)	68 (83)	35 (83)	
Heriditary	10 (8)	7 (9)	3 (7)	
Anorexigen	3 (2)	3 (4)	0 (0)	
PVOD	7 (6)	3 (4)	4 (10)	
NYHA, n (%)				0.536
I/II	41 (33)	26 (32)	15 (36)	
III	72 (59)	47 (58)	25 (59)	
IV	10 (8)	8 (10)	2 (5)	
BSA, m^2	1.9 ± 0.2	1.9 ± 0.2	1.9 ± 0.2	0.172
Medical therapy, n (%)				0.319
Calcium antagonists	6 (5)	5 (6)	1 (2)	
ERA	50 (40)	31 (29)	19 (46)	
PDE 5I	13 (11)	7 (9)	6 (14)	
Prostacyclins	25 (20)	20 (24)	5 (12)	
Combination	29 (24)	18 (22)	11 (26)	

BSA = body surface area, NYHA = New York Heart Association, ERA = endothelin receptor antagonists, PDE 5I = phosphodiesterase 5 inhibitors.

Patient characteristics at baseline and follow-up

Table 2 shows the characteristics of the study population at baseline and after 1 year of follow-up. Patients with and without CMR measurements showed similar hemodynamics, NT-proBNP and exercise capacity (Supplement, Table A1). In the total study population, the mean reduction in PVR was -220 ± 409 dyne·s·cm^{-5} (mean relative change in PVR: -20 ± 42%), mean pulmonary artery pressure (mPAP) showed a decrease of -6 ± 12 mmHg and cardiac output (CO) was improved by 1.2 ± 1.6 L/min (all p <0.001 for change). RVEDV and RV mass remained unchanged but RVEF and SV showed on average small improvements (delta RVEF: 5 ± 11%, delta SV: 4 ± 8 mL/m^2, both p <0.001).

Table 2. Changes in hemodynamics, NT-proBNP, exercise capacity and CMR measurements.

Variable	Baseline (n = 123)	Follow-up (n = 123)	P-value
RHC			
mPAP, mmHg	54 ± 15	48 ± 14	<0.001
RAP, mmHg	9 ± 5	7 ± 6	<0.001
PVR, dyne·s·cm^{-5}	900 ± 439	662 ± 390	<0.001
PCWP, mmHg	8 ± 4	7 ± 3	0.030
CO, L/min	4.5 ± 1.4	5.7 ± 1.9	<0.001
CI, L/min/m^2	2.4 ± 0.7	3.1 ± 1.0	<0.001
Heart rate, bpm	82 ± 20	81 ± 15	0.647
SvO_2, %	64 ± 9	66 ± 9	0.013
NT-proBNP, ng/L*	1684 ± 1626	926 ± 1558	<0.001
6MWT			
Distance, m	437 ± 232	461 ± 121	0.393
CMR†			
RVEDV, ml/m^2	77 ± 20	75 ± 21	0.218
RVESV, ml/m^2	52 ± 19	47 ± 20	0.009
RV mass, g/m^2	52 ± 13	52 ± 16	0.820
RVEF, %	33 ± 10	38 ± 14	<0.001
SV, ml/m^2	25 ± 8	28 ± 9	0.003
LVEDV, ml/m^2	41 ± 13	46 ± 15	0.01
LVEF, %	63 ± 11	67 ± 12	0.001

6MWT = six-minute walk testing, CI = cardiac index, CMR = cardiac magnetic resonance imaging, CO = cardiac output, LVEDV = left ventricular end-diastolic volume, LVEF = left ventricular ejection fraction, mPAP = mean pulmonary artery pressure, NT-proBNP = N-terminal pro-brain natriuretic peptide, PCWP = pulmonary capillary wedge pressure, PVR = pulmonary vascular resistance, RAP = right atrial pressure, RHC = right heart catheterization, RVEDV = right ventricular end-diastolic volume, RVEF = right ventricular ejection fraction, RVESV = right ventricular end-systolic volume, SV = stroke volume, SvO_2 = mixed venous oxygen saturation. *NT-proBNP was measured in a subgroup of 74 patients, †CMR was performed in 81 patients.

Patient characteristics according to the three groups of changes in PVR

The three PVR groups had similar age, gender, type of diagnosis and NYHA functional class.

The relative change in PVR was not related to the type of single-agent therapy (comparisons between ERA, PDE5I and prostacyclins, all p >0.05). Of interest, 10% and 14% of the patients in groups 1 (unchanged/small PVR decrease) and 2 (modest PVR decrease) respectively received upfront combination therapies in contrast to 46% of the patients in group 3 (large PVR decrease) (group 3 vs. 1 and 2, both p<0.001) (Table 3).

Table 3. Demographics according to the three groups of PVR change.

Variable	1. Unchanged / small PVR decrease (n = 41)	2. Modest PVR decrease (n = 41)	3. Large PVR decrease (n = 41)
Age, yrs	50 ± 16	46 ± 18	48 ± 16
Female, n (%)	32 (78)	32 (78)	32 (78)
Diagnosis, n (%)			
Idiopathic	34 (83)	36 (87)	33 (81)
Other	7 (17)	5 (13)	8 (19)
NYHA, III/IV, n (%)	30 (73)	24 (59)	28 (68)
Medical therapy, n (%)			
Single agent	37 (90)	35 (85)	22 (54)
Combination	4 (10)	6 (15)	19 (46)*, †

*Group 3 compared to group 1: p <0.001, †group 3 compared to group 2: p <0.001.

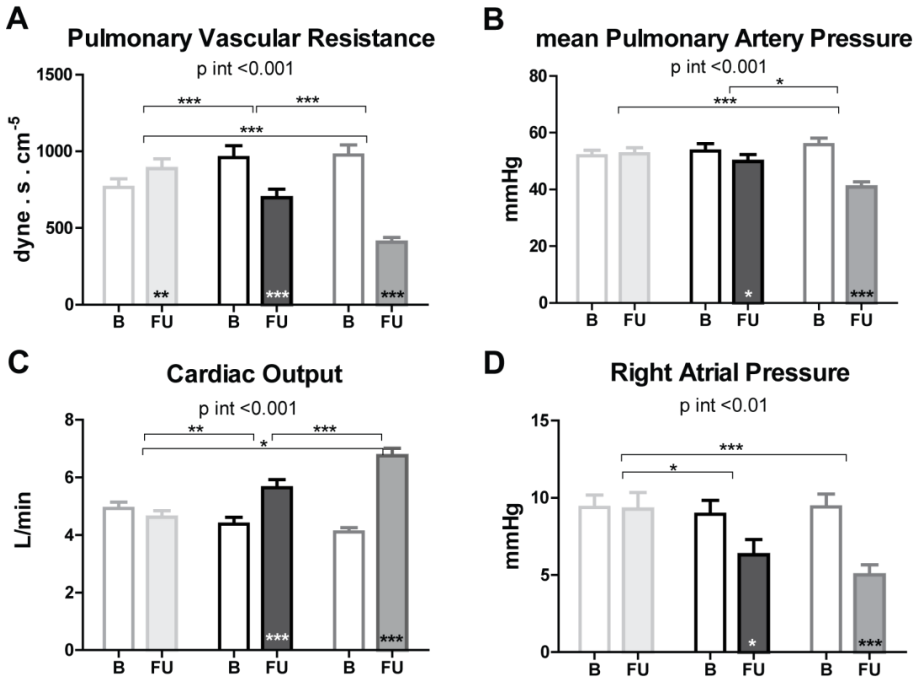

Figure 1. Hemodynamics according to the three groups of PVR.
(A) Pulmonary vascular resistance (PVR), **(B)** mean pulmonary artery pressure, **(C)** Cardiac output, **(D)** Right atrial pressure according to the three groups of PVR (licht grey: unchanged/small decrease PVR; black: modest PVR decrease; dark grey: large PVR decrease) at baseline (open bars) and after 1 year of follow-up (filled bars). Data are presented as mean ± SEM. In bars: *p <0.05, **p <0.01, ***p <0.001 for within group difference between baseline and follow-up. Above bars: *p <0.05, **p <0.01, ***p <0.001 for difference in change between groups. B = baseline, FU = follow-up.

Figure 1 shows the hemodynamics at baseline and at follow-up according to the three PVR groups. Group 1 showed a small, significant increase in PVR (delta PVR: 129 ± 233 dyne·s·cm^{-5}, p = 0.001) (Figure 1A) and no changes in other hemodynamics during follow-up. In comparison to group 2, group 3 showed stronger improvements in CO (delta CO: 2.7 ± 1.2 and 1.3 ± 1.2 L/min resp, p <0.001) and reduction in mPAP (delta mPAP: -15 ± 11 and -4 ± 9 mmHg resp., p <0.001) (Figure 1B, C). RAP was decreased in groups 2 and 3 (Figure 1D). In addition, heart rate was decreased in group 3 (delta heart rate: -8 ± 15 bpm; p = 0.017) but was unchanged in groups 1 and 2 (both: p >0.05). The distance at 6MWT was unchanged in all groups during follow-up (all p >0.05 for change) (data no shown). NT-proBNP was unchanged in groups 1 and 2 (both p >0.05) and was significantly decreased in group 3 (delta NT-proBNP: -1293 ± 1160 ng/L; p <0.001). In addition, RV volumes and mass showed a decrease in group 3 and were maintained in groups 1 and 2 (Figure 2A-C). There was no change of RV wall stress in group 1 but a trend towards a stronger decrease was observed in group 3 compared to group 2 (Figure 2D). SV and RVEF remained unchanged in groups 1 and 2 but the patients in group 3 showed a significant improvement (delta SV: -8 ± 8 ml/m^2, delta RVEF:14 ± 2%, both p <0.001) (Figure 2E, F).

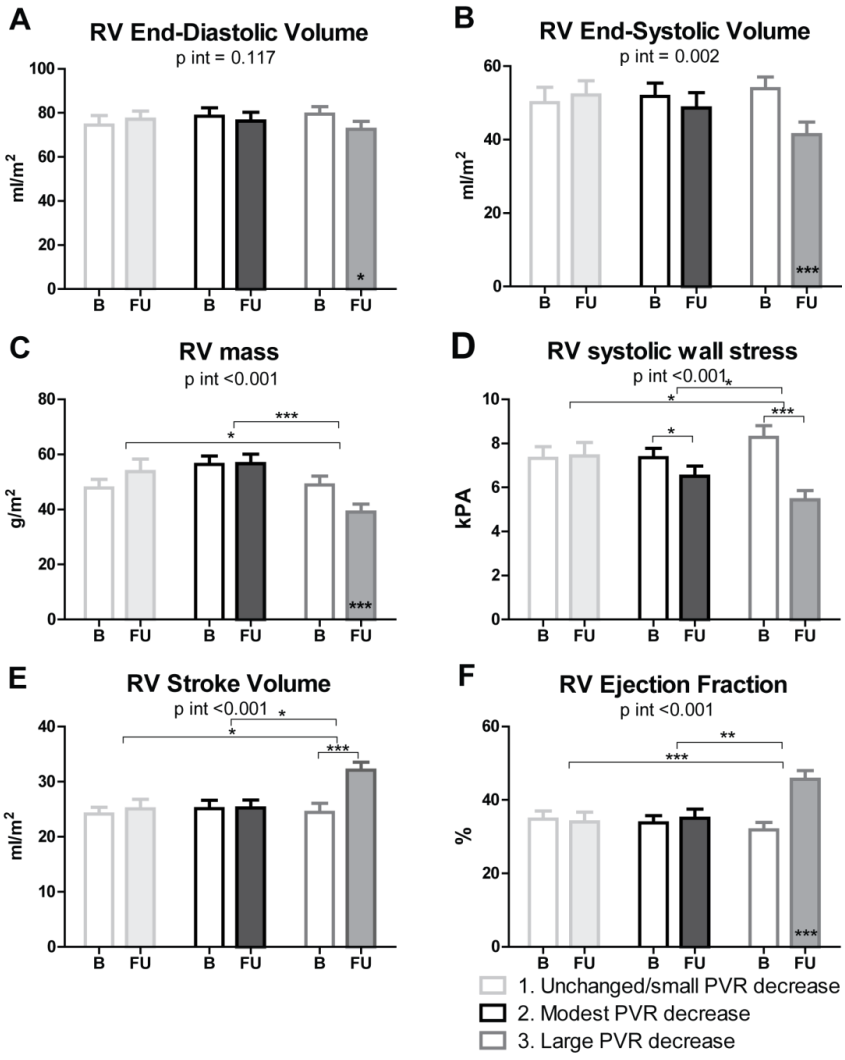

Figure 2. CMR measurements according to the three PVR groups.
(A) RV end-diastolic volume, **(B)** RV end-systolic volume, **(C)** RV mass**, (D)** RV wall stress, **(E)** RV Stroke Volume and **(F)** RV ejection fraction according to the three groups of PVR (light grey: unchanged/small PVR decrease, black: modest PVR decrease, dark grey: large PVR decrease) at baseline (open bars) and after 1 year of follow-up (filled bars). Data are presented as mean ± SEM. In bars: *p <0.05, ***p <0.001 for within group difference between baseline and follow-up. Above bars: *p <0.05, **p <0.01, ***p <0.001 for difference in change between groups.

Association between changes in PVR and survival

The mean change in PVR of -20% was not associated with survival (p = 0.140). ROC-analysis showed that a threshold decrease in PVR of -33% predicted survival with the combination of highest sensitivity (78%) and specificity (55%) (subsequent five-year survival = 75%, log-rank: p =

0.018). Figure 3 shows a landmark survival analysis of the three PVR groups. Mean survival rates were similar in patients with a small and modest PVR decrease (p = 0.861) but was significantly better in patients with a large PVR decrease (group 3: subsequent five-year survival = 85%, log-rank: p = 0.01 compared to groups 1 and 2). The large PVR decrease of group 3 predicted survival with 90% sensitivity and 42% specificity.

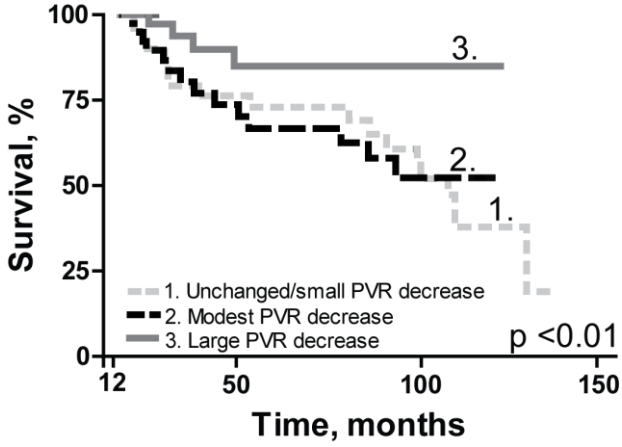

Figure 3. Landmark survival analysis according to the three groups of PVR. Group 1: unchanged/small PVR decrease (light grey), group 2: modest PVR decrease (black), group 3: large PVR decrease (dark grey).

Discussion

Our study shows in a large group of PAH patients that compared to a small or modest PVR reduction, a large PVR decrease of more than 42% after PAH targeted medical therapies is associated with normalisation of CO, improved RV volumes and function and is a sensitive predictor of long term survival. Of interest, more patients with a large PVR decrease received upfront medical combination therapy.

In correspondence with previous studies, we found that a modest PVR reduction after current medical therapies does hardly alter RV volumes and function[8-10]. Moreover, relatively modest PVR changes do not prevent RV deterioration in a subset of patients[8]. Current medical therapies have primarily vasodilator properties[18] but we hypothesize that only a large PVR reduction will cause a sufficient decrease in pulmonary pressures that could improve RV work and attain favourable direct cardiac effects[19,20]. We found that after a large PVR decrease, a stabilized RV function was guaranteed and more than 75% of the patients showed clinically significant improvements in RVEF and SV[21,22]. Furthermore only a large PVR

decrease was associated with lowered RV volumes and mass. Similar to the results in CTPEH patients who underwent pulmonary endarterectomy[17], we found that RV wall stress was reduced in the group of patients with a large PVR decrease and occurred coincidently with improvements in NT-proBNP and RVEF; known indicators of wall stress in PAH[23].
RV function was improved but not normalized after the strong PVR decrease. However the RV might cope better with the elevated load due to the fact that circumstances have been augmented. The tissue balance between oxygen supply and demand might have been improved in the group of patients with a strong PVR decrease as illustrated by the normalization of CO. Although future studies are required to quantify the changes in cardiac oxygen handling, we found that after a large PVR decrease the two main determinants of RV oxygen consumption in PAH *i.e.* pulmonary pressure and heart rate were reduced[24]. As a consequence RV mechanical efficiency, which is proportional to the ratio of RV power output and myocardial oxygen consumption may have been enhanced. This is supported by the finding that RVEF which is tightly related to mechanical efficiency, was only improved in the group of patients with a large PVR decrease[25].

In correspondence to the study of Sitbon et al., we found that in order to achieve a survival benefit, a large PVR decrease after medical treatment is required[12]. Small-modest PVR reductions were not independently related to survival[7,26]. Similar to Sitbon et al., we found that a PVR decrease of ~30% is associated with survival but larger PVR decreases were more sensitive indicators to accurately predict long-term survival[12].
In addition, our group has found similar results with respect to the change in NT-proBNP. Mauritz et al. showed that a threshold change in NT-proBNP rather than a change on a continuous scale was associated with survival[27]. Recently, it has been demonstrated that the mean change in distance at 6MWT did not provide sufficient clinical information but a threshold change of at least 42m might reduce the risk of clinical events[28]. These studies emphasize that clinical outcome can only be improved in case of significant treatment effects.

Although we did not observe significantly different effects between the various types of single agent therapies, our results demonstrated that a large PVR reduction was more frequently obtained after upfront application of combination therapies. These findings are in line with the study of Humbert et al. who showed that upfront combination of epoprostenol and bosentan resulted in a trend towards a stronger reduction in PVR compared to epoprostenol alone[29]. Recently these results were confirmed by Kemp et al. who observed a mean decrease in PVR of ~48% after upfront combination treatment and showed that these effects were sustained

during long term follow-up[30]. Furthermore, it has been shown that addition of sildenafil to background epoprostenol resulted in a stronger PVR decrease and longer time to clinical worsening[31]. Similarly, it was found that adding selexipag to patients stable on bosentan or sildenafil resulted in an additional 30% PVR reduction[32]. The study of van Wolferen et al. showed favourable effects on RV remodelling after combination treatment but did not assess hemodynamics[33].

We performed similar analyses in order to find the relevant change in mPAP (data not shown) but did not find a significant relationship between a threshold change in mPAP and survival. Comparable results were previously described by Sitbon et al.[12]. The explanation for this finding can be twofold. First, only few patients in the present study showed such a strong improvement in mPAP and therefore the analysis did not reach enough statistical power. Second, during end stage disease mPAP decreases and therefore does not reflect a treatment benefit. Another component of RV load *i.e.* compliance was not assessed in this study. However Lankhaar et al. showed that compliance and resistance are inversely related and that this relationship remains unchanged during medical treatment[34,35]. Therefore, at increased values of PVR, compliance is low and does not provide additional information.

Clinical implications

The results of the present study imply that in order to improve RV function and achieve long term survival, the PVR has to be substantially reduced after medical treatment. Our results show that a large PVR reduction is feasible after medical treatment and might be related to upfront combination therapy.

Study limitations

Treatment regimens were not controlled and therefore potential direct cardiac effects independent of the changes in PVR were not studied. However in correspondence with the EURO-MR study, we did not find different effects on RV volumes, mass and function between current types of therapy[36]. Similarly, most patients in the present study received either ERA or prostacyclin but we did not find different survival rates between these treatment regimens which is in line with previous studies[37]. In general, we did not find an association between type of medical treatment and prognosis.

Conclusions

In comparison to a small or modest PVR reduction, only a large PVR decrease of more than 42% after current PAH targeted medical therapies was related to normalized CO, improved RV function and was a sensitive predictor of long-term survival in patients with PAH. Of high interest, a large PVR decrease was associated with upfront medical combination therapies.

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References

1. McLaughlin VV, McGoon MD. Pulmonary arterial hypertension. Circulation. 2006; 114:1417-1431.
2. Voelkel NF, Quaife RA, Leinwand LA, et al. Right ventricular function and failure: report of a National Heart, Lung, and Blood Institute working group on cellular and molecular mechanisms of right heart failure. Circulation. 2006; 114:1883-1891.
3. Pasque MK, Trulock EP, Cooper JD, et al. Single lung transplantation for pulmonary hypertension. Single institution experience in 34 patients. Circulation. 1995; 92:2252-2258.
4. Reesink HJ, Marcus JT, Tulevski II, et al. Reverse right ventricular remodeling after pulmonary endarterectomy in patients with chronic thromboembolic pulmonary hypertension: utility of magnetic resonance imaging to demonstrate restoration of the right ventricle. J Thor Cardiovasc Surg. 2007; 133:58-64.
5. Kreitner KF, Ley S, Kauczor HU, et al. Chronic thromboembolic pulmonary hypertension: pre- and postoperative assessment with breath-hold MR imaging techniques. Radiology. 2004; 232:535-543.
6. Galie N, Manes A, Negro L, et al. A meta-analysis of randomized controlled trials in pulmonary arterial hypertension. Eur Heart J. 2009; 30:394-403.
7. Savarese G, Musella F, D'Amore C, et al. Haemodynamics, exercise capacity and clinical events in pulmonary arterial hypertension. Eur Respir J. 2012; 42:414-424.
8. van de Veerdonk MC, Kind T, Marcus JT, et al. Progressive right ventricular dysfunction in patients with pulmonary arterial hypertension responding to therapy. J Am Coll Cardiol. 2011; 58:2511-2519.
9. Chin KM, Kingman M, de Lemos JA, et al. Changes in right ventricular structure and function assessed using cardiac magnetic resonance imaging in bosentan-treated patients with pulmonary arterial hypertension. Am J Cardiol. 2008; 101:1669-1672.
10. Roeleveld RJ, Vonk-Noordegraaf A, Marcus JT, et al. Effects of epoprostenol on right ventricular hypertrophy and dilatation in pulmonary hypertension. Chest. 2004; 125:572-579.
11. Provencher S, Sitbon O, Humbert M, et al. Long-term outcome with first-line bosentan therapy in idiopathic pulmonary arterial hypertension. Eur Heart J. 2006; 27:589-595.
12. Sitbon O, Humbert M, Nunes H, et al. Long-term intravenous epoprostenol infusion in primary pulmonary hypertension: prognostic factors and survival. J Am Coll Cardiol. 2002; 40:780-788.

13. Galie N, Hoeper MM, Humbert M, et al. Guidelines for the diagnosis and treatment of pulmonary hypertension: the Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS), endorsed by the International Society of Heart and Lung Transplantation (ISHLT). Eur Heart J. 2009; 30:2493-2537.
14. van Wolferen SA, Marcus JT, Boonstra A, et al. Prognostic value of right ventricular mass, volume, and function in idiopathic pulmonary arterial hypertension. Eur Heart J. 2007; 28:1250-1257.
15. ATS statement: guidelines for the six-minute walk test. Am J Respir Crit Care Med. 2002; 166:111-117.
16. Gan CT, McCann GP, Marcus JT, et al. NT-proBNP reflects right ventricular structure and function in pulmonary hypertension. Eur Respir J. 2006; 28:1190-1194.
17. Mauritz GJ, Vonk-Noordegraaf A, Kind T, et al. Pulmonary endarterectomy normalizes interventricular dyssynchrony and right ventricular systolic wall stress. J Cardiovasc Magn Reson. 2011; 14:5.
18. Handoko ML, de Man FS, Allaart CP, et al. Perspectives on novel therapeutic strategies for right heart failure in pulmonary arterial hypertension: lessons from the left heart. Eur Respir Rev. 2010; 19:72-82.
19. Westerhof N, Stergiopulos N, Noble M. Snapshots of Hemodynamics. An Aid for Clinical Research and Graduate Education. Second ed: Springer 2010.
20. Sniderman AD, Fitchett DH. Vasodilators and pulmonary arterial hypertension: the paradox of therapeutic success and clinical failure. International journal of cardiology. 1988; 20:173-181.
21. van Wolferen SA, van de Veerdonk MC, Mauritz GJ, et al. Clinically significant change in stroke volume in pulmonary hypertension. Chest. 2011; 139:1003-1009.
22. Bradlow WM, Hughes ML, Keenan NG, et al. Measuring the heart in pulmonary arterial hypertension (PAH): implications for trial study size. J Magn Reson Imaging. 2010; 31:117-124.
23. Vonk Noordegraaf A, Westerhof N. Right ventricular ejection fraction and NT-proBNP are both indicators of wall stress in pulmonary hypertension. Eur Respir J. 2007; 29:622-623.
24. Wong YY, Westerhof N, Ruiter G, et al. Systolic pulmonary artery pressure and heart rate are main determinants of oxygen consumption in the right ventricular myocardium of patients with idiopathic pulmonary arterial hypertension. Eur J Heart Fail. 2011; 13:1290-1295.
25. Wong YY, Ruiter G, Lubberink M, et al. Right ventricular failure in idiopathic pulmonary arterial hypertension is associated with inefficient myocardial oxygen utilization. Circ Heart Fail. 2011; 4:700-706.
26. Benza RL, Gomberg-Maitland M, Naeije R, et al. Prognostic factors associated with increased survival in patients with pulmonary arterial hypertension treated with subcutaneous treprostinil in randomized, placebo-controlled trials. J Heart Lung Transplant. 2011; 30:982-989.
27. Mauritz GJ, Rizopoulos D, Groepenhoff H, et al. Usefulness of serial N-terminal pro-B-type natriuretic peptide measurements for determining prognosis in patients with pulmonary arterial hypertension. Am J Cardiol. 2011; 108:1645-1650.
28. Gabler NB, French B, Strom BL, et al. Validation of 6-minute walk distance as a surrogate end point in pulmonary arterial hypertension trials. Circulation. 2012; 126:349-356.
29. Humbert M, Barst RJ, Robbins IM, et al. Combination of bosentan with epoprostenol in pulmonary arterial hypertension: BREATHE-2. Eur Respir J. 2004; 24:353-359.

30. Kemp K, Savale L, O'Callaghan DS, et al. Usefulness of first-line combination therapy with epoprostenol and bosentan in pulmonary arterial hypertension: an observational study. J Heart Lung Transplant. 2012; 31:150-158.
31. Simonneau G, Rubin LJ, Galie N, et al. Addition of sildenafil to long-term intravenous epoprostenol therapy in patients with pulmonary arterial hypertension: a randomized trial. Ann Intern Med. 2008; 149:521-530.
32. Simonneau G, Torbicki A, Hoeper MM, et al. Selexipag: an oral, selective prostacyclin receptor agonist for the treatment of pulmonary arterial hypertension. Eur Respir J. 2012; 40:874-880.
33. van Wolferen SA, Boonstra A, Marcus JT, et al. Right ventricular reverse remodelling after sildenafil in pulmonary arterial hypertension. Heart. 2006; 92:1860-1861.
34. Lankhaar JW, Westerhof N, Faes TJ, et al. Pulmonary vascular resistance and compliance stay inversely related during treatment of pulmonary hypertension. Eur Heart J. 2008; 29:1688-1695.
35. Lankhaar JW, Westerhof N, Faes TJ, et al. Quantification of right ventricular afterload in patients with and without pulmonary hypertension. Am J Physiol. 2006; 291:H1731-1737.
36. Peacock AJ, Crawley S, McLure L, et al. Changes in Right Ventricular Function Measured by Cardiac Magnetic Resonance Imaging in Patients Receiving Pulmonary Arterial Hypertension-targeted Therapy: The EURO-MR Study. Circ Cardiovasc Imaging. 2014; 7:107-114.
37. Sitbon O, McLaughlin VV, Badesch DB, et al. Survival in patients with class III idiopathic pulmonary arterial hypertension treated with first line oral bosentan compared with an historical cohort of patients started on intravenous epoprostenol. Thorax. 2005; 60:1025-1030.

Data supplement

Table 1. Comparison of hemodynamics of patients with and without CMR measurements.

Variable	Total study population (n = 123)	Patients with CMR (n = 81)	Patients without CMR (n = 42)	P-value
RHC				
mPAP, mmHg	54 ± 15	55 ± 15	51 ± 13	0.079
RAP, mmHg	9 ± 5	9 ± 5	10 ± 7	0.371
PVR, dyne·s·cm^{-5}	900 ± 439	938 ± 447	829 ± 438	0.185
PCWP, mmHg	8 ± 4	8 ± 4	8 ± 4	0.862
CO, L/min	4.5 ± 1.4	4.5 ± 1.3	4.6 ± 1.4	0.583
CI, L/min/m^2	2.4 ± 0.7	2.4 ± 0.7	2.4 ± 0.7	0.926
Heart rate	82 ± 20	85 ± 19	76 ± 19	0.052
SvO_2, %	64 ± 9	65 ± 9	63 ± 10	0.315
NT-proBNP, ng/L*	1684 ± 1626	1384 ± 1706	1918 ± 1684	0.216
6MWT				
Distance, m	428 ± 219	441 ± 238	377 ± 117	0.288

6MWT = six-minute walk testing, CI = cardiac index, CO = cardiac output, mPAP = mean pulmonary artery pressure, NT-proBNP = N-terminal pro-brain natriuretic peptide, PCWP = pulmonary capillary wedge pressure, PVR = pulmonary vascular resistance, RAP = right atrial pressure, SvO_2 = mixed venous oxygen saturation. *NT-proBNP was measured in a subgroup of n = 74 patients.

Chapter 7

Right ventricular dilatation precedes late clinical progression of initially stable patients with pulmonary arterial hypertension

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Submitted

Abstract

Introduction. Even after years of a stable response to therapy, patients with idiopathic pulmonary arterial hypertension (IPAH) may show an unexpected clinical deterioration due to progressive right ventricular (RV) failure. If the RV would already show progression during a stable condition, RV assessment might predict an ultimate deterioration. The aim of the study was to assess in five-year clinically stable IPAH patients whether initial differences or subsequent changes in RV volumes precede late clinical progression.
Methods. Included were 22 clinically stable patients, reflected by stable or improving NYHA-class II-III and exercise capacity during five years of follow-up. Twelve patients remained subsequently stable during a total follow-up of 10 years, while ten other patients showed late progression leading to death or lung-transplantation after a follow-up of 8 years. All patients underwent right heart catheterization and cardiac MRI at baseline, 1.5, 3.5, 6.5 and, when still alive, 10 years of follow-up.
Results. Baseline hemodynamics were comparable in both groups and remained on average unchanged during the entire follow-up period. Baseline RV end-systolic volume (RVESV) was higher and RV ejection fraction (RVEF) was lower in late-progressive patients. Late-progressive patients demonstrated a gradually increased RV end-diastolic volume (RVEDV) and RVESV and, a declined RVEF whereas long-term stable patients did not show any RV changes.
Conclusions. In five-year stable IPAH patients, subsequent disease progression is preceded by changes in RV volumes. Our results implicate that monitoring of RV volumes allows the anticipation to clinical worsening, even at the time of apparent clinical stability.

Introduction

In patients with pulmonary arterial hypertension (PAH), increased pulmonary vascular resistance (PVR) and pulmonary artery pressure ultimately result in right ventricular (RV) failure and death[1,2]. Various effective medical therapies have become available allowing prolonged clinical stability and survival[3-7]. Although a small group of patients may survive more than five years after diagnosis, overall long-term mortality rates are high[8]. Much is known about predictors of short-term survival[5,6] but predictors of ultimate clinical deterioration in patients with an initially favorable treatment response have not been identified. A clinically stable condition, defined as a stable or improving New York Heart Association (NYHA) functional class II-III and six-minute walking test (6MWT)[9], was not associated with better long-term survival[3,10-12]. Even patients who are in a seemingly sustained stable clinical condition may unexpectedly show rapid clinical deterioration due to progressive RV failure, which is associated with high mortality rates[13-15]. Current medical therapies may successfully improve 6MWT and cardiac output (CO), but do not necessarily slow RV failure progression[16-18]. If the RV would already show progressive adverse remodeling during a stable condition, assessment of RV remodeling parameters might predict an ultimate disease progression. Therefore, the aim of the present study was to assess whether initial differences or subsequent changes in RV volumes precede an ultimate clinical deterioration in idiopathic PAH (IPAH) and heritable PAH (HPAH) patients with a proven five-year stable clinical condition.

Methods

Patients

At the VU University Medical Center, patients were diagnosed as having PAH according to the guidelines, including a right heart catheterization (RHC)[19]. The present study was a retrospective analysis of an ongoing prospective study to assess the clinical value of cardiac magnetic resonance imaging (CMR) in PAH. The process of selection for the present study was made unaware of RHC and CMR results. Inclusion criteria were: (1) diagnosis of IPAH or HPAH, (2) age ≥18 years, (3) a proven clinical stable condition during the first five years of follow-up, defined as a stable NYHA class II-III and no reduction in the 6MWT ≥15%[9], (4) CMR, RHC, 6MWT, NYHA class at baseline and at regular follow-up intervals, (5) a total follow-up period of ten years. IPAH/HPAH patients diagnosed between February 1999 and February 2004 were selected for the study. In this period, 48 out of 58 patients were selected and followed until February

2013. Exclusion reasons: no regular CMR due to logistic reasons (n = 4) or claustrophobia (n = 1), follow-up in another hospital (n = 2), development of LV failure (n =1), a positive vasodilator challenge (n = 2)[19]. Twenty-four out of the 48 patients died and two patients showed early clinical disease progression within five years of follow-up (*i.e*. deterioration into NYHA IV and >15% reduction in the 6MWT). The other twenty-two patients fulfilled the inclusion criteria and were enrolled (Figure 1). The local Medical Ethics Committee approved the study without requirement of a consent statement because the study does not fall within the scope of the Medical Research Involving Human Subjects Act (WMO) (approval number 2012288).

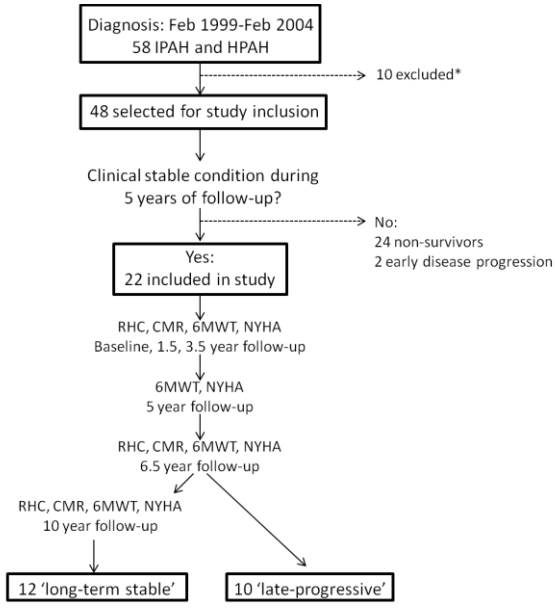

Figure 1. Study profile.
*Excluded due to: no regular CMR because of logistical reasons (n = 4) or claustrophobia (n = 1), follow-up in another hospital (n = 2), development of left ventricular failure (n = 1), a positive vasodilator challenge (n = 2).

Twelve out of the 22 patients remained in a clinically stable condition during ten years (IQR: 10-11 years) of follow-up and were considered to be “long-term stable” patients. The other ten patients showed late clinical disease progression, defined as progression into NYHA IV and a reduction in the 6MWT >15%[9] after five years of initial clinical stability. All ten patients subsequently died due to cardiopulmonary causes (n = 6) or underwent lung-transplantation (n = 4) by a median of eight years (IQR: 7-10 years) after the diagnosis and were considered “late-progressive” patients. All patients underwent a complete assessment consisting of RHC, CMR, 6MWT, and NYHA class at baseline and after 1.5 ± 0.4, 3.5 ± 0.9, 6.5 ± 1.0 years and, if still alive, after 10.0 ± 1.2 years of follow-up. Additional 6MWT

and NYHA class assessments were performed after 5.0 ± 0.4 years of follow-up. Furthermore, the number and reasons for acute hospitalizations during follow-up were recorded.
Application of PAH targeted medical therapies was performed in line with the guidelines[19] and according to the availability in the Netherlands. Before 2002, all NYHA class III-IV patients were initiated on prostacyclins. After 2002, NYHA II-III patients were treated with oral medical therapy consisting of endothelin receptor antagonists and/or phosphodiesterase-5 inhibitors, either as single agent therapy or as combination, while NYHA IV patients received prostacyclins with or without additional oral medical therapies. All patients received anticoagulation and diuretics.

Right heart catheterization
Hemodynamic assessment was performed with a 7F balloon tipped, flow directed Swan-Ganz catheter as described previously[20].

Cardiac magnetic resonance imaging
CMR was performed on a Siemens 1.5T Sonata or Avanto scanner. Data acquisition and post-processing were performed according to our routine protocol[20]. Briefly, during post-processing using dedicated software, a blinded observer assessed the left ventricular (LV) and RV volumes, mass and function by manual delineation of the endocardial and epicardial contours on short-axis images. Disc summation was performed according to Simpsons rule. Stroke volume (SV) was calculated as end-diastolic volume (EDV) – end-systolic volume (ESV). Ejection fraction was calculated as (SV/EDV)*100%. Ventricular relative wall thickness was calculated as ventricular mass divided by EDV[21]. Ventricular volumes and masses were indexed to body surface area (BSA).

Six-minute walking test
The 6MWT was performed according to American Thoracic Society guidelines[22].

Statistical analysis
Data are presented as mean ± SD, unless stated otherwise. Unpaired Student *t*-tests or Mann-Whitney tests were used to compare continuous variables and loglinear analysis was performed to compare categorical variables between the two groups at baseline. Linear Mixed Model analysis was applied to assess the differences between the groups over time. Residuals were normally distributed for every tested parameter. Model fit was evaluated and when necessary, random effects of time variables were corrected for intercepts and/or slopes. A sensitivity analysis was performed to test whether missing values influenced the results. Data were analyzed

using SAS (version 9.2 Inc, Cary, North-Carolina) and SPSS (version 20.0 Inc, Chicago, Illinois). P-values <0.05 were considered significant.

Results

Patient demographics

Table 1 shows similar demographics in the long-term stable and late-progressive patients. Baseline 6MWT was comparable in both groups (p = 0.321) and most patients were in NYHA class III. The 6MWT remained unchanged in both groups during follow-up, and both groups developed a stable NYHA class II-III during the application of medical treatment that persisted during five years of follow-up. After 6.5 years of follow-up, five late-progressive patients showed deterioration in NYHA class from II-III to IV (Figure 2).

Table 1. Baseline characteristics.

Variable	Long-term stable (n = 12)	Late-progressive (n = 10)	P-value
Diagnosis, n			0.781
IPAH	9	8	
HPAH	3	2	
Age, years	44 ± 14	37 ± 11	0.226
Gender, female, n	11	8	0.427
NYHA, II / III / IV, n	2 / 7 / 3	2 / 6 / 2	0.991
6MWT, distance, m	370 ± 151	439 ± 124	0.321

6MWT = six-minute walking test, HPAH = hereditary pulmonary arterial hypertension, IPAH = idiopathic pulmonary arterial hypertension, NYHA = New York Heart Association.

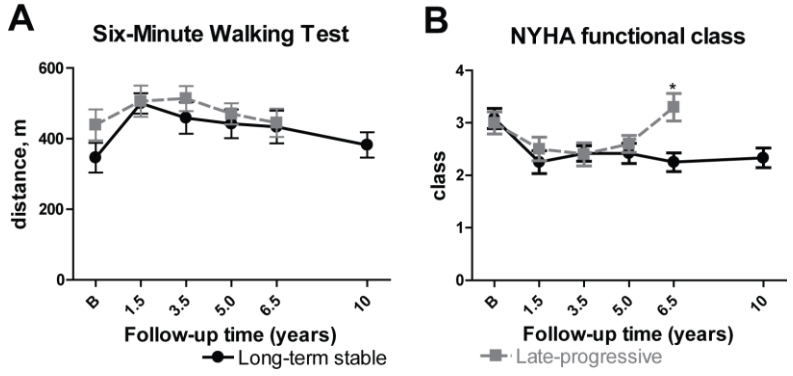

Figure 2.

(A) Six-minute walking test was comparable between the long-term stable (black) and late-progressive patients (grey). (**B**) NYHA functional class improved in both groups after the initiation of medical therapies and persisted during five years of follow-up. After 6.5 years of follow-up, NYHA class deteriorated in late-progressive patients. Data are presented as mean ± SEM. $^{*}p < 0.05$ between groups.

During the initial five years of clinical stability, no acute hospitalizations were necessary for RV failure progression and no intravenous diuretics were administered. During this time of follow-up, five long-term stable and three late-progressive patients received mono-therapy, five stable and six progressive patients switched from mono to dual-therapy and one stable patient switched from mono to dual to triple-therapy because of lack of clinical improvement. Furthermore, one long-term stable patient received dual-therapy and one late-progressive patient switched from dual to triple-therapy. Overall, treatment regimens were balanced between groups ($p = 0.992$).

Baseline characteristics

Baseline hemodynamics were similar in the two groups (Table 2). RV end-diastolic volume (RVEDV), RV mass and RV wall thickness were comparable between groups. Late-progressive patients showed higher RV end-systolic volume (RVESV) and lower RV ejection fraction (RVEF) compared to long-term stable patients (Table 2). No differences in LV parameters were observed between both groups.

The 22 patients included in this study showed similar baseline demographics, NYHA class, 6MWT and hemodynamics compared to the 10 patients who survived five years of follow-up but were excluded from the study selection (all $p > 0.07$) (not shown).

Table 2. Baseline hemodynamics and cardiac measures.

Variable	Long-term stable	Late-progressive	P-value
RHC			
mPAP, mmHg	53 ± 15	59 ± 14	0.327
PVR, dyne·s·cm^{-5}	920 ± 470	1140 ± 372	0.244
RAP, mmHg	8 ± 3	8 ± 4	0.891
PAWP, mmHg	6 ± 3	8 ± 6	0.411
CO, L·min^{-1}	4.6 ± 1.4	3.8 ± 0.9	0.090
HR, bpm	79 ± 14	75 ± 15	0.657
SvO_2, %	65 ± 7	62 ± 5	0.281
CMR			
RV remodeling			
RVEDV, ml·m^{-2}	73 ± 19	83 ± 10	0.166
RVESV, ml·m^{-2}	45 ± 11	59 ± 12	0.015
RV mass, g·m^{-2}	57 ± 13	58 ± 16	0.782
RV relative wall thickness	0.81 ± 0.23	0.70 ± 0.14	0.216
RV function			
RVEF, %	37 ± 9	29 ± 8	0.038
SV, ml·m^{-2}	28 ± 11	24 ± 5	0.352
LV remodeling			
LVEDV, ml·m^{-2}	42 ± 17	44 ± 10	0.824
LVESV, ml·m^{-2}	14 ± 7	19 ± 8	0.138

LV mass, $g \cdot m^{-2}$	55 ± 14	58 ± 10	0.627
LV relative wall thickness	1.42 ± 0.48	1.39 ± 0.44	0.861
LV function			
LVEF, %	66 ± 7	58 ± 13	0.079

CMR = cardiac magnetic resonance imaging, CO = cardiac output, HR = heart rate, LV = left ventricular, LVEDV = left ventricular end-diastolic volume, LVEF = left ventricular ejection fraction, LVESV = left ventricular end-systolic volume, mPAP = mean pulmonary artery pressure, PAWP = pulmonary arterial wedge pressure, PVR = pulmonary vascular resistance, RAP = right atrial pressure, RHC = right heart catheterization, RV = right ventricular, RVEDV = right ventricular end-diastolic volume, RVEF = right ventricular ejection fraction, RVESV = right ventricular end-systolic volume, SV = stroke volume, SvO_2 = mixed venous oxygen saturation.

Changes in hemodynamics during follow-up

Mean pulmonary artery pressure (mPAP) remained unchanged in both groups during follow-up (Figure 3). Although absolute values of pulmonary vascular resistance (PVR) and cardiac output (CO) were different between groups at 1.5 years follow-up (both $p < 0.05$), the changes in both parameters during 1.5 years of follow-up were comparable (PVR: p-interaction = 0.547, CO: p-interaction = 0.821). Furthermore, absolute values of PVR and CO were similar in both groups at 3.5 and 6.5 years follow-up. Long-term stable patients showed a stronger decrease in right atrial pressure (RAP) compared to late-progressive patients during the first 3.5 years of follow-up (p-interaction = 0.003) but the change in RAP was not different between both groups during the overall follow-up period of 6.5 years (p-interaction = 0.274). In both groups, pulmonary arterial wedge pressure, heart rate and mixed venous oxygen saturation remained on average comparable and unchanged during the total follow-up period (not shown).

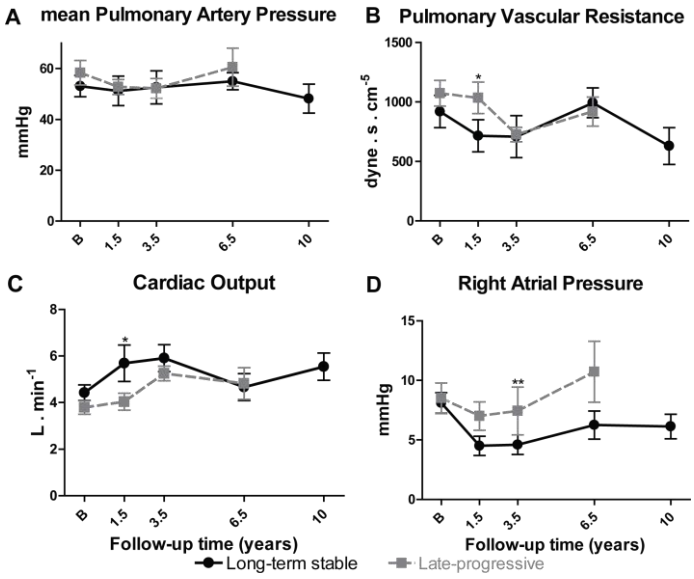

Figure 3.

(A) mean pulmonary artery pressure was comparable between the long-term stable (black) and late-progressive patients (grey) during follow-up. **(B)** Pulmonary vascular resistance, **(C)** cardiac output and **(D)** right atrial pressure showed a temporary difference between the two groups during the first years of follow-up but became equal during the subsequent follow-up intervals. Data are presented as mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$ between groups.

Changes in cardiac wall thickness, volumes and function during follow-up

Figure 4 demonstrates that during 6.5 years of follow-up, RVEDV and RVESV were continuously increased in the progressive patients (within group both, $p < 0.001$) but remained unchanged in the stable patients (within group both, $p > 0.597$) (both, p-interaction < 0.006). No differences were observed between groups in the relative RV wall thickness over time.

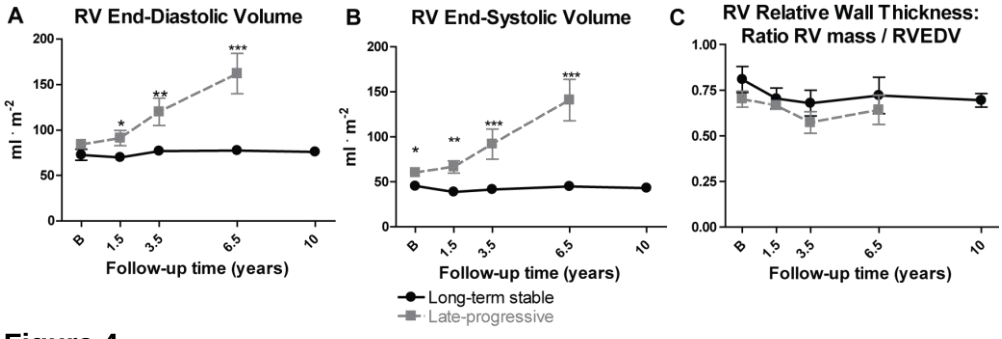

Figure 4.

(A) Right ventricular (RV) end-diastolic volume and **(B)** RV end-systolic volume increased progressively in the late-progressive patients (grey) but remained unchanged in the long-term stable patients (black) (both, p-interaction < 0.01). **(C)** RV relative wall thickness was

comparable in both groups during follow-up. Data are presented as mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ between groups.

SV remained initially unchanged in both groups but became lower in late-progressive than in long-term stable patients after 6.5 years of follow-up (Figure 5). Late-progressive patients showed a decline in RVEF during 6.5 years of follow-up (p = 0.008), which was different compared to the long-term stable patients who showed an initial increase during the first 3.5 years of follow-up and a subsequently stable RVEF during ten years of follow-up (p-interaction = 0.006).

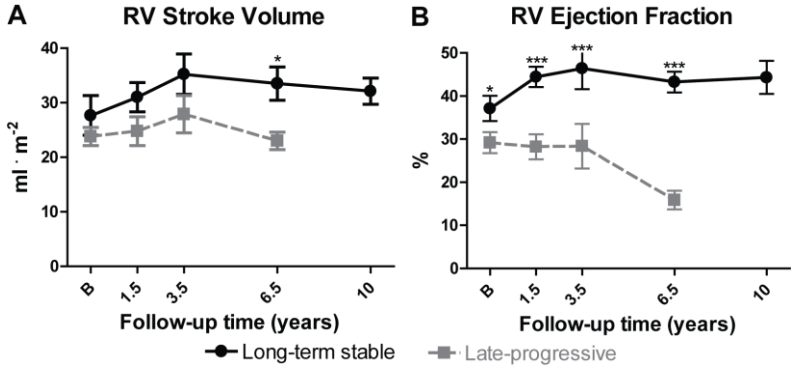

Figure 5.
(A) Stroke Volume was comparable in the long-term stable (black) and late-progressive groups (grey) during the first 3.5 years of follow-up and became lower in the late-progressive patients after 6.5 years of follow-up. **(B)** RV ejection fraction (RVEF) was lower at baseline and showed a gradual decline in the late-progressive patients during 6.5 years of follow-up whereas RVEF increased in long-term stable patients during the first 3.5 years of follow-up that persisted throughout ten years of follow-up (p-interaction = 0.006). Data are presented as mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ between groups.

LV end-diastolic volume (LVEDV) and the relative LV wall thickness remained comparable in both groups during follow-up (Figure 6). LV end-systolic volume (LVESV) increased in the late-progressive patients during 6.5 years of follow-up (p = 0.016) and remained unchanged in the long-term stable patients (p = 0.298). However, the changes in LVESV were not different in both groups during the overall follow-up period (p-interaction = 0.253). LVEF remained stable over time in the long-term stable patients and decreased in the late-progressive patients during 6.5 years of follow-up (within group, p = 0.042). Although the absolute values of LVEF were lower in the late-progressive patients than in the long-term stable patients at all time points, the changes in LVEF were not significantly different between groups (p-interaction = 0.196).

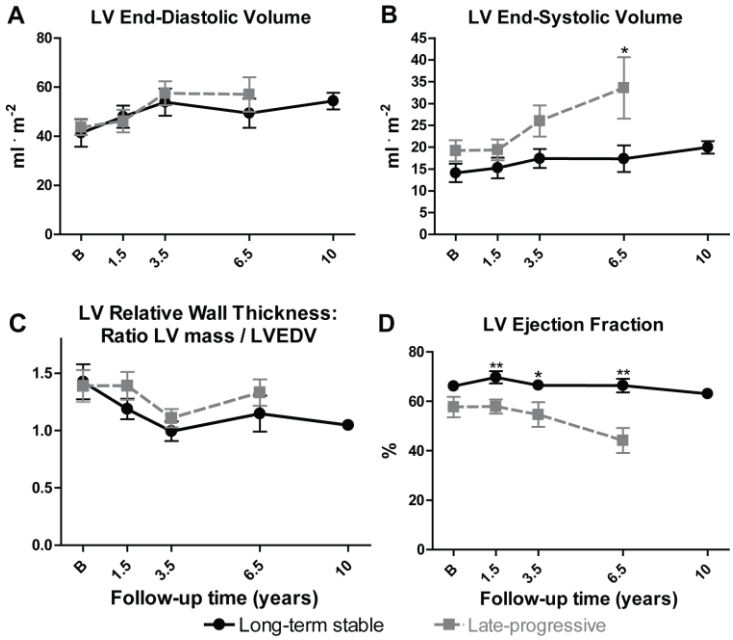

Figure 6.
(A) Left ventricular (LV) end-diastolic volume remained low and unchanged in the long-term stable (black) and late-progressive patients (grey). **(B)** LV end-systolic volume increased in the late-progressive patients during the last years of follow-up and remained unchanged in the long-term stable patients. **(C)** LV relative wall thickness was similar in the two groups during follow-up. **(D)** LV ejection fraction was higher in the long-term stable patients compared to the late-progressive patients at every follow-up interval. Data are presented as mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$ between groups.

Discussion

In a unique cohort of long-term surviving PAH patients, we show that RV remodeling can be progressive even in patients who are seemingly clinical stable during 5-10 years of follow-up. Moreover, we show that an ultimate disease progression is preceded by changes in RV volumes and RVEF but not by changes in NYHA class, exercise capacity or hemodynamics.

This study provides the first phenotypic descriptions of PAH patients who showed an initially favorable treatment response and survived for at least five years after diagnosis. Corresponding to previous results, we showed that improvements in CO and RAP during the first years of follow-up were associated with survival[23]. However in line with former studies, we show that patients with an initially favorable treatment response show no further hemodynamic changes during long-term follow-up, regardless of the final outcome[24,25]. These findings might suggest that long-term treatment with

PAH specific vasodilator therapies might halt some of the progressive pulmonary vascular remodeling and may account for the improved outcomes in the current treatment era[7,25].

Corresponding to former studies[16,20,26], we show that increased RV volumes during follow-up provide strong prognostic information. The most important finding of the present study was that increasing RV volumes during a clinically stable period preceded ultimate clinical deterioration. The absolute differences in RV volumes between long-term stable and late-progressive PAH patients gradually increased during follow-up which demonstrates their incremental prognostic relevance.
The importance of progressive cardiac remodeling during clinically stable disease is supported by previous findings. In studies focusing on LV failure, increased LV volumes in asymptomatic patients were independent predictors of the development of symptomatic heart failure and mortality[27-29]. Other LV studies have found that LV remodeling might progress despite the application of medical therapies aiming to preserve CO and clinical stability[18,30]. Similarly, it has been demonstrated that current PAH vasodilator medical therapies significantly improve CO but might have limited effects on RV adaptation and remodeling[17].
We observed a lower baseline RVEF and a gradual RVEF decline in the late-progressive patients during follow-up but not in the long-term stable patients. These findings correspond to previous studies showing that a low and decreasing RVEF were strong prognostic predictors[16,31,32]. During long-term follow-up, the changes in RVEF did not show incremental prognostic relevance and did not provide additional information to measures of RV volumetric remodeling.

Assessment of RV remodeling is not only of prognostic importance but is also of physiological interest. It has been proposed that an increase in EDV might be a compensatory mechanism to normalize SV in the setting of contractile dysfunction[33,34]. However an increase in RVEDV can only be beneficial when RVESV remains unchanged but this was not the case in the late-progressive PAH patients. An elevated RVESV is a poor physiological sign since it is associated with a right shift of the pressure-volume loop and lower RV contractility. According to the Law of Laplace, elevated RV volumes lead to increased RV wall stress. In addition, RV wall thickness remained unchanged during follow-up and was probably insufficient to lower the wall stress[35].
In the patients with a long-term stable condition, there was no progressive RV remodeling despite a similar elevation in pulmonary pressures as observed in the late-progressive patients. A possible explanation could be that these patients showed better intrinsic RV adaptation mechanism to the increased afterload with preserved RV-arterial coupling[36] or other factors

could play a role such as metabolism, neurohormones, inflammation, ischemia and genetics[36,37].

Compared to LV reference values[38] and in line with previous studies in PAH, LV filling dimensions in the two currently studied PAH patient groups were low[20,39-41]. Impaired LV filling might be explained by direct ventricular interaction due to interventricular dyssynchrony and leftward septum bowing[41-43], or by a low RV output resulting in LV underfilling. Strikingly, at every measured follow-up time point, LV systolic function was lower in the progressive patients compared to the stable patients. This might be a result of reduced LV filling but previous studies have also demonstrated by LV strain imaging that LV contractility and LV torsion were impaired in PAH patients[39,41]. Furthermore, according to the LV pressure-volume relationship, an increased LVESV is associated with impaired LV contractility.

Clinical implications

We show that in long-term PAH survivors, a clinically stable profile and preserved CO may mask RV failure progression, and that changes in RV volumes may be sensitive parameters to predict an ultimate deterioration, even at the time of clinical stability. Our results implicate that evaluation of RV volumes and RVEF are important in order to detect early heart failure development and to permit timely intervention. Our results raise the question whether prognosis can be improved by a goal-oriented strategy using RV rather than clinical parameters as treatment goal. The findings of an ultimate similarity in PVR in long-term stable and late-progressive patients also begs the question whether late disease progression could have been prevented by more aggressive vasodilator treatment or rather by a treatment specifically improving RV adaptation.

In contrast to patients who died soon after the diagnosis[5,6], the late-progressive patients did not show a severely disturbed hemodynamic profile, not even in the last measurement before death. Because hemodynamic progression can be very rapid in end-stage disease, it is likely that had RHC been performed in the days-weeks prior to death, results would have been much worse. In contrast, the considerable RV dilatation observed in the late-progressive patients is infrequently encountered in patients with shorter survival periods[16,20,26]. This suggests that extensive RV remodeling takes years to develop. Importantly, these observations imply that the long-term follow-up results of the present study cannot be extrapolated to patients with a more severe hemodynamic profile shortly after the initial diagnosis.

Limitations

Although we included a small patient population, clear differences between both study groups were found with high levels of statistical significance. Previously, Addetia et al. demonstrated that in order to detect specific changes in RV volumes and function by CMR in PAH patients, a small sample size is sufficient in order to achieve adequate statistical power[44]. Death and lung transplantation together were used as composite endpoint to define late-progression. When only non-survivors were included in the analysis, we found similar results with more pronounced absolute differences between the long-term stable and late-progressive groups (not shown).

During the first years of follow-up, measurements of N-terminal pro-brain natriuretic peptide (NT-proBNP) were unavailable in the Netherlands and therefore not included. Former studies have shown that although NT-proBNP contains prognostic information, a sufficient increase is required in order to be of clinical relevance. Further studies are required to test the sensitivity of early changes in NT-proBNP to detect ultimate disease progression[11,45].

Conclusions

RV volumes and RVEF can deteriorate in apparently stable IPAH/HPAH patients and changes in these parameters precede ultimate disease progression and mortality. Our results imply that monitoring of RV remodeling is essential in order to detect early development of heart failure and to permit timely intervention, even at the time of clinical stability.

References

1. McLaughlin VV, McGoon MD. Pulmonary arterial hypertension. Circulation. 2006; 114:1417-1431.
2. Voelkel NF, Quaife RA, Leinwand LA, et al. Right ventricular function and failure: report of a National Heart, Lung, and Blood Institute working group on cellular and molecular mechanisms of right heart failure. Circulation. 2006; 114:1883-1891.
3. Savarese G, Paolillo S, Costanzo P, et al. Do changes of 6-minute walk distance predict clinical events in patients with pulmonary arterial hypertension? A meta-analysis of 22 randomized trials. J Am Coll Cardiol. 2012; 60:1192-1201.
4. Benza RL, Miller DP, Barst RJ, et al. An evaluation of long-term survival from time of diagnosis in pulmonary arterial hypertension from the REVEAL Registry. Chest. 2012; 142:448-456.
5. Humbert M, Sitbon O, Chaouat A, et al. Survival in patients with idiopathic, familial, and anorexigen-associated pulmonary arterial hypertension in the modern management era. Circulation. 2010; 122:156-163.
6. Benza RL, Miller DP, Gomberg-Maitland M, et al. Predicting survival in pulmonary arterial hypertension: insights from the Registry to Evaluate Early and Long-Term

Pulmonary Arterial Hypertension Disease Management (REVEAL). Circulation. 2010; 122:164-172.
7. Galie N, Manes A, Negro L, et al. A meta-analysis of randomized controlled trials in pulmonary arterial hypertension. Eur Heart J. 2009; 30:394-403.
8. Gomberg-Maitland M, Dufton C, Oudiz RJ, et al. Compelling evidence of long-term outcomes in pulmonary arterial hypertension? A clinical perspective. J Am Coll Cardiol. 2011; 57:1053-1061.
9. McLaughlin VV, Archer SL, Badesch DB, et al. ACCF/AHA 2009 expert consensus document on pulmonary hypertension a report of the American College of Cardiology Foundation Task Force on Expert Consensus Documents and the American Heart Association developed in collaboration with the American College of Chest Physicians; American Thoracic Society, Inc.; and the Pulmonary Hypertension Association. J Am Coll Cardiol. 2009; 53:1573-1619.
10. Souza R, R C, Delcroix M, Galiè N, et al. Association Between WHO Functional Class and Long-term Prognosis in Patients With Pulmonary Arterial Hypertension: Data From SERAPHIN, A Randomized Controlled Study of Macitentan. Chest. 2013; 144(Abstr).
11. Fritz JS, Blair C, Oudiz RJ, et al. Baseline and follow-up 6-min walk distance and brain natriuretic peptide predict 2-year mortality in pulmonary arterial hypertension. Chest. 2013; 143:315-323.
12. Sitbon O, Humbert M, Nunes H, et al. Long-term intravenous epoprostenol infusion in primary pulmonary hypertension: prognostic factors and survival. J Am Coll Cardiol. 2002; 40:780-788.
13. Haddad F, Peterson T, Fuh E, et al. Characteristics and outcome after hospitalization for acute right heart failure in patients with pulmonary arterial hypertension. Circ Heart Fail. 2011; 4:692-699.
14. Sztrymf B, Souza R, Bertoletti L, et al. Prognostic factors of acute heart failure in patients with pulmonary arterial hypertension. Eur Respir J. 2010; 35:1286-1293.
15. Greyson CR. Pathophysiology of right ventricular failure. Crit Care Med. 2008; 36: S57-65.
16. van de Veerdonk MC, Kind T, Marcus JT, et al. Progressive right ventricular dysfunction in patients with pulmonary arterial hypertension responding to therapy. J Am Coll Cardiol. 2011; 58:2511-2519.
17. Handoko ML, de Man FS, Allaart CP, et al. Perspectives on novel therapeutic strategies for right heart failure in pulmonary arterial hypertension: lessons from the left heart. Eur Respir Rev. 2010; 19:72-82.
18. Cohn JN, Ferrari R, Sharpe N. Cardiac remodeling--concepts and clinical implications: a consensus paper from an international forum on cardiac remodeling. Behalf of an International Forum on Cardiac Remodeling. J Am Coll Cardiol. 2000; 35:569-582.
19. Galie N, Hoeper MM, Humbert M, et al. Guidelines for the diagnosis and treatment of pulmonary hypertension: the Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS), endorsed by the International Society of Heart and Lung Transplantation (ISHLT). Eur Heart J. 2009; 30:2493-2537.
20. van Wolferen SA, Marcus JT, Boonstra A, et al. Prognostic value of right ventricular mass, volume, and function in idiopathic pulmonary arterial hypertension. Eur Heart J. 2007; 28:1250-1257.
21. Gaasch WH, Zile MR. Left ventricular structural remodeling in health and disease: with special emphasis on volume, mass, and geometry. J Am Coll Cardiol. 2011; 58:1733-1740.
22. ATS statement: guidelines for the six-minute walk test. Am J Respir Crit Care Med. 2002; 166:111-117.

23. Savarese G, Musella F, D'Amore C, et al. Haemodynamics, exercise capacity and clinical events in pulmonary arterial hypertension. Eur Respir J. 2013; 42:414-424.
24. Rich S, Pogoriler J, Husain AN, et al. Long-term effects of epoprostenol on the pulmonary vasculature in idiopathic pulmonary arterial hypertension. Chest. 2010; 138:1234-1239.
25. Mauritz GJ, Kind T, Marcus JT, et al. Progressive changes in right ventricular geometric shortening and long-term survival in pulmonary arterial hypertension. Chest. 2012; 141:935-943.
26. Vasan RS, Larson MG, Benjamin EJ, et al. Left ventricular dilatation and the risk of congestive heart failure in people without myocardial infarction. New Engl J Med. 1997; 336:1350-1355.
27. Migrino RQ, Young JB, Ellis SG, et al. End-systolic volume index at 90 to 180 minutes into reperfusion therapy for acute myocardial infarction is a strong predictor of early and late mortality. The Global Utilization of Streptokinase and t-PA for Occluded Coronary Arteries (GUSTO)-I Angiographic Investigators. Circulation. 1997; 96:116-121.
28. Lauer MS, Evans JC, Levy D. Prognostic implications of subclinical left ventricular dilatation and systolic dysfunction in men free of overt cardiovascular disease (the Framingham Heart Study). Am J Cardiol. 1992; 70:1180-1184.
29. Packer M. Long-term strategies in the management of heart failure: looking beyond ventricular function and symptoms. Am J Cardiol. 1992; 69:G150-154.
30. Nickel N, Golpon H, Greer M, et al. The prognostic impact of follow-up assessments in patients with idiopathic pulmonary arterial hypertension. Eur Respir J. 2013; 39:589-596.
31. Kawut SM, Al-Naamani N, Agerstrand C, et al. Determinants of right ventricular ejection fraction in pulmonary arterial hypertension. Chest. 2009; 135:752-759.
32. Zafrir N, Zingerman B, Solodky A, et al. Use of noninvasive tools in primary pulmonary hypertension to assess the correlation of right ventricular function with functional capacity and to predict outcome. Int J Cardiovasc Imaging. 2007; 23:209-215.
33. MacIver DH, Dayer MJ. An alternative approach to understanding the pathophysiological mechanisms of chronic heart failure. Int J Cardiol. 2011; 154:102-110.
34. Gaudron P, Eilles C, Ertl G, et al. Compensatory and noncompensatory left ventricular dilatation after myocardial infarction: time course and hemodynamic consequences at rest and during exercise. Am Heart J. 1992; 123:377-385.
35. Simon MA, Deible C, Mathier MA, et al. Phenotyping the right ventricle in patients with pulmonary hypertension. Clin Transl Sci. 2009; 2:294-299.
36. Rain S, Handoko ML, Trip P, et al. Right ventricular diastolic impairment in patients with pulmonary arterial hypertension. Circulation. 2013; 128:2016-2025.
37. Voelkel NF, Gomez-Arroyo J, Abbate A, et al. Mechanisms of right heart failure-A work in progress and a plea for failure prevention. Pulm Circ. 2013; 3:137-143.
38. Natori S, Lai S, Finn JP, et al. Cardiovascular function in multi-ethnic study of atherosclerosis: normal values by age, sex, and ethnicity. AJR Am J Roentgenol. 2006; 186:S357-365.
39. Hardegree EL, Sachdev A, Fenstad ER, et al. Impaired left ventricular mechanics in pulmonary arterial hypertension: identification of a cohort at high risk. Circ Heart Fail. 2013; 6:748-755.
40. Tonelli AR, Plana JC, Heresi GA, et al. Prevalence and prognostic value of left ventricular diastolic dysfunction in idiopathic and heritable pulmonary arterial hypertension. Chest. 2012; 141:1457-1465.
41. Puwanant S, Park M, Popovic ZB, et al. Ventricular geometry, strain, and rotational mechanics in pulmonary hypertension. Circulation. 2010; 121:259-266.

42. Marcus JT, Gan CT, Zwanenburg JJ, et al. Interventricular mechanical asynchrony in pulmonary arterial hypertension: left-to-right delay in peak shortening is related to right ventricular overload and left ventricular underfilling. J Am Coll Cardiol. 2008; 51:750-757.
43. Gan C, Lankhaar JW, Marcus JT, et al. Impaired left ventricular filling due to right-to-left ventricular interaction in patients with pulmonary arterial hypertension. Am J Physiol. 2006; 290:H1528-1533.
44. Addetia K, Bhave NM, Tabit CE, et al. Sample Size and Cost Analysis for Pulmonary Arterial Hypertension Drug Trials Using Various Imaging Modalities to Assess Right Ventricular Size and Function Endpoints. Circ Cardiovasc Imaging. 2014; 7:115-124.
45. Mauritz GJ, Rizopoulos D, Groepenhoff H, et al. Usefulness of serial N-terminal pro-B-type natriuretic peptide measurements for determining prognosis in patients with pulmonary arterial hypertension. Am J Cardiol. 2012; 108:1645-1650.

Chapter 8

Cardiac MRI and PET scanning in right ventricular failure

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Introduction

Right ventricular (RV) failure may be defined as a complex clinical syndrome that can result from any structural or functional cardiac disorder that impairs the ability of the right heart to fill or eject appropriately[1]. In pulmonary arterial hypertension (PAH); a progressive pulmonary vascular disease resulting in chronic pressure overload, patients die due to the consequences of RV failure[2,3]. In the past, the RV has been largely understudied and the pathophysiology of RV failure has remained incompletely understood. It has become clear that increased pulmonary pressures are insufficient to explain the development of RV failure[3-7]. Furthermore, it is intriguing that end stage RV failure can be completely reversed as is observed after lung transplantation[8]. Recent advanced, non-invasive imaging techniques have been developed that can directly study RV myocardial tissue processes and may increase the insights into the factors contributing to the development of chronic RV failure. Cardiac magnetic resonance imaging (MRI) and positron emission tomography (PET) allow *in vivo* assessment of RV morphology, function, tissue characterization, perfusion and blood flow, metabolism, neurohormonal activation and other molecular processes. These techniques may help to identify factors which determine risk and prognosis and may allow assessment of therapeutic effects on RV function in PAH patients.

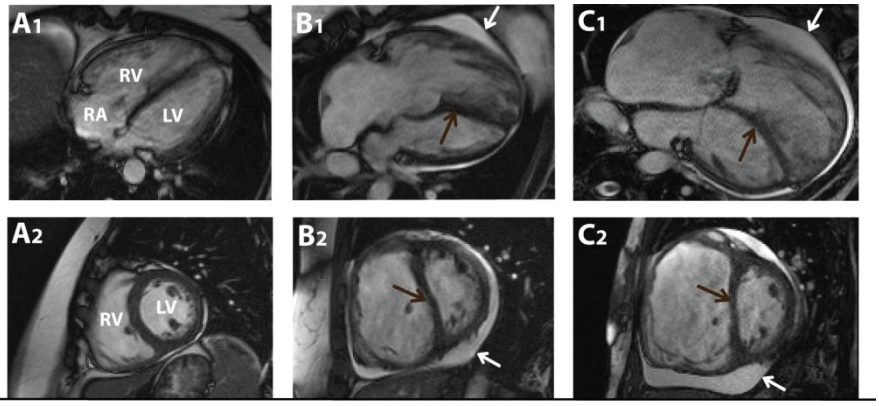
Cardiac MRI provides high resolution imaging, does not need geometric assumptions and lacks ionizing radiation. It has been shown that two-dimensional and three-dimensional cardiac MRI measures are highly accurate[9,10] and reproducible[11]. MRI has become the gold standard to noninvasively measure RV mass, volumes and function[12] and has the ability to image perfusion and cellular and molecular tissue characteristics. Although the role of MRI in establishing the diagnosis of PAH is limited, it allows complete RV phenotyping and is especially valuable in the monitoring of therapeutic effects. However because MRI is relative expensive and requires operator expertise, this methodology is not widely used. Furthermore, scan times are quite long end ferromagnetic objects such as pacemakers and defibrillators are incompatible. Nevertheless, its (clinical) relevance has been clearly established[12]. PET is a rapidly developing technique allowing perfusion imaging, assessment of molecular and physiologic processes and evaluation of the cardiac nervous system. Limitations of PET are it's costs and labor intensity. Furthermore, due to the relatively low spatial resolution, current clinical PET-scanners are combined with computed tomography (CT). In PAH, PET has only been recently used and broader application can be expected in the near future. In addition, rapid developments in hybrid scanners consisting of PET and MRI are taking place in order to allow integrated cardiac assessment.

Here we provide an overview of current and future applications of cardiac MRI and PET for assessment of chronic RV failure and review potential contributing factors that could help considerably to understand the pathophysiology of RV failure in the setting of chronic pressure overload. Furthermore, we demonstrate the potential clinical relevance of these imaging techniques in patients with PAH.

RV structural and functional imaging

- *RV remodeling and wall stress*

Figure 1 demonstrates cardiac MRI cine images obtained over time from a 35 year old, female patient who was diagnosed as having idiopathic PAH, New York Heart Association (NYHA) functional class II. Mean pulmonary artery pressure was 45 mmHg and cardiac output (CO) was 4.1 L/min. At the time of diagnosis, she showed cardiac compensation with a maintained crescent shape of the RV, RV hypertrophy with an increased RV mass, relatively preserved cardiac dimensions (but a small increase in RV volume), no pericardial effusion and moderate RV and left ventricular (LV) function (Figure 1A). Despite unchanged pulmonary pressures after 7 years of follow-up, she developed symptoms of progressive RV failure, even though the CO remained relatively stable. Figure 1 demonstrates the characteristics of the progression to RV failure by showing the development of a spherical RV shape, progressively enlarged RV cross sectional area, apical ballooning, thinning of the RV wall, bulging of the interventricular septum (IVS) into the LV, under filling of the LV, an increased right atrium, tricuspid insufficiency, pericardial effusion and severely impaired biventricular function.



	Baseline	3 year follow-up	7 year follow-up
Hemodynamics			
PAP, m/s/d, mmHg	45 / 71 / 26	-	44 / 67 / 35
CO, L/min	4.1	4.2	3.4
MRI			
RVEDV, ml	140	279	449
RV mass, g	78	128	170
SV, ml	49	40	34
RVEF, %	39	18	8
LVEF, %	62	52	29

Figure 1. Cardiac magnetic resonance imaging (MRI) four-chamber **(A1-C1)** and short axis cine images **(A2-C2)** obtained in a patient with pulmonary arterial hypertension (PAH) over time.

The course from right ventricular (RV) structural compensation to end-stage RV failure is demonstrated. This patient was diagnosed with an elevated mean pulmonary artery pressure (PAP) of 45 mmHg and a cardiac output (CO) of 4.1 L/min. At baseline, the RV showed a concentric RV remodeling pattern with increased RV mass, a crescent RV shape, small amounts of dilatation, modest RV function (right ventricular ejection fraction (RVEF): 39%) and preserved left ventricular (LV) function (left ventricular ejection fraction (LVEF): 62%) **(A1, A2)**. During 7 years of follow-up, PAP was unchanged and CO remained relatively stable. However, the RV remodeling pattern has changed **(B, C)**. RV end-diastolic volume (RVEDV) showed a progressive increase from 140 to 449ml after 7 years of follow-up. Smaller increases in RV mass were observed. Furthermore, the RV developed a spherical shape, apical ballooning, bulging of the interventricular septum into the LV (dark arrows), LV underfilling, an enlarged right atrium, tricuspid insufficiency, pericardial effusion (white arrows). In addition, RVEF and LVEF showed a progressive decline to 8% and 29%, respectively. PAP (m / s/ d) = pulmonary artery pressure (mean / systole / diastole); SV = stroke volume.

The combined assessment of multiple RV imaging parameters provides insights into RV remodeling. According to a basic physiological principle of Guyton and Hall, the heart primarily functions as an on-demand pump to maintain CO in order to fulfill the needs of sufficient oxygen delivery to the body[13]. In the setting of chronic RV pressure overload, the RV compensates enduringly (*i.e.* by Frank-Starling mechanism), RV dilatation, RV hypertrophy, tachycardia, changes in contractility) in order to sustain

the CO. However, in the long run RV compensation mechanisms may get exhausted resulting in disturbed intrinsic RV structure and ultimately RV dysfunction and failure. Importantly, imaging of the RV might be of clinical relevance since it is likely that early changes in the RV structure could predict an ultimate RV functional deterioration (Figure 1).

Based on the combined MRI assessment of RV mass, shape and volumes and by measures of RV pressure, ventricular wall stress can be estimated using the law of Laplace (*i.e.* wall stress = intraluminal pressure times chamber internal radius, divided by wall thickness)[14]. According to Laplace, RV hypertrophy is considered part of the adaptive remodeling response to increase RV pumping effectiveness by unloading of the individual muscle fibres and lowering ventricular wall stress. In contrast, an increased RV mass measured by cardiac MRI is associated with an increased mortality in scleroderma PAH patients[15] and might have some prognostic relevance in idiopathic PAH[16]. These still ambiguous findings might be explained by several aspects. First, although RV mass in patients with PAH measured by MRI can be more than 2.5 times increased when compared to normal reference values[17], the RV often faces at rest a four-fold increase in pulmonary artery pressures indicating that the amount of RV hypertrophy may or may not be insufficient to lower the wall stress and protect RV function. This is in keeping with several MRI studies showing that, in PAH patients, RV mass and pulmonary pressures are only modestly related[15,18,19]. Furthermore, it has been observed that patients with PAH associated with the Eisenmenger syndrome showed a higher amount of RV hypertrophy despite a similar elevation in pulmonary pressure compared to idiopathic PAH patients; in those patients RV hypertrophy was associated with better RV function and survival[20,21]. However, in RV autopsy tissue of PAH patients and in rat models with pulmonary hypertension (PH) it has been found that an increase in RV mass is not necessarily accompanied by a similar increase in the number of contractile elements[22]. Second, using MRI with gadolinium contrast it has been demonstrated that degenerative changes occur in the hypertrophic RV of PAH patients. It was found that delayed contrast enhancement (DCE) appears as a unique pattern at the IVS insertion points and might be a reflection of focal fibrosis[23-26]. The extent of DCE was most strongly correlated with increased RV mass, volumes and pulmonary pressures (*i.e.* RV wall stress)[23,24]. Third, RV hypertrophy is associated with maladaptive changes in RV metabolism and blood flow (see below).

During chronic pressure overload, the RV phenotype changes from a more concentric (Figure 1A) towards an eccentric remodeling pattern (Figure 1B, C). The RV shape becomes more like the LV. In contrast to the crescent shape and bellows-like contraction pattern of the normal RV wall, a spherical RV shape in PAH might tolerate high pressure without generating high wall stress due to a decreased RV wall curvature radius at end-

systole[27]. In addition, the RV dilates and it has been found by MRI that increased RV end-diastolic volume at baseline and progressive dilatation during follow-up were among the strongest predictors of mortality in PAH[16]. Although RV dilatation might be beneficial at first resulting in increased preload to increase contractility and sustain CO, this compensatory mechanism eventually fails and results in a maladaptive course of remodeling with increased wall stress, disturbed RV function and ultimately depressed RV output.

- *RV contractility*

Load independent RV contractility can be assessed by right heart catheterization according to the single beat method[28-32]. Non-invasive MRI approaches have also been developed to estimate RV contractility[33,34]. In PAH patients, RV contractility is increased compared to controls. However, paradoxically global RV systolic function (e.g. determined by RV ejection fraction and stroke volume) is significantly decreased in PAH. This paradox is explained by the fact that in PAH, RV hypercontractility augmentation is insufficient for the degree of pressure overload, a phenomenon which has been described as ventriculo-arterial uncoupling[28,29,33,34]. In addition to a systolic impairment, it was recently demonstrated that RV diastolic stiffness is increased in PAH patients and this was related to disease severity[28].

- *RV function*

Imaging parameters describing global systolic RV function are all load dependent and therefore reflect RV-arterial coupling rather than intrinsic RV contractility[35]. In fact, global RV systolic function is determined by multiple factors: preload, afterload, contractility, ventricular synchrony, valvular regurgitation and shunt fraction[3,36]. Low stroke volume and RV ejection fraction (RVEF) at baseline and a further decrease during follow-up were associated with poor survival[4,16,37]. Furthermore, stroke volume determined by MRI has been validated as imaging parameter to monitor therapeutic effects[38]. In addition, after current medical therapies, it has been found that the changes in RVEF were only modestly related to the changes in RV load (measured by pulmonary vascular resistance (PVR)). Moreover, despite the fact that PVR is reduced by treatment, RVEF can deteriorate. The decrease in RVEF was associated with poor outcome, independent of the reduction in PVR (Figure 2)[4].

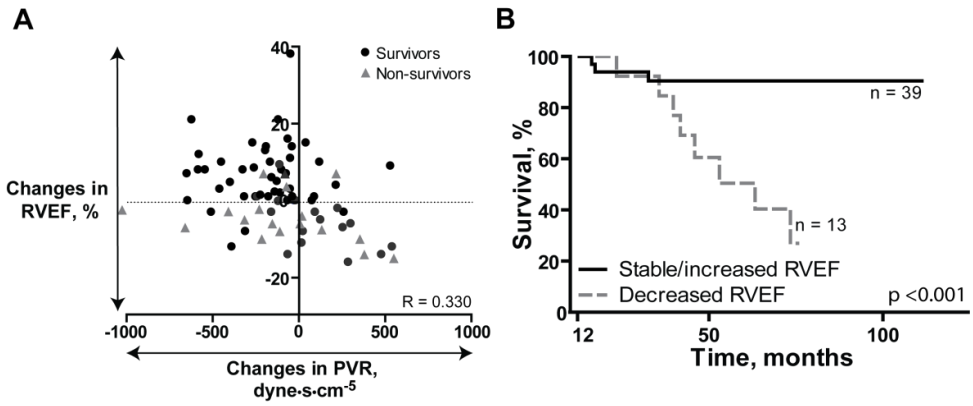


Figure 2. Relationship between changes in PVR and changes in RVEF after medical treatment.
(A) After 1 year of current PAH targeted medical treatment, the changes in pulmonary vascular resistance (PVR) were modestly related to the changes in RVEF. **(B)** In the PAH patients with a reduced PVR after therapy, a decrease in RVEF was associated with a worse survival compared to patients with a stable/improved RVEF. Both groups showed a similar decrease in PVR after treatment (mean PVR reduction: 284 ± 248 dyne·s·cm^5) (Printed with permission[4]).

The determination of RV ejection fraction is relatively time consuming whereas stroke volume can be more easily obtained by phase contrast mapping MRI[39]. Other more readily applicable measures of RV function have also been explored. RV shortening in a longitudinal plane (tricuspid annular plane excursion or TAPSE) and transverse plane provided simple estimates of RV ejection fraction of which transverse shortening showed the strongest correlation[40]. In addition, Mauritz et al. showed that assessment of both parameters are valuable, but that changes in transverse wall motion rather than changes in TAPSE reflect RV dysfunction during end-stage disease[37].
Regional ventricular wall deformation and ventricular synchrony are important aspects of RV function. MRI tagging techniques are considered as the reference technique to measure the relative amount of myocardial wall deformation (segmental strain), the velocity of deformation (strain rate) and synchrony (*i.e.* timing of mechanical activation and relaxation between wall segments); all of these parameters can be determined in a circumferential, longitudinal and radial axis. It has been found that in the healthy RV, there is a predominant longitudinal rather than circumferential wall deformation, resulting in a bellows-like or peristaltic action. Normal wall deformation is in general larger at the basal and apical segments than at the mid-segment[41]. Patients with PH showed an altered pattern with a globally reduced longitudinal and circumferential wall deformation[42]. Furthermore, it has been found that regional longitudinal wall deformation can already be disturbed at the time that global RV function is still intact,

implying that changes in regional measures could be sensitive parameters to detect early RV dysfunction in PAH[43].
In addition, it has been shown by MRI that RV asynchrony may contribute to the development of impaired cardiac function. In PAH patients, RV mechanical contraction is prolonged and continues after the pulmonary valve closes (resulting in a so-called post systolic contraction period) rendering RV contraction inefficient[44,45]. In addition, at the time that the RV still contracts, the LV in PAH is already in its relaxation phase, leading to leftward septum bowing, a low LV end-diastolic volume and decreased stroke volume[45-47]. In fact, a low LV end-diastolic volume appears to be a strong predictor of poor prognosis[16].

RV molecular and perfusion imaging

- *RV metabolic remodeling*

PET studies have demonstrated that in LV cardiomyopathies a switch takes place from primarily fatty acid metabolism to glucose metabolism in order to maintain the adenosine triphosphate (ATP) supply[48,49]. In the failing human RV under chronic pressure overload, single photon emission computed tomography (SPECT) has demonstrated a decreased RV uptake of fatty acids which was associated with impaired RV function and poor prognosis[50]. Fatty acid uptake can also be estimated using PET with ^{11}C-palmitate tracers[48,51] but such studies have yet to be performed in PAH. In addition, RV myocardial glucose metabolism can be measured by PET with ^{18}F-2-deoxy-2-fluoro-D-Glucose (^{18}FDG) tracers[52]. Some studies have demonstrated an increase in the ratio of RV to LV glucose uptake in PAH patients. However it remains unclear whether this ratio is explained by an increased RV glucose uptake[53-55] or a lowered LV uptake[56]. Furthermore, inconsistent results have been reported with respect to the relevance of glucose metabolism in relationship with RV load and function[52,55-59]. The differences between study results are perhaps due to differences in patient populations, scanning protocols or data analysis. At this moment, it remains unclear which changes in metabolism occur in the RV of PAH patients and whether these changes can be regarded as adaptive or indicative of pathological remodeling. This area of imaging of RV metabolism will likely be further developed in the coming years.

- *RV blood flow and oxygen balance*

Using PET with a combination of ^{15}O-labeled tracers (^{15}O-H_2O, ^{15}O-CO, and ^{15}O-O_2) or by a more practical method using ^{11}C-acetate tracers, RV myocardial oxygen consumption (MVO_2) can be estimated[60,61]. It has been demonstrated that resting MVO_2 is significantly elevated in patients with PAH (Figure 3) and this finding is primarily determined by increased

pulmonary pressures and an increased heart rate[59,62]. PAH patients in NYHA class III show a higher MVO_2 compared to NYHA class II patients, despite a similar RV power output, which implies that the RV of these patients becomes mechanically inefficient (Figure 4)[59].

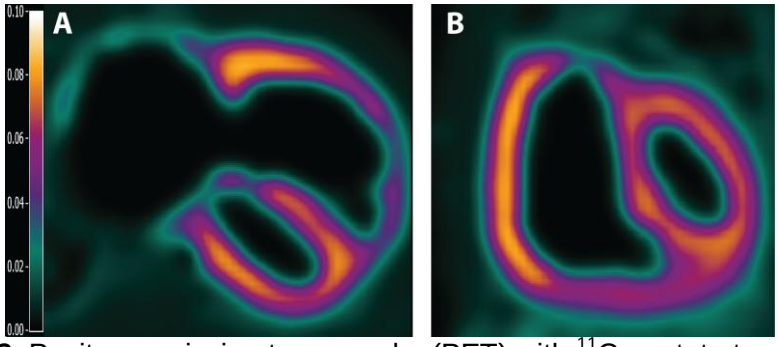


Figure 3. Positron emission tomography (PET) with ^{11}C-acetate tracers of the heart in a patient with PAH.
(A) A four chamber **(B)** and short-axis images obtained in the same patient with end-stage RV failure as in Figure 1. It is demonstrated that part of the RV wall shows an increased myocardial oxygen consumption (MVO_2) (bright) that can be even higher compared to the LV.

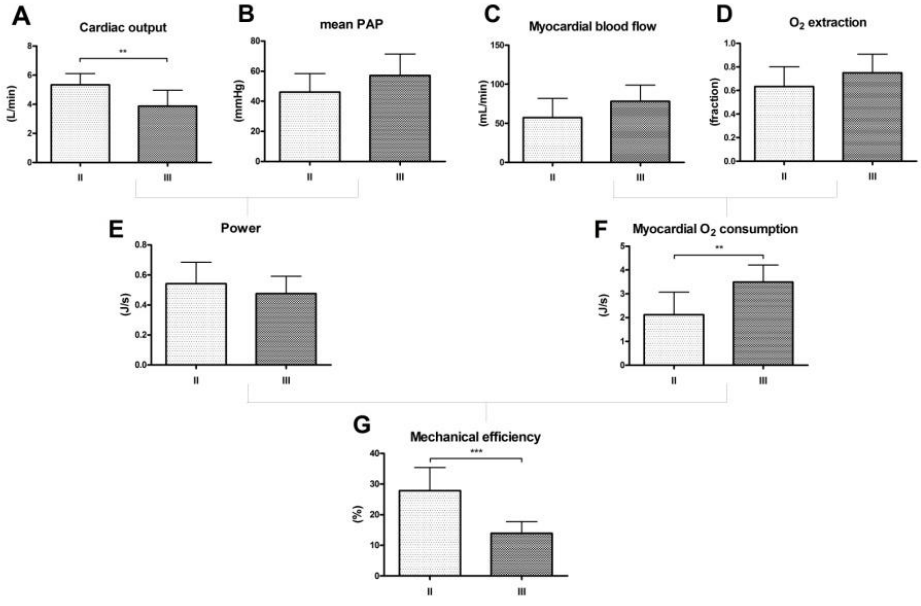


Figure 4. Impaired RV mechanical efficiency in patients with PAH is primarily determined by increased MVO_2.
Light grey bars = New York Heart Association (NYHA) Functional class II patients, dark bars = NYHA functional class III patients. **(A)** Patients in NYHA II showed a higher cardiac output (CO) **(B)** and similar mean pulmonary artery pressure (mPAP) compared to NYHA III

patients. **(E)** RV power output (*i.e.* product of CO and mean PAP) was similar in both groups. **(C)** RV myocardial blood flow determined by positron emission tomography (PET) with $H_2{}^{15}O$ tracers and **(D)** oxygen (O_2) extraction fraction estimated by PET using $^{15}O_2$ tracers were not statistically different in both groups. **(F)** However. there was a significantly higher MVO_2 per gram of myocardial tissue in NYHA III compared to NYHA II patients. **(G)** RV mechanical efficiency was reduced by ~50% in NYHA III in comparison with NYHA II as a result of a similar RV power output but higher MVO_2 (Printed with permission[59]).

The normal RV has two mechanisms which generate an increased MVO_2 during stress: (1) coronary flow reserve and (2) oxygen extraction fraction (OEF) reserve[63-66]. In patients with PAH during resting conditions, the OEF is already elevated whereas the mean resting myocardial blood flow is similar in PAH compared to controls[59,67,68]. In addition, PAH patients showed an impaired perfusion reserve since myocardial blood flow did neither increase during adenosine induced stress MRI[67] nor during PET imaging with cycling exercise[69]. Surprisingly, the change in myocardial blood flow during exercise was unrelated to resting OEF. Especially in patients with a high resting OEF, an attenuated increase in blood flow during exercise was observed and was related to a poorer clinical, hemodynamic and RV condition[67,69]. The findings of impaired myocardial blood flow reserve might be explained by multiple factors. First, although mean resting coronary blood flow was unchanged, coronary flow was inversely related to the amount of RV hypertrophy[68]. Transmural blood flow may be impaired, perhaps as a consequence of a reduced capillary density of the failing RV tissue[22]. Indeed, impaired angiogenesis might play a role in the mismatch between hypertrophic myocytes and capillaries. Second, the resting coronary blood flow and perfusion reserve are both negatively associated with increased pulmonary pressures[67,68], which could imply that these extravascular compressive forces may restrict an increase in blood flow. Other explanations could be impaired autoregulatory flow mechanisms[70] and systemic hypotension resulting in reduced coronary driving pressures. However, van Wolferen et al. showed that the latter factor is probably not a major determinant of impaired RV flow in PAH[68].
To summarize, the increased RV oxygen demand in patients with PAH is not adequately compensated for by an increased oxygen supply. As a potential consequence, Gomez et al. observed RV ischemia in PAH patients by SPECT imaging[71].

The near future of RV molecular imaging

- *RV angiogenesis*

In PH animal models, impaired angiogenesis in the setting of RV hypertrophy has been demonstrated, which might lead to RV ischemia and fibrosis[36,72]. Vascular endothelial growth factor (VEGF) is a major

contributor to angiogenesis and integrins serve as important mediators of angiogenis. Novel imaging strategies have been developed that can directly measure angiogenesis *in vivo* using PET with tracers such as ^{64}CU-labeled $VEGF_{121}$ or ^{18}F arginine-glycine-aspartic acid peptide (RGD) with affinity for the $\alpha_v\beta_3$ integrins. Angiogenic imaging has successfully been performed in rat models with myocardial infarction[73-75] and in a patient 2 weeks after myocardial infarction[76]. These imaging techniques may become important in the assessment of pathophysiological mechanisms of RV disease processes *in vivo* in PAH.

- *RV apoptosis*

Apoptosis is defined as programmed cell death may play a causal role in the development of cardiac hibernation and heart failure[77]. Radiolabeled annexin can be used as an imaging protein that binds to phosphatidylserine (PS); a phospholipid expressed on the outer cell membrane during apoptosis which serves as a marker for macrophages to remove apoptotic cells[78]. It has been demonstrated by SPECT that increased ^{99}Tc-Labeled annexin V uptake was associated with the deterioration of LV function in patients with LV failure[79] and related to allograft rejection in cardiac transplant recipients[80]. Such studies have not yet been performed in PAH patients but in PH animal models increased levels of SPECT cardiac annexin V uptake have been detected[81].
In addition, activated caspases are involved in the signal transduction of apoptosis and when radiolabeled, they can be used for PET measurements of apoptosis *in vivo*. In mouse models of dilated cardiomyopathies, it has been shown that although the overall rate of myocyte apoptosis was low, persistent levels can overwhelm the limited regenerative capacity of the myocardium contributing to heart failure[82].

- *RV neurohormonal system*

Preclinical studies have demonstrated that dysfunctional neuronal signaling might be an important hallmark in the development of RV failure in PAH[83]. PET imaging with radiolabeled norepinephrine (NE) analogs such as ^{11}C-meta-hydroxyephedrine (HED) can be applied to estimate presynaptic sympathetic function and ^{11}C-CGP-12177 or ^{11}C-CGP-12388 tracers are used to estimate levels of postsynaptic sympathetic β-adrenoceptors. HED reuptake and β-adrenoceptor density were reduced in patients with LV cardiomyopathies and associated with impaired LV function and worse survival[84-86].
The renin-angiotensin-aldosterone system (RAAS) activity in PAH is increased and associated with disease severity[87]. By use of PET imaging with ^{11}C-KR31173 tracers, levels of cardiac angiotensin receptors have been detected in healthy volunteers[88]. Such data might be of pathophysiological interest also in patients with PAH.

- *Magnetic resonance spectroscopy*

Magnetic resonance spectroscopy (MRS) is a technically difficult technique but can provide insight into cardiac metabolism *in vivo* without requirements of an external tracer. There is limited expertise in the field of PAH[89] but multiple studies have been performed in patients with LV failure. Multiple ^{31}Phophorus MRS studies have demonstrated that LV levels of creatine and ATP were decreased in advanced heart failure and related to survival[90].

- *Hybrid PET-MRI*

Currently, hybrid PET-CT has become commonplace in clinical practice and research settings and has significantly contributed to our insights into pathophysiological processes in cardiovascular diseases[91]. Although CT allows detailed structural imaging it is less useful in RV functional assessment. Hybrid PET-MRI is an innovative, rapidly accepted technique which does not only allow (simultaneous) combination of PET with anatomic imaging but also provides functional imaging, perfusion imaging, tissue characterization and flow imaging that might provide improved insights into the pathophysiology of RV failure. However one limitation of hybrid systems with MRI is that information required for attenuation correction of nuclear images is not provided. Recently, the first hybrid PET-MRI results of patients with myocardial infarction have been published and have demonstrated high image qualities (Figure 5)[92-94].

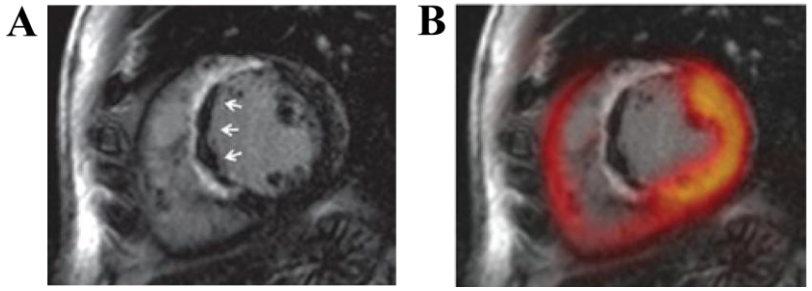


Figure 5. Short-axis images obtained by hybrid PET-MRI in a patient with myocardial infarction of the septal and anteroseptal wall.
(A) MRI with late gadolinium enhancement (LGE) demonstrates the infarct zone with a no-reflow phenomenon (arrows). **(B)** Fusion of ^{18}F-2-deoxy-2-fluoro-D-Glucose (^{18}FDG) PET and MRI LGE images demonstrates absent ^{18}FDG uptake in the infarcted area (Printed with permission[92]).

Summary and conclusions

We predict that *in vivo* cardiac MRI and PET will significantly contribute to a better understanding of the pathophysiological processes which lead to the development of chronic RV failure. Imaging studies have demonstrated that in the setting of chronic pressure overload, the RV compensates enduringly to sustain CO by an increase in wall mass, dilatation and contractility and marked changes in the RV shape. With passage of time, these compensation mechanisms fail, resulting in increased wall stress and impaired global RV function. Other factors that might contribute to disturbed RV function are a reduced wall deformation and an inefficient RV contraction pattern. The resulting interventricular asynchrony is associated with leftward septum bowing, impaired LV filling and decreased stroke volume. Furthermore, the RV becomes mechanically insufficient: more oxygen is required for a comparable power output. At the same time, RV oxygen delivery is impaired and tissue oxygenation is reduced. Alterations in myocardial metabolism have been observed in PAH, but their overall relevance and cause of consequence of RV failure remain unclear. In the near future it can be expected that the importance of changes in cellular functions and signaling pathways will become clear and ‘imageable’. This might allow a regional and quantifiable analysis of processes such as angiogenesis, apoptosis and neurohormonal factors. Table 1 provides an overview of currently available clinical imaging tracers that could be relevant for the assessment of molecular processes of RV diseases in patients. In addition, recent developments in (hybrid) PET and MRI might allow an integrated RV assessment *in vivo.* They will likely provide an important basis for simultaneous measurements of multiple myocardial disease processes.

Table 1. Overview of applicable tracers for molecular imaging in patients with heart failure.

Function	Tracer MRI	Tracer PET
Angiogenesis		^{18}F-arginine-glycine-aspartic acid peptide (RGD) $\alpha_v\beta_3$ integrins[76*]
Apoptosis	Iron labeled Annexin V[95*] Synaptotagmin C2A[96*]	^{18}F-Annexin V[97*]
Metabolism	Adenosine triphosphate (ATP), phosphocreatinine[89,98*, #]	^{11}C-palmitate[51*] ^{18}F-fluoro-2-deoxy-D-glucose[59]
Oxygen consumption		^{11}C-acetate[60] ^{15}O-H_2O, ^{15}O-CO, ^{15}O-O_2[59,61]
Neuroreceptors		
Sympathic signaling		^{11}C- hydroxyephedrine (HED)[85,86*] ^{11}C-CGP-12177[86,99*]
Parasympathic signaling		^{11}C-CGP-12388[100*] ^{11}C-MQNB[101*]
Renin-Angiotensin-Aldosterone-System (RAAS)		^{11}C-KR31173[88*]

*Previously performed in patients with left ventricular failure but yet has not been applied in patients with right ventricular failure. #Using ^{31}Phosphorus-magnetic resonance spectroscopy.

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References

1. Hunt SA, Abraham WT, Chin MH, et al. 2009 Focused update incorporated into the ACC/AHA 2005 Guidelines for the Diagnosis and Management of Heart Failure in Adults A Report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines Developed in Collaboration With the International Society for Heart and Lung Transplantation. J Am Coll Cardiol. 2009; 53:e1-90.
2. McLaughlin VV, McGoon MD. Pulmonary arterial hypertension. Circulation. 2006; 114:1417-1431.
3. Voelkel NF, Quaife RA, Leinwand LA, et al. Right ventricular function and failure: report of a National Heart, Lung, and Blood Institute working group on cellular and molecular mechanisms of right heart failure. Circulation. 2006; 114:1883-1891.
4. van de Veerdonk MC, Kind T, Marcus JT, et al. Progressive right ventricular dysfunction in patients with pulmonary arterial hypertension responding to therapy. J Am Coll Cardiol. 2011; 58:2511-2519.

5. Champion HC, Michelakis ED, Hassoun PM. Comprehensive invasive and noninvasive approach to the right ventricle-pulmonary circulation unit: state of the art and clinical and research implications. Circulation. 2009; 120:992-1007.
6. Bristow MR, Zisman LS, Lowes BD, et al. The pressure-overloaded right ventricle in pulmonary hypertension. Chest. 1998; 114:S101-106.
7. Morrison D, Goldman S, Wright AL, et al. The effect of pulmonary hypertension on systolic function of the right ventricle. Chest. 1983; 84:250-257.
8. Pasque MK, Trulock EP, Cooper JD, et al. Single lung transplantation for pulmonary hypertension. Single institution experience in 34 patients. Circulation. 1995; 92:2252-2258.
9. Shors SM, Fung CW, Francois CJ, et al. Accurate quantification of right ventricular mass at MR imaging by using cine true fast imaging with steady-state precession: study in dogs. Radiology. 2004; 230:383-388.
10. Katz J, Whang J, Boxt LM, et al. Estimation of right ventricular mass in normal subjects and in patients with primary pulmonary hypertension by nuclear magnetic resonance imaging. J Am Coll Cardiol. 1993; 21:1475-1481.
11. Grothues F, Moon JC, Bellenger NG, et al. Interstudy reproducibility of right ventricular volumes, function, and mass with cardiovascular magnetic resonance. Am Heart J. 2004; 147:218-223.
12. Pennell DJ, Sechtem UP, Higgins CB, et al. Clinical indications for cardiovascular magnetic resonance (CMR): Consensus Panel report. Eur Heart J. 2004; 25:1940-1965.
13. Hall JE. Guyton and Hall Textbook of Medical Physiology. 12th ed: Elsevier Health Sciences 2010.
14. Mauritz GJ, Vonk-Noordegraaf A, Kind T, et al. Pulmonary endarterectomy normalizes interventricular dyssynchrony and right ventricular systolic wall stress. J Cardiovasc Magn Reson. 2012; 14:5.
15. Hagger D, Condliffe R, Woodhouse N, et al. Ventricular mass index correlates with pulmonary artery pressure and predicts survival in suspected systemic sclerosis-associated pulmonary arterial hypertension. Rheumatology. 2009; 48:1137-1142.
16. van Wolferen SA, Marcus JT, Boonstra A, et al. Prognostic value of right ventricular mass, volume, and function in idiopathic pulmonary arterial hypertension. Eur Heart J. 2007; 28:1250-1257.
17. Kawut SM, Lima JA, Barr RG, et al. Sex and race differences in right ventricular structure and function: the multi-ethnic study of atherosclerosis-right ventricle study. Circulation. 2011; 123:2542-2551.
18. Roeleveld RJ, Marcus JT, Boonstra A, et al. A comparison of noninvasive MRI-based methods of estimating pulmonary artery pressure in pulmonary hypertension. J Magn Reson Imaging. 2005; 22:67-72.
19. Saba TS, Foster J, Cockburn M, et al. Ventricular mass index using magnetic resonance imaging accurately estimates pulmonary artery pressure. Eur Respir J. 2002; 20:1519-1524.
20. Hopkins WE, Waggoner AD. Severe pulmonary hypertension without right ventricular failure: the unique hearts of patients with Eisenmenger syndrome. Am J Cardiol. 2002; 89:34-38.
21. Hopkins WE, Ochoa LL, Richardson GW, et al. Comparison of the hemodynamics and survival of adults with severe primary pulmonary hypertension or Eisenmenger syndrome. J Heart Lung Transplant. 1996; 15: 100-105.
22. Ruiter G, Ying Wong Y, de Man FS, et al. Right ventricular oxygen supply parameters are decreased in human and experimental pulmonary hypertension. J Heart Lung Transplant. 2013; 32:231-240.
23. Sanz J, Dellegrottaglie S, Kariisa M, et al. Prevalence and correlates of septal delayed contrast enhancement in patients with pulmonary hypertension. Am J Cardiol. 2007; 100:731-735.

24. McCann GP, Gan CT, Beek AM, et al. Extent of MRI delayed enhancement of myocardial mass is related to right ventricular dysfunction in pulmonary artery hypertension. AJR Am J Roentgenol. 2007; 188:349-355.
25. McCann GP, Beek AM, Vonk-Noordegraaf A, et al. Delayed contrast-enhanced magnetic resonance imaging in pulmonary arterial hypertension. Circulation. 2005; 112:e268.
26. Blyth KG, Groenning BA, Martin TN, et al. Contrast enhanced-cardiovascular magnetic resonance imaging in patients with pulmonary hypertension. Eur Heart J. 2005; 26:1993-1999.
27. Greyson CR. Pathophysiology of right ventricular failure. Crit Care Med. 2008; 36:S57-65.
28. Rain S, Handoko ML, Trip P, et al. Right Ventricular Diastolic Impairment in Patients with Pulmonary Arterial Hypertension. Circulation. 2013; 128:2016-2025.
29. Tedford RJ, Mudd JO, Girgis RE, et al. Right Ventricular Dysfunction in Systemic Sclerosis Associated Pulmonary Arterial Hypertension. Circ Heart Fail. 2013; 6:953-963.
30. Trip P, Kind T, van de Veerdonk MC, et al. Accurate assessment of load-independent right ventricular systolic function in patients with pulmonary hypertension. J Heart Lung Transplant. 2013; 32:50-55.
31. Brimioulle S, Wauthy P, Ewalenko P, et al. Single-beat estimation of right ventricular end-systolic pressure-volume relationship. Am J Physiol. 2003; 284:H1625-1630.
32. Sunagawa K, Yamada A, Senda Y, et al. Estimation of the hydromotive source pressure from ejecting beats of the left ventricle. IEEE Trans Biomed Eng. 1980; 27:299-305.
33. Sanz J, Garcia-Alvarez A, Fernandez-Friera L, et al. Right ventriculo-arterial coupling in pulmonary hypertension: a magnetic resonance study. Heart. 2012; 98:238-243.
34. Kuehne T, Yilmaz S, Steendijk P, et al. Magnetic resonance imaging analysis of right ventricular pressure-volume loops: in vivo validation and clinical application in patients with pulmonary hypertension. Circulation. 2004; 110:2010-2016.
35. Guihaire J, Haddad F, Boulate D, et al. Non-invasive indices of right ventricular function are markers of ventricular-arterial coupling rather than ventricular contractility: insights from a porcine model of chronic pressure overload. Eur Heart J Cardiovasc Imaging. 2013; 14:1140-1149.
36. Bogaard HJ, Abe K, Vonk Noordegraaf A, et al. The right ventricle under pressure: cellular and molecular mechanisms of right-heart failure in pulmonary hypertension. Chest. 2009; 135:794-804.
37. Mauritz GJ, Kind T, Marcus JT, et al. Progressive changes in right ventricular geometric shortening and long-term survival in pulmonary arterial hypertension. Chest. 2012; 141:935-943.
38. van Wolferen SA, van de Veerdonk MC, Mauritz GJ, et al. Clinically significant change in stroke volume in pulmonary hypertension. Chest. 2011; 139:1003-1009.
39. Mauritz GJ, Marcus JT, Boonstra A, et al. Non-invasive stroke volume assessment in patients with pulmonary arterial hypertension: left-sided data mandatory. J Cardiovasc Magn Reson. 2008; 10:51.
40. Kind T, Mauritz GJ, Marcus JT, et al. Right ventricular ejection fraction is better reflected by transverse rather than longitudinal wall motion in pulmonary hypertension. J Cardiovasc Magn Reson. 2010; 12:35.
41. Petitjean C, Rougon N, Cluzel P. Assessment of myocardial function: a review of quantification methods and results using tagged MRI. J Cardiovasc Magn Reson. 2005; 7:501-516.

42. Fayad ZA, Ferrari VA, Kraitchman DL, et al. Right ventricular regional function using MR tagging: normals versus chronic pulmonary hypertension. Magn Reson Med. 1998; 39:116-123.
43. Shehata ML, Harouni AA, Skrok J, et al. Regional and global biventricular function in pulmonary arterial hypertension: a cardiac MR imaging study. Radiology. 2013; 266:114-122.
44. Mauritz GJ, Marcus JT, Westerhof N, et al. Prolonged right ventricular post-systolic isovolumic period in pulmonary arterial hypertension is not a reflection of diastolic dysfunction. Heart. 2011; 97:473-478.
45. Marcus JT, Gan CT, Zwanenburg JJ, et al. Interventricular mechanical asynchrony in pulmonary arterial hypertension: left-to-right delay in peak shortening is related to right ventricular overload and left ventricular underfilling. J Am Coll Cardiol. 2008; 51:750-757.
46. Gan CT, Holverda S, Marcus JT, et al. Right ventricular diastolic dysfunction and the acute effects of sildenafil in pulmonary hypertension patients. Chest. 2007; 132:11-17.
47. Vonk-Noordegraaf A, Marcus JT, Gan CT, et al. Interventricular mechanical asynchrony due to right ventricular pressure overload in pulmonary hypertension plays an important role in impaired left ventricular filling. Chest. 2005; 128:S628-630.
48. de las Fuentes L, Herrero P, Peterson LR, et al. Myocardial fatty acid metabolism: independent predictor of left ventricular mass in hypertensive heart disease. Hypertension. 2003; 41:83-87.
49. Davila-Roman VG, Vedala G, Herrero P, et al. Altered myocardial fatty acid and glucose metabolism in idiopathic dilated cardiomyopathy. J Am Coll Cardiol. 2002; 40:271-277.
50. Nagaya N, Goto Y, Satoh T, et al. Impaired regional fatty acid uptake and systolic dysfunction in hypertrophied right ventricle. J Nucl Med. 1998; 39:1676-1680.
51. Bergmann SR, Weinheimer CJ, Markham J, et al. Quantitation of myocardial fatty acid metabolism using PET. J Nucl Med. 1996; 37:1723-1730.
52. Bokhari S, Raina A, Rosenweig EB, et al. PET imaging may provide a novel biomarker and understanding of right ventricular dysfunction in patients with idiopathic pulmonary arterial hypertension. Circ Cardiovasc Imaging. 2011; 4:641-647.
53. Lundgrin EL, Park MM, Sharp J, et al. Fasting 2-deoxy-2-[18F]fluoro-D-glucose positron emission tomography to detect metabolic changes in pulmonary arterial hypertension hearts over 1 year. Ann Am Thorac Soc. 2013; 10:1-9.
54. Hagan G, Southwood M, Treacy C, et al. (18)FDG PET imaging can quantify increased cellular metabolism in pulmonary arterial hypertension: A proof-of-principle study. Pulm Circ. 2011; 1:448-455.
55. Oikawa M, Kagaya Y, Otani H, et al. Increased [18F]fluorodeoxyglucose accumulation in right ventricular free wall in patients with pulmonary hypertension and the effect of epoprostenol. J Am Coll Cardiol. 2005; 45:1849-1855.
56. Kluge R, Barthel H, Pankau H, et al. Different mechanisms for changes in glucose uptake of the right and left ventricular myocardium in pulmonary hypertension. J Nucl Med. 2005; 46:25-31.
57. Can MM, Kaymaz C, Tanboga IH, et al. Increased right ventricular glucose metabolism in patients with pulmonary arterial hypertension. Clin Nucl Med. 2011; 36:743-748.
58. Fang W, Zhao L, Xiong CM, et al. Comparison of 18F-FDG uptake by right ventricular myocardium in idiopathic pulmonary arterial hypertension and pulmonary arterial hypertension associated with congenital heart disease. Pulm Circ. 2012; 2:365-372.

59. Wong YY, Ruiter G, Lubberink M, et al. Right ventricular failure in idiopathic pulmonary arterial hypertension is associated with inefficient myocardial oxygen utilization. Circ Heart Fail. 2011; 4:700-706.
60. Wong YY, Raijmakers P, van Campen J, et al. 11C-Acetate clearance as an index of oxygen consumption of the right myocardium in idiopathic pulmonary arterial hypertension: a validation study using 15O-labeled tracers and PET. J Nucl Med. 2013; 54:1258-1262.
61. Knaapen P, van Campen LM, de Cock CC, et al. Effects of cardiac resynchronization therapy on myocardial perfusion reserve. Circulation. 2004; 110:646-651.
62. Wong YY, Westerhof N, Ruiter G, et al. Systolic pulmonary artery pressure and heart rate are main determinants of oxygen consumption in the right ventricular myocardium of patients with idiopathic pulmonary arterial hypertension. Eur J Heart Fail. 2011; 13:1290-1295.
63. Tune JD, Gorman MW, Feigl EO. Matching coronary blood flow to myocardial oxygen consumption. J Appl Physiol. 2004; 97:404-415.
64. Hart BJ, Bian X, Gwirtz PA, et al. Right ventricular oxygen supply/demand balance in exercising dogs. Am J Physiol. 2001; 281:H823-830.
65. Saito D, Tani H, Kusachi S, et al. Oxygen metabolism of the hypertrophic right ventricle in open chest dogs. Cardiovasc Res. 1991; 25:731-739.
66. Kusachi S, Nishiyama O, Yasuhara K, et al. Right and left ventricular oxygen metabolism in open-chest dogs. Am J Physiol. 1982; 243:H761-766.
67. Vogel-Claussen J, Skrok J, Shehata ML, et al. Right and left ventricular myocardial perfusion reserves correlate with right ventricular function and pulmonary hemodynamics in patients with pulmonary arterial hypertension. Radiology. 2011; 258:119-127.
68. van Wolferen SA, Marcus JT, Westerhof N, et al. Right coronary artery flow impairment in patients with pulmonary hypertension. Eur Heart J. 2008; 29:120-127.
69 Wong YY, Raijmakers PG, Knaapen P, et al. Supine-exercise-induced oxygen supply to the right myocardium is attenuated in patients with severe idiopathic pulmonary arterial hypertension. Heart. 2011; 97:2069-2074.
70. Murray PA, Vatner SF. Reduction of maximal coronary vasodilator capacity in conscious dogs with severe right ventricular hypertrophy. Circ Res. 1981; 48:25-33.
71. Gomez A, Bialostozky D, Zajarias A, et al. Right ventricular ischemia in patients with primary pulmonary hypertension. J Am Coll Cardiol. 2001; 38:1137-1142.
72. Sutendra G, Dromparis P, Paulin R, et al. A metabolic remodeling in right ventricular hypertrophy is associated with decreased angiogenesis and a transition from a compensated to a decompensated state in pulmonary hypertension. J Mol Med. 2013; 91:1315-1327.
73. Rodriguez-Porcel M, Cai W, Gheysens O, et al. Imaging of VEGF receptor in a rat myocardial infarction model using PET. J Nucl Med. 2008; 49:667-673.
74. Higuchi T, Bengel FM, Seidl S, et al. Assessment of alphavbeta3 integrin expression after myocardial infarction by positron emission tomography. Cardiovasc Res. 2008; 78:395-403.
75. Meoli DF, Sadeghi MM, Krassilnikova S, et al. Noninvasive imaging of myocardial angiogenesis following experimental myocardial infarction. The J Clin Invest. 2004; 113:1684-1691.
76. Makowski MR, Ebersberger U, Nekolla S, et al. In vivo molecular imaging of angiogenesis, targeting alphavbeta3 integrin expression, in a patient after acute myocardial infarction. Eur Heart J. 2008; 29:2201.
77. Foo RS, Mani K, Kitsis RN. Death begets failure in the heart. J Clin Invest. 2005; 115:565-571.

78. Danial NN, Korsmeyer SJ. Cell death: critical control points. Cell. 2004; 116:205-219.
79. Kietselaer BL, Reutelingsperger CP, Boersma HH, et al. Noninvasive detection of programmed cell loss with 99mTc-labeled annexin A5 in heart failure. J Nucl Med. 2007; 48:562-567.
80. Narula J, Acio ER, Narula N, et al. Annexin-V imaging for noninvasive detection of cardiac allograft rejection. Nat Med. 2001; 7:1347-1352.
81. Paffett ML, Hesterman J, Candelaria G, et al. Longitudinal in vivo SPECT/CT imaging reveals morphological changes and cardiopulmonary apoptosis in a rodent model of pulmonary arterial hypertension. PloS one. 2012; 7:e40910.
82. Wencker D, Chandra M, Nguyen K, et al. A mechanistic role for cardiac myocyte apoptosis in heart failure. J Clin Invest. 2003; 111:1497-1504.
83. de Man FS, Handoko ML, Guignabert C, et al. Neurohormonal axis in patients with pulmonary arterial hypertension: friend or foe? Am J Respir Crit Care Med. 2013; 187:14-19.
84. Caldwell JH, Link JM, Levy WC, et al. Evidence for pre- to postsynaptic mismatch of the cardiac sympathetic nervous system in ischemic congestive heart failure. J Nucl Med. 2008; 49:234-241.
85. Pietila M, Malminiemi K, Ukkonen H, et al. Reduced myocardial carbon-11 hydroxyephedrine retention is associated with poor prognosis in chronic heart failure. Eur J Nucl Med. 2001; 28:373-376.
86. Schafers M, Dutka D, Rhodes CG, et al. Myocardial presynaptic and postsynaptic autonomic dysfunction in hypertrophic cardiomyopathy. Circ Res. 1998; 82:57-62.
87. de Man FS, Tu L, Handoko ML, et al. Dysregulated renin-angiotensin-aldosterone system contributes to pulmonary arterial hypertension. Am J Respir Crit Care Med. 2013; 186:780-789.
88. Fukushima K, Bravo PE, Higuchi T, et al. Molecular hybrid positron emission tomography/computed tomography imaging of cardiac angiotensin II type 1 receptors. J Am Coll Cardiol. 2012; 60:2527-2534.
89. Spindler M, Schmidt M, Geier O, et al. Functional and metabolic recovery of the right ventricle during Bosentan therapy in idiopathic pulmonary arterial hypertension. J Cardiovasc Magn Reson. 2005; 7:853-854.
90. Hudsmith LE, Neubauer S. Magnetic resonance spectroscopy in myocardial disease. JACC Cardiovasc Imaging. 2009; 2:87-96.
91. Kaufmann PA, Di Carli MF. Hybrid SPECT/CT and PET/CT imaging: the next step in noninvasive cardiac imaging. Semin Nucl Med. 2009; 39:341-347.
92. Nensa F, Poeppel TD, Beiderwellen K, et al. Hybrid PET/MR Imaging of the Heart: Feasibility and Initial Results. Radiology. 2013; 268:366-373.
93. Schlosser T, Nensa F, Mahabadi AA, et al. Hybrid MRI/PET of the heart: a new complementary imaging technique for simultaneous acquisition of MRI and PET data. Heart. 2013; 99:351-352.
94. Ibrahim T, Nekolla SG, Langwieser N, et al. Simultaneous positron emission tomography/magnetic resonance imaging identifies sustained regional abnormalities in cardiac metabolism and function in stress-induced transient midventricular ballooning syndrome: a variant of Takotsubo cardiomyopathy. Circulation. 2012; 126:e324-326.
95. Sosnovik DE, Schellenberger EA, Nahrendorf M, et al. Magnetic resonance imaging of cardiomyocyte apoptosis with a novel magneto-optical nanoparticle. Magn Reson Med. 2005; 54:718-724.
96. Zhao M, Beauregard DA, Loizou L, et al. Non-invasive detection of apoptosis using magnetic resonance imaging and a targeted contrast agent. Nat Med. 2001; 7:1241-1244.
97. Keen HG, Dekker BA, Disley L, et al. Imaging apoptosis in vivo using 124I-annexin V and PET. Nucl Med Biol. 2005; 32:395-402.

98. Conway MA, Allis J, Ouwerkerk R, et al. Detection of low phosphocreatine to ATP ratio in failing hypertrophied human myocardium by 31P magnetic resonance spectroscopy. Lancet. 1991; 338:973-976.
99. Naya M, Tsukamoto T, Morita K, et al. Myocardial beta-adrenergic receptor density assessed by 11C-CGP12177 PET predicts improvement of cardiac function after carvedilol treatment in patients with idiopathic dilated cardiomyopathy. J Nucl Med. 2009; 50:220-225.
100. de Jong RM, Willemsen AT, Slart RH, et al. Myocardial beta-adrenoceptor downregulation in idiopathic dilated cardiomyopathy measured in vivo with PET using the new radioligand (S)-[11C]CGP12388. Eur J Nucl Med Mol Imaging. 2005; 32:443-447.
101. Le Guludec D, Cohen-Solal A, Delforge J, et al. Increased myocardial muscarinic receptor density in idiopathic dilated cardiomyopathy: an in vivo PET study. Circulation. 1997; 96:3416-3422.

Chapter 9

Conclusions and future perspectives

Mariëlle C. van de Veerdonk

Conclusions

Based on the findings presented in this thesis, we may conclude that in patients with pulmonary arterial hypertension (PAH):

- Right heart remodeling is a spatial heterogeneous process. Right ventricular (RV) trabeculae show more hypertrophy than the RV free wall. The interventricular septum (IVS) shows a different remodeling pattern compared to the RV free wall.
- RV dysfunction can progress despite a reduction in pulmonary vascular resistance (PVR) after current medical therapies. A decrease in RV function is associated with poor survival, regardless of the changes in PVR.
- Only if PVR is reduced more than 42% after medical treatment, stabilization of RV function and better survival are guaranteed.
- Male patients show a worse RV function compared to female patients despite a similar elevated afterload. This might explain the worse survival rates of males compared to females.
- In longstanding seemingly clinical stable patients under medical treatment, changes in RV volumes precede an ultimate clinical deterioration.

Future perspectives

RV parameters as endpoint in clinical trials

In order to accurately study RV structural remodeling in PAH patients, RV trabeculae cannot be neglected from the RV mass assessment (**Chapter 2**) but the IVS should be not be included (**Chapter 3**). Although RV mass is of physiological importance, the clinical relevance remains uncertain because RV mass might be an insensitive measure to assess disease severity due to the modest observer-reproducibility and relative small changes in RV mass during follow-up.

Changes in RV volumetric and functional parameters reflect disease severity and might be sensitive measures to predict clinical worsening and survival (**Chapters 4-7**)[1]. These results raise the question whether RV volumes and function could be valuable endpoints in clinical trials. As a first step, future studies are required to find the extent of change in RV measures that will be of clinical importance for monitoring therapeutic effects during follow-up. The clinical important difference in stroke volume has been determined and was found to be 10ml[2]. In addition, a change in RV ejection fraction (RVEF) >3% could be of clinical relevance (**Chapters 4 and 5**). Although the repeatability of RV end-diastolic volume was found to be 10ml[3], the clinical important difference has to be determined. To date

RV structural and functional parameters have not served as endpoints in clinical trials studying new therapies, but it is time to use RV imaging parameters as an endpoint in the near future.

Raising the bars in the treatment of PAH

Despite the application of current medical therapies, survival remains unsatisfactory[4,5]. This might be explained by the fact that in a substantial number of PAH patients, the RV shows progressive dysfunction despite a decrease in PVR and is associated with poor survival (**Chapter 4**). Preservation of RV function is the yardstick for long-term survival. Therefore, we have prospectively initiated the "*Goal Oriented Strategy to Preserve Ejection fraction trial*" (GOSPEL) (to assess whether a goal oriented strategy to preserve RV function will result in improved clinical outcome of PAH patients. A stable/improved RVEF is considered as the primary outcome parameter.

Although the mean PVR reduction after current therapies might be insufficient to improve RV function, a strong decrease in PVR guaranteed a stabilized/increased RVEF and was related to long-term survival. In correspondence with previous studies[5-8], a strong PVR decrease was associated with the application of (upfront) combination therapies (**Chapter 6**). In line with these findings, we hypothesize in our prospective study that RV function can only be preserved when early and aggressive combination therapies result in a significant PVR reduction. When this strategy not only result in a preserved CO but also leads to a decreased PAP, RV work will be decreased relieving stress on the RV and contributing to RV preservation[9].

Improve insights and treatment of the RV

The development of RV dysfunction is perhaps not fully explained by the elevated load. It remains insufficiently understood why some patients show progressive RV dysfunction despite a decrease in PVR after therapy (**Chapter 4**). Alternatively, it remains unclear why some patients with a long-term stable clinical condition do not show progressive RV remodeling during ten years of follow-up despite a significantly elevated afterload **(Chapter 7)**. RV function is profoundly influenced by the sex of the individual (**Chapter 5**) and other factors might play an additional role[10-12]. Future studies are required to improve our knowledge in the underlying processes of the development of RV failure. Load-independent RV measures might contribute to improved insights in intrinsic RV properties[13]. Advanced imaging of the RV by (hybrid) MRI and PET might lead to better pathophysiological understanding in the near future (**Chapter 8**).

At the same time it is pertinent to think about direct RV targeted therapies. Several experimental studies have suggested a potential benefit of betablocker therapies in PAH[14-17]. In a recent retrospective clinical study, it

was demonstrated that betablocker therapies did not adversely affect survival[18]. At the VU Medical Center, a phase-II prospective clinical study has been initiated to assess the safety and effects of bisoprolol on RV function in PAH patients.

In short, the results of this thesis clearly showed that the RV is not only a mere bystander in PAH but an important determinant of the symptoms and outcome of the patient. Therefore rigorous monitoring of the RV and the search for RV targeted therapy are important tools to improve outcome in PAH in the near future.

References

1. Peacock AJ, Crawley S, McLure L, et al. Changes in Right Ventricular Function Measured by Cardiac Magnetic Resonance Imaging in Patients Receiving Pulmonary Arterial Hypertension-targeted Therapy: The EURO-MR Study. Circulation. 2014; 7:107-114.
2. van Wolferen SA, van de Veerdonk MC, Mauritz GJ, et al. Clinically significant change in stroke volume in pulmonary hypertension. Chest. 2011; 139:1003-1009.
3. Bradlow WM, Hughes ML, Keenan NG, et al. Measuring the heart in pulmonary arterial hypertension (PAH): implications for trial study size. J Magn Reson Imaging. 2010; 31:117-124.
4. Benza RL, Miller DP, Gomberg-Maitland M, et al. Predicting survival in pulmonary arterial hypertension: insights from the Registry to Evaluate Early and Long-Term Pulmonary Arterial Hypertension Disease Management (REVEAL). Circulation. 2010; 122:164-72.
5. Humbert M, Barst RJ, Robbins IM, et al. Combination of bosentan with epoprostenol in pulmonary arterial hypertension: BREATHE-2. The Eur Respir J. 2004; 24:353-359.
6. Kemp K, Savale L, O'Callaghan DS, et al. Usefulness of first-line combination therapy with epoprostenol and bosentan in pulmonary arterial hypertension: an observational study. J Heart Lung Transplant. 2012; 31:150-158.
7. Jacobs W, Boonstra A, Marcus JT, et al. Addition of prostanoids in pulmonary hypertension deteriorating on oral therapy. J Heart Lung Transplant. 2009; 28:280-284.
8. Simonneau G, Rubin LJ, Galie N, et al. Addition of sildenafil to long-term intravenous epoprostenol therapy in patients with pulmonary arterial hypertension: a randomized trial. Ann Intern Med. 2008; 149:521-530.
9. Handoko ML, de Man FS, Allaart CP, et al. Perspectives on novel therapeutic strategies for right heart failure in pulmonary arterial hypertension: lessons from the left heart. Eur Respir Rev. 2010; 19:72-82.
10. Voelkel NF, Gomez-Arroyo J, Abbate A, et al. Mechanisms of right heart failure-A work in progress and a plea for failure prevention. Pulm Circ. 2013; 3:137-143.
11. Kawut SM, Al-Naamani N, Agerstrand C, et al. Determinants of right ventricular ejection fraction in pulmonary arterial hypertension. Chest. 2009; 135:752-759.
12. Voelkel NF, Quaife RA, Leinwand LA, et al. Right ventricular function and failure: report of a National Heart, Lung, and Blood Institute working group on cellular and molecular mechanisms of right heart failure. Circulation. 2006; 114:1883-1891.
13. Rain S, Handoko ML, Trip P, et al. Right ventricular diastolic impairment in patients with pulmonary arterial hypertension. Circulation. 2013; 128:2016-2025.

14. de Man FS, Handoko ML, van Ballegoij JJ, et al. Bisoprolol delays progression towards right heart failure in experimental pulmonary hypertension. Circ Heart Fail. 2012; 5:97-105.
15. Bogaard HJ, Natarajan R, Mizuno S, et al. Adrenergic receptor blockade reverses right heart remodeling and dysfunction in pulmonary hypertensive rats. Am J Respir Crit Care Med. 2010; 182:652-660.
16. Ishikawa M, Sato N, Asai K, et al. Effects of a pure alpha/beta-adrenergic receptor blocker on monocrotaline-induced pulmonary arterial hypertension with right ventricular hypertrophy in rats. Circ J. 2009; 73:2337-2341.
17. Usui S, Yao A, Hatano M, et al. Upregulated neurohumoral factors are associated with left ventricular remodeling and poor prognosis in rats with monocrotaline-induced pulmonary arterial hypertension. Circ J. 2006; 70:1208-1215.
18. So PP, Davies RA, Chandy G, et al. Usefulness of beta-blocker therapy and outcomes in patients with pulmonary arterial hypertension. Am J Cardiol. 2010; 109:1504-1509.

Summary

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Summary

Pulmonary arterial hypertension (PAH) is a progressive disease of the small pulmonary arteries that results in increased pulmonary vascular resistance (PVR) and pulmonary artery pressure (PAP). As a consequence, the right ventricle (RV) has to cope with the increased afterload in order to provide sufficient cardiac output (CO). The RV develops hypertrophy, dilates and shows changes in metabolism. However, the RV is not suitable to cope with longstanding pressure overload, resulting in RV maladaptation, decreased RV function and ultimately RV failure and death. The current medical treatment strategy is aimed to improve RV function by reducing the afterload. During the last decade, multiple medical therapies have been developed that have mainly pulmonary vasodilator effects resulting in reduced PVR and improved CO. Despite these therapeutic effects, survival remains unsatisfactory. Therefore more insights are warranted in how RV dysfunction evolves structurally and functionally during the course of disease and under medical treatment.
The aims of this thesis are twofold. The first aim was to provide improved insights in RV structural remodeling to pressure overload. Secondly, we aimed to assess the changes in RV adaptation and function over time in relationship to the changes in afterload under current PAH targeted medical therapies. In this thesis, we performed RV assessment by magnetic resonance imaging (MRI), which is considered the gold standard to measure RV mass, volumes and function.

In **Chapter 2**, we studied the hypertrophic response of the RV in PAH patients in comparison to control subjects. The RV muscle mass does not only consist of the RV free wall but also includes many trabeculae: small muscle bundles that support the RV free wall. However, RV trabeculae have been generally ignored in previous literature. Therefore, by use of MRI and right heart catheterization (RHC), we studied in 50 PAH patients and 20 control subjects the hypertrophic response of both the RV free wall and trabeculae to pressure overload and assessed the changes in these RV mass compartments during one year of medical treatment. We found that the RV trabeculae showed a larger contribution to total RV mass in PAH patients (~35%) compared to controls (~25%) ($p < 0.001$). Moreover, in PAH patients the changes in mass of the RV trabeculae were stronger related to the changes in PVR and PAP after one year of medical treatment than the mass of the RV free wall. These results implicate that trabeculae are important contributors to RV adaptation to chronic pressure overload in PAH.

During chronic RV pressure overload, interventricular dyssynchrony is associated with leftward bowing of the interventricular septum (IVS) and

disturbed IVS function. However, it is unknown what tissue alterations occur in the IVS and if they are comparable to the changes in the RV free wall. In **Chapter 3**, we studied in 17 PAH patients by MRI and PET the mass and glucose metabolism of the RV, IVS and left ventricle (LV). In addition, we studied in more detail whether cellular alterations of the IVS were comparable to the RV free wall in a monocrotoline pulmonary hypertension (PH) rat model. In PAH patients, we found that the changes in IVS mass and glucose metabolism were not comparable to the changes in the RV free wall (both p for difference <0.001). In addition, the cardiomyocyte cross-sectional area and capillary density remained preserved in the IVS but were impaired in the RV free wall of PH rats (both p interaction <0.001). Furthermore, we found that although fibrosis and inflammation were increased in both the IVS and the RV in PH rats, the magnitude of increase was significantly lower for the IVS (both p interaction <0.001). These results demonstrate that despite a similar pressure overload in PAH, IVS morphology and metabolism remain better preserved and do not resemble the remodeling of the RV free wall.

Despite a reduction in PVR accomplished by current medical therapies, survival of PAH patients remains grim. This might be explained by the fact that this PVR reduction does not automatically result in improved RV function. Therefore, in **Chapter 4** we studied the relationship between the changes in PVR and changes in RV function and survival after medical treatment. A large group of PAH patients underwent MRI to measure RV ejection fraction (RVEF) as a measure of systolic RV function and RHC to measure PVR at baseline and after one year of treatment. We found that the changes in PVR were moderately related to the changes in RVEF (R = 0.33; p = 0.001). 68% of the patients showed a reduced PVR after medical treatment. In 75% of these therapeutic responders, this was associated with a stable or improved RV function and favorable survival. However, 25% of these patients showed despite a similar decrease in PVR, progressive RV dysfunction which was associated with poor survival.

Since male gender is associated with a poor survival, we assessed in **Chapter 5** whether this could be explained by a distinct vascular or RV response to medical treatment. At baseline, we observed that RVEF and PVR were comparable between males and females. Both genders showed a similar reduction in PVR after medical treatment (p for difference = 0.63). However, females showed an improved RVEF while males showed a deterioration in RVEF after medical treatment (p <0.001 after correction for confounders) which was associated with a poor survival. One third of the effects of gender on survival was mediated by the differences in RVEF, which indicates that also other factors could play an important role in the gender disparity in PAH.

In **Chapter 6**, we hypothesized that a strong reduction in PVR after medical treatment will result in an guaranteed improvement of RV function and survival. In this chapter, we investigated the relationships between baseline PVR, PVR-response, changes in RV adaptation and function and survival after current PAH therapies. we demonstrated that a threshold decrease in the relative PVR change of more than 42% was feasible in a substantial number of PAH patients and was associated with an improvement in RV function, reduction of RV dilatation and long-term survival. A strong PVR decrease was related to the application of upfront combination therapies.

Even after years of a stable response to medical treatment (defined by a stable New York Heart Association (NYHA) functional class II-III and six-minute walking test), PAH patients may show an unexpected rapid clinical deterioration due to progressive RV failure, which is associated with a high mortality rate. If the RV would already demonstrate signs of progressive adverse remodeling during the initial stable clinical condition, assessment of RV remodeling parameters might predict an ultimate disease progression. In **Chapter 7**, we assessed in 22 five-year clinically stable patients with PAH whether differences in RV volumes, precede an ultimate disease progression. We compared regularly obtained RHC and MRI measures between twelve patients who remained stable during 10 year of follow-up and ten other patients who showed late disease progression leading to death or lung transplantation after a median duration of eight years. We found that RV remodeling can be progressive, even in PAH patients who are seemingly clinically stable during 5-10 years of follow-up. Moreover, we showed that an ultimate disease progression is preceded by changes in RV volumes and RVEF and not by differences in NYHA functional class, exercise capacity or hemodynamic parameters.

In **Chapter 8**, we provided an overview of advanced *in vivo* imaging of the RV by cardiac MRI and PET. We show that imaging of RV hypertrophy, shape, dilatation, wall stress, global function, contractility, dyssynchrony and IVS bowing, perfusion and metabolism have significantly contributed to a better understanding of the pathophysiological processes that contribute to the development of chronic RV failure in PAH. Furthermore, we explained that in the near future it can be expected that the importance of changes in tissue processes such as angiogenesis, apoptosis and neurohormonal factors will become more clear and ‘imageable’. In addition, recent advances in hybrid PET and MRI might allow integrated assessment of the RV in vivo and will be one of the most important future developments.

Summary (Dutch)

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Het aanpassingsvermogen en functioneren van de rechter hartkamer in pulmonale arteriële hypertensie

Pulmonale arteriële hypertensie (PAH) is een chronisch, progressieve aandoening waarbij de bloedvaten in de longen zijn veranderd en vernauwd. Hierdoor ontstaat een verhoogde weerstand en bloeddruk in de longvaten. De diagnose kan worden gesteld wanneer de bloeddruk in de longvaten hoger is dan 25 mmHg, invasief gemeten tijdens een rechter hartkatheterisatie. De aandoening is zeldzaam en de oorzaak is vaak onbekend (idiopathisch) maar kan ook erfelijk zijn of geassocieerd met andere aandoeningen zoals aangeboren hartafwijkingen, bindweefsel ziekten, drugs of medicijnen. PAH komt twee keer zo vaak bij vrouwen voor. De leeftijd waarop de diagnose wordt gesteld, is gemiddeld 40 jaar.
Het gevolg van de verhoogde longvaatweerstand en bloeddruk, is dat het de rechter hartkamer hindert in het pompen van bloed richting de longen. De rechter hartkamer probeert zich aan te passen door meer spiermassa te ontwikkelen en door uit te zetten. Ook treden er bijvoorbeeld veranderingen in metabolisme op. De rechter hartkamer die normaal niet berekend is op deze hoge longvaatweerstand en bloeddruk, is onvoldoende in staat om zich langdurig aan te passen waardoor het in conditie achteruit zal gaan. Dit uit zich bij patiënten in klachten van kortademigheid bij inspanning en vermoeidheid. Uiteindelijk overlijden PAH patiënten ten gevolge van het falen van de rechter hartkamer.
In de afgelopen 15 jaar zijn er meerdere medicijnen ontwikkeld die zich richten op het verlagen van de longvaatweerstand en daarmee de rechter hartkamer proberen te verbeteren. Met behulp van deze medicijnen zijn de klachten van patiënten verminderd en is de overlevingsduur verlengd, niettemin blijft PAH een ongeneeslijke ziekte. Daarom is meer begrip nodig in het structurele en- functionele aanpassingsvermogen van de rechter hartkamer aan de chronische overbelasting ten gevolge van de verhoogde longvaatweerstand. Tevens is meer inzicht noodzakelijk in de effecten van huidige medicatie op de rechter hartkamer.

Dit proefschrift heeft twee doelen: enerzijds meer inzicht verkrijgen in de aanpassing van de rechter hartkamer aan de verhoogde longvaatweerstand van patiënten met PAH. Anderzijds was het doel om de veranderingen in de aanpassing en het functioneren van de rechter hartkamer over tijd te bestuderen in relatie met de veranderingen in de longvaatweerstand die teweeg worden gebracht met behulp van de huidige medicijnen.
Voor dit proefschrift maakten we gebruik van magnetische resonantie imaging (MRI). Dit is beeldvormend onderzoek waarmee de spiermassa,

mate van uitzetting en functie van de rechter hartkamer het meest precies en betrouwbaar kunnen worden gemeten.

De spiermassa van de rechter hartkamer bestaat niet alleen uit de wand maar ook uit vele trabecula (kleine bundeltjes spierweefsel die extra steun geven aan de wand). Dit laatste aspect wordt echter vaak buiten beschouwing gelaten. In **Hoofdstuk 2** hebben we daarom niet alleen naar de veranderingen in spiermassa van de wand maar ook van trabecula gekeken in relatie tot de chronische overbelasting op de rechter hartkamer voor en na medicamenteuze behandeling. In dit onderzoek bestudeerden we 50 patiënten met PAH en 20 gezonde controles. We vonden dat de trabecula een grotere bijdrage leverden aan de totale spiermassa van de rechter hartkamer in PAH patiënten (~35%) dan in de controlegroep (~25%). Verder zagen we dat in behandelde PAH patiënten, de veranderingen in bloeddruk en weerstand van de longvaten sterker waren gerelateerd aan veranderingen van de massa van trabecula dan aan de veranderingen in massa van de wand van de rechter hartkamer. Deze resultaten impliceren dat de trabecula mogelijk een belangrijke bijdrage leveren aan het aanpassingsvermogen van de rechter hartkamer in PAH. Trabecula moeten daarom niet genegeerd worden in onderzoek naar de rechter hartkamer.

Het doorbuigen richting de linker hartkamer van het septum, het tussenschot dat de rechter en de linker hartkamer van elkaar scheidt, is kenmerkend voor patiënten met PAH. Het septum heeft in het gezonde hart een substantiële bijdrage aan de normale functie van de rechter hartkamer. Het is bekend dat het septum in PAH niet optimaal kan functioneren en dat de septum doorbuiging zorgt voor belemmering van de functie van de linker hartkamer. Er is echter nog nooit onderzoek gedaan naar onderliggende, structurele veranderingen in het septum in PAH. In **Hoofdstuk 3** onderzochten we of er onderliggende structurele veranderingen optreden in het septum en of deze lijken op de veranderingen zoals deze ook worden gezien in de wand van de rechter hartkamer in patiënten met PAH. Ook keken we naar de veranderingen in metabolisme met behulp van positron emissie tomografie beeldvorming (PET). We deden niet alleen onderzoek in patiënten met PAH, maar verrichten ook onderzoek in een ratten-model met pulmonale hypertensie om te kijken welke veranderingen er op celniveau optreden. We vonden in PAH patiënten dat het septum geen toename liet zien in spiermassa, in tegenstelling tot de wand van de rechter hartkamer. Ook zagen we dat het septum qua metabolisme niet leek op de rechter hartkamer. Op celniveau vonden we in ratten met pulmonale hypertensie dat de grootte van de hartspiercellen en het aantal bloedvaatjes in het septum niet veranderden ten opzichte van gezonde ratten. In de rechter hartkamer van ratten met pulmonale hypertensie

waren de hartspiercellen echter sterk vergroot en het aantal bloedvaatjes afgenomen. Ondanks het feit dat ratten met pulmonale hypertensie wel een toename in bindweefsel en ontsteking lieten zien in het septum, was deze toename significant kleiner dan in de wand van de rechter hartkamer. Op basis van dit onderzoek kunnen we concluderen dat het septum in PAH beter bestand is tegen de verhoogde vaatweerstand en bloeddruk in de longen en dat het niet dezelfde veranderingen laat zien als de wand van de rechter hartkamer.

De discrepantie tussen het gunstige effect van de medicijnen op de longvaatweerstand en de blijvend slechte overleving van patiënten met PAH, doet de vraag oproepen of deze longvaatweerstand verlaging wel automatisch leidt tot een verbetering van de rechter hartkamer functie. Dit hebben we in **Hoofdstuk 4** onderzocht. Met behulp van MRI hebben we de functie van de rechter hartkamer gemeten en door middel van een rechter hartkatheterisatie hebben we de longvaatweerstand gemeten voor en na 1 jaar medicamenteuze behandeling. Deze studie toonde aan dat de verandering in de rechter hartkamerfunctie na behandeling matig was gerelateerd aan de veranderingen in longvaatweerstand. We vonden dat in de meeste patiënten inderdaad een verlaging van de vaatweerstand teweeg werd gebracht met behulp van medicijnen. In 75% van deze patiënten was dit geassocieerd met een verbetering van de rechter hartkamerfunctie. In 25% van de PAH patiënten was dit echter niet het geval en was er sprake van een korte overlevingsduur.

Ondanks het feit dat PAH vaker voorkomt onder vrouwen, hebben mannen een kortere overlevingsduur. In **Hoofdstuk 5** onderzochten we of dit verschil in overleving verklaard kan worden door een verschillende respons op behandeling. We lieten zien dat voor de start van behandeling mannen en vrouwen een vergelijkbare longvaatweerstand en rechter hartkamerfunctie hadden. Na 1 jaar behandeling lieten beide groepen een vergelijkbare daling in longvaatweerstand zien. Vrouwen lieten daarop een verbetering van de functie van de rechter hartkamer zien, terwijl deze functie bij mannen verslechterde. Deze verslechtering in hartfunctie was gerelateerd aan de kortere overlevingsduur in mannen met PAH.

In **Hoofdstuk 6** vroegen we ons af of de achteruitgang van de rechter hartkamerfunctie ondanks een verlaging in longvaatweerstand verklaard zou kunnen worden door het feit dat de huidige behandelingen gemiddeld een te kleine verandering in longvaatweerstand teweeg brengen om de rechter hartkamer de kans te geven om te verbeteren. In deze studie vergeleken we verschillende maten van dalingen in de longvaatweerstand en onderzochten de effecten daarvan op de functie van de rechter hartkamer en overleving. In dit hoofdstuk lieten we zien dat alleen wanneer

de longvaatweerstand met meer dan 42% werd verlaagd met behulp van medicatie, dit gegarandeerd leidde tot een stabilisatie/verbetering van de rechter hartkamerfunctie en geassocieerd was met een langdurige overleving.

Een belangrijk probleem in de kliniek is dat na een lange stabiele situatie onder medicamenteuze behandeling, een patiënt met PAH soms heel onverwachts kan verslechteren ten gevolge van achteruitgang van de rechter hartkamer. In **Hoofdstuk 7** hebben we onderzocht aan welke parameters we zouden kunnen herkennen welke patiënt uiteindelijk zal gaan verslechtering en bij welke patiënt dit niet zal gebeuren. In dit hoofdstuk toonden we aan dat ondanks een stabiele klinische situatie, de rechter hartkamer al veranderingen kan laten zien in het aanpassingsvermogen en dan vooral in de mate van uitzetting (dilatatie). De mate van uitzetting was een voorspeller voor een toekomstige klinische achteruitgang. Deze uiteindelijke achteruitgang kon niet voorspeld door de initiële klinische conditie, inspanningsvermogen en hoogte van de longvaatweerstand en bloeddruk. De resultaten van deze studie impliceren dat het in de gaten houden van het aanpassingsvermogen van de rechter hartkamer belangrijk is om de eerste tekenen van hartfalen te kunnen ontdekken om daarmee een uiteindelijke verslechtering te kunnen voorkomen.

In **Hoofdstuk 8** geven we een overzicht van de mogelijke manieren om de rechter hartkamer te onderzoeken met behulp van MRI en PET. Ook beschrijven we methoden die nog in ontwikkeling zijn maar uiteindelijk een belangrijke rol kunnen gaan spelen in het verkrijgen van nieuwe inzichten in de ontwikkeling en preventie van hartfalen in patiënten met PAH.

Conclusies

Samenvattend heeft dit proefschrift inzicht gegeven in het structurele en functionele aanpassingsvermogen van de rechter hartkamer op de verhoogde druk en weerstand in de longvaten van patiënten met PAH. Op basis van dit proefschrift kunnen we in patiënten met PAH de volgende conclusies trekken:

- Tijdens chronische drukbelasting tonen de trabecula een sterkere toename in spiermassa dan de wand van de rechter hartkamer. Het septum laat niet dezelfde veranderingen als de wand van de rechter hartkamer zien, ondanks dezelfde drukbelasting.
- De functie van de rechter hartkamer kan afnemen ondanks een verlaging van de weerstand in de longen na medicamenteuze behandeling. Een verslechtering in de functie van de rechter

hartkamer, ongeacht de veranderingen in longvaatweerstand, is geassocieerd met een korte overlevingsduur.

- Mannen hebben een slechtere functie van de rechter hartkamer dan vrouwen ondanks een vergelijkbare longvaatweerstand en bloeddruk. Dit verschil kan de kortere overlevingsduur van mannen ten opzichte van vrouwen verklaren.
- Alleen een sterke verlaging van de longvaatweerstand garandeert een stabilisatie/verbetering van de rechter hartkamerfunctie en is geassocieerd met een langdurige overleving.
- In patiënten met een langdurig stabiele respons op medicamenteuze behandeling kan een uiteindelijke verslechtering in een vroeg stadium worden voorspeld op basis van veranderingen in de mate van uitzetting van de rechter hartkamer.

List of abbreviations

6MWT	= six-minute walking test
APAH	= associated pulmonary arterial hypertension
ATP	= adenosine triphosphate
BMI	= body mass index
BSA	= body surface area
CMR	= cardiac magnetic resonance imaging
CO	= cardiac output
CI	= cardiac index
CSA	= cross sectional area
CT	= computed tomography
DCE	= delayed contrast enhancement
ERA	= endothelin receptor antagonist
^{18}FDG	= ^{18}F-2-deoxy-2-fluoro-D-Glucose
GFR	= glomerular filtration rate
HPAH	= heritable pulmonary arterial hypertension
HR	= heart rate
IPAH	= idiopathic pulmonary arterial hypertension
IVS	= interventricular septum
LV	= left ventricle or left ventricular
LVEDV	= left ventricular end-diastolic volume
LVEF	= left ventricular ejection fraction
LVESV	= left ventricular end-systolic volume
mPAP	= mean pulmonary artery pressure
MRglu	= myocardial glucose uptake
MRI	= magnetic resonance imaging
MRS	= magnetic resonance spectroscopy
MVO_2	= myocardial oxygen consumption
NT-proBNP	= N-terminal pro-brain natriuretic peptide
NYHA	= New York Heart Association
OEF	= oxygen extraction fraction
PAH	= pulmonary arterial hypertension
PAP	= pulmonary artery pressure
PAWP	= pulmonary arterial wedge pressure
PCWP	= pulmonary capillary wedge pressure
PDE-5I	= phosphodiesterase five inhibitor
PET	= positron emission tomography
PH	= pulmonary hypertension
PAH	= pulmonary arterial hypertension
PVOD	= pulmonary veno-occlusive disease
PVR	= pulmonary vascular resistance
RAP	= right atrial pressure
RHC	= right heart catheterization

ROC	= receiver operating characteristic curve analysis
RV	= right ventricle or right ventricular
RVEDV	= right ventricular end-diastolic volume
RVEF	= right ventricular ejection fraction
RVESV	= right ventricular end-systolic volume
RVFW	= right ventricular free wall
SPECT	= single photon emission computed tomography
SV	= stroke volume
SvO_2	= mixed venous oxygen saturation
TAPSE	= tricuspid annular plane systolic excursion
TPM	= trabeculae and papillary muscles
WHO FC	= world health organization functional class

List of publications

Full papers

Contractile dysfunction of left ventricular cardiomyocytes in patients with pulmonary arterial hypertension

E. Manders, H.J. Bogaard, M.L. Handoko, **M.C. van de Veerdonk**, A. Keogh, N. Westerhof, G.J.M. Stienen, C. G. dos Remedios, M. Humbert, P. Dorfmüller, E. Fadel, C. Guignabert, J. van der Velden, A. Vonk Noordegraaf, F. S. de Man, C.A. C. Ottenheijm. *J Am Coll Cardiol 2014; in press.*

The interventricular septum in pulmonary hypertension does not show features of right ventricular failure

G. Ruiter*, **M.C. van de Veerdonk***, H.J. Bogaard, J.T. Marcus, A.A. Lammertsma, N. Westerhof, W.J. van der Laarse, F.S. de Man, A. Vonk Noordegraaf. *Int J Cardiol 2014; epub ahead of print.*

Right ventricular dilatation precedes late clinical progression of initially stable patients with pulmonary arterial hypertension

M.C. van de Veerdonk, J.T. Marcus, H.J. Bogaard, F.S. de Man, A. Boonstra, M.W. Heijmans, N. Westerhof, A. Vonk Noordegraaf. *Submitted.*

Cardiac MRI and PET scanning in right ventricular failure (*Book chapter*).

M.C. van de Veerdonk, J.T. Marcus, H.J. Bogaard, A. Vonk Noordegraaf. *2014; in press.*

State of the Art: Advanced Imaging of the Right Ventricle and Pulmonary Circulation in Humans (*Review*).

M.C. van de Veerdonk, J.T. Marcus, H.J. Bogaard, A. Vonk Noordegraaf. *Pulm Circ 2014; in press.*

The importance of trabecular hypertrophy in right ventricular adaptation to chronic pressure overload.

M.C. van de Veerdonk, S.A. Dusoswa, J.T. Marcus, O.A. Spruijt, T. Kind, H.J. Bogaard, A. Boonstra, N. Westerhof, A. Vonk Noordegraaf. *Int J Cardiovasc Im 2014;* 30:357-365.

The right ventricle explains sex differences in survival in idiopathic pulmonary arterial hypertension patients

W. Jacobs, **M.C. van de Veerdonk**, P. Trip, F.S. de Man, M.W. Heijmans, J.T. Marucs, S.M. Kawut, H.J. Bogaard, A. Boonstra, A. Vonk Noordegraaf. *Chest 2013; epub ahead of print.*

Changes in Right Ventricular Function Measured by Cardiac Magnetic Resonance Imaging in Patients Receiving Pulmonary Arterial Hypertension-targeted Therapy; The EURO-MR Study.

A.J. Peacock, S. Crawley, L. McLure, K. Blyth, C.D. Vizza, R. Poscia, M. Francone, I. Iacucci, H. Olschewski, G. Kovacs, A. Vonk Noordegraaf, J.T. Marcus, **M.C. van de Veerdonk**, F.P. Oosterveer. *Circ Cardiovasc Im 2014; 7:107-114.*

Prognostic relevance of exercise parameters in pulmonary arterial hypertension.

H. Groepenhoff, A. Vonk Noordegraaf, **M.C. van de Veerdonk**, A. Boonstra, N. Westerhof, H.J. Bogaard. *PLoS One 2013; 8:e72013.*

Effects of Diaphragm Plication on Pulmonary Function and Cardiopulmonary Exercise Parameters.

W.N. Welvaart, P.M.C. Jak, **M.C. van de Veerdonk**, J.T. Marcus, C.A.C. Ottenheijm, M.A. Paul, A. Vonk Noordegraaf. *Eur J Cardiothorac Surg 2013; 44:643-647.*

Accurate assessment of right ventricular systolic function in patients with pulmonary hypertension.

P. Trip, T. Kind, **M.C. van de Veerdonk**, J.T. Marcus, F.S. de Man, N. Westerhof, A. Vonk Noordegraaf. *J Thor Heart Lung 2012; 32:50-55.*

Progressive Right Ventricular Dysfunction in Patients with Pulmonary Arterial Hypertension Responding to Therapy.

M.C. van de Veerdonk, T. Kind, J.T. Marcus, G.J. Mauritz, M.W. Heijmans, H.J. Bogaard, A. Boonstra, K.M.J. Marques, N. Westerhof, A. Vonk-Noordegraaf. *J Am Coll Cardiol 2011; 58:2511-2519.*

Progressive Changes in Right Ventricular Geometric Shortening and Long-term Survival in Pulmonary Arterial Hypertension.

G.J. Mauritz, T. Kind, J.T. Marcus, H.J. Bogaard, **M. van de Veerdonk**, P.E. Postmus, N. Westerhof, A. Vonk-Noordegraaf. *Chest 2011; 141:934-943.*

Clinically significant change in stroke volume in pulmonary hypertension.

S.A. Van Wolferen, **M.C. van de Veerdonk**, G.J. Mauritz, W. Jacobs, J.T. Marcus, K.M. Marques, J.G. Bronzwaer, M.W. Heymans, A. Boonstra, P.E. Postmus, N. Westerhof, A. Vonk Noordegraaf. *Chest 2010;* 139:1003-1009.

Right ventricular ejection fraction is better reflected by transverse rather than longitudinal wall motion in pulmonary hypertension.
T. Kind, G.J. Mauritz, J.T. Marcus, **M. Van de Veerdonk**, N. Westerhof, A. Vonk Noordegraaf. J Cardiovasc Magn Reson 2010; 12:35.

Maggot excretions inhibit biofilm formation on biomaterials.
G. Cazander, **M.C. van de Veerdonk**, C. M. Vandenbroucke-Grauls, M.W. Schreurs, G. N. Jukema. Clin Orthop Relat Res 2010; 468:2789-2796.

Oral presentations

MRI and right ventricular function.
M.C. van de Veerdonk. PAH meeting. *Oslo, Norway, 2013.*

Improved right ventricular function after substantial afterload reduction in patients with pulmonary arterial hypertension.
M.C. van de Veerdonk, J.T. Marcus, H.J. Bogaard, N. Westerhof, A. Vonk Noordegraaf. *European Respiratory Society Conference*, *Vienna, Austria 2012.*

In pulmonary hypertension changes in right ventricular ejection fraction better predict mortality than changes in pulmonary vascular resistance.
M.C. van de Veerdonk, J.T. Marcus, N. Westerhof, A. Vonk-Noordegraaf. *European Respiratory Society Conference, Barcelona, Spain 2010.*

MRI one stop shop for severe pulmonary arterial hypertension.
M.C. van de Veerdonk. Multimodality imaging and decision making in cardiology. La Sapienza University, Rome, Italy, 2013.

MRI one stop shop for severe pulmonary arterial hypertension.
M.C. van de Veerdonk. Multimodality imaging and decision making in cardiology. La Sapienza University, Rome, Italy, 2012.

Imaging the right ventricle in pulmonary arterial hypertension.
M.C. van de Veerdonk. Stanford University, San Francisco, USA, 2012.

The relationship between right ventricular function and afterload in pulmonary arterial hypertension.

M.C. van de Veerdonk. CPC-INSERM Retreat, Munich, Germany, 2012.

The cardiorenal syndrome in pulmonary arterial hypertension.

M.C. van de Veerdonk. Erasme Hospital, Free University, Brussels, Belgium, 2011.

Poster presentations

Characteristics of a stable and satisfactory condition in long-term survivors of pulmonary arterial hypertension.

M.C. van de Veerdonk, J.T. Marcus, H.J. Bogaard, F.S. de Man, A. Boonstra, N. Westerhof, A. Vonk Noordegraaf. *European Respiratory Society Conference, Barcelona, Spain, 2013.*

Heart function and survival improve after a strong reduction in afterload in patients with pulmonary arterial hypertension.

M.C. van de Veerdonk, J.T. Marcus, H.J. Bogaard, F.S. de Man, N. Westerhof, A. Vonk Noordegraaf. *American Thoracic Society Conference, Philadelphia, USA, 2013.*

Changes in right ventricular mass are related to changes in pulmonary pressures in pulmonary arterial hypertension.

M.C. van de Veerdonk, S.A. Dusoswa, T. Kind, J.T. Marcus, N. Westerhof, A. Vonk Noordegraaf. *European Respiratory Society Conference, Amsterdam, The Netherlands, 2011*

Association of renal dysfunction with cardiac output and right atrial pressure in pulmonary arterial hypertension.

M.C. van de Veerdonk, A. Boonstra, A. Vonk Noordegraaf. *European Respiratory Society Conference, Amsterdam, The Netherlands, 2011*.

Changes in Right Ventricular Ejection Fraction rather than Pulmonary Vascular Resistance Predict Outcome in Pulmonary Arterial Hypertension.

M.C. van de Veerdonk, J.T. Marcus, N. Westerhof, A. Vonk-Noordegraaf. *American Thoracic Society Conference, New Orleans, USA, 2010.*

Right Ventricular Function is a Strong Prognostic Predictor of Mortality in Pulmonary Arterial Hypertension.

M.C. van de Veerdonk, T. Kind, G.J. Mauritz, N. Westerhof, J.T. Marcus, A. Vonk-Noordegraaf. *European Respiratory Society Conference, Vienna, Austria, 2009.*

De interactie van madenexcreten en bacteriële biofilms op traumatologisch-orthopedische materialen.

G. Cazander, **M.C. van de Veerdonk**, C.M.J.E. Vandenbroucke-Grouls, G.N. Jukema. *Chirurgendagen, Veldhoven, the Netherlands, 2009.*

Grants

2013 ATS Scholarship. **M.C. van de Veerdonk.** *American Thoracic Society Conference*, Philadelphia, USA, 2013.

2010 ERS Young Scientist Sponsorship. **M.C. van de Veerdonk.** *European Respiratory Society Conference*, Barcelona, 2010.

Curriculum vitae

Mariëlle Carolijn van de Veerdonk was born in Hilversum, the Netherlands on February 19, 1986. In 2004 she completed secondary school (vwo) at the Erfgooiers College in Huizen. After first being eliminated by numerous clausus from medical school she started the study Psychology and finished her propaedeutic in 2005. Subsequently she started studying Medicine at the VU University of Amsterdam. In 2008 she obtained the Bachelor of Science degree. In the fourth year of medical school, she started with scientific research on pulmonary arterial hypertension at the department of pulmonary diseases of the VU University Medical Center, under supervision of prof. dr. A. Vonk Noordegraaf. After 6 months of a research internship, she continued her research as a student assistant during the internship years of medicine. She performed her final internship at the department of cardiology at the Flevoziekenhuis, Almere under supervision of dr. H. Verheul. She graduated from medical school in July 2011. Subsequently, she commenced full-time PhD research on her topic of pulmonary arterial hypertension. In January 2014, she started to work as a specialist not in training at the department of cardiology at the Sint Lucas Andreas Hospital under supervision of dr. J. Schroeder-Tanka.

Mariëlle Carolijn van de Veerdonk werd geboren te Hilversum, Nederland op 19 februari 1986. In 2004 slaagde zij voor haar eindexamen VWO op het Erfgooiers College in Huizen. Nadat zij werd uitgeloot tijdens de numerus fixus voor de geneeskunde-opleiding, begon zij aan de studie psychologie waarvan zij de propedeuse behaalde in 2005. Daarna kon zij alsnog starten met de studie geneeskunde aan de Vrije University van Amsterdam. In 2008 verkreeg zij de graad Bachelor of Science. Tijdens het vierde jaar van de studie geneeskunde begon zij aan haar wetenschappelijke stage naar pulmonale arteriële hypertensie op de afdeling longziekten van het VU medisch centrum onder begeleiding van prof. Dr. A. Vonk Noordegraaf. Na afloop van deze stage continueerde zij haar onderzoek tijdens haar co-schappen. Haar oudste co-schap deed ze op de afdeling cardiologie van het Flevoziekenhuis in Almere, onder begeleiding van dr. H. Verheul. In juli 2011 behaalde zij haar artsexamen. Aansluitend continueerde zij haar wetenschappelijk onderzoek naar pulmonale arteriële hypertensie maar nu op fulltime basis in het kader van een promotietraject. Sinds januari 2014 werkt zij als arts-assistent niet in opleiding op de afdeling cardiologie van het Sint Lucas Andreas ziekenhuis onder leiding van dr. J. Schroeder-Tanka.

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