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Pulmonary hypertension related to heart failure and hypoxia

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Experiences from Skåne University Hospital in Lund

JAKOB LUNDGREN

FACULTY OF MEDICINE | LUND UNIVERSITY



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Mechanisms, new treatment strategies and impact on
mortality following heart transplantation.
Experiences from Skåne University Hospital in Lund

Jakob Lundgren, M.D.



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DOCTORAL DISSERTATION

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To be defended at Segerfalksalen, BMC, Lund University, Lund.

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Prof. Jean-Luc Vachiéry, M.D., Ph.D., Dept. of Cardiology, CUB,

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Title Pulmonary hypertension related to heart failure and hypoxia – Mechanisms, new treatment strategies and impact on mortality following heart transplantation. Experiences from Skåne University Hospital in Lund.		
Abstract <p>Pulmonary hypertension due to left heart disease, caused by passive congestion of the pulmonary circulation is associated with poor prognosis. If long-standing, endothelial damage and subsequent vasoconstriction may occur, in some cases further complicated by vascular remodeling of the pulmonary vessels. Such remodeling may induce fixed elevated pulmonary vascular resistance, potentially impairing outcome after heart transplantation. Knowledge on physiological alterations after heart transplantation, both with regards to hemodynamics and the plasma concentrations of vasoactive substances, is scarce. There are furthermore few options for treating pulmonary hypertension due to left heart disease and hypoxic pulmonary vasoconstriction, the latter a condition which may aggravate pulmonary hypertension due to left heart disease. These issues were the focus of the present thesis.</p> <p>First, in a porcine model of hypoxic pulmonary vasoconstriction, soluble guanylate cyclase stimulation alone or in combination with dual endothelin receptor blockade was found to completely reverse acute hypoxic pulmonary vasoconstriction without affecting oxygen consumption.</p> <p>Second, in a retrospective review of the patients' heart transplanted and post-operatively followed at Skåne University Hospital in Lund, Sweden between 1988 and 2010, the impact of pre-operative and post-operative pulmonary hypertension on long-term survival was investigated. Invasive hemodynamics during slight exercise were furthermore studied to identify the hemodynamic response to exercise after heart transplantation. The findings suggest that with careful patient selection and care, pre-operative pulmonary hypertension may not be a strict contraindication for heart transplantation. Pulmonary hypertension one year after heart transplantation was, however, associated with impaired survival. Also, whereas the post-operative response to slight exercise with regards to cardiac output was adequate after heart transplantation, the patients' exhibited abnormally high ventricular filling pressures during exercise. The reason for the elevated filling pressures is probably multifactorial and possibly related to transplanted hearts being more dependent of the Frank Starling-mechanism to maintain adequate cardiac output, as well as decreased diastolic compliance.</p> <p>Finally, in a prospective cohort of consecutive heart transplanted patients without severe cardiopulmonary complications, we analyzed plasma concentrations of substances in the nitric oxide pathway prior to and after heart transplantation. The L-Arginine/ADMA-ratio, a ratio previously associated with disease severity and outcome in heart failure, was found to be markedly improved after heart transplantation and inversely correlated to pulmonary vascular resistance, suggesting an improved nitric oxide mediated vasodilatation.</p>		
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Jakob Lundgren, M.D.



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Coverphoto – *A pulmonary artery wedge pressure curve from a patient with pulmonary hypertension.* Designed with assistance from Christian Reitan.

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To Sigrid

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List of Publications

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals.

- I.** **Lundgren J**, Kylhammar D, Hedelin P, Rådegran G. sGC stimulation totally reverses hypoxia-induced pulmonary vasoconstriction alone and combined with dual endothelin-receptor blockade in a porcine model. *Acta Physiol (Oxf)*. 2012 Nov;206(3):178-94.
- II.** **Lundgren J**, Rådegran G. Hemodynamic Characteristics Including Pulmonary Hypertension at Rest and During Exercise Before and After Heart Transplantation. *J Am Heart Assoc*. 2015 Jul 21;4(7).
- III.** **Lundgren J**, Algotsson L, Kornhall B, Rådegran G. Preoperative pulmonary hypertension and its impact on survival after heart transplantation. *Scand Cardiovasc J*. 2014 Feb;48(1):47-58.
- IV.** **Lundgren J**, Söderlund C, Rådegran G. Impact of postoperative pulmonary hypertension on outcome after heart transplantation. *Scand Cardiovasc J*. 2017 Jun;51(3):172-181.
- V.** **Lundgren J**, Sandqvist A, Hedeland M, Bondesson U, Wikström G, Rådegran G. Alterations in plasma L-arginine and methylarginines in heart failure and after heart transplantation. *Scand Cardiovasc J*. 2018 Aug;52(4):196-204.

The following review forms the basis for parts of the introduction of the thesis.

Lundgren J, Rådegran G. Pathophysiology and potential treatments of pulmonary hypertension due to systolic left heart failure. *Acta Physiol (Oxf)*. 2014 Jun;211(2):314-33.

Abbreviations

ACR	Acute cellular rejection
ADMA	Asymmetric dimethylarginine
CO	Cardiac output
Cpc-PH	Combined pre-capillary and post-capillary PH
CTEPH	Chronic thromboembolic PH
DPG	Diastolic pressure gradient
ECMO	Extracorporeal membrane oxygenation
EMB	Endomyocardial biopsy
ESC	European Society of Cardiology
F _i O ₂	Inspiratory oxygen fraction
HPV	Hypoxic pulmonary vasoconstriction
HR	Heart rate
HT	Heart transplantation
Ipc-PH	Isolated post-capillary PH
ISHLT	International Society for Heart and Lung Transplantation
LVAD	Left ventricular assist device
MAP	Mean arterial pressure
MPAP	Mean pulmonary artery pressure
MRAP	Mean right atrial pressure
NO	Nitric oxide
NYHA	New York Heart Association

PAH	Pulmonary arterial hypertension
PAWP	Pulmonary artery wedge pressure
PDE5i	Phosphodiesterase type 5 inhibitor
PH	Pulmonary hypertension
PH-LHD	Pulmonary hypertension due to left heart disease
PVR	Pulmonary vascular resistance
RHC	Right heart catheterization
S_{AO_2}	Aortic O_2 saturation
sGC	Soluble guanylate cyclase
S_{PAO_2}	Pulmonary artery O_2 saturation
SV	Stroke volume
TPG	Transpulmonary gradient
TPR	Total pulmonary resistance

Abstract

Pulmonary hypertension due to left heart disease, caused by passive congestion of the pulmonary circulation, is associated with poor prognosis. If long-standing, endothelial damage and subsequent vasoconstriction may occur, in some cases further complicated by vascular remodeling of the pulmonary vessels. Such remodeling may induce fixed elevated pulmonary vascular resistance, potentially impairing outcome after heart transplantation. Knowledge on physiological alterations after heart transplantation, both with regards to hemodynamics and the plasma concentrations of various vasoactive substances, is scarce. There are furthermore few options for treating pulmonary hypertension due to left heart disease and hypoxic pulmonary vasoconstriction, the latter a condition which may aggravate pulmonary hypertension due to left heart disease. These issues were the focus of the present thesis.

First, in a porcine model of hypoxic pulmonary vasoconstriction, soluble guanylate cyclase stimulation alone or in combination with dual endothelin receptor blockade was found to completely reverse acute hypoxic pulmonary vasoconstriction without affecting oxygen consumption.

Second, in a retrospective review of the patients' heart transplanted and post-operatively followed at Skåne University Hospital in Lund, Sweden between 1988 and 2010, the impact of pre-operative and post-operative pulmonary hypertension on long-term survival was investigated. Invasive hemodynamics during slight exercise were furthermore studied to identify the hemodynamic response to exercise after heart transplantation. The findings suggest that with careful patient selection and care, pre-operative pulmonary hypertension may not be a strict contraindication for heart transplantation. Pulmonary hypertension one year after heart transplantation was, however, associated with impaired survival. Also, whereas the post-operative response to slight exercise with regards to cardiac output was adequate after heart transplantation, the patients' exhibited abnormally high ventricular filling pressures during exercise. The reason for the elevated filling pressures is probably multifactorial and possibly related to transplanted hearts being more dependent of the Frank Starling-mechanism to maintain adequate cardiac output, as well as decreased diastolic compliance.

Finally, in a prospective cohort of consecutive heart transplanted patients without severe cardiopulmonary complications, we analyzed plasma concentrations of

substances in the nitric oxide pathway prior to and after heart transplantation. The L-Arginine/ADMA-ratio, a ratio previously associated with disease severity and outcome in heart failure, was found to be markedly improved after heart transplantation and inversely correlated to pulmonary vascular resistance, suggesting an improved nitric oxide mediated vasodilatation.

Swedish summary

Högt tryck i lungkretsloppet – pulmonell hypertension – kan uppstå till följd av ett stort antal sjukdomstillstånd. Vänstersidig hjärtsjukdom, som i västvärlden främst orsakas av olika former av hjärtsvikt och i mindre utsträckning av sjukdomar i hjärtats klaffar, är den vanligaste orsaken till pulmonell hypertension och dess förekomst vid vänstersidig hjärtsjukdom är förenat med dålig prognos. I ett första skede är den pulmonella hypertensionen en direkt följd av den vänstersidiga hjärtsjukdomen. Denna resulterar i en passiv stockning av blod bakåt i lungkretsloppet, med förhöjda lungartärstryck som följd. Om förhöjda tryck kvarstår under en längre tid kan skada på blodkärlens väggar uppstå, med ökad kärlsammandragning som följd. Denna kan sedan ge upphov till en ombyggnad av kärlväggen, vilken medför försämrad kärlvidgningsförmåga. Sådan ombyggnad är särskilt allvarlig och kan i samband med hjärttransplantation leda till akut svikt av höger hjärthalvas funktion. Detta då det nya hjärtat inte är anpassat till den ökade belastning som förändringarna i lungkretsloppet medför. Om sådan svikt uppstår är risken för tidig död efter hjärttransplantation förhöjd. Kunskapsläget kring vilka patienter med pulmonell hypertension som har försämrad prognos är dock dåligt med varierande resultat från tidigare studier. Dessutom är förståelsen för fysiologiska förändringar efter hjärttransplantation bristfällig, både vad gäller hjärtats pumparbete och normalnivåer av kärlaktiva substanser. Vidare saknas specifik medicinsk behandling för pulmonell hypertension på basen av vänstersidig hjärtsjukdom. Medicinsk behandling saknas också vid pulmonell hypertension orsakad av ett antal andra tillstånd, däribland så kallad hypoxisk pulmonell vasokonstriktion. Vid hypoxisk pulmonell vasokonstriktion drar blodådrorna i lungan ihop sig till följd av låg syrehalt i den inandade luften eller till följd av nedsatt lufttillförsel till delar av lungan. Det senare ses bland annat vid kroniskt obstruktiv lungsjukdom (KOL), en sjukdom som inte sällan utgör ett samttillstånd vid vänstersidig hjärtsjukdom. Kombinationen av vänstersidig hjärtsjukdom och KOL kan därigenom förvärra den pulmonella hypertensionen ytterligare. Syftet med den aktuella avhandlingen var att undersöka fysiologiska förändringar efter hjärttransplantation, både vad gäller hjärtats pumparbete och kärlvidgande substanser, samt utvärdera hur pulmonell hypertension påverkar överlevnaden efter transplantation. Vi undersökte också om kärlvidgande behandling skulle kunna användas för behandling av hypoxisk pulmonell vasokonstriktion.

I **delarbete I** studerades, i en grismodell, effekten av två typer av kärlaktiva substanser vid akut hypoxisk pulmonell vasokonstriktion. Substanserna, en sGC-stimulerare som förmedlar kväveoxids kärlvidgande egenskaper och en dubbel endotelinreceptorblockerare som hindrar kärlsammandragning, kunde häva den hypoxi-orsakade pulmonella hypertentionen och akuta kärlsammandragningen utan allvarliga sidoeffekter. Fynden kan dock ej appliceras på andra former av pulmonell hypertention, inklusive pulmonell hypertention på basen av vänstersidig hjärtsjukdom. Faktum är att ett antal kärlaktiva substanser i ovan undersökta läkemedelsgrupper testats i kliniska studier på vänstersidig hjärtsjukdom. Den stora majoriteten av dessa studier har varit negativa, varför denna typ av behandling ej rekommenderas i nuläget.

I **delarbete II-IV** genomfördes en journalgenomgång av samtliga patienter som hjärttransplanterats vid Skånes universitetssjukhus i Lund 1988-2010. I studierna inkluderades de vuxna patienter som följts i Lund och som genomgått invasiva vilomätningar av hjärtats pumparbete och tryckförhållanden – så kallad högersidig hjärkateterisering – före och efter hjärttransplantation. I **delarbete II** studerades patienter som utöver vilomätningar även genomgått mätningar vid lätt arbete före och efter hjärttransplantation. Vi fann att hjärtats pumparbete i vila ej nämnvärt skiljer sig från vad som förväntas bland friska individer och att hjärtminutvolymen vid lätt ansträngning efter hjärttransplantation även den är adekvat. Trots detta kunde vi konstatera att hjärttransplanterade patienter, vid fysisk aktivitet, har kraftigt förhöjda tryck i vänster förmak. Sannolikt kan detta förklaras av en fysiologisk anpassning till följd av att det transplanterade hjärtat saknar nervförsörjning. Huruvida denna avvikelse har betydelse för överlevnaden efter hjärttransplantation är ej studerat och framtida arbeten krävs för att utröna om det finns incitament för medicinsk behandling av de förhöjda trycken i vänster förmak. I **delarbete III-IV** undersöktes hur pulmonell hypertention före och efter hjärttransplantation påverkar långtidsöverlevnaden efter densamma. I vårt material sågs ingen skillnad i postoperativ överlevnad baserat på om patienterna hade preoperativ pulmonell hypertention, eller ej. Dessa fynd innebär inte att pulmonell hypertention är ofarligt i samband med hjärttransplantation utan belyser snarare svårigheterna att identifiera patienterna med ovan beskrivna ombyggnad av lungkärnen. Med god patientselektion och vård är pulmonell hypertention i gruppen som helhet alltså inget absolut hinder för hjärttransplantation. Detta utesluter inte att en subgrupp med kraftig kärlombyggnad har försämrad överlevnad. Vi fann däremot att pulmonell hypertention vid upprepade mätningar under, eller kvarstående efter, första året efter hjärttransplantation är förenat med försämrad långtidsöverlevnad.

Kväveoxid är centralt för kärlvidgning och produceras från aminosyran L-Arginin, en process som hämmas av substansen ADMA. Det är välkänt att kväveoxidberoende kärlvidgning är minskad vid flera tillstånd, inklusive vänstersidig

hjärtsjukdom. Vid vänstersidig hjärtsvikt har man också funnit att kvoten mellan L-Arginin och ADMA är ett bra mått på kväveoxid-orsakad kärlvidgning. Denna kvot har dessutom visat sig vara korrelerad till såväl svårighetsgrad av, som överlevnad vid, vänstersidig hjärtsvikt. För att studera förändring av kväveoxid-förmedlad kärlvidgning efter hjärttransplantation genomfördes **delarbete V** i vilket plasmanivåerna av dessa substanser studerades i 12 på varandra följande hjärttransplantationspatienter utan hjärt-kärl komplikationer. Jämfört med kontrollpersoners plasmanivåer konstaterades att L-Arginin var lågt och ADMA högt hos patienter före hjärttransplantation, vilket resulterade i en låg L-Arginin/ADMA-kvot. Efter transplantation normaliserades L-Arginin-nivåerna medan ADMA var oförändrat. Följaktligen förbättrades även L-Arginin/ADMA-kvoten, dock utan att nå normalnivåer. Denna kvot uppvisade också en omvänd korrelation till resistansen i lungkärnen efter hjärttransplantation, vilket tyder på att L-Arginin/ADMA-kvoten reflekterar lungkärnstonus efter hjärttransplantation samt att den kväveoxid-beroende kärlvidgningen är förbättrad, men ej normaliserad. Vilken betydelse dessa fynd har för behandling av patienter efter hjärttransplantation är oklart och framtida arbeten krävs alltså för att utvärdera om dessa patienter har nytta av kärlvidgande behandling.

Sammanfattningsvis bidrar denna avhandling med utökad insikt i den komplexa situation, framförallt i relation till pulmonell hypertension, som en hjärttransplantation utgör. I genomförda arbeten noteras en tydlig fysiologisk skillnad i hjärtarbete vid lätt ansträngning efter hjärttransplantation, jämfört med vad som förväntas hos friska individer. Överlevnad efter transplantation har vidare utvärderats baserat på preoperativ pulmonell hypertension. I detta arbete hade patienter med pulmonell hypertension i gruppen som helhet inte påverkad överlevnad och pulmonell hypertension utgör således inget absolut hinder för hjärttransplantation. Däremot har patienter med pulmonell hypertension efter hjärttransplantation försämrade långtidsöverlevnad. Det är också möjligt att en subgrupp av patienter med preoperativ PH och kärlombyggnad har ökad dödlighet. På grund av detta, och utformning samt storlek av studierna i denna avhandling, krävs framtida arbeten för att bekräfta resultaten och för att utarbeta definitioner som tydligt identifierar patienter med ombyggnad av lungkärnen. Framtida arbeten krävs också för att utvärdera om behandling med substanser som, i lungkretsloppet, har kärlvidgande egenskaper medför gynnsamma effekter vid pulmonell hypertension på basen av vänstersidig hjärtsjukdom eller efter hjärttransplantation.

Introduction

Heart Transplantation

More than 50 years have passed since the first orthotopic human heart transplantation (HT) was performed by Christian Barnard's team at Groote Schuur Hospital in Cape Town, South Africa (1). Despite successful surgery, the patient died from pneumonia 18 days after HT. In the years that followed, a few hundred HTs were performed worldwide (2). The initial results were discouraging (2), partly due to poor understanding of issues related to rejection and immunosuppression. The field was, however, revolutionized by the introduction of endomyocardial biopsies (EMB), allowing the possibility of monitoring rejections (3). This was followed by the discovery of cyclosporine, an immunosuppressive agent decreasing t-cell activity by inhibiting calcineurin (4). The discovery of cyclosporine was, in turn, followed by a number of newer immunosuppressive drugs (5). After a dramatic increase in the number of HTs performed yearly after the introduction of cyclosporine in the 1980's, the number of HTs worldwide has remained relatively constant, around 4000-5000 per year, during the last decades (Figure 1) (6).

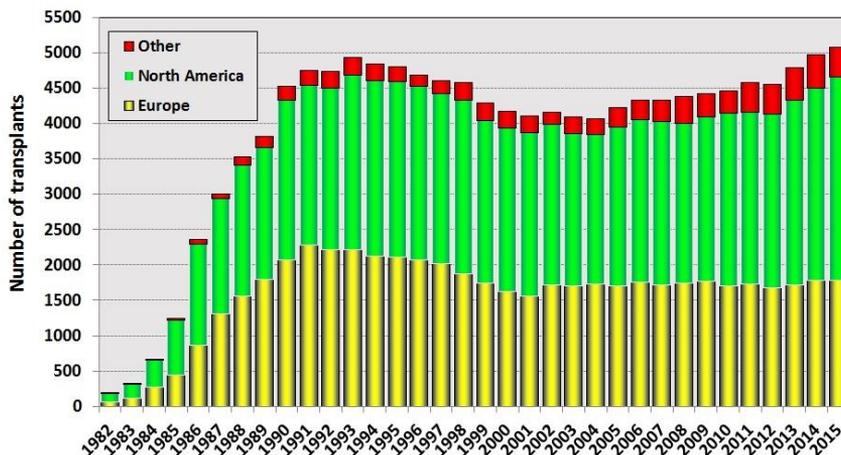


Figure 1. Heart Transplantations 1982-2015

Worldwide heart transplantations reported to ISHLT. Printed with permission from ISHLT and UNOS, JHLT. 2017 Oct;36(10): 1037-1079. (6)

Today, the most common maintenance immunosuppression regimen includes a combination of the calcineurin inhibitor tacrolimus, the antimetabolite mycophenolate mofetil and steroids (6). As new immunosuppressants have been introduced, the post-HT survival has steadily improved (Figure 2). However, in the last decades the main improvement in survival has been during the first post-operative year, whereas survival beyond then has remained relatively constant (Figure 2). Despite improved therapies, long-term complications related to immunosuppression, such as malignancies, infections and renal failure are among the most common causes of cumulative death after HT (6).

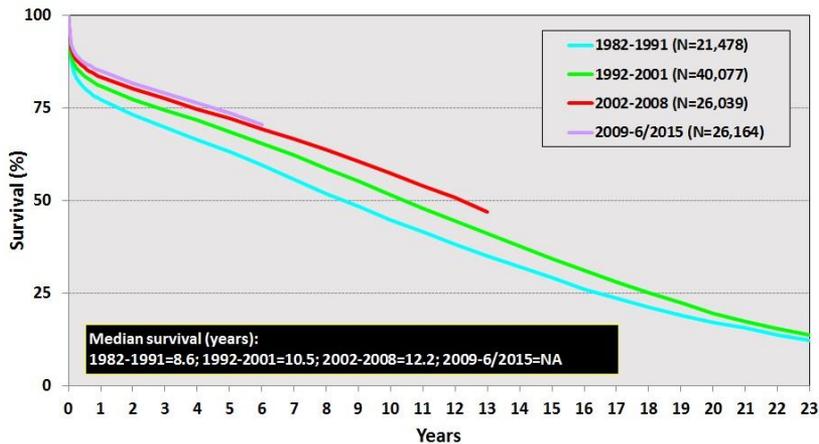


Figure 2. Survival After Heart Transplantation 1982-2015
Worldwide survival after heart transplantation as reported by ISHLT. Printed with permission from ISHLT and UNOS, JHLT. 2017 Oct;36(10): 1037-1079. (6)

Hemodynamics in Heart Transplantation

Pulmonary Hypertension in Relation to Heart Transplantation

Severe pulmonary hypertension (PH) with pulmonary vascular remodeling and “fixed” elevated pulmonary vascular resistance (PVR) increases the risk for acute right heart failure after HT. In this setting, the right ventricle of the transplanted heart is not adapted to the persistently increased resistance in the pulmonary circuit after HT and may therefore fail to pump against it. Acute right heart failure due to pre-operative PH has shown to be a common cause of early mortality after HT (7;8). Based on early reports where pre-operative PH was associated with worse post-operative outcome (9;10), PH with vascular remodeling is considered a relative contraindication for HT according to the International Society for Heart and Lung Transplantation (ISHLT) (7). However, the continuous improvement in survival during the first year after HT suggests that pre-, intra- and post-operative care has improved over the past decades. It is therefore likely that the thoracic intensive care units of today are able to handle many of the potential issues related to pre-operative

PH and that previous reports may be outdated. In support of this, in the most recent report from ISHLT, patients who underwent HT 2004-2015 had similar post-HT survival, irrespective of pre-operative PVR levels (Figure 3) (6). Yet, early mortality remains high and with present hemodynamic definitions it is difficult to identify the patients with an increased risk for acute right heart failure after HT (11-14). Therefore, a good clinical evaluation is essential for adequate patient selection, to further improve survival and decrease the risk of post-operative complications.

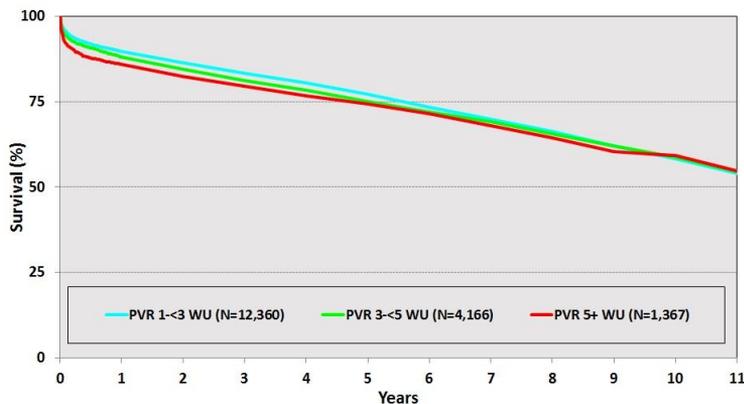


Figure 3. PVR and Survival After Heart Transplantation 2004-2015

Survival after heart transplantation based on pre-operative PVR. Printed with permission from ISHLT and UNOS, JHLT. 2017 Oct;36(10): 1037-1079. (6)

It is well established that pre-operative PH may persist early after HT (15). In most cases it does, however, resolve with time (16). In contrast to multiple previous studies on the impact of pre-operative PH on post-HT survival, the knowledge on post-HT PH and its effect on long-term outcome is scarce. A few studies (17;18) have found that persistently elevated PVR and mean pulmonary artery pressure (MPAP) one year after HT is associated with worse prognosis. In these reports, the common definition of PH (MPAP \geq 25 mmHg) was not used and the impact of post-operative PH therefore remains to be thoroughly investigated.

Exercise Hemodynamics After Heart Transplantation

Most reports on exercise after HT have used non-invasive methods and focused on either exercise capacity or a potential reinnervation of the transplanted heart (19-28). In contrast, complete invasive data on hemodynamic response to exercise after HT is scarce (29-35). It has, however, been suggested that ventricular filling pressures are elevated during exercise after HT (33;35). The precise reason is unknown and has been attributed to factors such as increased dependence of the Frank Starling Mechanism (31;36;37) and diastolic dysfunction secondary to hypertension, myocardial ischemia or rejection (15;16;33;35). Fluid retention, lack

of heart rate reserve and lack of the Bainbridge effect have also been suggested to contribute to the increased ventricular filling pressures (15;16;34;35).

Mechanical Circulatory Support

Over the past decades various mechanical circulatory supports, primarily implantable left ventricular assist devices (LVADs), have been used as bridge-to-transplant in severely ill patients. The ISHLT Mechanical Circulatory Support Registry reports a 3-year survival with modern LVADs above 60% (38). With improving devices and increasing organ shortage, the number of patients transplanted from mechanical support has steadily increased, worldwide reaching more than 50 % in recent years (Figure 4 a) (6). In the most recent report from the ISHLT, no difference in post-operative survival was observed based on long-term intracorporeal LVAD or not prior to HT, whereas the pre-operative use of extracorporeal membrane oxygenation (ECMO) was associated with worse post-HT survival (Figure 4 b) (6). In current ISHLT guidelines on mechanical circulatory support, heart failure patients at high risk of one-year mortality should be referred for HT or mechanical circulatory support, if there are no contraindications for such therapy (39). The same guidelines support mechanical circulatory support both as bridge-to-transplantation and as destination therapy. The latter is, however, still a matter of debate, currently being investigated in Sweden in the SweVAD trial (ClinicalTrials.gov Identifier: NCT02592499).

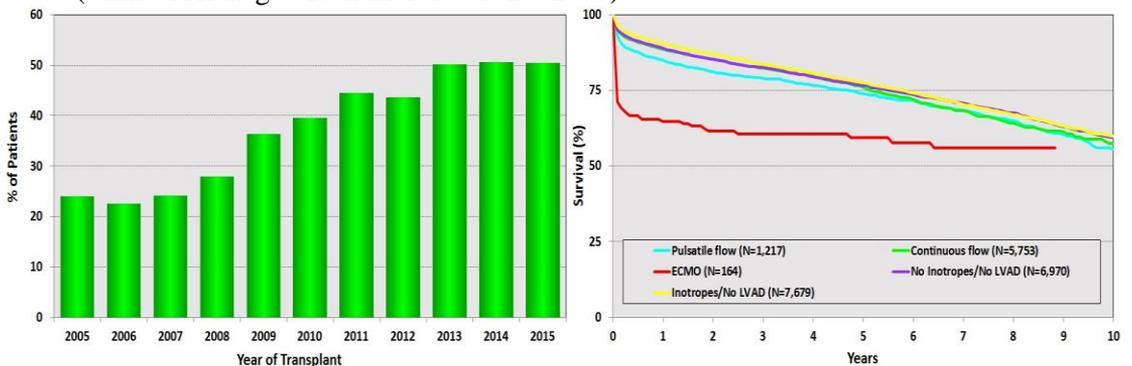


Figure 4 (a) and (b). Bridge-to-Transplantation and Post-Operative Survival
 (a) % of HT patients bridged to transplantation with LVAD, (b) Post-operative survival based on pre-operative inotropes and LVAD. Printed with permission from ISHLT and UNOS, JHLT. 2017 Oct;36(10): 1037-1079. (6)

LVAD is also used in patients with pharmacologically irreversible PH, as long term LVAD may reverse fixed pulmonary pressures and resistances in these patients (40-49). The positive hemodynamic effects with LVAD have been shown to remain stable after HT (44;49). In patients with irreversible PH, mechanical circulatory support as bridge to candidacy is therefore recommended by the ISHLT (50). In patients where PH does not resolve with LVAD, concomitant phosphodiesterase

type 5 inhibitor (PDE5i) may be considered (39), as this has been shown to reverse PH during LVAD therapy in a single center study (51). The SOPRANO trial is currently evaluating the dual endothelin receptor antagonist macitentan in the same setting (ClinicalTrials.gov Identifier: NCT02554903).

An analysis of a large multi-national registry has shown that in over 20 % of patients, LVAD-implantation is complicated by acute right heart failure within 30 days of surgery (52), as the failing right ventricle is not able to cope with the increased flow generated by the device. Previously, several risk factors for right heart failure has been presented (53-57), but adequate risk stratification has been lacking. Recently, a new right heart failure score based on data from the EUROMACS database was presented (52) and outperformed previously presented risk scores (54;57).

Although LVAD therapy improve quality of life as well as survival (58-61) and despite the fact that modern devices out-perform older generations (60;61), adverse events, resulting in hospitalization, remain common (38;62). Therefore LVAD speed optimization and close hemodynamic monitoring are of great importance (63-65). When using the ramp test described by Uriel et al. (63) to optimize hemodynamics, diastolic pressure gradient (DPG) > 5 mmHg is still common in LVAD patients. In these patients, DPG > 5 mmHg is furthermore associated with increased risk of heart failure readmission and death. In contrast, in a univariate analyze of the same material, PVR did not predict death or heart failure readmission (66). Previously, PVR has commonly been used as a therapeutic target in LVAD patients, but recent data consequently suggests that DPG may be more appropriate (66).

The Clinical and Research Programs for Heart Transplantation and Mechanical Circulatory Support in Lund, Sweden

In February 1988, one month after the concept of brain death was formally enacted by Swedish law, the first HT in Lund was carried out by the thoracic surgeon Jan Otto Solem. Since 2011, Lund and Gothenburg serve as the two Swedish referral centers for HT. At present, approximately 25-30 HTs are being performed yearly in Lund.

After the first HT performed in Lund more than 30 years ago, a HT follow-up program was initiated by cardiologist Stig Persson. During the years that have passed, Dr. Björn Kornhall has further refined this extensive program. The follow-up has included frequent visits to health care providers, blood tests and diagnostic investigations. In the first year after HT, around 14 routine EMBs and 4-5 hemodynamic evaluations with right heart catheterizations (RHC) have been performed (Figure 5 a).



Figure 5 (a-c). The Hemodynamic Lab in Lund and Left Ventricular Assist Devices as Bride-to-Transplantation (a) The Hemodynamic lab at Skåne University Hospital in Lund, (b) The patient with the first LVAD (Heartmate) implanted at Skåne University Hospital, Lund, in 1993 out walking with a large control unit and (c) A patient followed at Skåne University Hospital, Lund, currently being bridged to HT with a Heartmate III, visualizing today's small portable control unit. Printed with written permission of the patient.

With regard to LVADs, the patients have been followed in a similar matter to that of the HT patients, including frequent blood tests and echocardiographic evaluations. The first LVAD implantation in Lund was performed in 1993. Initially the HeartMate IP and HeartMate VE were the most commonly used devices. Thereafter, when the second generation of LVADs became available, the HeartMate II and, less frequently, HeartWare were used for many years. In the most recent years, the HeartMate III has been the device most commonly used (Figure 5 b and c). Today approximately 40 % of the HT patients at Skåne University Hospital are bridged to HT with a LVAD (Figure 6).

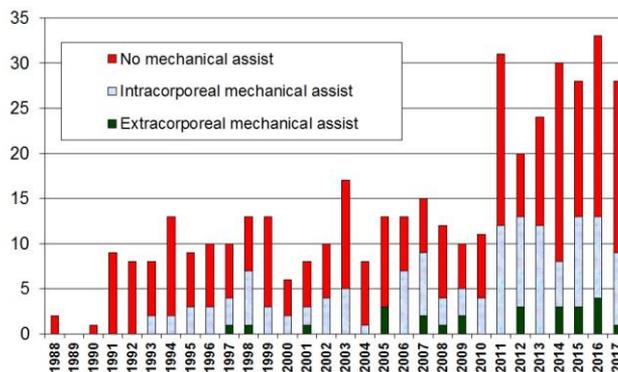


Figure 6. Heart Transplantations in Lund 1988-2017 Number of heart transplantations performed per year in Lund, including those bridged with LVAD. Designed by and printed with the permission of Dr. Björn Kornhall.

The present thesis was performed within Lund Heart Transplantation and Pulmonary Hypertension Research Network, utilizing data from the Lund Heart Transplantation Research Register and blood samples collected at the Hemodynamic Lab in Lund, stored in the Lund Cardio Pulmonary Register cohort of Region Skåne’s Biobank. The network and registers have been established by Assoc. Prof. Göran Rådegran to facilitate clinical research in HT and PH, based on data assembled at the Hemodynamic Lab at Skåne University Hospital in Lund.

Pulmonary Hypertension

Pulmonary hypertension is divided into five groups depending on the disorder causing the condition (14). PH group 1, pulmonary arterial hypertension (PAH), results from remodeling and obstruction of the small pulmonary arteries. In contrast, the two largest groups of PH namely group 2, PH due to left heart disease (PH-LHD), and group 3, PH due to lung diseases and/or hypoxia, occur as a result of the underlying condition and is often related to the severity of the corresponding disease. PH group 4, chronic thromboembolic PH (CTEPH), is instead caused by chronic obstruction of the pulmonary arteries due to fibrotic transformation of a pulmonary arterial thrombus. Finally, PH group 5 is caused by unknown or multifactorial mechanisms (Table 1).

Table 1. Clinical Classification of Pulmonary Hypertension

Adapted from Galiè et al. 2016 (14)

1.	PULMONARY ARTERIAL HYPERTENSION (PAH)
1'.	PULMONARY VENO-OCCLUSIVE DISEASE AND/OR PULM CAPILLARY HEMANGIOMATOSIS
1''.	PERSISTENT PULMONARY HYPERTENSION OF THE NEWBORN (PPHN)
2.	PULMONARY HYPERTENSION DUE TO LEFT HEART DISEASE
2.1.	Left ventricular systolic dysfunction
2.2.	Left ventricular diastolic dysfunction
2.3.	Valvular disease
2.4.	Congenital/acquired left heart inflow/outflow tract obstruction and congenital cardiomyopathies
2.5.	Congenital/acquired pulmonary veins stenosis
3.	PULMONARY HYPERTENSION DUE TO LUNG DISEASE AND/OR HYPOXIA
3.1.	Chronic obstructive pulmonary disease
3.2.	Interstitial lung disease
3.3.	Other pulmonary diseases with mixed restrictive and obstructive pattern
3.4.	Sleep-disordered breathing
3.5.	Alveolar hypoventilation disorders
3.6.	Chronic exposure to high altitude
3.7.	Developmental lung diseases
4.	CHRONIC THROMBOEMBOLIC PULMONARY HYPERTENSION (CTEPH)
5.	PULMONARY HYPERTENSION WITH UNCLEAR AND/OR MULTIFACTORIAL MECHANISMS

Pulmonary Arterial Hypertension

Over the years, research in PH has primarily been devoted to PAH where the prognosis, if untreated, is very poor with an estimated survival of approximately 1-2.8 years (67). PAH is a rare and complex pulmonary vasculopathy, primarily affecting the small pulmonary arteries (68;69). Although it has been established that several pathways including metabolic signaling, growth factors, cytokines etc. are involved (70;71), the precise pathological processes that initiate PAH are largely unknown. Initially, the disease is dominated by excessive vasoconstriction resulting from endothelial dysfunction and imbalance between vasoactive substances including, but not limited to, endothelin, nitric oxide (NO) and prostacyclin. As the disease progresses, vascular remodeling characterized by medial hypertrophy, intimal proliferation and fibrosis as well as adventitial thickening, become increasingly prominent (68). Additional hallmarks of PAH include vascular inflammation, the formation of plexiform lesions and in situ thrombosis, which cause further obstruction of the vascular lumen (68;70;71). The reduced vascular lumen and stiffening of the arteries result in increased PVR and subsequent right ventricular overload, leading to right ventricle hypertrophy followed by dilatation. The increased load causes right ventricular failure, syncope and ultimately death (72).

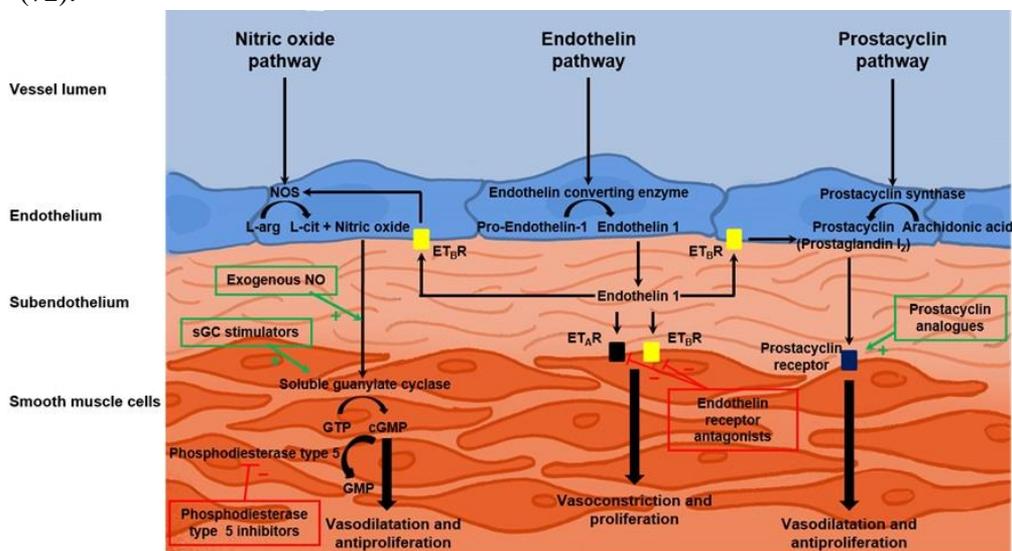


Figure 7. Therapeutic Targets in PAH
Printed with permission from ACTA Physiologica (73).

The scientific focus on PAH has resulted in improved pathophysiological understanding and development of medical therapies targeting the endothelin, NO and prostacyclin pathways (Figure 7) (14). These drugs primarily serve to counteract the excessive pulmonary vasoconstriction and to slow disease

progression which, however, cannot be entirely stopped even with modern therapy. The disease consequently still carries a poor prognosis (74) and lung transplantation remains the ultimate treatment available. Although strong evidence is lacking for the use of PAH drugs in other forms of PH, off label use, particularly in CTEPH, has been common (14;75).

The Endothelin Pathway

Endothelin-1 is a potent vasoconstrictor mediating its effects via endothelin A and B receptors on vascular smooth muscle cells. Endothelin B receptors are also located on endothelial cells where they mediate vasodilatory effects through the NO and prostacyclin pathways (76;77). The constrictive effects does however seem to be the most pronounced and clinically important (78).

In PAH, single and dual endothelin receptor blockade has been investigated in multiple trials (79-82) and found to improve six minute walking distance (79;80) and reduce hospitalizations as well as disease progression (81;82). Endothelin receptor blockade has consequently become a cornerstone in the medical management of PAH (14). In CTEPH, the dual endothelin receptor blocker bosentan therapy was investigated in the BENEFIT trial in which PVR and six minute walking distance were co-primary endpoints. In BENEFIT, treatment with bosentan improved PVR but not six minute walking distance (83). In the recent MERIT trial in inoperable CTEPH, the novel dual endothelin receptor blocker macitentan resulted in significant reduction of the primary endpoint PVR (84).

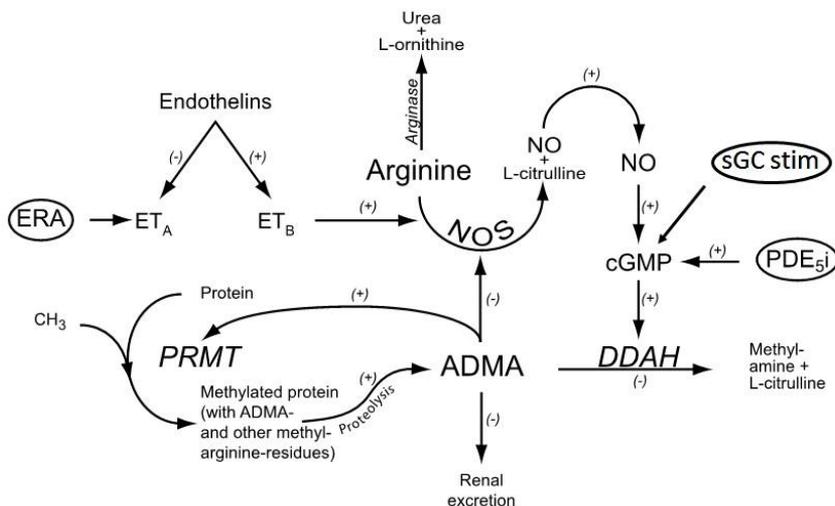


Figure 8. The L-Arginine, NO and ADMA Pathway

ERA – Endothelin receptor antagonist, sGC stim – sGC stimulator and PDE5i – Phosphodiesterase type 5 inhibitor. Printed with permission from Heart & Vessels (85).

The Nitric Oxide Pathway

Nitric oxide is produced from L-Arginine (86) and exerts pulmonary vasodilatory effects (87) by activation of soluble guanylate cyclase (sGC) (Figure 8). sGC in turn catalyzes the conversion of guanosine triphosphate to the second messenger cyclic guanosine monophosphate which causes vasodilatation (88;89). The half-life of cyclic guanosine monophosphate is short and the substance degraded by phosphodiesterases. In the pulmonary circulation, PDE5 is the most abundant isoform (90) and inhibition of PDE5 consequently results in vasodilatation. Apart from the vasodilatory effects, NO influences vasoproliferation, angiogenesis, cellular adhesion and platelet aggregation (91).

The conversion of L-Arginine to NO and Citrulline is catalyzed by NO synthase (86) and inhibited by the competitive NO synthase inhibitor asymmetric dimethylarginine (ADMA). ADMA, in turn, is primarily metabolized and to a lesser extent excreted by the kidneys (91). ADMA has shown to be elevated in several cardiovascular conditions including PAH and heart failure (85;92-96) and is consequently thought to play a central part in the decreased NO production in these conditions.

With regard to medical therapies in PAH, PDE5i's have demonstrated improvement in six minute walking distance in several trials (97;98). In combination with single endothelin receptor blockade with ambrisentan, the PDE5i tadalafil has also shown to reduce hospitalizations in PAH (82). The beneficial effects of substances targeting the NO pathway in PAH have also been highlighted in trials with the sGC stimulator riociguat, where therapy improved both primary and secondary endpoints including six minute walking distance, functional class and quality of life (99;100). Together with endothelin receptor blockade, substances targeting the NO pathway are central in treatment of PAH (14).

Of note, in CTEPH riociguat improved six minute walking distance, hemodynamics, biomarkers and functional class (101) and is currently the only recommended medical therapy for this condition (14).

The Prostacyclin Pathway

Prostacyclin is a cyclooxygenase derived prostaglandin with vasodilatory, anti-inflammatory, anti-proliferative, anti-platelet and anti-thrombotic effects. The pulmonary vasodilatation is caused by the binding of prostacyclin to prostacyclin-receptors on vascular smooth muscle cells, thereby increasing cyclic adenosine monophosphate (102).

Prostacyclin analogues were the first drugs available for the treatment of PAH (103;104) and can be administered as intravenous or subcutaneous infusions, as inhalations or orally (71). These drugs are effective in improving outcomes in PAH (103;104) but due to short half-life, complex routes of admission and frequent adverse effects, they are rarely used as first line therapy in patients with WHO class

II and III (14). Recently, the orally administered prostacyclin receptor agonist selexipag, with a longer half-life than previous prostacyclin analogues, was proven to reduce PAH related hospitalizations and disease progression in the GRIPHON trial (105). Based on the beneficial effects and more convenient route of administration, the use of substances targeting the prostacyclin pathway may consequently increase (14;105). In the ongoing TRITON trial, it is investigated whether initial triple combination with selexipag in combination with the endothelin receptor antagonist macitentan and PDE5i tadalafil is superior to a dual combination of the latter two drugs (ClinicalTrials.gov Identifier: NCT02558231).

Pulmonary Hypertension due to Left Heart Disease

Pulmonary hypertension due to left heart disease is by far the most common cause of PH, accounting for up to 80 % of all cases (106). In developed countries, it is primarily caused by systolic or diastolic heart failure and to a lesser extent by valvular heart disease (14). As the prevalence of heart failure is increasing (107), consequently so is PH-LHD. However, the definite prevalence of PH in left heart disease is unknown and vary considerably depending on the method used but is likely at least 50% (106;108). In a cohort of patients with systolic and diastolic heart failure referred for RHC, PH was identified in 54-80 % depending on the underlying diagnosis (109). PH-LHD is indeed a complex and heterogeneous condition associated with poor prognosis in heart failure (110;111), yet PH is often overlooked in the setting of left heart disease (112).

The precise pathophysiology and pathobiology of PH-LHD is multifactorial and not completely understood. In acute left heart failure, increased left ventricular pressures are transmitted to the pulmonary circuit, resulting in passive congestion of the pulmonary veins, capillaries and arteries (113). In present guidelines this passive PH is referred to as isolated post-capillary PH (Ipc-PH) (Figure 9) (14). The acutely elevated capillary pressure causes increased stress on the capillary wall and subsequent edema (106). These effects are initially reversible but may, if long-standing, result in structural changes including thickened of the alveolar-capillary membranes and thereby decreased diffusion capacity of the lungs (114). Furthermore, long-standing elevation in pulmonary artery pressures may result in endothelial damage and alterations in vasoactive substances, including increased endothelin production (115-117) and decreased NO production (118). These changes result in impaired smooth muscle relaxation and excessive pulmonary vasoconstriction (117;118). Such “active” PH with a pre-capillary component denotes as combined pre-capillary and post-capillary PH (Cpc-PH) (Figure 9) (14).

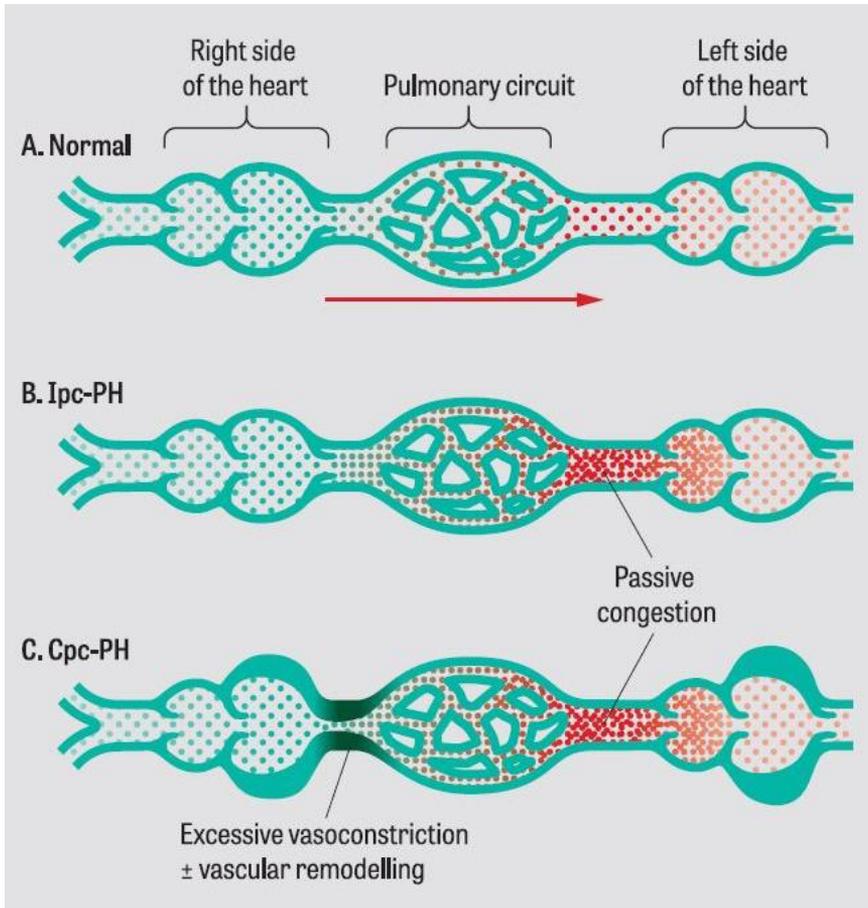


Figure 9. Pathophysiology in Pulmonary Hypertension due to Left Heart Disease
IpcPH – Isolated post-capillary PH, Cpc-PH – Combined pre-capillary and post-capillary PH

The vasoconstriction may, over time, result in remodeling of the pulmonary vasculature (119). The remodeling primarily seems to affect the pulmonary resistance arteries. Changes observed include medial hypertrophy, as well as intimal thickening and fibrosis but also, to a lesser extent, adventitial fibrosis (119;120). The vascular remodeling in PH-LHD is consequently, in parts, similar to the one seen in PAH. Plexiform lesions are however rarely observed in left heart disease (119;120).

Hemodynamic testing

The Nobel Prize in physiology and medicine 1956 was awarded for the development of RHC during the first half of the 20th century (121;122), allowing invasive hemodynamic monitoring of patients with heart disease. The subsequent introduction of the Swan Ganz catheter in 1970 (123), with which the investigator

can estimate left atrial pressure by inflating a balloon and temporarily obstruct the pulmonary artery [pulmonary artery wedge pressure – PAWP], has further improved the diagnostic value provided by RHC. Consequently, RHC has been central for the increased understanding of the circulation and improved care for these diseases. To date, RHC remains an important tool in the diagnosis of severe heart disease and pulmonary hypertension.

In 1958 Paul Wood first defined PH as systolic pulmonary artery pressure above 30 mmHg and diastolic pulmonary artery pressure above 15 mmHg (124). In this pioneer work, Wood also sub-classified post-capillary PH into a passive and a reactive “out-of-proportion” group. The term “out-of-proportion” was used for many years, referring to cases where MPAP was more pronouncedly increased than what would be expected based on the levels of PAWP, thereby suggesting an active pulmonary vascular component. The term, however, caused confusion, so it was abandoned in the most recent European Society of Cardiology (ESC) guidelines (14;108).

Over the years, several hemodynamic parameters have been proposed as markers for disease severity and for the identification of patients with active pulmonary vascular disease. For many years, the transpulmonary gradient (TPG) was the parameter of choice, included in the ESC guidelines (Figure 10 a) (125). In the most recent version of the guidelines, TPG was, however, removed and replaced with a combination of DPG and PVR. The reason for this was that DPG had shown to be less affected by factors such as left atrial pressure and flow, as compared to the TPG, thereby being pathophysiologically attractive in PH-LHD (126). However, retrospective studies in heart failure have thereafter provided conflicting results in relation to the impact of DPG on outcome (12;120;127-141), so PVR was added as an indirect marker of right ventricular function (Figure 10 b) (14).

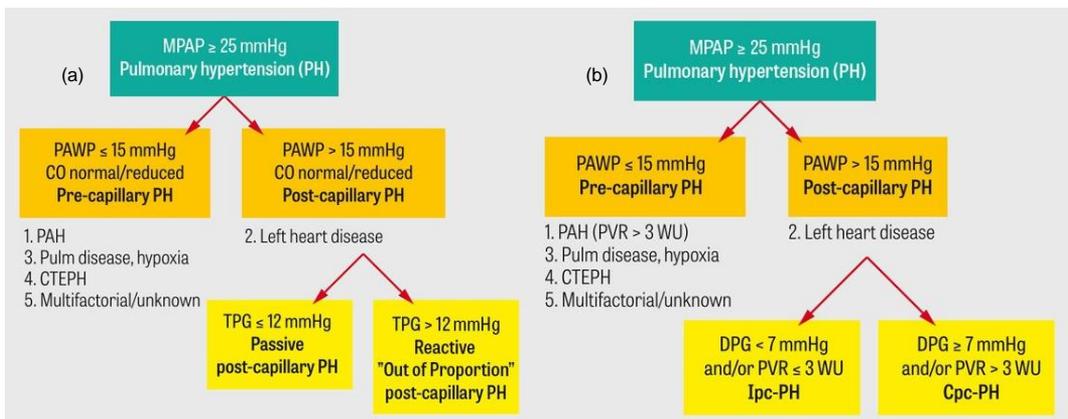


Figure 10 a and b. ESC's Hemodynamic Definition of Pulmonary Hypertension

(a) Previous definition of PH according to ESC's guidelines from 2009, (b) ESC's current definition of PH (2015). Adapted from Galiè et al. 2009 and 2016 (14;125).

Hemodynamic exercise testing

Exercise measurements during invasive hemodynamic assessments are commonly performed. Exercise provides additional information on disease severity (142-147) and improve early diagnosis of heart failure (148), as well as PH (149). It has been established that, in health, an increase of cardiac output (CO) by $1 \text{ L}\cdot\text{min}^{-1}$ is accompanied by, on average, an increase in MPAP by 1 mmHg (150;151) and that these parameters rapidly return to resting values (152) after exercise. Large inter-individual alterations in the flow-pressure relationship have, however, been observed (153).

PH during exercise has previously been defined as MPAP > 30 mmHg (154). In recent guidelines this definition was removed as sufficient evidence for the hemodynamic thresholds which indicates exercise PH has been lacking (14;125) and since MPAP has been found to increase beyond 30 mmHg in healthy individuals (144). An adequate definition of exercise PH is also complicated by the fact that hemodynamic alterations due to increasing age are more pronounced during exercise than at rest (144;155;156) and that invasive data are particularly scarce in the elderly. Patients with severe lung disease also represent a challenge, as marked alterations in intrathoracic pressures during the respiratory cycle may complicate the interpretation. Total pulmonary resistance (TPR = MPAP/CO) is affected by alterations in respiratory pressure to a lesser extent than previously used parameters and therefore seems to be a more reliable marker for diagnosis of exercise PH also in this population (157). Moreover, the pressure-flow relationship assessed by TPR does not seem to exceed 3 WU in healthy individuals (153;158)

With regards to PVR, an increase during exercise can be either due to pulmonary vascular disease or congestion of the pulmonary circuit due to left heart disease (153;158). PVR during exercise therefore has a poor diagnostic value in left heart disease whereas the prognostic value in pulmonary vascular disease is very good (149). Due to the limitations of MPAP and PVR, TPR has been suggested to be a more adequate predictor of exercise PH. TPR > 3 WU has indeed been documented to be an excellent discriminator for exercise PH alone or in combination with MPAP > 30 mmHg (149;159), although findings may differ depending on how the measurements are performed (159). Based on these findings, the European Respiratory Society has released an official statement suggesting that exercise PH should be defined as MPAP > 30 mmHg and TPR > 3 WU in the absence of MPAP ≥ 25 mmHg at rest (157). The natural history of exercise PH is not known and needs further investigation (157).

Acute vasodilatation test

Resting hemodynamics is often insufficient to sub-define post-capillary PH and identify patients with severe pulmonary vascular remodeling. As stated previously, this is of major importance when evaluating patients for HT, as fixed elevated PVR

may increase the risk for acute right heart failure after HT. Therefore, acute vasodilatory testing is recommended in the work-up for HT when the systolic pulmonary artery pressure is ≥ 50 mmHg and either TPG is ≥ 15 mmHg and/or PVR is > 3 WU (7). A PVR which remains high without severe systemic hypotension is considered a relative contraindication for HT (7). In an attempt to sub-define post-capillary PH, an acute vasodilatation test is also included in the ISHLT definition of PH (Figure 11) (11). Such a test is, however, lacking in the ESC definition of PH. In fact, in recent studies using the novel ESC definition, acute vasodilatation tests have failed to identify a sub group of patients with fixed PVR and worse prognosis (134;141), suggesting that the current value of these tests, outside pre-HT evaluations, is limited.

With regards to the drugs used for the vasodilatation test, the effects of several vasoactive agents on pulmonary hemodynamics have been reported (160). Among these substances, intravenous infusion of nitroprusside and inhaled NO are probably best documented (13;160) and also commonly used.

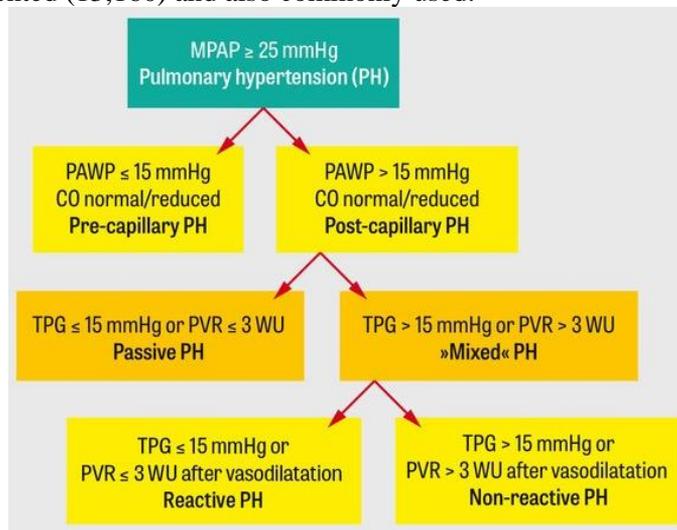


Figure 11. ISHLT's Hemodynamic Definition of Pulmonary Hypertension
Adapted from Fang et al 2012 (11).

Therapies in Pulmonary Hypertension due to Left Heart Disease

There are no specific therapies for PH-LHD. Treatment instead focuses on optimizing the underlying cause of the disease, as appropriate (14;161). This includes standard heart failure medications as well as implantable devices and surgery (161). Several studies, including observational single center reports and large clinical trials, have investigated the use of pulmonary vasoactive drugs targeting the endothelin, NO and prostacyclin pathways in left heart disease. Some of these trials have exclusively included patients with post-capillary PH although most have not.

Several trials with endothelin receptor antagonists have been carried out in systolic left heart failure with and without PH. None of these have shown beneficial effects of the treatment. Instead, safety concerns have arisen, with a tendency of increased fluid retention using endothelin receptor antagonists (162-164). At present, a trial with the endothelin receptor antagonist macitentan is carried out in patients with diastolic heart failure and pulmonary vascular disease (ClinicalTrials.gov Identifier: NCT03153111), a condition previously not investigated. This is of great importance as the clinical manifestations of PAH and PH due to diastolic heart failure may be similar. Consequently, if this trial is negative even more effort has to be put into finding robust markers for differentiation between PAH and PH due to diastolic heart failure.

With regards to the NO pathway several observational studies as well as small randomized single-center trials have reported beneficial effects on hemodynamics, quality of life and functional class with PDE5i's, primarily sildenafil, in left heart disease with or without PH. Despite these positive results and the potentially large patient population available for these drugs, large clinical trials have, until recently, been lacking. In recent years the trials RELAX and SIOVAC have been published, both failing to meet their primary end-point (165;166). In fact, in SIOVAC where study participants with persistent PH after valvular surgery were randomized to PDE5i or placebo, treatment with PDE5i resulted in an increased risk for the primary end-point, a composite of death, heart failure hospitalization, change in WHO functional class and quality of life. Moreover, the sGC stimulator riociguat, available for the treatment of PAH and CTEPH, has been investigated in systolic and diastolic heart failure, also without a clear improvement in the primary end-point change in MPAP. Riociguat did, however, improve several other hemodynamic parameters in systolic heart failure (167;168), suggesting that sGC stimulators may be beneficial in this condition. A novel sGC stimulator with longer half-life, vericiguat, has therefore been evaluated in phase II trials on systolic and diastolic heart failure (169;170). In SOCRATES-REDUCED, high doses of vericiguat improved the primary end-point, change in NT-proBNP (169).

Finally, prostacyclin was evaluated for the treatment of severe heart failure in the FIRST trial. Despite previous results suggesting beneficial effects of prostacyclin in heart failure (171), FIRST was terminated early due to increased mortality in patients receiving prostacyclin (172).

As randomized trials in general have failed to demonstrate beneficial effects of PAH specific drugs in left heart disease, with or without PH, off label use outside clinical trials should be avoided (Table 2) (14).

Table 2. Completed Clinical Trials in Pulmonary Hypertension due to Left Heart Disease

Drug Class	Study acronym and substance	Study population	PH inclusion criteria	Primary endpoint	Outcome
ERA					
	REACH-1 (162): Bosentan	LVEF<35%, NYHA IIIb-IV (n = 370)	No	Change in clinical status.	Stopped prematurely due to increased hepatic transaminases. Less adverse events after 3 months, in patients who completed the study.
	ENABLE-1 & 2 (163): Bosentan	LVEF<35%, NYHA IIIb-IV (n = 1613)	No	All-cause mortality or heart failure hospitalization.	No difference in primary endpoint. Increased risk of worsening HF, likely due to fluid retention.
	MELODY-1 (164): Macitentan	LVEF≥30%, NYHA II-III (n = 63)	Yes, Cpc-PH	Significant fluid retention or worsening NYHA class.	Trend towards higher incidence of significant fluid retention.
PDE5i					
	RELAX (165): Sildenafil	LVEF≥50%, NYHA II-IV (n = 216)	No	Change in peak oxygen consumption.	No improvement in primary endpoint.
	SIOVAC (166): Sildenafil	Successful valvular replacement/repair > 1 year before inclusion.	Yes, MPAP≥30 mmHg.	Composite of death, heart failure hospitalization, WHO class and QoL.	Increased risk of primary endpoint.
sGC stim					
	LEPHT (167): Riociguat	LVEF≤40% (n = 201)	Yes, MPAP≥25 mmHg	Change in MPAP.	No improvement in primary endpoint. Improvement in CI and PVR. Well tolerated.
	DILATE (168): Riociguat	HFpEF (n = 39)	Yes, post-capillary PH	Change in MPAP.	No change in primary endpoint. Well tolerated.
	SOCRATES-REDUCED (169): Vericiguat	LVEF<45% Worsening heart failure (n = 456)	No	Change in NT-proBNP	No improvement in primary endpoint in pooled analysis. Improvement in primary endpoint with high doses of Vericiguat.
	SOCRATES-PRESERVED (170): Vericiguat	LVEF≥45%, NYHA II-IV (n = 477)	No	Change in NT-proBNP and left atrial volume	No improvement in primary endpoint. Improved QoL.
PGI2					
	FIRST (172): Epoprostenol	HFrEF, NYHA IIIb-IV (n = 471)	No	All-cause mortality	Terminated early due to a strong trend towards increased mortality.

Hypoxic Pulmonary Vasoconstriction

Hypoxic pulmonary vasoconstriction (HPV) was first described in an *in vivo* cat model in 1946 (173) and a year later in human (174). HPV mainly affects the smooth muscle cells of the pulmonary resistance arteries (175;176) and is primarily caused by a decreased partial pressure of oxygen in alveolar air. Lower mixed venous saturation does also, to a lesser extent, contribute to HPV (177). HPV in focal hypoxia is beneficial and contribute to optimized ventilation-perfusion matching and gas exchange. In global hypoxia, e.g. during rapid ascent to high altitude it may, however, be detrimental. HPV may then limit exercise capacity (178) as well as contribute to the development of high altitude pulmonary edema and right heart failure by increasing pulmonary pressures and resistances (179-181). Whereas acute HPV is fully reversible (173), chronic hypoxia may result in pulmonary arterial and venous remodeling, as well as PH (182-184). The remodeling of the pulmonary arteries is characterized by medial hypertrophy as well as intimal and adventitial thickening. In severe cases plexiform lesions may also occur (183). Interestingly, the pulmonary veins furthermore undergo arterIALIZATION and intimal fibrosis (184). As in PAH and PH-LHD, the endothelin, NO and prostacyclin pathways are also central in HPV and in the development of hypoxic PH, including vascular remodeling (185) (Figure 12).

Figure 12. Chacaltaya Ski Lodge in Bolivia at Approximately 5300 m Above Sea Level.

The partial pressure of oxygen at this altitude corresponds to a F_iO_2 of approximately 0.10 causing pronounced hypoxic pulmonary vasoconstriction, thereby impairing exercise capacity. Photographed by and printed with the permission of assoc. prof. Göran Rådegran.



Therapies in Hypoxic Pulmonary Vasoconstriction

Treatment of acute global hypoxia focuses, apart from removing the cause of hypoxia, on increasing systemic oxygen supply via ventilation or ECMO. In acute hypoxia, reports have shown beneficial effects with PDE5i and inhaled NO but not with endothelin receptor antagonists or prostacyclin (185). In PH due to chronic lung diseases, PAH drugs have shown to improve hemodynamics without improving exercise capacity. In fact, several trials have resulted in further impairment in arterial saturation and quality of life as they interfere with HPV, thereby aggravating ventilation-perfusion mismatching (185-190). Guidelines therefore advise against the use of drugs targeting the endothelin, NO and prostacyclin pathways in PH due to lung diseases and/or hypoxia (14).

Aims

The overall objectives of the present thesis were to characterize patients prior to and after heart transplantation with regard to hemodynamics and blood borne biomarkers, as well as to evaluate vasoactive substances in the treatment of acute hypoxic pulmonary vasoconstriction, which may aggravate pulmonary hypertension due to left heart disease.

The specific aims of the included papers were to investigate:

- The effects of two pulmonary vasodilators, namely the soluble guanylate cyclase stimulator BAY 41-8543 and the dual endothelin receptor blocker tezosentan, in acute hypoxic pulmonary vasoconstriction.
- The hemodynamic characteristics after heart transplantation, including exercise response.
- The impact of pre-operative and post-operative pulmonary hypertension on outcome after heart transplantation.
- Plasma concentrations of substances related the nitric oxide pathway and nitric oxide dependent pulmonary vasodilatation prior to and after heart transplantation.

Materials and Methods

Paper I

***In Vivo* Pig Model of Acute Hypoxic Pulmonary Vasoconstriction**

An *in vivo* pig model of acute hypoxic HPV was established by our group (191;192). Experiments were carried out at the Department of Experimental Medicine and Surgery at the Panum Institute, University of Copenhagen, Denmark (Figure 13). Trained animal technicians performed animal care and surgical procedures.



Figure 13. Pig Model of Acute Hypoxic Pulmonary Vasoconstriction

Animals, Anesthesia and Ventilation

18 female pigs (mean±SEM weight of 31.1±0.4 kg) were fasted overnight with free access to water. All pigs were pre-medicated intramuscularly with 1 mL·10 kg⁻¹ of a mixture of Narcoxyl vet, Ketaminol vet, Metadon and Turbogestic in Zoletil 50 vet Tørstof. Anesthesia was maintained with intravenous infusion of Propofol and Fentanyl throughout the experiment. After intubation, the lungs were mechanically ventilated with a respirator and the inspiratory oxygen fraction (F_IO₂) was monitored using an oxygen sensor and a medical oxygen apparatus (OM871). After the experiments, the animals were euthanized by a mixture of Pentobarbital and

Lidocainhydrochloride. Further details can be found in the original publication (193).

Invasive Procedures, Measurements and Hemodynamic Surveillance

Two skin incisions were made, one along the medial line of the throat and one in the groin, to locate the internal jugular vein and the common carotid artery, as well as the femoral artery, respectively. An infusion catheter was inserted through the internal jugular vein and advanced into the right atrium. Catheters for arterial pressure measurements were positioned in the aortic arch, through the common carotid artery, as well as in the femoral artery. The femoral arterial catheter was used as a back-up, if problems with the aortic catheter would occur (193).

A Swan Ganz catheter (Edwards Lifesciences, Irvine, CA, USA), introduced through the right internal jugular vein, was used to measure mean right atrial pressure (MRAP), pulmonary artery pressures and PAWP, as well as CO in triplicates by thermodilution.

Electrocardiogram, heart rate (HR), mean arterial pressure (MAP), MRAP and MPAP, were monitored and recorded throughout the experiment. PAWP was measured intermittently. Non-invasive saturation was monitored by a pulse oximetry probe placed on the tails of the pigs. PVR, systemic vascular resistance and stroke volume (SV) were calculated using the following formulae: $PVR = (MPAP - PAWP) / CO$, systemic vascular resistance = $(MAP - MRAP) / CO$, and $SV = CO / HR$.

Blood samples from the pulmonary artery and the aortic arch were used for immediate blood gas analysis, determining blood aorta (A_O) and pulmonary artery (PA) O_2 saturation ($S_{A_OO_2}$, S_{PAO_2}), hemoglobin concentration (Hb_{A_O} , Hb_{PA}), O_2 -pressure ($p_{A_OO_2}$, p_{PAO_2}), pH_{A_O} and pH_{PA} . This allowed calculation of aorta and pulmonary artery O_2 -content ($C_{A_OO_2}$, C_{PAO_2}): $C_{A_OO_2} = (Hb_{A_O} \cdot 1.34 \cdot S_{A_OO_2}) + (0.0031 \cdot p_{A_OO_2})$, and $C_{PAO_2} = (Hb_{PA} \cdot 1.34 \cdot S_{PAO_2}) + (0.0031 \cdot p_{PAO_2})$, O_2 -extraction = $C_{A_OO_2} - C_{PAO_2}$, and O_2 -consumption = $CO \cdot (C_{A_OO_2} - C_{PAO_2})$. In the formulae, SO_2 is expressed as a fraction of 1.0 and not %, Hb as $g \cdot dL^{-1}$, pO_2 as mmHg, and the correction factors 1.34 as $mL \cdot g^{-1}$ and 0.0031 as $mL \cdot dL^{-1} \cdot mmHg^{-1}$.

Induction of Hypoxia

Hypoxia was induced by slowly lowering the oxygen supply and airflow on the respirator from $F_{iO_2} \sim 0.21$, to a stable level at ~ 0.15 and ~ 0.10 , respectively. The respiratory rate and tidal volume were kept constant throughout the experiments. Hemodynamic measurements and blood sampling were performed towards the end of normoxia, 5 minutes into stable hypoxia at $F_{iO_2} \sim 0.15$ and 15 minutes into stable hypoxia at $F_{iO_2} \sim 0.10$ (hypoxia baseline). During the induction of hypoxia, 1000 mL of sodium chloride was administered to each animal to avoid sudden systemic hypotension.

Six pigs were used for control measurements, where the stability of the HPV response over time was evaluated. In this protocol, hemodynamic measurements and blood sampling were performed after 5, 15, 22.5, 30, 37.5, 60, 67.5, 75, and 82.5 min of sustained hypoxia at $F_iO_2 \sim 0.10$, corresponding to the time-points when measurements were performed in the intervention protocols described below.

Drugs

Twelve pigs were used to study the effects of the sGC stimulator BAY 41-8543 (Bayer Scheering Pharma AG, Wuppertal, Germany), alone (n=6) or in combination with dual ET-receptor blockade with tezosentan (Actelion, Allschwil, Switzerland, n=6).

In the first intervention protocol, the sGC stimulator BAY 41-8543 was infused at increasing doses of 1, 3, 6, 9 and 12 $\mu\text{g}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$ for ~ 7.5 minutes each. Measurements were performed at steady state, 5-7.5 min after starting infusion of the sGC stimulator at each dose.

In the second protocol, 5 $\text{mg}\cdot\text{kg}^{-1}$ body weight of the dual endothelin A receptor and B receptor antagonist tezosentan was delivered as a manual bolus injection, followed by infusion of BAY 41-8543 at 1, 3 and 6 $\mu\text{g}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$ for ~ 7.5 minutes each. Measurements were performed 5, 15, 30 and 60 min after injection of tezosentan and at steady state between 5-7.5 min after starting infusion of the sGC stimulator at each dose.

All drugs were administered in the right atrium during sustained hypoxia at $F_iO_2 \sim 0.10$. An infusion pump (Model 11plus, Harvard apparatus, Maryland, USA) was used to administer BAY 41-8543.

Paper II-IV

Study Population and Data Collection

Medical records from the 215 HT patients followed at Skåne University Hospital in Lund, Sweden, 1988-2010 were retrospectively reviewed. A total of 219 HTs were included, out of which 214 (98%) were first-time HTs. Five (2%) were re-HTs; three within seven days, one 175 days and one 18 years after HT. 218 of the HTs had been performed in Lund. One pediatric first time HT had been performed abroad. 72 (33%) of the patients received a mechanical assist prior to HT (Table 3). Children under the age of 18 (n=21), re-HTs (n=5) and patients referred from, and subsequently evaluated at, other hospitals (n=83) and/or with incomplete data prior

to HT (n=12) were excluded. 94 adults evaluated at rest, at our hemodynamic lab, prior to HT remained for analysis (Table 3).

Table 3. Baseline Characteristics of the Entire HT Population in Lund 1988-2010 and the 94 Included Patients

Risk factor category and group	Entire population characteristics				Study population characteristics			
	Mean	SD +/-	N	%	Mean	SD +/-	N	%
Recipient characteristics								
Gender of recipient			215				94	
Male			147	68.4			70	74.4
Female			68	31.6			24	25.6
Age of recipient (years)	44.6	17.2	215		51.6	9.9	94	
≤10			9	4.2			0	0
11-20			24	11.1			1	1.1
21-30			8	3.7			4	4.3
31-40			17	7.9			6	6.4
41-50			42	19.5			21	22.3
51-60			85	39.5			45	47.9
61≤			30	14.0			17	18.1
Recipient length (cm)	167.3	25.3	197		173.4	7.9	93	
Recipient weight (kg)	68.4	18.9	198		75.3	15.0	93	
Recipient blood group			215				94	
O			71	33.0			31	33.0
A			109	50.7			46	48.9
B			19	8.8			8	8.5
AB			16	7.4			9	9.6
Time on waiting list (days)	151.3	200.4	219		148.6	179.6	94	
Indication for HT			215				94	
ARVD			3	1.4			0	0
Cardiac Tumor			1	0.5			0	0
CHD			11	5.1			1	1.1
CVR			1	0.5			0	0
DCM			98	45.6			46	48.9
DCM (Adria)			3	1.4			1	1.1
DCM + AI/AS			9	4.2			5	5.3
HCM			10	4.7			8	8.5
Myocarditis			13	6.0			6	6.4
IHD			58	27.0			24	25.6
RCM			6	2.8			3	3.2
RHF + VT/VF			2	0.9			0	0
Donor characteristics								
Gender of donor			219				94	
Male			138	63.0			58	61.7
Female			81	37.0			36	38.3
Age of donor (years)	40.0	15.7	219		43.9	13.7	94	
Donor length (cm)	172.4	18.7	210		176.3	9.5	92	
Donor weight (kg)	74.1	18.5	211		79.4	13.5	93	
Ischemic time (min)	184.7	63.2	201		182.6	61.5	86	
Recipient-donor matching								
Age difference (years)	4.6	15.9	219		7.7	14.7	94	
Sex matching			219				94	
Sex-matched			160	73.1			71	75.5
Sex-mismatched			59	26.9			23	24.5
ABO-matching			219				94	
Identical			184	84.0			82	87.2
Compatible			32	14.6			12	12.8
Incompatible			3	1.4			0	0

Paper II

The 94 patients were hemodynamically characterized at rest prior to and after HT. 32 of the 94 patients exercised prior to and one year after HT. They were subsequently characterized into two groups, one with patients ≤ 50 years ($n=12$) and one with patients >50 years ($n=20$), at the time of HT. This sub classification was performed to compare our findings to those presented in healthy individuals published in previous systematic reviews, as these have shown that the hemodynamic response to exercise differ for healthy individuals aged ≤ 50 years vs. >50 years (144;155).

Table 4. Previous Publications on Pre-Operative Pulmonary Hypertension and Their Reported Outcomes After Heart Transplantation

Author/Period	Characterization	Results
Costard-Jäckle and Fowler (9) 1980-1988	1. "Low risk": PVR <2.5 or PVR ≤ 2.5 and sAP ≥ 85 with vasodilatation 2. "High risk": PVR ≥ 2.5 or PVR ≤ 2.5 but sAP ≤ 85 with vasodilatation	- Increased 3- month mortality in high risk group
Murali et al. (10) 1980-1991	1. TPG <15 and PVR <5 2. TPG <15 and PVR ≥ 5 3. TPG ≥ 15 and PVR <5 4. TPG ≥ 15 and PVR ≥ 5	- Increased 0-2 day mortality in severe PH pre-HT (group 4) - Increased 30-day mortality with TPG >15
Lindelöw et al. (32) 1988-1990	1. "Low PVR": PVR ≤ 3 2. "High PVR": PVR ≤ 3 and MAP ≥ 70 with vasodilatation	- No difference in mortality between groups
Delgado et al. (194) 1991-1996	1. No PH: TPG ≤ 12 and PVR ≤ 2.5 2. PH: TPG >12 and/or PVR >2.5	- Increased 30-day mortality with PH
Klotz et al. (195) 1998-2001	1. No PH: TPG <12 and PVR <2.5 2. PH: TPG ≥ 12 and/or PVR ≥ 2.5	- No difference in 1 year mortality between patients without PH vs reversible PH
Chang et al. (196) 1983-2000	1. No PH: PVR <2.5 2. Mild/moderate PH: PVR 2.5-4.9 3. Severe PH: PVR ≥ 5	- Increased 1, 3 and 6 month survival with mild/moderate PH - Increased 1 year mortality with severe PH, otherwise unchanged
Goland et al. (17) 1989-2004	1. No PH: PVR <3 , TPG <10 2. Mild-Moderate PH: PVR 3-6, TPG 10-20 3. Severe PH: PVR >6 , TPG >20	- No difference in mortality with pre-HT PH - Increased long term mortality with sustained PH after HT
Klotz et al. (197) 1996-2005	1. No PH: TPG ≤ 12 and PVR ≤ 2.5 2. Rev PH: TPG ≤ 12 and PVR ≤ 2.5 with vasodilatation	- No difference in mortality between groups
Gude et al. (18) 1983-2007	1. No PH: 2-3 of: sPAP ≤ 50 , TPG ≤ 10 and PVR ≤ 2.5 2. PH: 2-3 of: sPAP ≤ 50 , TPG ≤ 10 and PVR ≤ 2.5 with vasodilatation	- No difference in 1 year mortality with pre-HT PH - Increased long term mortality with sustained PH after HT
	1. No PH: 2-3 of: SPAP ≤ 50 , TPG ≤ 10 and PVR ≤ 2.5 2. PH: 2-3 of: SPAP ≤ 50 , TPG ≤ 10 and PVR ≤ 2.5 with vasodilatation	- No difference in long-term mortality with pre-HT PH - Increased long-term mortality with sustained PH after HT

Paper III

The 94 included patients were characterized according to: i. previously published hemodynamic risk criteria for outcome after HT (Table 4) (9;10;17;18;18;32;194-197); ii. ESC's guidelines from 2009 (125); iii. ISHLT's State of art summary statement from 2012 (11); iv. ISHLT's relative contraindications for HT from 2006 (7) and v. ISHLT's criteria for increased risk of right heart failure and early death after HT from 2006 (7) (Table 5). To separate the patients into groups according to ISHLT's State of art summary statement, the criteria for passive and reactive mixed PH were modified, i.e. defined as $TPG \leq 15$ mmHg and $PVR \leq 3$ WU, instead of $TPG \leq 15$ mmHg or $PVR \leq 3$ WU. Survival was compared between the subgroups of each study.

Table 5. Previous Definitions of Pulmonary Hypertension and a High Risk Population According to ESC and ISHLT

Definition/Author	Characterization
PH- definitions according to ESC-guidelines from 2009. Galiè et al. (125)	<ol style="list-style-type: none"> 1. No PH: $mPAP < 25$ 2. Pre-capillary PH: $MPAP \geq 25$ and $PCWP \leq 15$ and CO normal or reduced 3. Post-capillary PH: $MPAP \geq 25$ and $PCWP > 15$ and CO normal or reduced <ol style="list-style-type: none"> 3a. Passive: $TPG \leq 12$ 3b. Reactive: $TPG > 12$
ISHLT's State of art summary statement. Fang et al. (11)	<ol style="list-style-type: none"> 1. No PH: $mPAP < 25$ 2. Passive PH: $MPAP \geq 25$ and $PCWP > 15$ and $TPG \leq 15$ or $PVR \leq 3.0$ 3. Mixed PH: $MPAP \geq 25$ and $PCWP > 15$ and $TPG > 15$ or $PVR > 3.0$ <ol style="list-style-type: none"> 3a. Reactive PH (with vasodilatation) : $TPG \leq 15$ or $PVR \leq 3.0$ 3b. Non-reactive PH (with vasodilatation): $TPG > 15$ or $PVR > 3.0$
ISHLT-guidelines for performing vasodilation during RHC. Mehra et al. (7)	<ol style="list-style-type: none"> 1. $sPAP \geq 50$ and either $TPG \geq 15$ or $PVR > 3.0$ while maintaining $sAP > 85$ with vasodilatation
ISHLT's defined relative contraindications for HT. Mehra et al.	<ol style="list-style-type: none"> 1. $PVR > 5$ or $PVRI > 6$ or $TPG > 16-20$
ISHLT's defined increased risk of right heart failure and early death after HT. Mehra et al. (7)	<ol style="list-style-type: none"> 1. $sPAP > 60$ and either $PVR > 5$ or $PVRI > 6$ or $TPG > 16-20$ 2. $PVR > 2.5$ with vasodilatation 3. $PVR < 2.5$ but sAP falls to < 85 with vasodilatation

Paper IV

Of the 94 patients, 89 were post-operatively evaluated with routine RHC at our hemodynamic laboratory. The five patients who did not perform RHC after HT died early after transplantation: three within one day, one after 14 days, and one after 25 days. All were men and three had pre-operative PH. No additional, clinically indicated, hemodynamic evaluations were included in the study.

The patients were grouped depending on their hemodynamic data at the post-operative evaluations. Survival was compared between the groups and hazard ratios for death were estimated with a Cox regression analysis adjusted for gender, age at the time of HT and kidney function one year after HT.

Right Heart Catheterization and Endomyocardial Biopsies

Right heart catheterization (Figure 14 a) was performed at rest prior to HT, as well as one and four weeks, three and six months and one year after HT. In all patients capable to exercise, hemodynamic measurements were also performed during supine bicycle exercise prior to HT, as well as four weeks, three and six months and one year after HT. Men exercised at 50 W and women at 30 W, maintaining steady revolutions at 60 per minute. Measurements were conducted at steady state, approximately five minutes after initiation of exercise. There was no graded increase in workload. If patients had more than one RHC prior to HT, the one closest to HT, or assist implantation, was analyzed. Pre-operative RHC was on average performed 143.1 ± 113.3 days prior to HT.

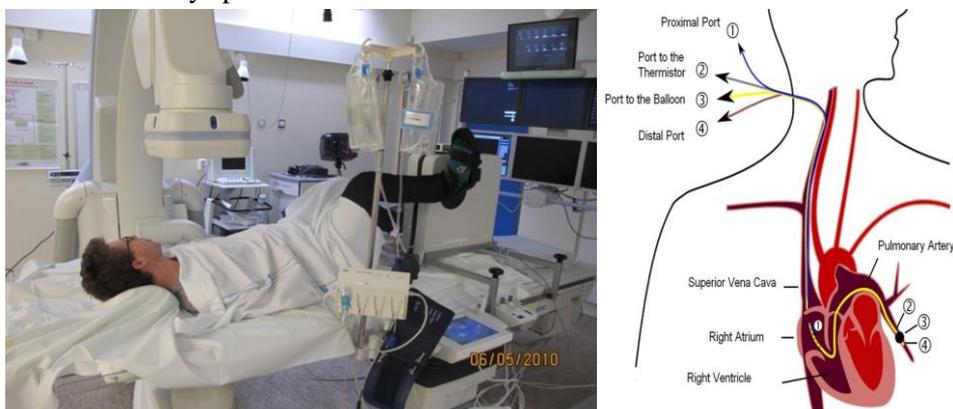


Figure 14 (a) and (b). Right Heart Catheterization and Swan Ganz Catheter
(a) The hemodynamic lab at Skåne University Hospital, Lund, (b) Schematic illustration of a Swan Ganz catheter

RHC was predominantly performed via the right internal jugular vein, using a Swan Ganz catheter (Figure 14 b) (Baxter Health Care Corp, Santa Ana, CA). During RHC MPAP, PAWP, MRAP and MAP, were measured. HR was recorded from electrocardiography. CO was measured by thermodilution. TPG, DPG, SV, cardiac index, stroke volume index, PVR, pulmonary vascular resistance index and TPR were calculated by the following formulae: $TPG = MPAP - PAWP$, $DPG = \text{diastolic pulmonary artery pressure} / PAWP$, $SV = CO / HR$, $CI = CO / \text{body surface area}$, $SVI = SV / \text{body surface area}$, $PVR = TPG / CO$, $PVRI = TPG / CI$ and $TPR = MPAP / CO$. Left ventricular stroke work index and right ventricular stroke work index were calculated using different formulae depending on recommendations at the time of each study, i.e. left ventricular stroke work index = $(MAP - PAWP) \cdot SV$ index and right ventricular stroke work index = $(MPAP - MRAP) \cdot SV$ index (paper II) or left ventricular stroke work index = $(MAP - PAWP) \cdot SV$ index $\cdot 0.0136$ and right ventricular stroke work index = $(MPAP - MRAP) \cdot SV$ index $\cdot 0.0136$, where 0.0136 is a conversion factor (paper III).

To monitor acute cellular rejection (ACR), EMBs were performed from the right interventricular septum on a routine basis on 14 occasions during the first post-operative year (week 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, 24, 32, 40, and 52 after HT). Some of the EMBs were performed in connection with the RHCs described above. From 2005 onwards, EMBs were graded according to both the 1990 and the 2004 ISHLT grading systems (198;199). Prior to 2005, the EMBs were solely graded according to the 1990 system (198).

With regard to renal function, glomerular filtration rate was measured at one year after HT using the iohexol clearance method. In patients where iohexol clearance measurements were missing, the creatinine-based CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation recommended by KDIGO (200), was used.

Drugs and Devices

A vasodilator test or mechanical assist was used when necessary to assess acute and sustained vaso-reactivity, respectively. Of the 94 included patients, 23 patients were exposed to a vasodilator, either intravenous nitroprusside (Nitropress, Hospira Inc, Lake Forest, USA) or nitric oxide (NO, INOmax, INO Therapeutics AB, Lidingö, Sweden) inhalation (INOvent, INO Therapeutics AB, Lidingö, Sweden) during RHC. The doses for nitroprusside were ~0.5, 1, 2, 4 and 8 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ and for NO 20 or 40 parts per million.

21 of the 94 patients received a mechanical assist as a bridge to transplantation. Of these, five were HeartMate IP (Thoratec, Pleasanton, CA, USA), five HeartMate VE (Thoratec, Pleasanton, CA, USA), three Jarvik 2000 (Jarvik Heart Inc., New York, NY, USA), one Abiomed (Abiomed Inc, Danvers, MA, USA) and seven HeartMate II (Thoratec, Pleasanton, CA, USA). 12 patients with mechanical assist were hemodynamically re-evaluated with the device prior to HT, three had a HeartMate IP, four HeartMate VE, two Jarvik 2000 and three HeartMate II. Four patients with mechanical assist exercised prior to HT and one year thereafter.

Paper V

Study Population

Clinical data was gathered from medical records of all 43 patients who underwent HT at Skåne University Hospital in Lund June 2012 through February 2014. All patients were included in a prospective cohort study and 12 patients were included in the present investigation. The 12 patients were selected as they were

hemodynamically evaluated at our lab, at rest, prior to HT, as well as four weeks and six months after HT, and as they at the four week and six month evaluations; i. did not have rejections requiring specific therapy; ii. did not have post-operative PH and; iii. had left ventricular ejection fraction $\geq 50\%$ (Table 6).

12 healthy, age-matched, non-smokers without drug treatment and no symptoms or signs of common cold were used for comparison.

Table 6. Baseline Characteristics of the Included Patients and Controls

Parameter	Patients		Healthy subjects	
	n	median (IQR)	n	median (IQR)
Clinical features				
Sex, male/female	12	10/2	12	5/7
Age, years	12	50 (45-60)	12	51 (46-60)
Weight, kg	12	80 (71-92)		N/A
Length, cm	12	179 (176-180)		N/A
BSA, m ²	12	2.0 (1.9-2.1)		N/A
NYHA class, IIIb/IV		3/9		N/A
Etiology, DCM/HCM/IHD	12	8/1/3		N/A
Diabetes mellitus, yes/no	12	0/12	12	0/12
Hypertension, yes/no	12	0/12	12	0/12
AF, yes/no	12	2/10	12	0/12
History of smoking, yes/no	12	3/9	12	0/12
Medical history				
Betablockers, %	12	12 (100)		-
ACEi/ARB, %	12	8/4 (100)		-
MRA, %	12	8 (67)		-
Anticoagulants, %	12	10 (83)		-
Statins, %	12	3 (25)		-
Diuretics, %	12	12 (100)		-
Biochemical indicators				
L-Arginine, μM	12	56 (48-60)	12	86 (78-92) *
ADMA, μM	12	0.57 (0.47-0.71)	12	0.39 (0.32-0.42) *
SDMA, μM	12	0.85 (0.68-1.15)	12	0.41 (0.36-0.45) *
L-Arginine/ADMA	12	86 (68-128)	12	225 (203-273) *
P-Urea, $\text{mmol}\cdot\text{L}^{-1}$	12	9 (8-13)		N/A
P-Homocysteine, $\mu\text{M}\cdot\text{L}^{-1}$	12	16 (13-27)		N/A
P-NT-proBNP, $\text{ng}\cdot\text{L}^{-1}$	12	4058 (3348-7408)		N/A
P-creatinine, $\mu\text{M}\cdot\text{L}^{-1}$	12	106 (95-131)		N/A
eGFR, $\text{mL}\cdot\text{min}^{-1}\cdot 1.73\text{m}^{-2}$	12	61 (44-78)		N/A

Plasma Samples and Biomarker Analysis

Plasma samples of patients collected from the superior vena cava during RHC were stored at -80°C in the Lund Cardio Pulmonary Register cohort of Region Skåne's biobank. The samples were analyzed for plasma concentrations of ADMA, symmetric dimethylarginine, L-Arginine, L-Ornithine and L-Citrulline with liquid chromatography – tandem mass spectrometry (LC-MS/MS), at the Swedish National Veterinary Institute in Uppsala, Sweden. The L-Arginine/ADMA-ratio, the L-Arginine/L-Ornithine-ratio and the global arginine bioavailability ratio (L-Arginine/(L-Ornithine+L-Citrulline)) were calculated.

Control samples collected at Uppsala University Hospital and stored at -20°C were analyzed for L-Arginine, ADMA and symmetric dimethylarginine. The analysis of control samples was performed prior to the analysis of patient samples, at which point the L-Ornithine and L-Citrulline analyzes were not available. Consequently, L-Ornithine and L-Citrulline levels are lacking in controls. The methods used for the analysis of L-Arginine, ADMA and symmetric dimethylarginine were identical in patients and controls.

Ethics

The experiments in paper I were ethically approved by Dyreforsøgstilsynet, Copenhagen, Denmark (2009/561-1621) and performed in accordance with the guidelines of the Department of Experimental Medicine and Surgery at the Panum Institute, Copenhagen, Denmark.

Papers II-V were performed with approval from the local ethics boards in Lund (Approval nos. 2014/92, 2011/777, 2011/368 and 2010/114 for papers II-IV and 2010/114, 2010/442, 2011/368, 2011/777, 2015/270 for paper V) and Uppsala (Approval no. 2010/343, paper V). Papers II-V performed in accordance with the Declarations of Helsinki and Istanbul.

Statistical Analyses

Parametric or non-parametric statistics were used, as appropriate, depending on the distribution of data, i.e. Paired t-test or Wilcoxon Signed rank test, respectively, when comparing one group prior to, and after, an intervention; One Way Repeated Measures ANOVA (Tukey-test) or One Way Repeated Measures ANOVA on Ranks (Tukey-test), respectively, when the same group was compared over time; t-test or Mann-Whitney Rank Sum test, respectively, when two groups were compared and Pearson- or Spearman Correlation, respectively, when measuring the association between two variables. The χ^2 test was used to compare the proportion of EMBs with ACR between two groups. Survival curves were plotted using the Kaplan-Meier method, differences between groups were investigated using the Log-Rank test and hazard ratios of death were estimated with Cox proportional hazards regression analysis. In papers III and IV, last day of follow-up was 30th of June 2012 (mean 8.2±5.8 years).

Continuous variables are presented as mean±SEM (paper I), mean±SD (paper II-IV) or median and interquartile range (paper V), unless otherwise indicated. Categorical variables are expressed as counts and percentages. With the exception of the Cox proportional hazards regression analysis in paper IV, performed in SPSS

(IBM SPSS Statistics 20, IBM Corp., Armonk, NY, USA) all statistical analyses were performed using SigmaStat/SigmaPlot 11 (Systat Software Inc., San José, CA, USA). A p value < 0.05 was considered statistically significant throughout.

Results

Paper I

Hypoxia

Induction of Hypoxia

In a pooled analysis of all 18 pigs, hypoxia gradually, and maximally, increased MPAP by 14.2 ± 0.6 mmHg, PVR by 2.8 ± 0.3 WU, MRAP by 2.4 ± 0.6 mmHg, PAWP by 1.8 ± 0.5 mmHg and CO by 0.7 ± 0.2 L \cdot min $^{-1}$. Hypoxia also maximally decreased SVR by 5.6 ± 1.3 WU, S_{AO_2} by 21.7 ± 2.8 % and S_{PAO_2} by 17.8 ± 3.5 %. MAP, HR and blood- O_2 -consumption were not significantly altered by induction of hypoxia (Figure 15).

Hypoxia protocol

During sustained hypoxia at $F_{iO_2} \sim 0.10$ for 82.5 min, all hemodynamic variables as well as blood- O_2 -consumption remained stable, whereas blood S_{AO_2} and S_{PAO_2} decreased by 13.9 ± 3.1 % and 14.0 ± 2.4 %, respectively.

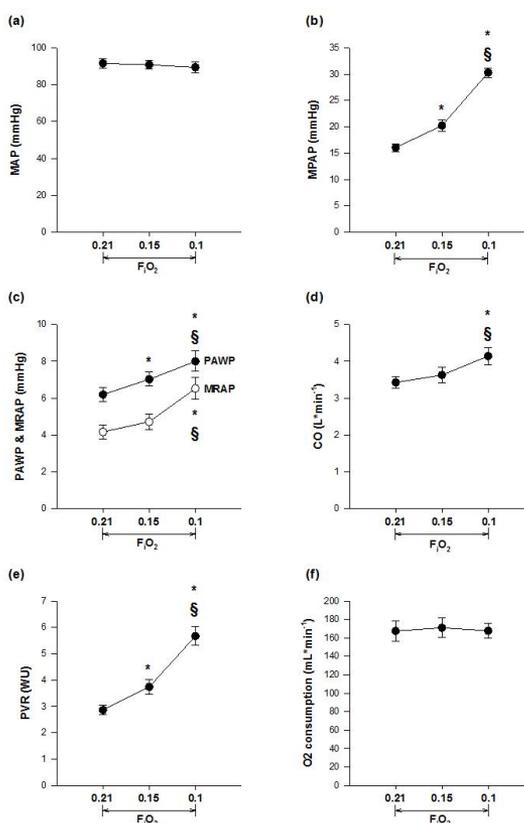


Figure 15. Hemodynamic and O_2 -consumption Response to Hypoxia

* Indicates statistical significance as compared to normoxia, § Indicates statistical significance as compared to F_{iO_2} 0.15.

Effects of Soluble Guanylate Cyclase Stimulation

Soluble Guanylate Cyclase stimulation with BAY 41-8543 dose-dependently, maximally, decreased MPAP by 15.0 ± 1.2 mmHg and PVR by 4.7 ± 0.7 WU, both below the normoxic level, reaching a steady state at $6-12 \mu\text{g} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$. MRAP decreased to normal levels at doses off at $3-6 \mu\text{g} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$, being unaltered

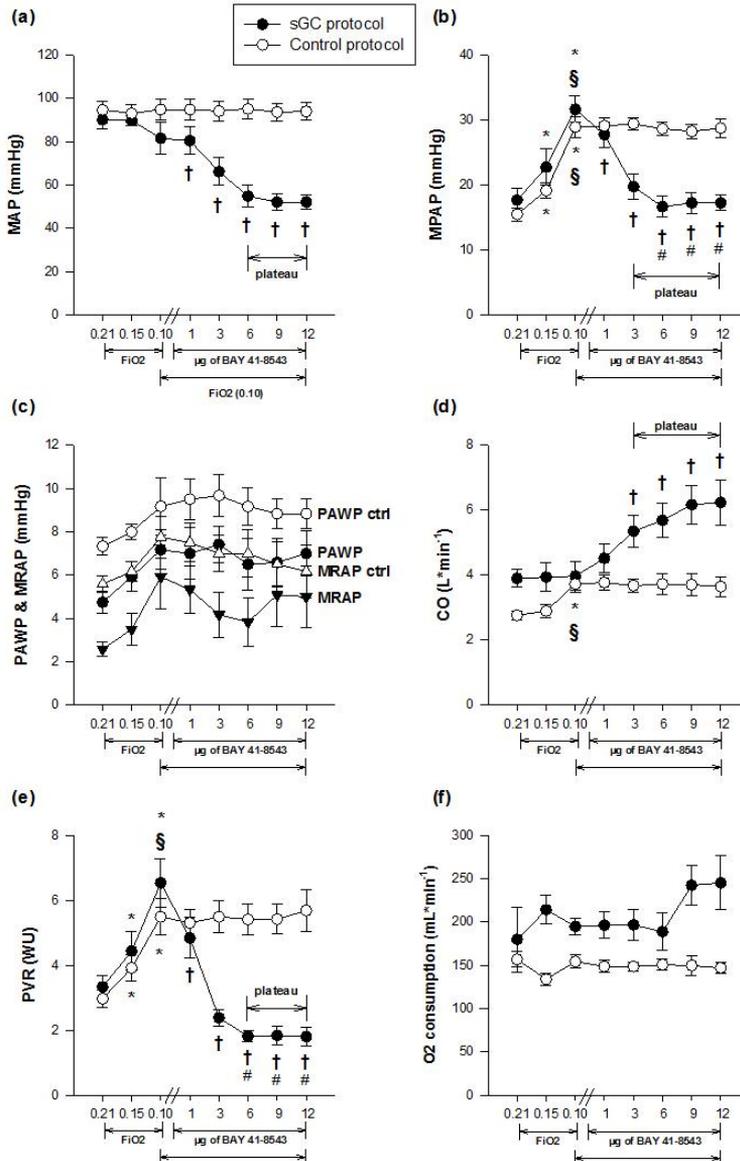


Figure 16. Hemodynamic and O₂-consumption Response to sGC Stimulation

During hypoxia induction * indicates statistical significance as compared to normoxia and § Indicates statistical significance as compared to FIO₂ 0.15. † indicates statistical significance as compared to FIO₂ 0.10 without BAY 41-8543 and # indicates values below the normoxic level. Statistical indications for MRAP are shown in the original publication (193).

thereafter. Furthermore, BAY 41-8543 dose-dependently decreased MAP by 26.8 ± 3.3 mmHg, levelling off at $6-12 \mu\text{g} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ and increased CO by maximally $1.4 \pm 0.3 \text{ L} \cdot \text{min}^{-1}$, levelling off at $3-12 \mu\text{g} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$, as well as S_{PAO_2} by 12.4 ± 5.7 % at doses of $12 \mu\text{g} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$. PAWP, S_{AO_2} and blood- O_2 -consumption remained unaltered (Figure 16).

Effects of Soluble Guanylate Cyclase Stimulation in Combination with Dual Endothelin Receptor Blockade

Dual Endothelin Receptor Blockade

Dual ET-receptor blockade with tezosentan completely normalized PVR and MRAP and maximally decreased MPAP by 11.8 ± 1.2 mmHg, reaching a steady state 30-60 minutes after bolus injection of tezosentan for MPAP and PVR and after 15-60 min for MRAP. Furthermore, tezosentan maximally decreased MAP by 27.2 ± 2.8 mmHg, levelling off 30-60 minutes after injection and decreased S_{PAO_2} by 6.3 ± 2.3 % after 60 minutes. PAWP, CO, blood S_{AO_2} , and blood O_2 -consumption were unaltered by tezosentan injection (Figure 17).

Soluble Guanylate Cyclase Stimulation on Top of Dual Endothelin Receptor Blockade

Among the variables decreased by tezosentan, BAY 41-8543 further, dose-dependently decreased MPAP to normoxic levels as well as PVR and MAP by 1.9 ± 0.4 WU and 20.5 ± 2.9 mmHg, respectively, to levels below those in normoxia, levelling off at infusion rates of $3-6 \mu\text{g} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ for all three variables. MRAP, PAWP, CO, S_{AO_2} , S_{PAO_2} and blood O_2 -consumption remained unaltered with BAY 41-8543 infusion in addition to tezosentan (Figure 17).

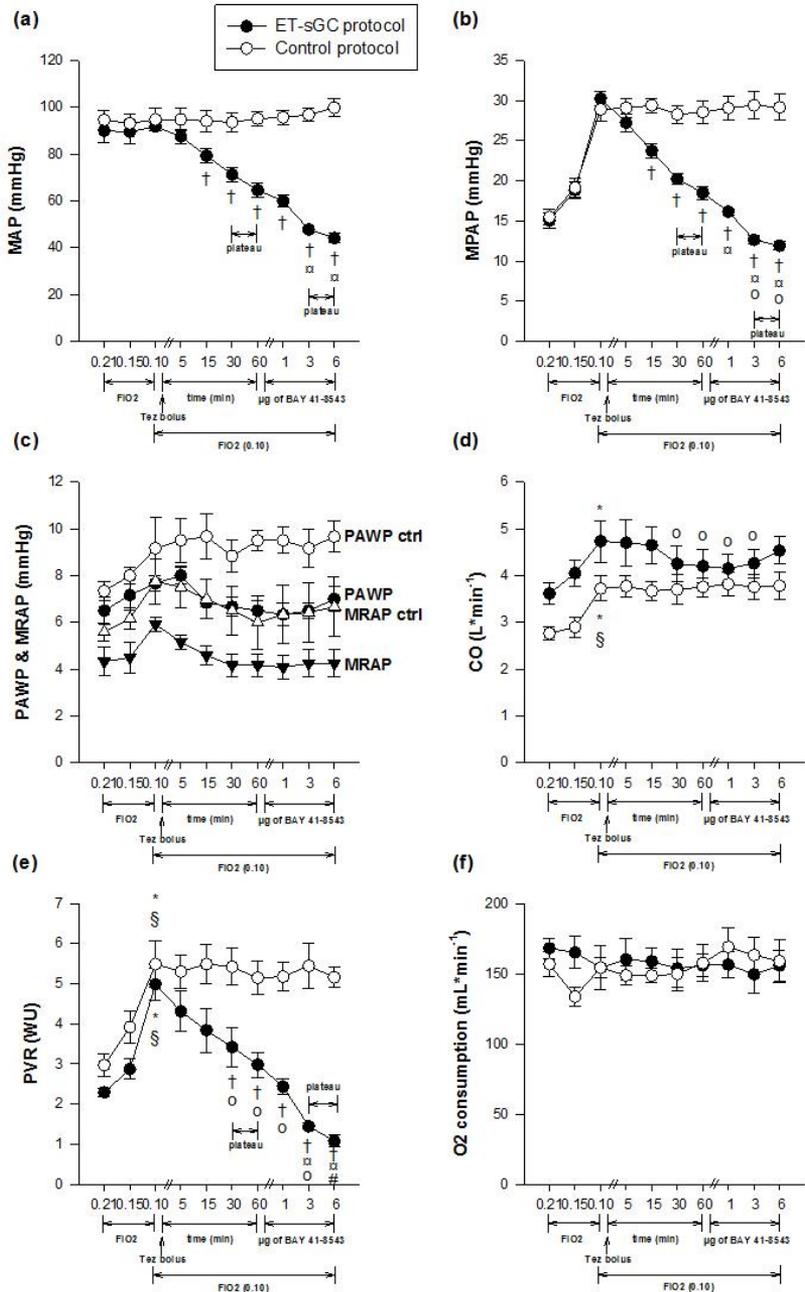


Figure 17. Hemodynamic and O₂-consumption Response to sGC Stimulation in Combination with Endothelin Receptor Blockade

During hypoxia induction * indicates statistical significance as compared to normoxia and § indicates statistical significance as compared to F_IO₂ 0.15. During sustained F_IO₂ 0.10, † indicates statistical significance as compared to F_IO₂ 0.10 without tezosentan, α indicates statistical significance with BAY 41-8543 as compared to hypoxia 60 min after tezosentan bolus infusion, ○ indicates return to normoxic values and # indicates values below the normoxic level. Statistical indications for MRAP are shown in the original publication (193).

Paper II-IV

Pre-operative Hemodynamics

At rest, in all 94 patients, pre-operative MPAP was 30.1 ± 8.8 mmHg, PAWP 20.5 ± 8.2 mmHg, TPG 9.6 ± 3.5 mmHg, DPG -1.2 ± 4.6 mmHg, MRAP 7.9 ± 6.1 mmHg, SV 46.7 ± 17.1 mL \cdot beat $^{-1}$, CO 3.7 ± 1.0 L \cdot min $^{-1}$ and PVR 2.8 ± 1.3 WU.

32 patients exercised during RHC prior to HT and one year after HT. During the pre-operative assessment, exercise, compared to rest, increased MPAP to 44.4 ± 10.5 mmHg, PAWP to 30.5 ± 9.5 mmHg, TPG to 14.0 ± 5.3 mmHg, MRAP to 16.3 ± 7.4 mmHg, HR to 111.9 ± 19.6 beat \cdot min $^{-1}$, SV to 60.2 ± 21.6 mL \cdot beat $^{-1}$ and CO to 6.4 ± 1.7 L \cdot min $^{-1}$, whereas PVR and TPR remained unchanged (Figure 18).

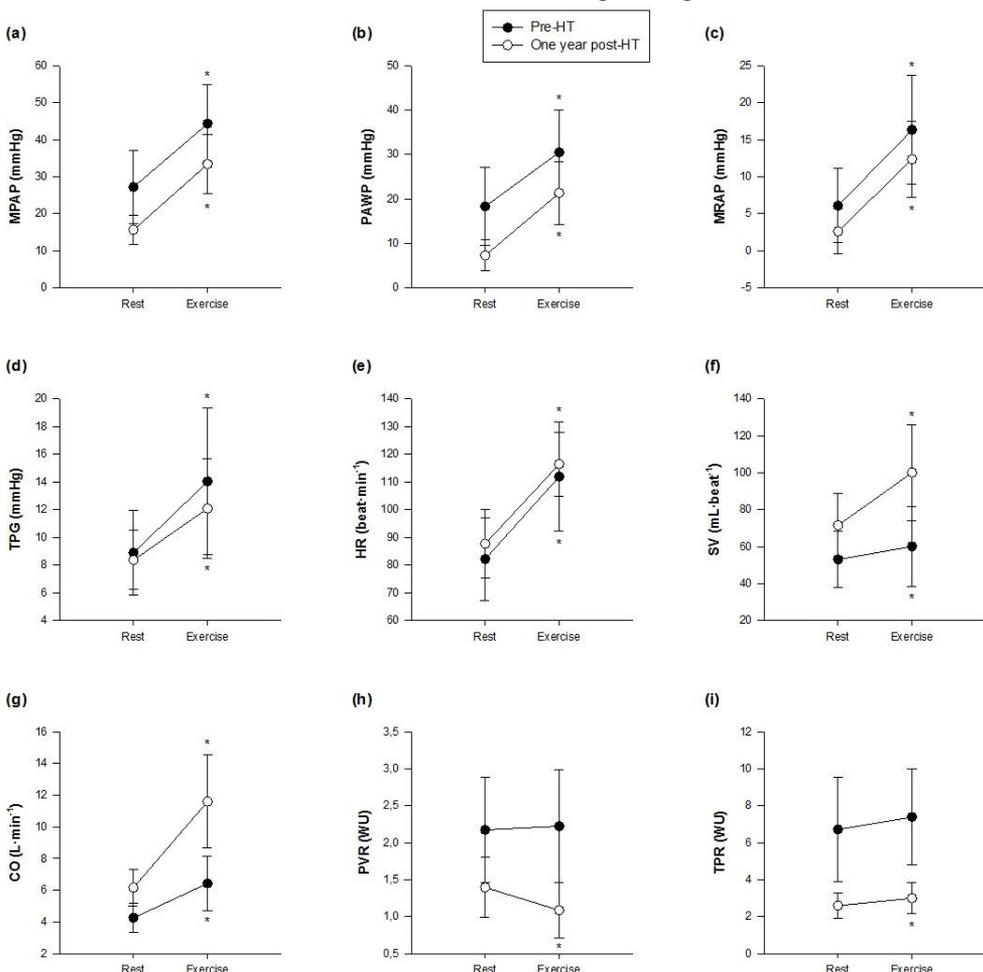


Figure 18. Hemodynamic Response to Exercise Prior to and After Heart Transplantation

* Indicates statistical significance as compared to rest.

With regard to age dependent differences, none of the hemodynamic parameters above differed for patients ≤ 50 years and >50 years, neither at rest nor during exercise. The percentage change did furthermore not differ between the groups.

Pre-operative Pulmonary Hypertension

Sixty-six of the 94 patients had PH prior to HT. Of these, 63 had post-capillary PH and three had pre-capillary PH according to present definitions (14;125). 50 of the patients with post-capillary PH had passive post-capillary PH and 13 had a reactive post-capillary PH, according to ESC's guidelines from 2009 (125). $DPG \geq 7$ mmHg was present in three patients.

Acute Vasoreactivity Test

The 23 patients exposed to acute vasodilatation during RHC prior to HT showed a decrease in MPAP by 4.6 ± 10.4 mmHg and PVR by 1.6 ± 1.3 WU. PVR did not decrease to <2.5 WU in 12 patients. In four patients where PVR decreased to <2.5 WU, SAP decreased to <85 mmHg.

Hemodynamic Response to Left Ventricular Assist Device

Twelve patients were hemodynamically re-evaluated 77 ± 47 days after assist-implantation. Prior to implantation, MPAP in these patients was 29.3 ± 7.8 mmHg, PAWP was 21.8 ± 8.3 mmHg, MRAP was 6.7 ± 7.7 mmHg, DPG was -2.3 ± 5.4 mmHg, CO was 3.4 ± 0.7 L·min⁻¹ and PVR was 2.2 ± 0.7 WU. At re-evaluation, MPAP, PAWP, and MRAP were normalized. DPG increased to 2.0 ± 4.2 mmHg and CO increased to 4.3 ± 1.1 L·min⁻¹ whereas PVR remained unaltered. Five of the 12 patients still had $PVR > 2.5$ WU at the re-evaluation prior to HT.

Post-operative Hemodynamics

Hemodynamic Response to Heart Transplantation

Seventy-one patients performed RHC at rest one week after HT, 72 at four weeks after HT, 63 at three months after HT, 72 at six months after HT and 78 at one year after HT.

Compared to prior to HT, at rest, one week after HT, there was a decrease in MPAP, PAWP, PVR and TPR, along with an increase in CO and SV. MRAP and HR were unaltered (Figure 19).

HR and PVR remained constant throughout the first year after HT, whereas MPAP, PAWP, MRAP, SV and TPR further decreased and CO further increased (Figure 19).

With regards to exercise, four weeks after HT there was a decrease in MPAP, PAWP, HR, PVR and TPR compared to prior to HT, along with an increase in CO and SV. MRAP was unaltered early after HT. Whereas MPAP, PAWP, SV and TPR remained stable throughout the first year after HT, HR returned to pre-operative levels, CO further increased, and MRAP as well as PVR decreased, one year after HT (Figure 19).

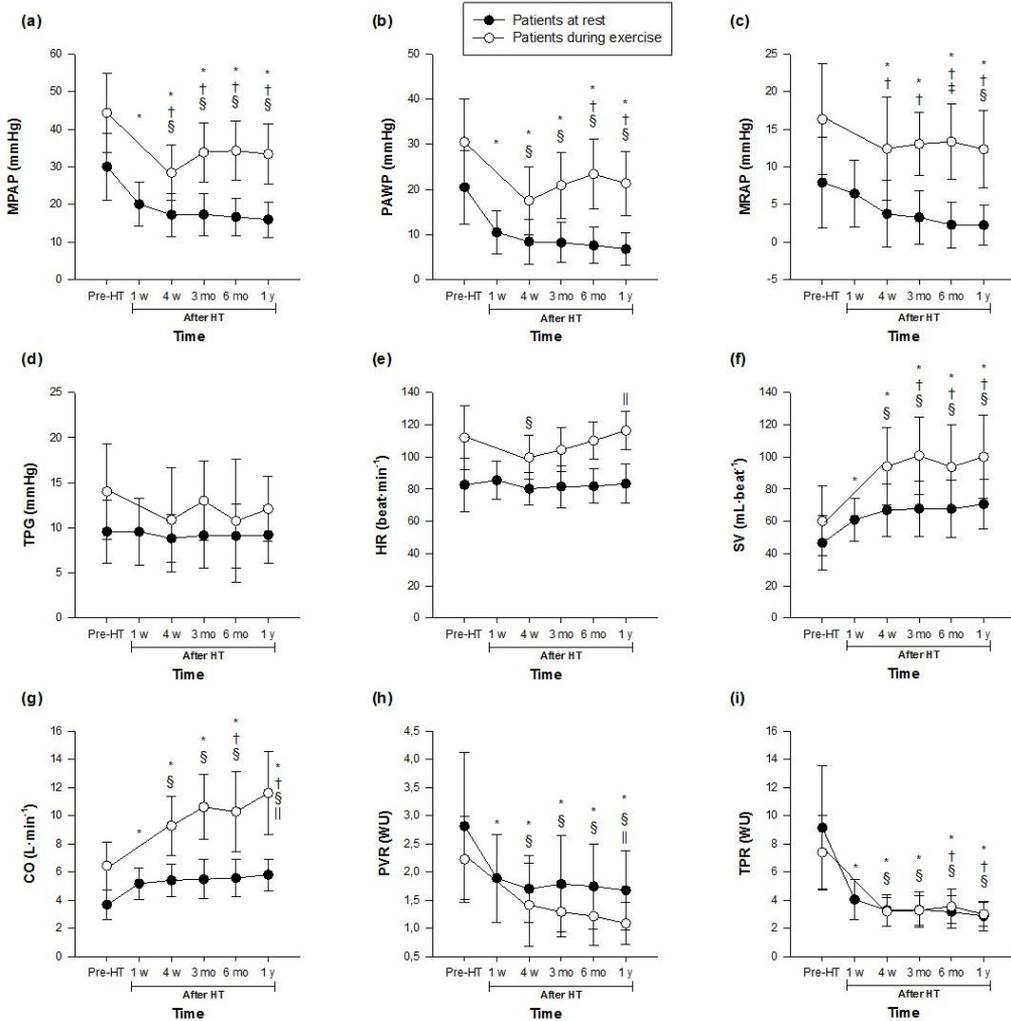


Figure 19. Hemodynamic Response to Exercise Prior to and After Heart Transplantation

* Indicates statistical significance as compared with prior to HT, † indicates statistical significance at rest as compared with one week after HT, ‡ indicates statistical significance at rest as compared with 4 weeks after HT, § indicates statistical significance during exercise compared with prior to HT and || indicates statistical significance during exercise compared with 4 weeks after HT.

Hemodynamics during Exercise One Year After Heart Transplantation

For the 32 patients who exercised during RHC one year after HT, exercise, compared to rest, increased MPAP by 17.8 ± 6.1 mmHg, PAWP by 14.1 ± 5.5 mmHg, MRAP by 9.8 ± 4.3 mmHg, TPG by 3.7 ± 3.1 mmHg, HR by 28.7 ± 9.1 6 beat·min⁻¹, SV by 28.5 ± 15.6 mL·beat⁻¹, CO by 5.4 ± 2.4 L·min⁻¹ and TPR by 0.4 ± 0.7 WU, whereas PVR decreased by 0.3 ± 0.3 WU (Figure 18).

Resting TPR was higher in patients >50 years compared to patients ≤50 years. In contrast, during exercise, not only TPR, but also MPAP and PVR were higher in patients >50 years, compared to those ≤50 years.

Post-operative Pulmonary Hypertension

Sixty-three of the 89 patients who were hemodynamically evaluated after HT had pre-operative PH. 53 (84 %) of these performed RHC at one week after HT. In 37 (70 %), the PH was reversed and in 16 (30 %) it persisted. None of the three patients with DPG ≥ 7 mmHg prior to HT had elevated DPG one week after HT. Four weeks after HT nine patients had PH, of which eight had pre-operative PH. The corresponding number of patients with PH at three months, six months and one year were eight (seven pre-operative), six (five) and eight (seven), respectively.

Out of the eight patients with PH one year after HT, four had pre-capillary PH, one had combined pre-capillary and post-capillary PH, and three had isolated post-capillary PH, according to ESC's guidelines from 2015 (14). Moreover, seven patients had DPG ≥ 7 mmHg, four had PVR > 3 WU and nine had TPG ≥ 12 mmHg.

Twenty-nine patients (33 %) exhibited PH at least once during the first year after HT. Of these, 18 had PH at a single examination and 11 had PH at two or more examinations. Sixteen of the patients with PH at one measurement and 10 of the patients with PH at two or more measurements had pre-operative PH.

The number of post-operative catheterizations was similar in patients without post-operative PH, in patients with PH at one post-operative evaluation and in patients with PH at repeated evaluations (n = 4.0, n = 3.9, and n = 4.3, respectively).

Pre-operative Hemodynamic Comparisons Based on Post-operative Pulmonary Hypertension

Pre-operative hemodynamic data were compared for patients who i. did not have post-operative PH, ii. had PH at one post-operative examination, and iii. had PH at multiple examinations. Pre-operative MPAP, PAWP, MRAP, and PVR were significantly higher in patients with PH at one post-operative examination, compared to those without post-operative PH. Moreover, pre-operative MPAP and PAWP were significantly higher in patients with PH at repeated examinations compared to patients without post-operative PH. In contrast, pre-operative hemodynamics were similar for patients with PH at one and repeated post-operative

evaluations, except for CO which was lower in those with PH at one evaluation (Table 7).

Finally, there was a significant positive correlation between pre-operative MPAP and post-operative MPAP, which decreased with time. The R^2 -values were 0.39 at one week, 0.22 at four weeks, 0.19 at three months, and 0.06 one year after HT. There was no significant correlation between pre-operative MPAP and MPAP at the six month evaluation.

Table 7. Pre-Operative Hemodynamics Based on Post-Operative Pulmonary Hypertension

* Indicates pre-operative statistical significance between patients with no PH post-HT and those with PH at one examination, § indicates pre-operative statistical significance between patients with no PH post-HT and those with PH at repeated examinations and # indicates pre-operative statistical significance between patients with PH at one examination post-HT and those with PH at repeated examinations

Parameter	All patients		No PH post-HT		PH at one examination post-HT		PH at repeated examinations post-HT		Sign
	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	
Patients	89		60		18		11		
MAP, mmHg	73.9	13.6	74.7	15.4	71.2	8.5	74.1	10.2	
MPAP, mmHg	30.3	8.8	27.9	8.6	34.6 *	7.0	35.8 §	8.4	*, §
PAWP, mmHg	20.6	8.1	18.8	7.8	24.1 *	7.7	24.4 §	8.0	*, §
TPG, mmHg	9.7	3.5	9.1	3.4	10.4	3.1	11.5	4.3	
DPG, mmHg	-1.2	4.4	-1.1	4.5	-1.8	4.6	0.0	3.5	
MRAP, mmHg	7.9	6.0	6.6	5.3	12.0 *	6.9	8.2	5.0	*
CO, L·min⁻¹	3.7	1.1	3.7	1.0	3.2	1.0	4.2 #	1.0	#
PVR, WU	2.8	1.3	2.6	1.0	3.7 *	1.8	2.8	1.3	*

Acute Cellular Rejections and Post-operative Renal Function

A total of 1,376 EMBs were performed during the first post-operative year. There were a total of 172 EMB with $ACR \geq 2$ (12.5 %) according to the ISHLT grading system from 1990 (198). Of these, 110 (8.0 %) were grade 2 and 62 (4.5 %) were grade 3A/3B. There was no significant difference in the proportion of EMBs with $ACR \geq 2$ or $\geq 3A/3B$ in patients with and without post-operative PH. Nor was there a significant difference in the proportion of EMBs with $ACR \geq 2$ or $3A/3B$ in patients with PH at one or repeated post-operative evaluations (Table 8).

One year after HT, measurements of renal function were available in 84 patients of whom 57 did not have post-operative PH. 17 had PH at a single post-operative evaluation and 10 had PH at repeated evaluations. Glomerular filtration rate was significantly lower in patients with PH at repeated evaluations ($36.9 \pm 13.2 \text{ mL} \cdot \text{min}^{-1} \cdot 1.73\text{m}^{-2}$) compared to patients without PH ($56.8 \pm 18.4 \text{ mL} \cdot \text{min}^{-1} \cdot 1.73\text{m}^{-2}$) and patients with PH at one evaluation ($52.8 \pm 20.2 \text{ mL} \cdot \text{min}^{-1} \cdot 1.73\text{m}^{-2}$).

Table 8. Post-Operative Pulmonary Hypertension in Relation to Acute Cellular Rejection

				P-value	
% of EMBs with ACR \geq grade 2 or 3A/3B in patients <i>without</i> PH (n=60)		vs.	% of EMBs with ACR \geq grade 2 or 3A/3B in patients with PH at <i>one</i> RHC (n=18)		
• ≥ 2	8.5 % (79/933)		• ≥ 2	5.9 % (16/270)	0.217
• $\geq 3A/3B$	5.0 % (47/933)		• $\geq 3A/3B$	3.0 % (8/270)	0.203
			% of EMBs with ACR \geq grade 2 or 3A/3B in patients with PH at <i>repeated</i> RHCs (n=11)		
			• ≥ 2	8.7 % (15/173)	0.952
			• $\geq 3A/3B$	4.0 % (7/173)	0.716
% of EMBs with ACR \geq grade 2 or 3A/3B in patients with PH at <i>one</i> RHC (n=18)		vs.	% of EMBs with ACR \geq grade 2 or 3A/3B in patients with PH at <i>repeated</i> RHCs (n=11)		
• ≥ 2	5.9 % (16/270)		• ≥ 2	8.7 % (15/173)	0.361
• $\geq 3A/3B$	3.0 % (8/270)		• $\geq 3A/3B$	4.0 % (7/173)	0.730

Survival

Mean survival among the 94 patients was 13.7 years with 1, 5 and 10 year survival of 92.6 %, 81.1 % and 71.1 %, respectively. The survival did not significantly differ from all 215 patients (Figure 20 a). Post-operative survival in patients with pre-operative LVAD was 13.7 years, identical to the 94 patients.

With regards to early mortality, three of the 94 patients died within 24 hours after HT, another two within 30 days, one after 330 days and one after 332 days, resulting in seven deaths (7.4 %) within the first year post-HT. Four of these patients had post-capillary PH prior to HT and three did not.

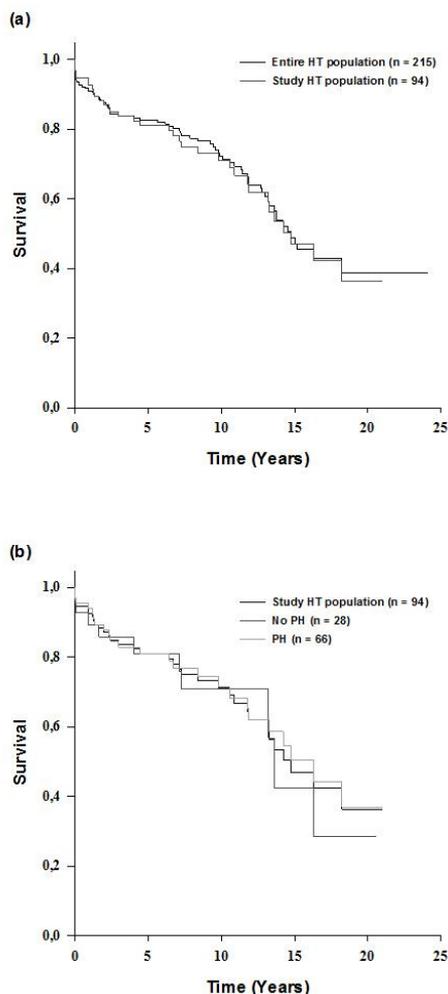


Figure 20 a and b. Survival After Heart Transplantation
 (a) All 215 patients vs study population, (b) Pre-operative PH vs no PH

Pre-operative Pulmonary Hypertension

Long-term survival did not differ between those with and without pre-operative PH (Figure 20 b), nor did it differ when the patients were grouped based on sub-classifications from ESC's guidelines (2009), ISHLT's state of art summary statement (2012), ISHLT's risk criteria (2006) and hemodynamic criteria from previous studies.

At the end of follow-up, two of the three patients with pre-operative $DPG \geq 7$ mmHg were alive with a mean follow-up of 3.2 ± 1.9 years. The third patient died 6.7 years after HT due to acute pulmonary embolism.

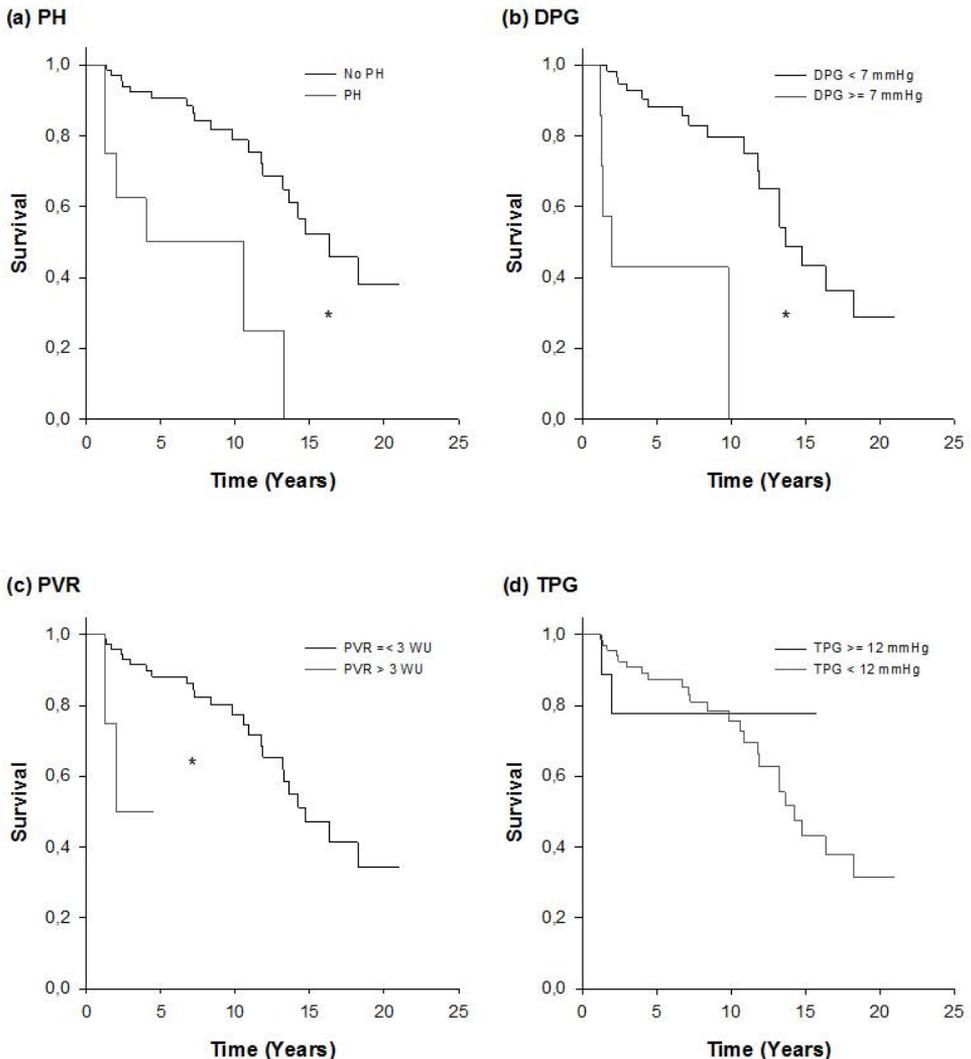


Figure 21. Survival Based on Hemodynamics One Year After HT
* Indicates statistical significance between the investigated groups.

Post-operative Pulmonary Hypertension

Long-term survival was lower among the eight patients with PH one year after HT, compared to those without PH (estimated survival 7.0 vs. 15.1 years) (Figure 21 a). When adjusted for gender and age at the time of transplantation, MPAP at one year after HT was associated with a higher risk of death in the Cox regression model (hazard ratio 1.2, 95 % CI 1.1–2.3).

Survival of the seven patients with $DPG \geq 7$ mmHg one year after HT was furthermore significantly lower than in those with $DPG < 7$ mmHg (estimated survival 5.0 vs. 14.2 years). Survival was also lower for patients with $PVR > 3$ WU compared to those with $PVR \leq 3$ WU (estimated survival 3.1 vs. 14.5 years). In contrast, there was no significant difference in survival for patients with $TPG \geq 12$ mmHg and those with $TPG < 12$ mmHg (Figure 21 b-d).

There was no significant difference in survival between the 29 patients with PH at least once during the first year after HT and the 60 patients without post-operative PH (estimated survival 12.4 vs. 15.7 years) (Figure 22 a). The survival was furthermore not significantly different in the 60 patients who did not exhibit PH and in the 18 patients who exhibited PH at one measurement (estimated survival 15.7 and 16.1 years, respectively), whereas survival was significantly lower in the 11 patients who exhibited PH at two or more examinations (estimated survival 7.8 years), both compared to patients without post-operative PH and those with PH at a single post-operative evaluation. In a multivariate Cox regression model adjusted for gender, age at the time of HT and renal function one year after HT, PH at repeated measurements was associated with a higher risk of death (hazard ratio 3.4, 95 % CI 1.4–8.0) than no PH and PH at a single examination after HT (Figure 22 b).

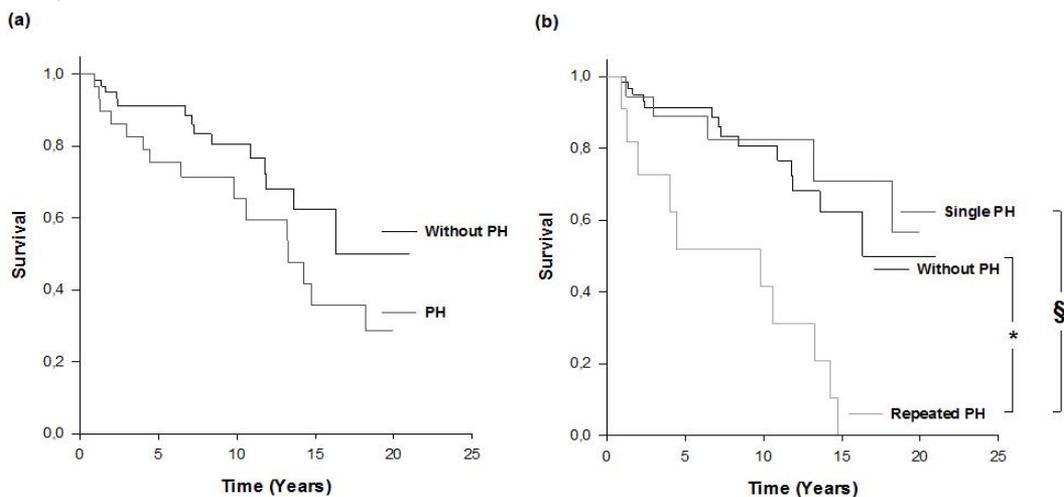


Figure 22 a and b. Survival Based on Post-Operative Pulmonary Hypertension

(a) PH vs No PH, (b) PH at a single and PH at repeated examinations vs No PH. * and § Indicates statistical significance between patients with PH at repeated evaluations and patients without PH or with PH at a single evaluation, respectively.

Forty-six of the 60 patients (77 %) without post-operative PH, 13 of the 18 patients (72 %) with PH at one post-operative examination, and one of the 11 patients (9 %) with PH at repeated post-operative evaluations were alive at the end of follow-up.

Cause of Death

At the end of follow-up, 34 patients had died. The most common cause of death was rejection (n = 7), i.e. acute cellular and humoral rejections as well as coronary allograft vasculopathy, followed by malignancy (n = 6), infection (n = 5) and intracranial hemorrhage (n = 4). Other causes of death included, amongst others, primary allograft dysfunction, acute coronary syndrome and pulmonary embolism.

Paper V

Pre-operative and Post-operative Characteristics

Two of the 12 patients were women and the median age was 50.0 years. The most common diagnosis was dilated cardiomyopathy (n=8) followed by ischemic heart disease (n=3). Three patients were ex-smokers and the remaining never smokers. Prior to HT, MPAP was 35.0 (26.5-41.5) mmHg, PAWP 25.0 (19.5-29.8) mmHg, MRAP 15.0 (11.5-18.5) mmHg, CO 3.4 (2.6-4.3) L·min⁻¹ and PVR 2.4 (1.9-3.0) WU. Nine of the 12 patients had pre-operative PH. Of these, three had combined pre-capillary and post-capillary PH and five had isolated post-capillary PH according to ESC's guidelines from 2015 (14).

Four weeks after HT there was an increase in CO to 6.0 (5.6-6.6) L·min⁻¹, as well as a decrease in MPAP to 17.0 (11.5-20.5) mmHg, PAWP to 8.0 (4.5-10.5) mmHg and PVR to 1.3 (1.1-1.5) WU, compared to prior to HT, remaining stable at the six month evaluation. Six months after HT MRAP decreased to 1.5 (0.0-4.0) mmHg. None of the patients had post-operative PH.

With regards to immunosuppression after HT, all patients were treated with a triple combination of corticosteroids, a calcineurin inhibitor (10 Tacrolimus, 2 Cyclosporine) and mycophenolate mofetil/sodium. In one patient an mTOR inhibitor was added prior to the six month evaluation due to renal dysfunction. Four weeks and six months post-HT three and two patients, respectively, were diagnosed with ACR grade 1R (according to 2005 system, corresponding to grade 1A/1B/2 in the 1990 system). None needed specific rejection treatment.

Plasma Levels of Substances in the Nitric Oxide Pathway

Prior to HT, the plasma concentration of L-Arginine was 56.0 (47.9-60.3) μM , ADMA 0.57 (0.47-0.71) μM and L-Arginine/ADMA-ratio 85.7 (68.3-128.3). L-Arginine was lower in patients than in controls, whereas ADMA was higher, resulting in a markedly lower L-Arginine/ADMA-ratio in patients (Figure 23).

Four weeks after HT there was an increase in the plasma concentration of; L-Arginine to 82.5 (64.7-101.2) μM and L-Arginine/ADMA-ratio to 164.0 (104.5-188.7), compared to prior to HT. All concentrations remained stable at the six month evaluation. After HT, L-Arginine was in line with healthy controls, whereas L-Arginine/ADMA, although improved, remained decreased. There was no change in ADMA plasma levels after HT (Figure 23).

Correlations

In a univariate analysis six months after HT, there was an inverse correlation between PVR and the L-Arginine/ADMA-ratio (R^2 : 0.39). There were no other significant correlations, neither at the pre-operative nor post-operative evaluations, between the L-Arginine/ADMA-ratio and the hemodynamic parameters, NT-proBNP, creatinine or urea. Moreover, neither the plasma levels of L-Arginine nor ADMA correlated to PVR, nor to any of the other investigated hemodynamic parameters, after HT.

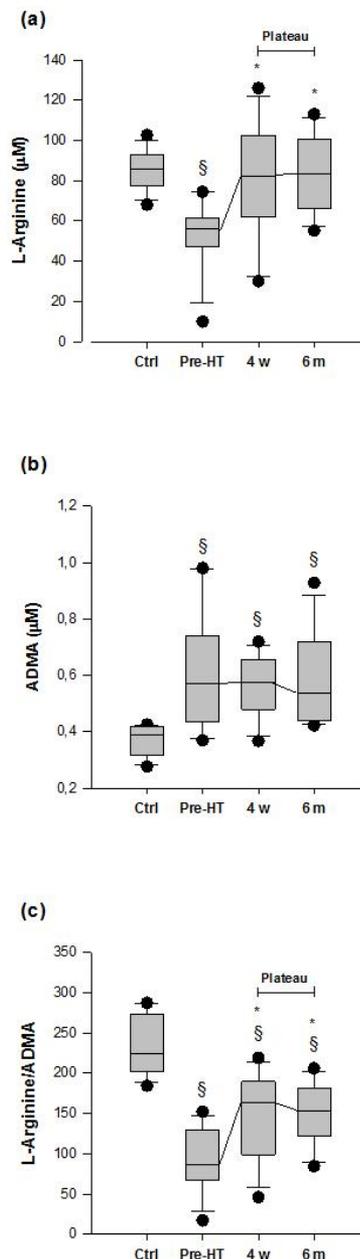


Figure 23. Plasma Levels of L-Arginine and ADMA Prior to and After Heart Transplantation

* Indicates statistical significance as compared to prior to HT and § indicates statistical significance as compared to controls.

Limitations

The limitations of the papers in this thesis are addressed in detail below.

Paper I

Despite porcine and human hearts sharing a close hemodynamic resemblance (201), there are interspecies variabilities in terms of the response to hypoxia and drugs, including tezosentan, which have to be taken into account when interpreting the results. Our findings can therefore not directly be applied in a human setting. Also, in a hospital setting the most common cause of HPV is various chronic pulmonary diseases including chronic obstructive pulmonary disease with emphysema, where dilatation of the pulmonary vessels may be detrimental, causing deoxygenation of the blood. Our findings in acute hypoxia therefore need confirmation in chronic HPV.

Paper II-IV

The retrospective design of paper II-IV has disadvantages compared to prospective studies. The long timespan and small number of patients in our cohort is furthermore a major limitation which could potentially influence the results. When evaluating the normal response to exercise in paper II it is also important to note that hemodynamic evaluations in healthy controls were not performed. Instead the results were compared to previously published systematic reviews on healthy individuals. Although this may not be optimal, due to ethical issues of performing heart catheterizations on healthy persons, we find our approach to be a valid alternative. The studies did furthermore only include patients who underwent HT. Pre-operative hemodynamic data may consequently not be applicable for patients with severe heart failure who do not undergo HT. The response to exercise prior to and after HT in paper II was, however, comparable to what has previously been described in heart failure (147). With regard to hemodynamics we therefore believe that the present observations are representative for patients with severe heart failure.

When investigating the impact of pre-operative PH on post-operative survival there is also a risk of selection bias as patients with clinical signs of severe pulmonary vascular disease may have been excluded from HT, irrespective of hemodynamics. In paper III we did furthermore not adjust survival data for potential confounders, which could influence the outcome. In paper IV we did not have the possibility to histologically examine the pulmonary vessels of the patients who died, so it cannot be determined whether repeated PH represented pulmonary vascular disease.

However, demographics and survival were similar for the 94 included patients and all 215 HT patients. Survival was furthermore constant during the entire period of transplantations and hemodynamic measurements were all performed routinely and in the same manner, mostly by the same physicians, minimizing the risk of investigator bias despite the long study period. In summary we believe the findings may be applicable on a larger HT population and are of interest, primarily to generate hypotheses for future trials. However, they must be interpreted cautiously and confirmation in larger prospective and controlled trials is needed.

Paper V

Although the small sample size was in line with previous pilot studies in HT, which has stimulated the initiation of larger trials, the study size is a major limitation. Furthermore, we did not measure NO and cGMP levels so we cannot be certain that their production was decreased prior to HT. However, the decreased L-Arginine/ADMA-ratio throughout our study, in combination with the fact that the ratio reflects pulmonary vascular tone, points in the direction of NO-dependent endothelial dysfunction prior to and after HT. Finally, medical therapies prior to and after HT were individually adjusted. Betablockers (202) and RAAS-blockade (203) have been shown to decrease circulating ADMA levels. These drugs are less frequently used after HT which, in theory, could increase plasma levels of ADMA. The use of these drugs does, however, represent a cornerstone in heart failure therapy and a true clinical situation, so our findings are relevant to generate hypotheses for future, larger, trials.

Discussion

Paper I

sGC stimulation with BAY 41-8543 totally reversed HPV during acute hypoxia. Dual endothelin receptor blockade with tezosentan furthermore markedly attenuated acute HPV. When the sGC stimulator was administered on top of dual endothelin receptor blockade, pulmonary pressures and resistances were even further decreased. Blood arterial O₂-saturation and O₂-consumption remained unaltered throughout the experiments, despite a marked systemic vasodilatation.

Response to Hypoxia

In our *in vivo* pig model there was a marked and stable increase in pulmonary artery pressures and resistances during sustained hypoxia for 82.5 min. These findings are similar to what has previously been described when various species have been exposed to severe hypoxia (176) and highlights the profound HPV in our study.

Interestingly, during induction of acute hypoxia, left ventricular filling pressure increased and was then unaltered throughout all experiments, suggesting that the increase was not solely a result of ventricular interdependence due to increased right heart load. Marked increases in left ventricular filling pressures upon exposure to acute hypoxia have not been observed in humans, whereas the capillary pressures increase and may contribute to high altitude pulmonary edema (204). On the other hand, patients with previous high altitude pulmonary edema have been shown to have high left ventricular filling pressures during exercise (205) and hypoxia has indeed been shown to cause temporary diastolic dysfunction, even in young, healthy, individuals (206). It consequently seems that the increase in left ventricular filling pressure observed in our study in parts may be species specific and possibly related to diastolic dysfunction during hypoxia.

Soluble Guanylate Cyclase Stimulation and Dual Endothelin Receptor Blockade Totally Reverses Acute Hypoxic Pulmonary Vasoconstriction

Stimulation of sGC, by intravenous infusion of BAY 41-8543, was found to totally reverse the pulmonary vasoconstrictor response to acute hypoxia and reduce the load on the right side of the heart, thereby protecting against right ventricular failure. If sustained, such benefits could potentially also prevent right ventricular failure in the chronic setting. Whether sGC stimulation could prohibit hypoxia induced pulmonary edema is unknown and needs further investigation. Considering the systemic effects of sGC stimulators, also observed in several human studies (99;101), it is however possible that the load of the left ventricle, and consequently pulmonary capillaries, may decrease with such therapies.

Treatment with endothelin receptor antagonists have proven beneficial in PAH (79-81). However, in PAH dual endothelin receptor blockade is seldom sufficient to fully normalize the increased pulmonary vascular tone (14). In our study it was therefore of importance to evaluate whether combination treatment, targeting several pathways, could be tolerated and of additional value. Dual ET-receptor blockade with tezosentan indeed effectively attenuated acute HPV. When adding sGC stimulation, pulmonary pressures and resistances further decreased to values as low as, or even lower than, those in normoxia. In recent years several studies on PAH have shown beneficial effects of combination therapy, as compared to mono therapy (81;82;105) and based on our findings such a combination treatment seems feasible and effective also in reversing acute hypoxia-induced PH. The pronounced reduction in systemic pressures observed in the present study may, however, be harmful in critically ill patients. Therefore, if treating patients with the present drugs, doses should be carefully titrated and systemic blood pressure closely monitored. It should, however, be noted that the decrease in systemic blood pressure with tezosentan in the present study has not been observed in humans (207) and is likely species-dependent.

As endothelin receptor blockers as well as sGC stimulators may, besides their vasodilating effects, also attenuate pulmonary vascular remodeling (208-210), our findings are of importance in the development of new treatment strategies for PH of various etiology, including PH-LHD and PH due to hypoxia. A few reports in patients with chronic lung disease, including chronic obstructive pulmonary disease, and fibrotic lung disease have, however, suggested otherwise (186-190). Although therapies targeting the nitric oxide and endothelin pathways may improve hemodynamics and decrease right ventricular load, they may also counter the beneficial effects of HPV in focal hypoxia, thereby impairing ventilation-perfusion matching and arterial oxygenation. Several negative studies have also been reported with drugs targeting these pathways in left heart disease (162-168), perhaps due to increased load on the left ventricle when dilating the pulmonary vessels. In contrast to the findings in left heart disease and chronic pulmonary disease, sGC stimulation

with the novel drug riociguat have shown beneficial effects in PAH and CTEPH (99-101;211) and is now used for treatment of these conditions. Trials with riociguat in systolic and diastolic left heart failure were also recently conducted. Riociguat did not decrease pulmonary artery pressures in either of the trials (167;168) but improved cardiac index and quality of life in systolic heart failure (167) and some hemodynamic parameters in diastolic heart failure (168), indicating potential beneficial effects in left heart disease. Therefore, the new sGC stimulator vericiguat is currently being tested in a phase III trial on systolic left heart failure (ClinicalTrials.gov Identifier: NCT02861534) after having shown to improve NT-proBNP (169). The same drug has also shown to improve quality of life in diastolic heart failure (170). At present, PAH targeted therapies are, however, not recommended in PH-LHD or lung disease (14).

When the present PhD project was initiated we planned for further studies with sGC stimulators in humans with PH-LHD. These plans were, however, abandoned when the above trials were initiated.

Paper II

Hemodynamic parameters during rest and slight supine exercise were characterized in patients undergoing HT. Hemodynamics markedly improved at rest and during exercise one and four weeks after HT, respectively. All parameters remained constant or improved even further throughout the first post-operative year. The post-HT functional response to exercise, with regards to cardiac output, was also adequate. Despite this, a profound increase in filling pressures was observed during exercise after HT. So, although the hemodynamics at rest were normalized after HT, the response to even slight exercise differed compared with the normal innervated heart.

Pre-operative Hemodynamics

Prior to HT, exercise resulted in a marked increase in both left and right ventricular filling pressures, indicating enhanced biventricular failure during exercise. Previous reports in healthy volunteers found a close relationship between exercise-induced increases in left and right ventricular filling pressures (150;151). Such a correlation was not observed prior to HT, suggesting that the increased load on the right ventricle is not solely due to increased left ventricular filling pressures, passive congestion of the pulmonary circuit and subsequent post-capillary PH, but also due to superimposed pulmonary artery pressures. The exaggerated increase in pulmonary pressure could be due to several factors, including HPV (29) related to

low mixed venous PO₂. Mixed venous PO₂ was indeed markedly decreased during exercise in the patients where these data were available.

With regards to exercise, the definition of exercise PH has been removed from ESC's guidelines on PH due to lack of robust data (14). However, the European Respiratory Society have published a statement suggesting that exercise PH should, at peak exercise, be defined as MPAP > 30 mmHg in combination with TPR > 3 WU, in absence of resting MPAP ≥ 25 mmHg (157). Recently it was also suggested that reference values for exercise should be individually determined, as the response to exercise was markedly altered by age (156). In contrast to previous findings in healthy individuals, (144;155;156) the hemodynamics in severe heart failure did not significantly differ between patients aged ≤ 50 and > 50 years during rest or exercise. In end-stage heart failure, age does consequently not seem to play a significant role in hemodynamic response to exercise. This is of importance as exercise testing may be beneficial in early detection of heart failure (148) and PH (149), as also suggested by our data. Exercise testing may furthermore aid in the differentiation between PAH and PH-LHD, especially when the latter is caused by diastolic dysfunction.

Post-operative Hemodynamics

Hemodynamics at rest after HT have been reported in several publications (15-18;32-34;194;196;212-217). In line with these reports, our study shows a dramatic hemodynamic improvement within the first week after HT, remaining stable or even further improving throughout the first year following transplantation. In contrast to the large number of studies on resting hemodynamics, publications on invasive exercise hemodynamics after HT are scarce (29-35). In a few small reports it has however been shown that MPAP and PAWP decrease early after HT (29) and that during exercise in denervated hearts, CO is initially increased by augmented preload and the Frank Starling mechanism and later by the effects of circulating catecholamines (31). These reports have, however, lacked longitudinal follow-up of patients. Our results therefore improve the understanding of post-operative hemodynamic development over time.

During exercise after HT pulmonary artery pressure and, in particular, bilateral ventricular filling pressures increased dramatically, despite normal values at rest. The increase was more pronounced than what would be expected in healthy individuals at the corresponding workload (144;155;156) and has been described previously (33;35). Several reasons for these elevated filling pressures have been suggested (15;16;31;33-37;218), including a prominent decrease in diastolic compliance in denervated hearts (218). Denervated hearts may also be more dependent on the Frank Starling mechanism than innervated hearts (31;36;37). The ultimate cause remains unclear and is probably multifactorial (33).

The HR in our study was, as expected due to denervation, slightly higher at rest than what would be expected in healthy individuals (156). During slight exercise, an increase to values as high as, or even slightly higher than, what has previously been described in health, was furthermore observed (144;155;156). As the increase in HR during slight exercise was similar to that of healthy persons, the chronotropic insufficiency described after HT (35) primarily seem to play a part in high intensity exercise.

Due to the increase in left ventricular filling pressures and subsequent increase in pulmonary pressures induced by exercise after HT, the post-operative response in PVR was comparable to what has been presented in healthy individuals (144;155). On the other hand, the response in TPR to exercise was still deranged at one year after HT. These findings suggest that TPR may give more adequate insight in the pulmonary circulation during exercise than PVR, as it reflects the increase in MPAP in relation to CO, independently of left ventricular filling pressures. Our findings consequently support the recent statement from the European Respiratory Society, suggesting that TPR should be used to define exercise PH (157).

Paper III

After HT no significant differences in survival was observed based on hemodynamic criteria used in previous publications, including ESC's guidelines from 2009 (14) and ISHLT's documents (7;11). Even though we consider severe pre-operative PH to be a risk factor for right heart failure after HT, previous hemodynamic criteria for PH seem insufficient to discriminate the impact of pulmonary hemodynamics on survival after HT. Recent reports have furthermore presented conflicting results on post-HT survival with regard to present ESC guidelines (14) and DPG (136-138). Consequently, new risk criteria including invasive hemodynamics as well as novel biomarkers and imaging techniques would be of great value.

Survival

Although it is well established that severe pre-operative PH with vascular remodeling increases the risk of acute right heart failure after HT, previous studies have used different hemodynamic parameters and thresholds when evaluating outcome after HT (7;9-11;17;18;32;125;194-197). Correct comparisons and predictions on survival are therefore difficult to make. Moreover, as survival during the first year after HT steadily rise (6), it is a reasonable assumption that today's post-operative care has greatly improved as compared to the care given in the early HT era. Therefore, early hemodynamic reports on outcome after HT may be

outdated, making it even harder to define robust hemodynamic criteria for acute right heart failure and early mortality after HT. The overall survival after HT was excellent in our cohort, despite most of the patients having some degree of pre-operative PH. The outcome of patients with PH was furthermore comparable to the patients without pre-operative PH, which may be attributed to the pre-, intra- and post-operative treatment at our thoracic intensive care unit, focusing on preventing fluid accumulation by aggressive diuretic therapy, still maintaining adequate perfusion pressure. Another possible explanation is that the patients in our cohort was likely to have PH caused by passive congestion and excessive vasoconstriction, rather than severe vascular remodeling. Our findings on post-operative outcome are in line with some recent reports (6;17;18;137;195;197;219;220), whereas they are in contrast to others (9;10;136;138;194). The diverging results indeed highlight the difficulty in identifying a high risk population. Which hemodynamic parameters that best predict outcome after HT and the precise magnitude at which they do so consequently remains unclear.

Diastolic Pressure Gradient

DPG ≥ 7 mmHg has been suggested to be a marker of severe pulmonary vascular disease and worse prognosis in PH-LHD (120). DPG was therefore introduced in the most recent ESC guidelines (14). As indicated previously there are, however, conflicting results on the clinical implications of DPG. Whereas some reports indeed have shown that DPG is a prognostic factor (120;127-134), others have not (12;135-141). In our material DPG ≥ 7 mmHg was only observed in three patients prior to HT. None of these patients had elevated DPG or acute right heart failure after HT and their survival was not significantly decreased. Diastolic pulmonary artery pressure has shown to be less affected by changes in flow and left ventricular filling pressures than MPAP (126), in theory making DPG an attractive marker in left heart disease. However, DPG is to a greater extent affected by HR, which has been presented as one of its major weaknesses (221;222). In patients with elevated DPG prior to HT, the HR was indeed higher than in the overall cohort. It is consequently likely that the high DPG in these patients was not due to pulmonary vascular disease, which could explain why DPG was normalized and outcome was not affected after HT. Finally, another complicating factor with DPG is the measurement of PAWP. Whereas diastolic pulmonary artery pressure is measured at end-diastole, measurements of PAWP vary potentially resulting in overestimation of PAWP and misclassification of patients. This was recently illustrated in a report where QRS-gated identified more patients with high DPG (12). In summary, there is a sound physiological rationale for DPG in sub-defining PH due to left heart failure, but there are several pitfalls which are not yet overcome. Consequently, the role of DPG is still debated and needs further evaluation.

With the controversies concerning DPG and PVR, another hemodynamic marker, namely the pulmonary arterial compliance, has recently been highlighted in the literature. In comparison to PVR, pulmonary arterial compliance accounts for the pulsatility of blood flow and is a sensitive marker for right ventricular afterload. Pulmonary arterial compliance was not investigated in our material but has indeed been shown to be an independent predictor of outcome in left heart failure with or without PH (135;141;223;224) and may consequently be implemented in future clinical practice.

Bridge-to-Transplantation

Our study confirms that mechanical assist prior to HT improved hemodynamics and normalized MPAP as well as PAWP. Moreover, none of the 21 patients who received an assist died the first year after HT, and their mean survival did not differ from the rest of the population. These findings suggest that LVAD therapy improve hemodynamics as well as survival, to levels similar to the general HT-population. These observations were confirmed in a recent publication where long-term LVAD use resulted in lowering of PVR which had previously been irreversibly elevated. The patients in this study subsequently underwent HT with outcome as good as other HT patients (49). Consequently, patients should not be disqualified from HT based purely on pre-LVAD PH. Of note, an ongoing trial is investigating the effects of the dual endothelin receptor antagonist macitentan in patients with persistent PH and PVR > 3 WU after LVAD implantation (ClinicalTrials.gov Identifier: NCT02554903). A previous study has furthermore shown that PDE5i may reverse PH that persists after LVAD implantation (51). It is therefore possible that PAH-therapies may be of value to optimize pulmonary vascular tone when the left ventricle is mechanically unloaded.

Paper IV

Patients with PH, DPG \geq 7mmHg or PVR > 3 WU one year after HT had worse long-term survival than patients without PH and those with low DPG or PVR, respectively. TPG at the commonly used cut-off at 12 mmHg did, however, not affect outcome. Of further interest, PH at repeated evaluations during the first post-operative year was associated with impaired long term survival as compared to both patients without PH and patients with PH at one post-operative examination, whereas outcome did not significantly differ for the latter two groups.

Survival

As compared to the large number of studies investigating the impact of pre-operative PH on outcome after HT, there has previously been scarce knowledge on the role of post-operative PH on survival. The few reports previously published have not used the common definition of PH (MPAP \geq 25 mmHg) (14). Therefore, although they showed that high MPAP and PVR after HT was associated with poor prognosis, the clinical implications of the findings have remained uncertain (17;18). Despite using a different definition (14), our findings were in line with the previous reports (17;18) emphasizing that abnormal pulmonary hemodynamics, including PH, one year after HT is associated with reduced long-term survival. For the first time we also reported post-operative survival based on results from repeated hemodynamic evaluations during the first year after HT. The findings suggest that PH at repeated examinations, but not at a single evaluation, is associated with impaired outcome, which have several potential explanations. One may be that hemodynamics can be temporarily affected by factors such as HPV, post-operative stress and therapies that may influence pulmonary vascular tone. Multiple assessments may therefore potentially decrease the risk of such transient confounders interfering with the clinical evaluations, thereby identifying patients with “true” pulmonary vascular disease. Performing repeated hemodynamic evaluations to sub-define post-capillary PH has, in fact, recently been suggested after a report where DPG was found not to affect survival (138;225).

As the pulmonary artery pressures were only moderately increased in patients with PH at repeated evaluations and the causes of death were not primarily cardiovascular, it is however unlikely that patients with repeated PH had severe pulmonary vascular disease. Instead it is probable that repeated PH was attributed to an overall worse condition. Nonetheless repeated PH was an independent marker of death when adjusted for age and gender as well as renal function one year after HT. ACR did furthermore not differ between the groups. Consequently, our findings warrant further investigations to define the actual cause of repeated PH. Interestingly, when investigating pre-operative hemodynamics based on whether patients had PH at one or repeated post-operative evaluations, no marked differences were identified. The lack of pre-operative differences indeed highlight the importance of close hemodynamic monitoring before and after HT to, if possible, identify a high-risk population at an earlier stage. To determine whether this population may benefit from vasoactive therapies, randomized controlled trials are urged for (73;106).

Finally, patients with DPG \geq 7mmHg or PVR $>$ 3 WU one year after HT had worse long-term survival than patients with low DPG and PVR, respectively. However, the groups were small and not all patients with high DPG or PVR had PH. Consequently, definite conclusions on the role of these parameters on outcome after HT cannot be made based on our findings. As described previously, conflicting

results on the impact of pre-operative DPG and PVR on outcome after HT have been presented in recent years. Due to the conflicting results, both DPG and PVR need further investigation, not only prior to HT but also thereafter.

Paper V

Plasma concentrations of substances related to the NO pathway were investigated prior to and after HT. Compared to controls, L-Arginine was low and ADMA high prior to HT, resulting in a low L-Arginine/ADMA-ratio. Although improved, the L-Arginine/ADMA-ratio was not normalized after HT. The inverse correlation to PVR furthermore suggest that the L-Arginine/ADMA-ratio reflects pulmonary vascular tone after HT and that NO-dependent endothelial function is still partly impaired. As all patients were free of post-operative PH, as well as ACR equal to or higher than grade 1R and had normal left heart function at all post-operative evaluations included, the findings may represent the normal plasma concentrations of these substances after HT.

Pre-operative Concentrations

In severe heart failure, L-Arginine was considerably lower than in controls. The levels also seemed to be lower than in patients with mild to moderate heart failure, recently reported by our group (85), possibly representing the progressive endothelial dysfunction and congestion of the pulmonary circuit with worsening heart failure. Similar results have been observed in other studies, where ADMA has been shown to correlate to NYHA functional class (92;95;226) and pulmonary pressures (227). Prior to HT, the plasma levels of the investigated substances were in fact in line with those of patients with PAH, in our previous report (85). There was also a non-significant trend towards worse states in our patients with, compared to without, PH. Further investigation is therefore encouraged to define whether or not there is a true difference between heart failure patients with and without PH and whether the concentrations represent pulmonary endothelial dysfunction.

Post-operative Concentrations

The L-Arginine/ADMA-ratio has been suggested to better reflect NO production and endothelial function, than L-Arginine and ADMA separately (228). The L-Arginine/ADMA-ratio has furthermore shown to be associated with disease severity (226), as well as mortality (229), in heart failure. Our results with improved yet not normalized L-Arginine/ADMA-ratio after HT where the ratio may also reflect

pulmonary vascular tone, as illustrated by its inverse correlation to PVR, suggest that NO-dependent endothelial function is still partly impaired early after HT. If using therapies targeting the NO-pathway prior to or after HT it consequently seems reasonable to use drugs which act independent of NO, such as sGC stimulators, rather than NO-dependent substances.

Arginase catalyzes the reaction where L-Arginine is converted to urea and L-Ornithine. Arginase has shown to be elevated in heart failure (230) and increases during oxidative stress and infections (231). Although L-Arginine increased after HT, L-Ornithine remained unaltered. Our findings do consequently not support that major alterations in arginase activity is the reason for increased L-Arginine concentrations following HT. L-Citrulline, produced together with NO, was also unaltered by HT. As NO and cyclic guanosine monophosphate-levels were not measured, we cannot be certain that NO production increased after transplantation. Most of the circulating L-Citrulline is, however, released from the intestines rather than from the endothelium (232). Therefore, an increased release of L-Citrulline from the endothelium may not be sufficient to significantly alter plasma concentrations of L-Citrulline. Unaltered plasma L-Citrulline levels does therefore not preclude increased NO production. Moreover, after HT CO increases, possibly reducing inflammation (233). With reduced inflammation it is reasonable to assume that the vascular sensitivity for NO is increased, leading to partly restored NO-mediated vasodilatation, irrespective of whether or not NO production is increased.

Conclusions

The present thesis includes a pig model of acute HPV, a retrospective review of heart transplanted patients and an analysis of plasma concentrations of substances related to the nitric oxide pathway in a prospective heart transplantation cohort. The following conclusions were drawn:

- sGC stimulation alone or in combination with dual endothelin receptor blockade, in an *in vivo* porcine model, dose-dependently attenuated acute HPV illustrated by normalization of MPAP and PVR, thereby reducing MRAP and consequently right ventricular afterload. There was a simultaneous systemic vasodilatation and reduction in MAP which, however, did not alter blood arterial O₂-saturation or O₂-consumption. Together, the findings suggest that sGC stimulation alone, or in combination with endothelin receptor blockade, may be used to prevent acute right heart failure in acute hypoxia.
- Hemodynamics during rest and slight exercise rapidly recovered after HT. The improvement in relevant hemodynamic parameters was maintained, or even further improved, throughout the first year after transplantation. Despite this improvement and despite an adequate functional response to exercise after HT, as illustrated by an increase in CO, there was a marked increase in ventricular filling pressures after HT, highlighted by elevated PAWP and MRAP as well as high MPAP. Such a pronounced increase has not previously been observed in healthy individuals, nor in patients with severe heart failure prior to HT. The alterations in exercise hemodynamics may partly be attributed to the fact that transplanted hearts lack innervation, being more dependent on the Frank Starling mechanism to increase CO, as compared to innervated hearts. However, the complete reason is probably multifactorial and a decreased diastolic compliance is also likely to play a part. If this is the case, the increased ventricular filling pressures may be the first sign of a post-operative diastolic dysfunction. Finally, age-dependent differences in the hemodynamic response to exercise have previously been described in healthy individuals. In end-stage heart failure, age did not significantly affect this response, an important finding as exercise criteria for PH may improve early diagnosis and outcome. Yet, such classifications

are currently lacking, partly due to previously described age-dependent alterations.

- Post-operative survival was not significantly influenced by pre-operative hemodynamic status, probably related to careful patient selection, as well as modern pre-, intra- and post-operative care. Excessive pulmonary vascular remodeling prior to HT may, however, still affect long-term outcome. Although patients with clinical signs of severe pulmonary vascular disease may have been excluded from HT, influencing the results, the findings highlight that previous and present hemodynamic definitions are insufficient to identify patients with vascular remodeling. The findings also show that LVAD is a feasible option for improving hemodynamics and bridging patients to HT. Consequently, PH *per se*, prior to mechanical unloading, is not an absolute contraindication for HT. Instead, with careful treatment of these patient, the potential complications caused by PH can, to a certain extent, be overcome and these patients transplanted without increased risk of early mortality.
- Patients with PH at one year after HT had impaired long-term survival, as compared to those without PH. Also, PH at repeated evaluations the first year after HT was associated with worse outcome than PH at a single examination. The PH in patients with PH at repeated measurements was moderate and is likely to be a result of an overall worse condition, rather than pulmonary vascular disease. Early and repeated hemodynamic measurements after HT may nonetheless be useful to identify patients with potentially impaired survival.
- Plasma concentrations of L-Arginine were low and ADMA concentrations high in severe heart failure, as compared to in healthy individuals. Whereas ADMA was unaltered by HT, L-Arginine rapidly returned to normal levels thereafter. Consequently, the L-Arginine/ADMA-ratio was improved, but not normalized and furthermore inversely correlated to PVR after HT. The findings thereby suggest that the L-Arginine/ADMA-ratio reflects post-operative pulmonary vascular tone and that NO-dependent endothelial vasodilation was improved although not normalized after HT. Whether this has implications on long-term outcomes remains to be investigated. Based on these findings, if targeting the NO-pathway after HT, NO independent substances such as sGC stimulators may be more appealing than NO-dependent vasodilators.

Future Perspectives

The incidence of heart failure is increasing and consequently, so is PH-LHD. Some patients with PH-LHD develop pulmonary vascular remodeling associated with impaired survival (120). In an attempt to improve diagnosis, ESC's guidelines were in 2015 updated with a new hemodynamic sub-classification of PH-LHD, where DPG was introduced (14). Subsequent retrospective studies on the prognostic value of DPG have, however, been conflicting and based on discussions at the Sixth World Symposium on PH it is likely that the definition again will be revised. The frequent revisions of the guidelines illustrate the complexity of the disease and difficulties in sub-defining post-capillary PH with present methods. Studies on invasive and non-invasive methods are needed to increase the understanding of disease progression in PH-LHD. A key aspect in this matter is the role of acute vasodilatation tests and exercise during invasive hemodynamic measurements. Inclusion of such variables in the guidelines may improve diagnosis and sub-classification of post-capillary PH, but studies on these aspects are lacking. Structured trials are therefore needed, in healthy individuals as well as in patients with heart failure, to increase the understanding of hemodynamics. A project on the influence of age on hemodynamic parameters at rest and during supine exercise was recently carried out in healthy individuals (156), stimulating further research. Hemodynamic monitoring is, however, unlikely to be sufficient to identify all patients with vascular remodeling. A structured work-up, also including non-invasive imaging and biomarkers, may improve adequate diagnosis. This needs to be investigated and relevant biomarkers identified.

Despite more than half a century has passed since the first HT, understanding of physiologic alterations after HT remain poor. Our results show a dramatic increase in filling pressures during exercise the first year after HT, but little is known of the long-term implications of these findings, as well as of the hemodynamic response to exercise late after HT. Whereas survival have steadily improved early after HT, survival beyond the first year has remained relatively constant (6). This highlights the importance of further research to increase the understanding of endothelial function as well as of long-term complications such as coronary allograft vasculopathy and diastolic dysfunction after HT. A key aspect is circulating biomarkers of endothelial function. We are currently investigating blood borne markers of inflammation and angiogenesis after HT but prospective multi-center

studies are needed to identify potential targets for medical therapies, which may slow the development of complications such as diastolic dysfunction, diabetes mellitus and renal impairment, thereby improving long-term survival.

For the time being, HT remains the ultimate treatment for end stage heart failure and specific medical therapies are lacking for the pulmonary component of PH-LHD. Several trials with medical therapies used to treat PAH, targeting the NO, endothelin and prostacyclin pathways, have been negative in left heart disease with or without PH (162-168;172), as well as in PH due to lung diseases (187-190), including chronic HPV. However, acute HPV may aggravate PH-LHD and in this setting knowledge on novel medical therapies is scarce. Interestingly, in contrast to previously negative findings the novel sGC stimulator vericiguat was, in a recent phase II trial on systolic heart failure, shown to reduce NT-proBNP (169) and a phase III trial is currently ongoing (ClinicalTrials.gov Identifier: NCT02861534). However, as hypotension often is a limiting factor in the treatment of heart failure, it remains to be seen whether this therapeutical approach has beneficial effects as compared to standard heart failure medications. It is instead plausible that the future in treatment of severe left heart disease, including PH-LHD, may lay in medical devices. In the present thesis the hemodynamic improvement with long-term use of LVADs as bridge to HT has been highlighted and it is clear that their long-term use may reverse pulmonary vascular changes. The first generations of these devices have been associated with a high rate of complications. There is, however, a steady technical improvement and in the two-year follow-up of the MOMENTUM 3 trial, Heartmate III was associated with a 19% absolute risk reduction in the composite primary end-point of survival free of disabling stroke or reoperation, as compared to Heartmate II (61). With further improvement and fully implantable devices, without percutaneous drive-lines, future generations of LVADs may be a long-term treatment option for patients with PH-LHD.

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sGC stimulation totally reverses hypoxia-induced pulmonary vasoconstriction alone and combined with dual endothelin-receptor blockade in a porcine model

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Abstract

Aim: Stimulation of soluble guanylate cyclase (sGC) with BAY 41-8543 was hypothesized to attenuate acute hypoxic pulmonary vasoconstriction alone and combined with dual endothelin (ET)-receptor antagonist tezosentan.

Methods: Measurements were taken in 18 anaesthetized pigs with a mean \pm SEM weight of 31.1 ± 0.4 kg, in normoxia ($\text{FiO}_2=0.21$) and hypoxia ($\text{FiO}_2=0.10$) without (control protocol, $n = 6$), and with right atrial infusion of BAY 41-8543 at 1, 3, 6, 9 and $12 \mu\text{g min}^{-1}$ per kg (protocol 2, $n = 6$) or tezosentan at 5 mg kg^{-1} followed by BAY 41-8543 at 1, 3 and $6 \mu\text{g min}^{-1}$ per kg (protocol 3, $n = 6$).

Results: Hypoxia ($n = 18$) increased ($P < 0.001$) mean pulmonary artery pressure (MPAP) and pulmonary vascular resistance (PVR) by 14.2 ± 0.6 mmHg and 2.8 ± 0.3 WU respectively. During sustained hypoxia without treatment, MPAP and PVR remained stable. BAY 41-8543 ($n = 6$) dose-dependently decreased ($P < 0.001$) MPAP and PVR by 15.0 ± 1.2 mmHg and 4.7 ± 0.7 WU respectively. Tezosentan ($n = 6$) decreased ($P < 0.001$) MPAP and PVR by 11.8 ± 1.2 mmHg and 2.0 ± 0.2 WU, respectively, whereafter BAY 41-8543 ($n = 6$) further decreased ($P < 0.001$) MPAP and PVR by 6.6 ± 0.9 mmHg and 1.9 ± 0.4 WU respectively. Both BAY 41-8543 and tezosentan decreased ($P < 0.001$) systemic arterial pressure and systemic vascular resistance. Blood- O_2 consumption remained unaltered ($P = \text{ns}$) during all interventions.

Conclusion: BAY 41-8543 totally reverses the effects of acute hypoxia-induced pulmonary vasoconstriction, and enhances the attenuating effects of tezosentan, without affecting oxygenation. Thus, sGC stimulation, alone or combined with dual ET-receptor blockade, could offer a means to treat pulmonary hypertension related to hypoxia and potentially other causes.

Keywords BAY 41-8543, hypoxia, pulmonary vasoconstriction, soluble guanylate cyclase, tezosentan.

Pulmonary hypertension (PH), defined by a mean pulmonary artery pressure (MPAP) ≥ 25 mmHg at rest (Galie *et al.* 2009), is a heterogeneous disease with severe consequences. According to the DANA POINT classification, PH may be related to (1) pulmonary arterial hypertension (PAH), (2) left heart disease, (3) lung diseases and/or hypoxia, (4) chronic thromboembolism, or be of (5) unclear and/or multifactorial mechanisms (Simmoneau *et al.* 2009). Even though groups 1–5 differ with regard to their aetiology, they may share similar pathological features.

Hypoxia, as exhibited in chronic obstructive and restrictive pulmonary diseases, as well as upon exposure to high altitude, is thus one factor involved in the pathogenesis of PH (Simmoneau *et al.* 2009). Whereas chronic hypoxia may induce structural and functional changes of the pulmonary arterial vascular bed, including remodelling with proliferation and migration of vascular smooth muscle cells and increased extracellular matrix formation (Humbert *et al.* 2004), acute hypoxia causes immediate pulmonary arteriolar vasoconstriction, known as hypoxic pulmonary vasoconstriction (HPV) (von Euler & Liljestrand 1946). Furthermore, as similar mediators may be involved in HPV and hypoxia-induced vascular remodelling, as in the pathogenesis of PAH, HPV has evolved as a means to evaluate factors that control pulmonary vascular tone.

Soluble guanylate cyclase (sGC) is a heterodimeric haeme protein, consisting of a large α -subunit and a small haeme-binding β -subunit (Boerrigter & Burnett 2010). It is the main enzyme activated by nitric oxide (NO) in the NO signalling pathway and catalyses the conversion of guanosine-5'-triphosphate into the second messenger cyclic guanosine-3'-5'-monophosphate (cGMP). cGMP in turn activates downstream effectors leading to several different cellular processes (Evgenov *et al.* 2006). There are both stimulators and activators of sGC, which can be either dependent or independent of NO (Stasch *et al.* 2011). The stimulators sensitize sGC to low levels of bioavailable NO, maintaining the enzyme in its active form, or increase sGC activity in the absence of NO (Stasch *et al.* 2011). sGC activators activate sGC when it is in its oxidized, or haeme-free, state (Stasch *et al.* 2011). Stimulation of sGC has, in this context, been suggested to, independently, as well as in synergy with NO, produce anti-aggregatory, anti-proliferative and vasodilator effects. sGC stimulation could thus offer a new means to treat PH of various causes (Evgenov *et al.* 2006). A prerequisite for the NO-induced activation of sGC is the presence of the reduced Fe^{2+} haeme moiety. Its removal or oxidation to Fe^{3+} , which may occur under oxidative stress, as for instance in hypoxia and atherosclerosis, leads to the formation of an NO-insensitive form (Ignarro *et al.* 1986). Such impairment of the NO-sGC-

cGMP pathway has, besides endothelin-1 (ET-1), been suggested to influence pulmonary vascular remodelling (Vermeersch *et al.* 2007). This implies that sGC also may be a fundamental mechanism that influences vascular structure and tone. Stimulation and/or activation of sGC could thus consequently offer a means to decrease pulmonary vascular tone, not only by vasodilatation, but also by inhibiting pulmonary vascular remodelling.

With respect to the effects of ET-receptor blockade on acute HPV, our laboratory recently showed, in a porcine model, that dual ET-receptor blockade with tezosentan, infused in the right atrium, markedly attenuated the acute hypoxia-induced increase in pulmonary arterial pressure and completely abolished the pulmonary vascular resistance (PVR) increase to hypoxia (Hedelin *et al.* 2011). In the present study, we utilized the same model of acute normobaric hypoxia in the pig, to study whether sGC stimulation alone, or on top of dual ET-receptor blockade, attenuates HPV. Such findings could be of importance to develop new single or combination treatments for PH related to hypoxia and potentially other causes, including PAH, as well as to clarify the potency of various endogenous factors that control pulmonary vascular tone. We hypothesized that right atrial infusion of the, NO-independent, sGC stimulator BAY 41-8543 (Stasch *et al.* 2002) alone, or on top of dual ET-receptor blockade with tezosentan (Clozel *et al.* 1999), would attenuate acute HPV.

Materials and methods

Animals and ethical approval

Eighteen female pigs of mixed Danish landrace and Yorkshire were fasted overnight with free access to water. They were subsequently studied, in accordance with the guidelines of the Department of Experimental Medicine and Surgery at the Panum Institute, Copenhagen, Denmark. The experiments were ethically approved by Dyreforsøgstilsynet, Copenhagen, Denmark (2009/561-1621).

Anaesthesia and ventilation

All pigs were pre-medicated intramuscularly with 1 mL 10 kg^{-1} of a mixture of 6.25 mL Narcoxyl vet (Xylazin 20 mg mL^{-1} ; Intervet, Roskilde, Denmark), 1.25 mL Ketaminol vet (Ketamin 100 mg mL^{-1} ; Intervet), 2 mL Metadon (10 mg mL^{-1} ; Nycomed, Roskilde, Denmark) and 2 mL Turbogesis (Butorphanol tartrat 10 mg mL^{-1} ; Scanvet, Fredensborg, Denmark) in Zoletil 50 vet Tørstof (Virbac, Kolding, Denmark). Anaesthesia was maintained with 1.5 mL

kg⁻¹ per h of Propofol (10 mg mL⁻¹; B Braun, Fredriksberg, Denmark) and 1 mL 10 kg⁻¹ per h of Fentanyl (50 µg mL⁻¹; Janssen-Cilag, Birkerød, Denmark) administered intravenously throughout the experiment with a B Braun Infusomat[®]Space and B Braun Perfusor[®]Compact infusion pump respectively. After intubation, the lungs were mechanically ventilated with a respirator (Dameca, Rødovre, Denmark) and the inspiratory oxygen fraction (F_IO₂) was monitored using an oxygen sensor and a medical oxygen apparatus OM871 (Dameca). After the experiments, the animals were killed by a 10–15 mL mixture of Pentobarbital (200 mg mL⁻¹) and Lidocainhydrochloride (20 mg mL⁻¹) (Veterinærapoteket, Copenhagen University, Copenhagen, Denmark).

Invasive procedures, measurements and haemodynamic surveillance

Two small skin incisions were made, one along the medial line of the throat and one in the groin, to locate the internal jugular vein and the common carotid artery, as well as the femoral artery respectively. An infusion catheter (Baby feeding tube No. 8; Unomedical, Birkerød, Denmark) was inserted through the internal jugular vein and advanced into the right atrium. Catheters for arterial pressure measurements (Baby feeding tube No. 8; Unomedical) were positioned in the aortic arch, through the common carotid artery, as well as in the femoral artery. The femoral arterial catheter was only used as a backup, if problems with the aortic catheter would occur.

A standard Swan Ganz catheter (931HF75 1.5 mL Cap; Edwards Lifesciences, Irvine, CA, USA), designed for use in adult humans, introduced through the right internal jugular vein, with its tip placed in the pulmonary artery, was used to measure right atrial-, pulmonary artery- and pulmonary capillary wedge pressures (PCWP), as well as CO in triplicates by thermodilution, infusing 10 mL bolus doses of cold saline (Pàvek *et al.* 1964). CO was calculated using a Baxter-Vigilance[®] monitor (Edwards Critical-Care, Saint-Prex, Switzerland).

ECG, heart rate (HR), mean aortic pressure (MAP), mean femoral artery pressure and mean right atrial pressure (MRAP) as well as MPAP were continuously monitored on a surveillance screen (Hewlett Packard, Berkshire, UK) and recorded using a PowerLab 8/30 system (AD Instruments, Oxfordshire, UK). PCWP was measured intermittently. By evaluating the pressure curves, it was ensured that the Swan Ganz catheter was correctly placed throughout the experiment. The data were processed using the PowerLab software Chart, version 5.5.6 (AD Instruments, Oxfordshire, UK). Non-invasive saturation was monitored by a pulseoximetry probe (Philips M1193A; Philips Medico

A/S, Copenhagen, Denmark) placed on the tails of the pigs, as a visual continuous feedback during the experiments. PVR, systemic vascular resistance (SVR) and stroke volume (SV) were calculated according to the formulas: PVR = (MPAP–PCWP)/CO, SVR = (MAP–MRAP)/CO, and SV = CO/HR.

Blood samples from the pulmonary artery and the aortic arch were used for immediate blood gas analysis with an ABL 700 Series apparatus (Radiometer, Copenhagen, Denmark), determining blood aorta (AO) and pulmonary artery (PA) O₂ saturation (S_{AO}O₂ and S_{PA}O₂), haemoglobin concentration (Hb_{AO} and Hb_{PA}), O₂ pressure (p_{AO}O₂ and p_{PA}O₂), pH_{AO} and pH_{PA}. This allowed calculation of aorta and pulmonary artery O₂ content (C_{AO}O₂ and C_{PA}O₂), where: C_{AO}O₂ = (Hb_{AO} · 1.34 · S_{AO}O₂) + (0.0031 · p_{AO}O₂), and C_{PA}O₂ = (Hb_{PA} · 1.34 · S_{PA}O₂) + (0.0031 · p_{PA}O₂), O₂ extraction = C_{AO}O₂–C_{PA}O₂ and O₂ consumption = CO · (C_{AO}O₂–C_{PA}O₂). In the formulas, SO₂ is expressed as a fraction of 1.0 and not%, Hb as g dL⁻¹, pO₂ as mmHg, and the correction factors 1.34 as mL g⁻¹ and 0.0031 as mL dL⁻¹ per mmHg.

Drugs

In protocol 2, 9000 µg of the sGC stimulator BAY 41-8543 (Bayer Schering Pharma AG, Wuppertal, Germany) was dissolved in a 50 mL solution consisting of glycerol/sterile water/polyethylene glycol 400 (60/100/949 = g g⁻¹ per g). The solutions were glycerol 99% (Sigma-Aldrich, Bornem, Belgium), sterile water and polyethylene glycol (Sigma-Aldrich). An infusion pump (Model 11plus; Harvard apparatus, Holliston, MA, USA) was utilized for right atrial infusion of BAY 41-8543. In protocol 3, 5 mg kg⁻¹ body weight of the dual ET A- and B-receptor antagonist tezosentan (Actelion Pharmaceuticals, Allschwil, Switzerland) was dissolved in 10 mL of sterile water. The tezosentan solution was delivered as a manual bolus injection in the right atrium during hypoxia. Tezosentan was chosen as a dual ET-receptor antagonist, as it has been developed for iv use and rapidly reaches a plateau in its effect. The half-life of tezosentan has been reported to be 0.5–2 h in various species (Clozel *et al.* 1999). Subsequently, an infusion pump (Model 11plus; Harvard apparatus) was utilized for right atrial infusion of BAY 41-8543, dissolved as described in protocol 2.

Induction of hypoxia

In 18 pigs, with a mean ± SEM weight of 31.1 ± 0.4 kg, hypoxia was induced by slowly lowering the oxygen supply and airflow on the respirator from F_IO₂–0.21, to a stable level at approx. 0.15 and

approx. 0.10 (hypoxia baseline) respectively. The respiratory rate and tidal volume were kept constant throughout the experiments. Haemodynamic measurements and blood sampling were performed at the end of normoxia, 5 min into stable hypoxia at F_{iO_2} -0.15 and 15 min into stable hypoxia at F_{iO_2} -0.10 (hypoxia baseline), corresponding to the altitudes of approx. 2800 and approx. 5500 m respectively. In protocol 1–3, approx. 1000 mL of sodium chloride was administered to each animal during the induction of hypoxia to avoid a too large drop in systemic blood pressure.

Subsequently, six pigs were used for control measurements, for this and related studies (protocol 1), where the stability of the HPV response over time was evaluated. Twelve pigs were used to study the effects of the sGC stimulator BAY 41-8543, alone (protocol 2, $n = 6$) or in combination with dual ET-receptor blockade with tezosentan (protocol 3, $n = 6$).

Protocol 1 – hypoxia control experiments

After having taken measurements in normoxia and hypoxia at F_{iO_2} -0.15 and approx. 0.10 (hypoxia baseline) at the above-mentioned time-points, in six pigs (30.2 ± 1.6 kg), haemodynamic measurements were taken and blood sampling were performed after 5, 15, 22.5, 30, 37.5, 60, 67.5, 75 and 82.5 min of sustained hypoxia at F_{iO_2} -0.10. These measurements correspond to the time-points when measurements were taken in protocol 2–3.

Protocol 2 – the effects of infusion of the sGC stimulator BAY 41-8543

After having taken measurements in normoxia and hypoxia at F_{iO_2} -0.15 and approx. 0.10 (hypoxia baseline) at the above-mentioned time-points, in six pigs (28.7 ± 0.3 kg), haemodynamic measurements were taken and blood sampling were performed during right atrial infusion of BAY 41-8543 at increasing doses of 1, 3, 6, 9 and $12 \mu\text{g min}^{-1}$ per kg for approx. 7.5 min each during sustained hypoxia at F_{iO_2} -0.10. Measurements were taken at steady state between 5 and 7.5 min after starting the sGC infusion at each dose. A stepwise increment was used to mimic a clinical situation of administering a drug, to reach a stable satisfactory response, eliminating 'on' and 'off' effects when infusing the drug.

Protocol 3 – the effects of dual ET-receptor blockade with tezosentan and subsequent infusion of the sGC stimulator BAY 41-8543

After having taken measurements in normoxia and hypoxia at F_{iO_2} -0.15 and approx. 0.10 (hypoxia

baseline) at the above-mentioned time-points, in six pigs (31.5 ± 0.6 kg), haemodynamic measurements were taken and blood sampling were performed during sustained hypoxia at F_{iO_2} -0.10 after 5, 15, 30 and 60 min from the time of right atrial bolus infusion of tezosentan at 5 mg kg^{-1} , and subsequently after infusion of BAY 41-8543 at 1, 3 and $6 \mu\text{g min}^{-1}$ per kg for approx. 7.5 min each. During infusion of BAY 41-8543, measurements were taken at steady state between 5 and 7.5 min after starting the sGC infusion at each dose.

Statistics and data analysis

A SigmaStat System (SigmaPlot 11.0) was used for statistical analysis. Parametric or non-parametric statistics were used depending on the distribution of data, that is, one-way repeated measures ANOVA (Tukey's test) or one-way repeated measures ANOVA on Ranks (Tukey's test). In specific, for the parameters in protocol 1, 2 and 3, an analysis was first performed between normoxia (F_{iO_2} -0.21) and hypoxia at F_{iO_2} -0.15 and F_{iO_2} -0.10, respectively, for each protocol, as well as for the pooled response of all pigs. In protocol 1, hypoxia baseline (F_{iO_2} -0.10) was then compared to the repeated measurements taken during sustained hypoxia at F_{iO_2} -0.10 to verify stability in the hypoxic response. In protocol 2, hypoxia baseline (F_{iO_2} -0.10) was compared with infusion of BAY 41-8543 at increasing infusion rates during sustained hypoxia at F_{iO_2} -0.10, and whether the response normalized to normoxic level. In protocol 3, hypoxia baseline (F_{iO_2} -0.10) was compared to the temporal response to infusion of tezosentan during sustained hypoxia at F_{iO_2} -0.10, and subsequently whether infusion of BAY 41-8543 could further alter the response during sustained hypoxia at F_{iO_2} -0.10, as compared to the effect of tezosentan, 60 min after bolus infusion, and whether normalization to a level as in normoxia occurred. A P value < 0.05 was considered statistically significant. $P = \text{ns}$ indicates not statistically significant. The values are mean \pm SEM, unless otherwise indicated.

Results

Induction of hypoxia

Haemodynamic measurements. As compared to normoxia, hypoxia at F_{iO_2} -0.10 ($n = 18$) increased MPAP by 14.2 ± 0.6 mmHg ($P < 0.001$), PVR by 2.8 ± 0.3 WU ($P < 0.001$), MRAP by 2.4 ± 0.6 mmHg ($P < 0.001$), PCWP by 1.8 ± 0.5 mmHg ($P < 0.001$), CO by 0.7 ± 0.2 L min^{-1} ($P < 0.002$) and SV by 4.9 ± 1.8 mL ($P < 0.012$), and decreased ($P < 0.05$) SVR by 5.6 ± 1.3 WU. MAP and HR were not altered

significantly ($P = ns$) by the induction of hypoxia. The specific haemodynamic responses to hypoxia for each protocol are shown in Figures 1–4.

Blood sampling. Compared to normoxia, hypoxia at F_{iO_2} -0.10 ($n = 18$), decreased blood S_{AO_2} by

$21.7 \pm 2.8\%$ -units ($P < 0.05$), S_{PAO_2} by $17.8 \pm 3.5\%$ -units ($P < 0.001$), p_{AO_2} by 9.6 ± 0.3 kPa ($P < 0.001$), p_{PAO_2} by 1.0 ± 0.2 kPa ($P < 0.001$), C_{AO_2} by 45.8 ± 6.1 mL L⁻¹ ($P < 0.05$), C_{PAO_2} by 34.7 ± 6.0 mL L⁻¹ ($P < 0.001$), O_2 extraction by 8.1 ± 3.4 mL L⁻¹ ($P < 0.015$), pH_{AO} by 0.05 ± 0.01

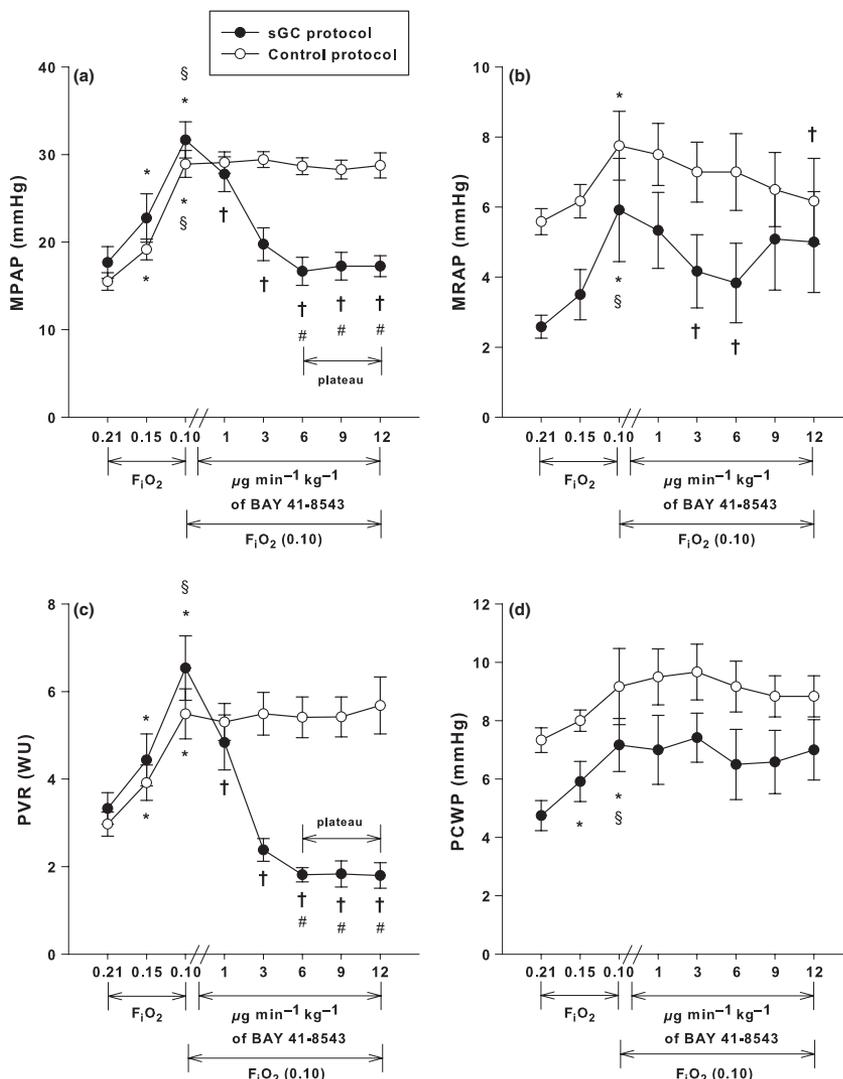


Figure 1 (a) Mean pulmonary artery pressure (MPAP), (b) mean right atrial pressure (MRAP), (c) pulmonary vascular resistance (PVR) and (d) pulmonary capillary wedge pressures (PCWP) during normoxia and hypoxia, without and with soluble guanylate cyclase (sGC) infusion. *During the induction of hypoxia indicates statistical significance as compared to normoxia, and §statistical significance as compared to hypoxia at F_{iO_2} -0.15. During sustained hypoxia at F_{iO_2} -0.10, †statistical significance as compared to hypoxia baseline without BAY 41-8543 and #statistical significance below the normoxic level.

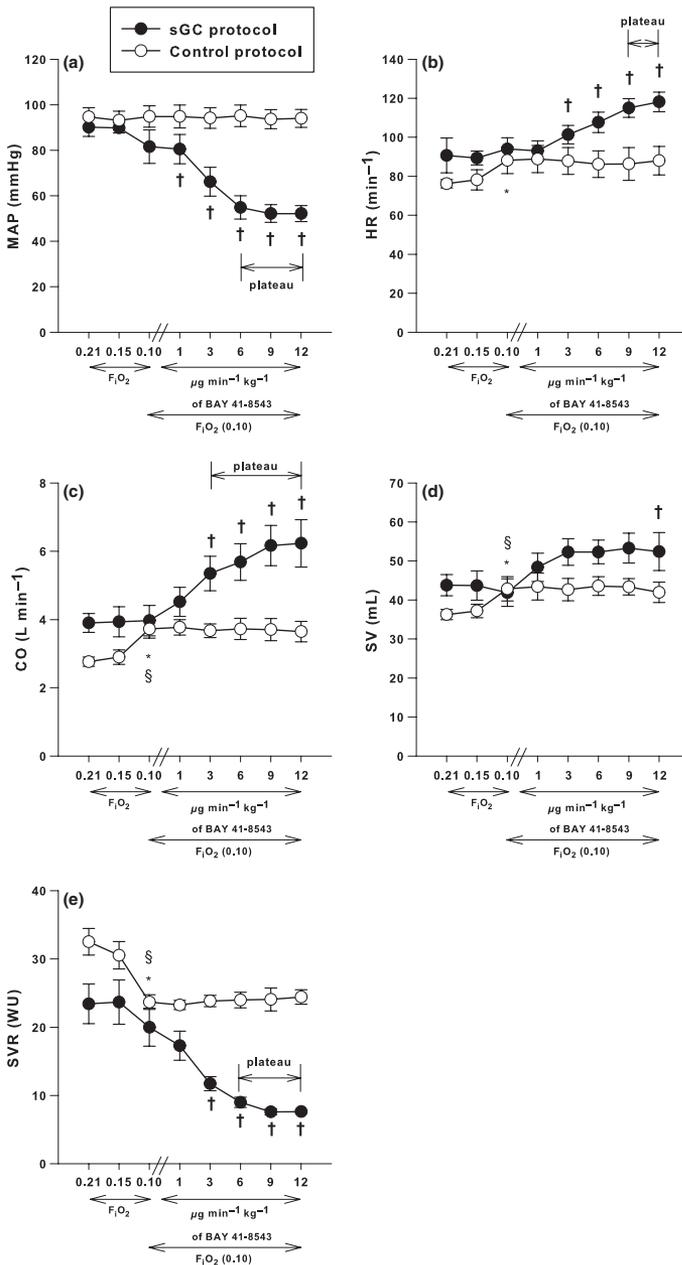


Figure 2 (a) Mean aortic pressure (MAP), (b) heart rate (HR), (c) CO, (d) stroke volume (SV) and (e) systemic vascular resistance (SVR) during normoxia and hypoxia, without and with soluble guanylate cyclase (sGC) infusion. *During the induction of hypoxia indicates statistical significance as compared to normoxia, and §statistical significance as compared to hypoxia at F_IO₂-0.15. During sustained hypoxia at F_IO₂-0.10, †statistical significance as compared to hypoxia baseline without BAY 41-8543.

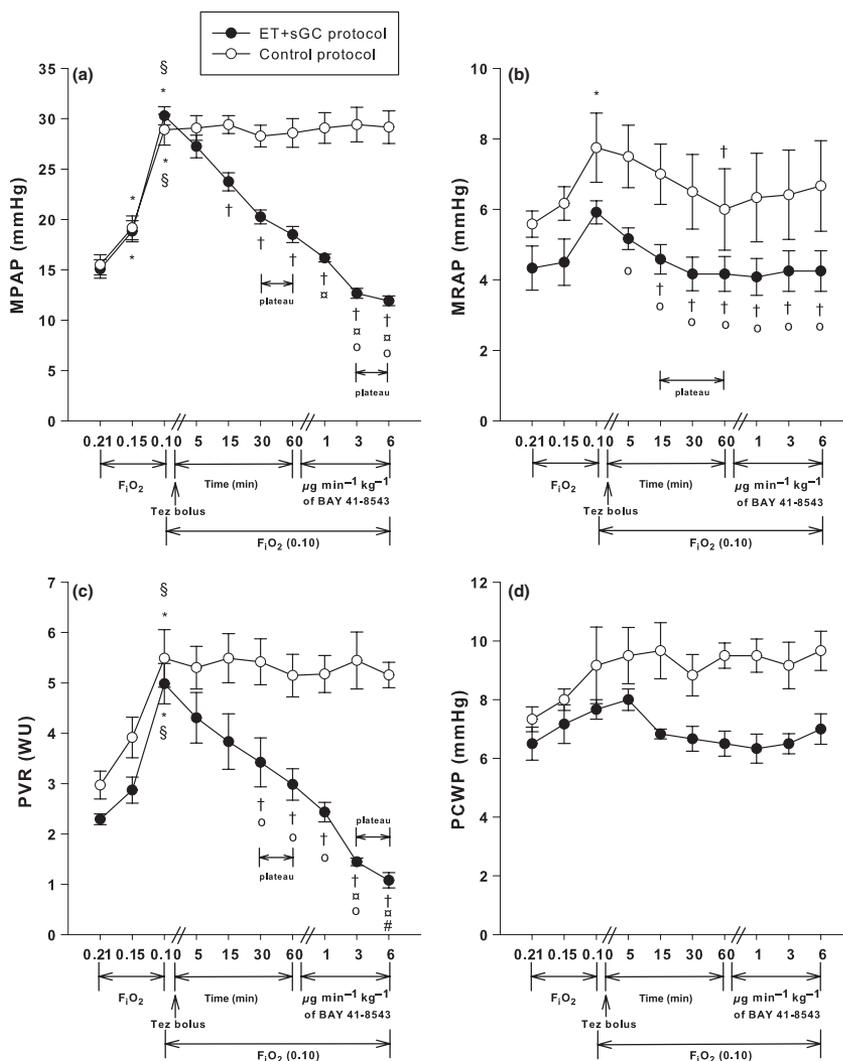


Figure 3 (a) Mean pulmonary artery pressure (MPAP), (b) mean right atrial pressure (MRAP), (c) pulmonary vascular resistance (PVR) and (d) pulmonary capillary wedge pressures (PCWP) during normoxia and hypoxia, without and with dual endothelin (ET)-receptor blockade, followed by soluble guanylate cyclase (sGC) infusion. *During the induction of hypoxia indicates statistical significance as compared to normoxia, and †statistical significance as compared to hypoxia baseline without tezosentan, ‡statistical significance with BAY 41-8543 as compared to hypoxia 60 min after tezosentan bolus infusion without BAY 41-8543, and ○not significantly different ($P = ns$) as compared to normoxia, that is, return to normoxia baseline. #Statistical significance below the normoxic level.

($P < 0.05$), pH_{PA} by 0.04 ± 0.01 ($P < 0.001$), Hb_{AO} by 1.2 ± 0.3 $g\ dL^{-1}$ ($P < 0.05$) and Hb_{PA} by 1.3 ± 0.2 $g\ dL^{-1}$ ($P < 0.001$), whereas blood- O_2

consumption was unchanged ($P = ns$) by the induction of hypoxia. The specific responses in the blood parameters for each protocol are shown in Tables 1–3.

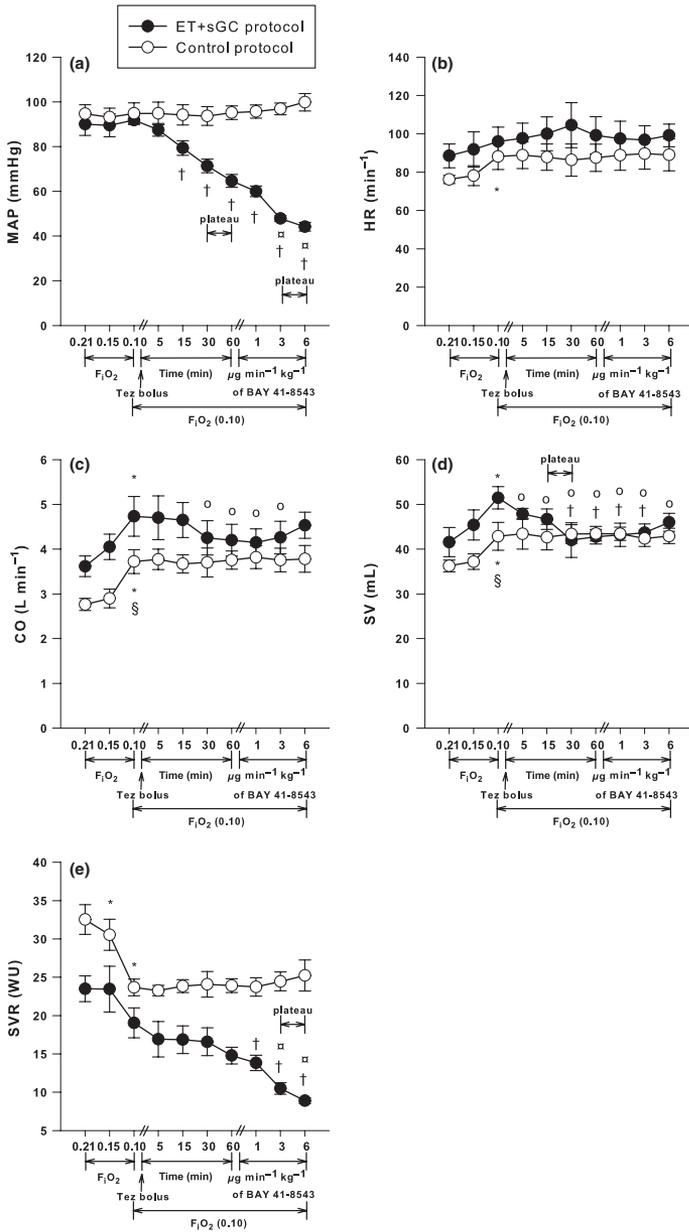


Figure 4 (a) Mean aortic pressure (MAP), (b) heart rate (HR), (c) CO, (d) stroke volume (SV) and (e) systemic vascular resistance (SVR) during normoxia and hypoxia, without and with dual endothelin (ET)-receptor blockade, followed by soluble guanylate cyclase (sGC) infusion. *During the induction of hypoxia indicates statistical significance as compared to normoxia, and †statistical significance as compared to hypoxia at F_iO₂ 0.10, ‡statistical significance as compared to hypoxia baseline without tezosentan, §statistical significance with BAY 41-8543 as compared to hypoxia 60 min after tezosentan bolus infusion without BAY 41-8543, and °not significant (P = ns) as compared to normoxia, that is, return to normoxia baseline.

Table 1 Blood aorta and pulmonary artery O₂ saturation, pO₂, O₂ content, pH, Hb, O₂ extraction and O₂ consumption during normoxia and hypoxia

	F _O ₂ -0.21	F _O ₂ -0.15	F _O ₂ -0.10 (baseline)	5 min (F _O ₂ -0.10)	15 min (F _O ₂ -0.10)	22.5 min (F _O ₂ -0.10)	30 min (F _O ₂ -0.10)	37.5 min (F _O ₂ -0.10)	60 min (F _O ₂ -0.10)	67.5 min (F _O ₂ -0.10)	75 min (F _O ₂ -0.10)	82.5 min (F _O ₂ -0.10)
S _{av} O ₂ (%)	97.7 ± 0.2	95.2 ± 0.4	77.1 ± 2.7*§	76.6 ± 3.5	73.5 ± 3.8	74.0 ± 3.4	74.0 ± 4.0	70.3 ± 4.6	69.8 ± 5.4	66.7 ± 5.2†	68.5 ± 6.6†	63.2 ± 5.6†
S _{pa} O ₂ (%)	56.6 ± 2.0	54.0 ± 2.2	40.7 ± 4.1*§	39.9 ± 4.5	37.2 ± 4.9	37.2 ± 5.2	36.7 ± 5.2	34.1 ± 5.8	33.5 ± 6.3†	30.7 ± 5.7*‡	30.7 ± 5.4*‡	26.7 ± 4.8*‡#
P _{av} O ₂ (kPa)	16.7 ± 0.4	11.3 ± 0.2*	6.9 ± 0.4*§	6.8 ± 0.5	6.6 ± 0.5	6.6 ± 0.4	6.3 ± 0.4	6.3 ± 0.5	6.4 ± 0.6	6.0 ± 0.5*‡#	6.3 ± 0.6	5.8 ± 0.5*‡#
P _{pa} O ₂ (kPa)	4.9 ± 0.1	4.8 ± 0.2	4.2 ± 0.2*§	4.1 ± 0.2	4.0 ± 0.3	4.0 ± 0.3	4.0 ± 0.3	3.8 ± 0.4	3.8 ± 0.4	3.7 ± 0.3*‡	3.7 ± 0.3*‡	3.5 ± 0.3*‡#
C _{av} O ₂ (mL L ⁻¹)	133.8 ± 3.2	119.1 ± 2.7*	93.2 ± 4.9*§	90.4 ± 3.9	85.7 ± 2.9	87.0 ± 2.5	86.5 ± 3.1	84.0 ± 3.5	84.4 ± 5.5	82.6 ± 5.2	81.8 ± 6.7†	85.9 ± 5.3*‡#
C _{pa} O ₂ (mL L ⁻¹)	78.8 ± 2.8	72.0 ± 3.5	50.8 ± 5.4*§	49.9 ± 6.3	44.6 ± 5.1	45.4 ± 5.4	45.1 ± 5.8	42.2 ± 6.9	42.3 ± 7.7	38.4 ± 6.7*‡	38.3 ± 6.2*‡	34.0 ± 5.8*‡#
O ₂ extraction (mL L ⁻¹)	57.0 ± 3.4	47.1 ± 3.7*	42.4 ± 3.4*	40.5 ± 3.9	41.2 ± 2.8	41.6 ± 3.3	41.4 ± 3.6	41.8 ± 3.8	42.1 ± 3.0	44.2 ± 1.7	43.5 ± 2.5	41.9 ± 1.9
O ₂ consumption (mL min ⁻¹)	156.7 ± 9.1	133.8 ± 6.9*	154.2 ± 7.5§	148.7 ± 7.0	155.6 ± 10.0	148.6 ± 5.2	149.7 ± 11.6	146.8 ± 6.8	157.6 ± 13.4	169.0 ± 13.5	163.2 ± 13.1	158.9 ± 15.0
pH _{av}	7.49 ± 0.02	7.48 ± 0.02	7.43 ± 0.02*§	7.43 ± 0.03	7.42 ± 0.03	7.42 ± 0.03	7.42 ± 0.03	7.42 ± 0.03	7.41 ± 0.03	7.41 ± 0.03†	7.41 ± 0.031†	7.41 ± 0.03†#
pH _{pa}	7.44 ± 0.02	7.43 ± 0.02	7.40 ± 0.02*§	7.39 ± 0.02	7.39 ± 0.03	7.38 ± 0.03	7.38 ± 0.03	7.38 ± 0.03	7.38 ± 0.03	7.37 ± 0.03	7.37 ± 0.03	7.4 ± 0.03†#
Hb _{av} (g dL ⁻¹)	10.1 ± 0.2	9.1 ± 0.2*	8.9 ± 0.4*	8.7 ± 0.3	8.6 ± 0.3	8.7 ± 0.3	8.6 ± 0.3	8.8 ± 0.3	9.0 ± 0.4	9.2 ± 0.4	8.9 ± 0.4	8.9 ± 0.4
Hb _{pa} (g dL ⁻¹)	10.2 ± 0.2	9.8 ± 0.3	9.2 ± 0.4*	9.2 ± 0.4	9.0 ± 0.4	9.1 ± 0.3	9.1 ± 0.3	9.2 ± 0.3	9.4 ± 0.4	9.3 ± 0.4	9.3 ± 0.3	9.4 ± 0.4

During the induction of hypoxia;

*Statistical significance as compared to normoxia, §Statistical significance compared to hypoxia at F_O₂-0.15.

During sustained hypoxia at F_O₂-0.10;

†Statistical significance compared to hypoxia baseline,

#Statistical significance compared to the 5 min measurement,

‡Statistical significance compared to the 15 min measurement,

§Statistical significance compared to the 22.5 min measurement,

■Statistical significance compared to the 30 min measurement, and

●Statistical significance compared to the 37.5 min measurement.

Table 2 Blood S_{AO_2} , S_{PAO_2} , P_{PAO_2} , P_{PAO_2} , C_{PAO_2} , C_{PAO_2} , O_2 consumption, pH_{AO} and pH_{PA} , during normoxia and hypoxia, without and with soluble guanylate cyclase infusion

	$F_{O_2-0.21}$	$F_{O_2-0.15}$	$F_{O_2-0.10}$ (baseline)	1 $\mu g \text{ min}^{-1}$ per kg ($F_{I_2O_2-0.10}$)	3 $\mu g \text{ min}^{-1}$ per kg ($F_{I_2O_2-0.10}$)	6 $\mu g \text{ min}^{-1}$ per kg ($F_{I_2O_2-0.10}$)	9 $\mu g \text{ min}^{-1}$ per kg ($F_{I_2O_2-0.10}$)	12 $\mu g \text{ min}^{-1}$ per kg ($F_{I_2O_2-0.10}$)
S_{AO_2} (%)	99.3 ± 0.47	95.1 ± 1.4*	68.1 ± 6.5*§	68.7 ± 7.9	67.8 ± 8.8	67.9 ± 8.8	69.6 ± 8.2	77.2 ± 8.2
S_{PAO_2} (%)	66.7 ± 2.5	60.0 ± 3.0*	33.3 ± 7.6*§	35.2 ± 8.1	38.0 ± 8.3	39.4 ± 7.5	42.0 ± 7.7	45.7 ± 7.3†
P_{PAO_2} (kPa)	15.1 ± 0.6	10.3 ± 0.3*	6.0 ± 0.4*§	6.0 ± 0.5	6.0 ± 0.5	5.9 ± 0.5	5.9 ± 0.3	6.2 ± 0.3
P_{PAO_2} (kPa)	5.5 ± 0.3	5.1 ± 0.2*	3.6 ± 0.3*§	3.6 ± 0.4	3.8 ± 0.4	3.9 ± 0.4	4.0 ± 0.4	4.1 ± 0.3
C_{AO_2} (mL L^{-1})	162.9 ± 11.1	156.1 ± 7.3	101.3 ± 12.2*§	98.9 ± 12.7	97.3 ± 13.8	88.6 ± 9.5	97.3 ± 10.4	102.1 ± 9.0
C_{PAO_2} (mL L^{-1})	114.3 ± 5.9	98.9 ± 5.8*	49.8 ± 11.8*§	53.3 ± 12.3	57.3 ± 12.6	55.6 ± 11.2	57.5 ± 10.2	61.2 ± 9.5
O_2 extraction (mL L^{-1})	46.6 ± 10.2	57.2 ± 6.5	51.4 ± 4.6	45.6 ± 5.6	40.0 ± 7.8	33.0 ± 2.2	39.7 ± 2.5	41.0 ± 5.3
O_2 consumption (mL min^{-1})	180.0 ± 37.5	214.0 ± 16.7	194.7 ± 9.7	196.2 ± 15.3	196.5 ± 18.3	188.5 ± 21.6	242.2 ± 22.6	245.0 ± 30.9
pH_{AO}	7.47 ± 0.04	7.44 ± 0.04	7.40 ± 0.05*§	7.39 ± 0.05	7.39 ± 0.05	7.38 ± 0.05	7.37 ± 0.05†	7.37 ± 0.05†
pH_{PA}	7.4 ± 0.04	7.4 ± 0.03*	7.4 ± 0.04*§	7.4 ± 0.04	7.4 ± 0.05	7.4 ± 0.05	7.3 ± 0.05	7.3 ± 0.05†
Hb_{AO} (g dL^{-1})	12.0 ± 0.8	12.0 ± 0.5	10.8 ± 0.4	10.6 ± 0.5	10.5 ± 0.3	9.8 ± 0.6	10.4 ± 0.3	9.8 ± 0.3
Hb_{PA} (g dL^{-1})	12.6 ± 0.4	12.1 ± 0.4	10.8 ± 0.4*§	11.0 ± 0.3	10.9 ± 0.3	10.2 ± 0.4	10.2 ± 0.3	10.0 ± 0.3

During the induction of hypoxia;

*Statistical significance as compared to normoxia,

§Statistical significance as compared to hypoxia at $F_{O_2-0.15}$.

During sustained hypoxia at $F_{O_2-0.10}$;

†Statistical significance compared to hypoxia baseline without BAY 41-8543.

Table 3 Blood S_{AO_2} , S_{PAO_2} , P_{AO_2} , P_{PAO_2} , C_{AO_2} , C_{PAO_2} , O_2 consumption, pH_{AO_2} , pH_{PAO_2} , Hb_{PAO_2} and Hb_{PAO_2} during normoxia and hypoxia, without and with dual endothelin (ET)-receptor blockade, followed by soluble guanylate cyclase (sGC) infusion

	$F_{O_2-0.21}$	$F_{O_2-0.15}$	$F_{O_2-0.10}$ (baseline)	ET-blockade 15 min ($F_{O_2-0.10}$)	ET-blockade 30 min ($F_{O_2-0.10}$)	ET-blockade 60 min ($F_{O_2-0.10}$)	SGC-stim 1 $\mu\text{g min}^{-1}$ per kg ($F_{O_2-0.10}$)	SGC-stim 3 $\mu\text{g min}^{-1}$ per kg ($F_{O_2-0.10}$)	SGC-stim 6 $\mu\text{g min}^{-1}$ per kg ($F_{O_2-0.10}$)
S_{AO_2} (%)	98.3 ± 0.4	96.1 ± 0.3	84.9 ± 0.8	85.8 ± 1.5	85.8 ± 1.7	82.7 ± 1.6	83.0 ± 2.3	82.6 ± 2.6	81.4 ± 3.0
S_{PAO_2} (%)	63.6 ± 1.4	62.1 ± 1.5	55.0 ± 1.9*	54.2 ± 2.8	53.4 ± 3.4	48.8 ± 3.7†	49.1 ± 4.2†	48.1 ± 3.7†	49.2 ± 3.2†
P_{AO_2} (kPa)	17.6 ± 0.6	11.2 ± 0.2	7.1 ± 0.2*§	7.4 ± 0.3	7.4 ± 0.2	6.8 ± 0.2	6.9 ± 0.2	6.9 ± 0.3	6.7 ± 0.3
P_{PAO_2} (kPa)	5.0 ± 0.2	5.0 ± 0.1	4.5 ± 0.1*§	4.5 ± 0.1	4.4 ± 0.2	4.1 ± 0.2	4.1 ± 0.2	4.1 ± 0.2†	4.2 ± 0.1
C_{AO_2} (mL L ⁻¹)	132.3 ± 3.7	120.3 ± 3.9*	99.1 ± 4.4*§	103.0 ± 2.4	102.6 ± 2.8	98.1 ± 3.4	97.2 ± 3.1	92.1 ± 3.0	90.4 ± 3.0
C_{PAO_2} (mL L ⁻¹)	85.1 ± 2.4	79.0 ± 3.1*	65.3 ± 2.1*§	67.7 ± 2.9	65.8 ± 3.9	59.6 ± 5.2	58.6 ± 5.3	55.7 ± 4.6†	55.7 ± 3.9†
O_2 extraction (mL L ⁻¹)	47.1 ± 2.7	41.3 ± 2.9	33.8 ± 4.2*	35.3 ± 3.6◊	36.8 ± 3.2◊	38.5 ± 4.0◊	38.6 ± 3.6◊	36.4 ± 3.9◊	34.7 ± 4.1◊
O_2 consumption (mL min ⁻¹)	168.1 ± 7.3	165.1 ± 11.5	154.3 ± 15.6	158.7 ± 9.3	153.8 ± 13.8	155.8 ± 8.2	156.5 ± 9.6	149.5 ± 13.2	155.8 ± 10.9
pH_{AO_2}	7.55 ± 0.02	7.53 ± 0.02	7.51 ± 0.01*	7.51 ± 0.01◊	7.52 ± 0.01◊	7.52 ± 0.01◊	7.52 ± 0.01◊	7.52 ± 0.01◊	7.52 ± 0.02◊
pH_{PAO_2}	7.50 ± 0.02	7.50 ± 0.02	7.48 ± 0.02*§	7.48 ± 0.01	7.48 ± 0.02◊	7.49 ± 0.01◊	7.49 ± 0.02◊	7.49 ± 0.02◊	7.48 ± 0.02◊
Hb_{PAO_2} (g dL ⁻¹)	9.7 ± 0.3	9.1 ± 0.3	8.6 ± 0.4*	8.8 ± 0.3◊	8.8 ± 0.3◊	8.7 ± 0.3◊	8.6 ± 0.3◊	8.2 ± 0.3‡	8.2 ± 0.3‡
Hb_{PAO_2} (g dL ⁻¹)	9.9 ± 0.3	9.4 ± 0.2	8.8 ± 0.4*	9.2 ± 0.3†◊	9.1 ± 0.3†◊	9.0 ± 0.3◊	8.8 ± 0.3‡	8.5 ± 0.3‡	8.3 ± 0.3†‡

During the induction of hypoxia;

*Statistical significance compared to normoxia,

§Statistical significance compared to hypoxia at $F_{O_2-0.15}$.

During sustained hypoxia at $F_{O_2-0.10}$;

†Statistical significance compared to hypoxia baseline without tezosentan,

‡Statistical significance with BAY 41-8543 as compared to hypoxia 60 min after tezosentan bolus infusion without BAY 41-8543, and

◊Not significantly different ($P = ns$) compared to normoxia, that is, return to normoxia baseline.

Protocol 1 – hypoxia control experiments

Haemodynamic measurements. At 5, 15, 22.5, 30, 37.5, 60, 67.5, 75 and 82.5 min duration of hypoxia at F_iO_2 -0.10 ($n = 6$), all haemodynamic variables remained stable ($P = ns$), as compared to hypoxia baseline at F_iO_2 -0.10, except MRAP at 60 min, which temporarily decreased ($P < 0.042$) by 1.8 ± 1.2 mmHg (Figs 1–4).

Blood sampling. Sustained hypoxia at F_iO_2 -0.10 ($n = 6$) for 82.5 min, decreased after 82.5 min blood S_{AO_2} by $13.9 \pm 3.1\%$ -units ($P < 0.001$), $S_{PA_2O_2}$ by $14.0 \pm 2.4\%$ -units ($P < 0.001$), p_{AO_2} by 1.1 ± 0.2 kPa ($P < 0.001$), $p_{PA_2O_2}$ by 0.7 ± 0.1 kPa ($P < 0.001$), C_{AO_2} by 17.3 ± 3.9 mL L⁻¹ ($P < 0.001$), $C_{PA_2O_2}$ by 16.8 ± 3.1 mL L⁻¹ ($P < 0.001$), pH_{AO} by 0.03 ± 0.01 ($P < 0.009$) and pH_{PA} by 0.03 ± 0.01 ($P < 0.05$), whereas blood-O₂ extraction, blood-O₂ consumption, Hb_{AO} and Hb_{PA} remained stable during sustained hypoxia for 82.5 min (Table 1).

Protocol 2 – the effects of sGC stimulation with BAY 41-8543 during hypoxia

Haemodynamic measurements. As compared to hypoxia baseline at F_iO_2 -0.10 ($n = 6$), BAY 41-8543 dose-dependently, maximally decreased MPAP and PVR by 15.0 ± 1.2 mmHg ($P < 0.001$) and 4.7 ± 0.7 WU ($P < 0.001$), respectively, both slightly below the normoxic level ($P < 0.001$), levelling off at $6\text{--}12$ $\mu\text{g min}^{-1}$ per kg. PCWP was unaltered ($P = ns$) and MRAP decreased ($P < 0.007$) by 2.1 ± 0.6 mmHg to values not different ($P = ns$) from normoxia, levelling off at $3\text{--}6$ $\mu\text{g min}^{-1}$ per kg, being unaltered ($P = ns$) for the other doses (Fig. 1). Furthermore, BAY 41-8543 dose-dependently, maximally decreased MAP by 26.8 ± 3.3 mmHg ($P < 0.001$) and SVR by 11.0 ± 2.1 WU ($P < 0.05$), levelling off at $6\text{--}12$ $\mu\text{g min}^{-1}$ per kg, increased HR by 21.0 ± 5.5 beats min⁻¹ ($P < 0.001$), CO by 1.4 ± 0.3 L min⁻¹ ($P < 0.001$) and SV by 6.5 ± 0.5 mL ($P < 0.05$), levelling off for HR at $9\text{--}12$ $\mu\text{g min}^{-1}$ per kg and CO at $3\text{--}12$ $\mu\text{g min}^{-1}$ per kg (Fig. 2).

Blood sampling. As compared to hypoxia baseline at F_iO_2 -0.10 ($n = 6$), BAY 41-8543 did not alter ($P = ns$) blood S_{AO_2} , p_{AO_2} , $p_{PA_2O_2}$, C_{AO_2} , $C_{PA_2O_2}$, Hb_{AO} and Hb_{PA} . $S_{PA_2O_2}$ was furthermore also unaltered ($P = ns$), except at 12 $\mu\text{g min}^{-1}$ per kg, where it increased by $12.4 \pm 5.7\%$ units ($P < 0.02$). Moreover, pH_{AO} and pH_{PA} were unaltered ($P = ns$) for the initial infusion rates, except for pH_{AO} at $9\text{--}12$ $\mu\text{g min}^{-1}$ per kg and for pH_{PA} at 12 $\mu\text{g min}^{-1}$ per kg, where it slightly decreased by 0.03 ± 0.01

($P < 0.004$) and 0.03 ± 0.01 ($P < 0.05$) respectively. BAY 41-8543 did neither alter ($P = ns$) blood-O₂ extraction or blood-O₂ consumption (Table 2).

Protocol 3 – the effects of dual ET-receptor blockade with tezosentan and subsequent infusion of the sGC stimulator BAY 41-8543

The effects of dual ET-receptor blockade during hypoxia. Haemodynamic measurements: As compared to hypoxia baseline (F_iO_2 -0.10, $n = 6$), dual ET-receptor blockade, maximally decreased MPAP by 11.8 ± 1.2 mmHg ($P < 0.001$), MRAP by 1.8 ± 0.4 mmHg ($P < 0.001$), PVR by 2.0 ± 0.2 WU ($P < 0.001$) and SV by 8.7 ± 2.7 mL ($P < 0.016$). For MPAP to levels slightly above those in normoxia ($P < 0.039$), and for PVR, MRAP and SV to normalize not different from the normoxic levels ($P = ns$). Levelling off occurred 30–60 min after tezosentan infusion for MPAP, PVR and SV and after 15–60 min for MRAP. CO was unaltered ($P = ns$) (Figs. 3 and 4). Furthermore, during hypoxia, tezosentan infusion maximally decreased MAP by 27.2 ± 2.8 mmHg ($P < 0.001$), levelling off 30–60 min after tezosentan bolus infusion. PCWP, SVR and HR were unaltered ($P = ns$) by tezosentan infusion however with a tendency ($P = ns$) for HR to increase and for PCWP to decrease (Figs 3 and 4).

Blood sampling: As compared to hypoxia baseline (F_iO_2 -0.10, $n = 6$), tezosentan did not alter ($P = ns$) blood S_{AO_2} , p_{AO_2} , $p_{PA_2O_2}$, C_{AO_2} , $C_{PA_2O_2}$, O₂ extraction, O₂ consumption, pH_{AO} , pH_{PA} and Hb_{AO} , whereas $S_{PA_2O_2}$ was decreased maximally by $6.3 \pm 2.3\%$ units ($P < 0.002$), 60 min after tezosentan infusion and Hb_{PA} temporarily increased, maximally by 0.2 ± 0.1 g dL⁻¹ ($P < 0.001$), to a level not different ($P = ns$) from in normoxia (Table 3).

The effects of sGC stimulation with BAY 41-8543 on top of dual ET-receptor blockade during hypoxia.

Haemodynamic measurements: Among the haemodynamic variables that were decreased by tezosentan, BAY 41-8543 infused at rates of 1, 3 and 6 $\mu\text{g min}^{-1}$ per kg further, dose-dependently, maximally decreased MPAP, PVR and MAP by 6.6 ± 0.9 mmHg ($P < 0.001$), 1.9 ± 0.4 WU ($P < 0.001$) and 20.5 ± 2.9 mmHg ($P < 0.001$) respectively. For MPAP to normalized values, not different ($P = ns$) from in normoxia, for PVR to a level slightly lower ($P < 0.048$) than in normoxia, and for MAP to values much lower ($P < 0.001$) than in normoxia, levelling off at infusion rates of $3\text{--}6$ $\mu\text{g min}^{-1}$ per kg for all three variables. SVR, which was unaltered during tezosentan infusion, maximally decreased by 5.9 ± 0.9 WU ($P < 0.001$), to levels much lower than in normoxia, levelling off at

infusion rates of 3–6 $\mu\text{g min}^{-1}$ per kg. MRAP, SV, PCWP, HR and CO remained unaltered ($P = \text{ns}$) with BAY 41-8543 infusion (Figs 3 and 4).

Blood sampling: Blood Hb_{PA} , which temporarily increased by dual ET-receptor blockade during hypoxia ($\text{F}_i\text{O}_2 \sim 0.10$), dose-dependently decreased, maximally by $0.7 \pm 0.07 \text{ g dL}^{-1}$ ($P < 0.001$), after subsequent infusion of the sGC stimulator BAY 41-8543. Among the variables that were unaltered by tezosentan bolus infusion, BAY 41-8543 infusion dose-dependently decreased blood Hb_{AO} , maximally by $0.6 \pm 0.01 \text{ g dL}^{-1}$ ($P < 0.002$), whereas blood $\text{S}_{\text{AO}}\text{O}_2$, $\text{p}_{\text{AO}}\text{O}_2$, $\text{p}_{\text{PA}}\text{O}_2$, $\text{C}_{\text{AO}}\text{O}_2$, $\text{C}_{\text{PA}}\text{O}_2$, O_2 extraction, O_2 consumption, pH_{AO} and pH_{PA} remained unaltered ($P = \text{ns}$) with BAY 41-8543 infusion. Blood $\text{S}_{\text{PA}}\text{O}_2$, which was decreased by tezosentan bolus infusion, remained unaltered ($P = \text{ns}$) with BAY 41-8543 infusion (Table 3).

Discussion

The present study shows that sGC stimulation with BAY 41-8543, during acute hypoxia at $\text{F}_i\text{O}_2 \sim 0.10$, dose-dependently attenuates and totally reverses the acute hypoxia-induced pulmonary vasoconstrictor response. This is manifested by the reduction of the hypoxia-induced increases in MPAP and PVR to values even slightly below those in normoxia. Our study furthermore confirms that dual ET-receptor blockade with tezosentan markedly attenuates the acute HPV response, reducing the hypoxia-induced increases in MPAP and PVR, for MPAP to levels slightly above those in normoxia and for PVR to normoxic levels. Subsequent sGC stimulation on top of dual ET-receptor blockade further dose-dependently decreases MPAP to 'low' normoxic values and PVR to values slightly below those in normoxia. Neither sGC stimulation nor dual ET-receptor blockade, or a combination of the two, altered blood arterial- O_2 saturation or blood- O_2 consumption. Both sGC stimulation and dual ET-receptor blockade, however, caused a marked systemic vasodilatation, as illustrated by the decreases in MAP and SVR, although during stable circulatory conditions. These results suggest that sGC stimulation alone, or in combination with dual ET-receptor blockade, could provide a rapid and effective means to reduce generalized acute hypoxia-induced pulmonary vasoconstriction, without affecting blood arterial oxygenation and blood- O_2 consumption.

Response to hypoxia

Hypoxic pulmonary vasoconstriction, first identified by von Euler and Liljestrand (1946), is primarily effective in the pre-capillary resistance arteries of the pul-

monary circulation (Kato & Staub 1966), although approx. 20% of the response may be related to the venous circuit. HPV may be beneficial in focal hypoxia, such as in atelectasis (Glasser *et al.* 1983), diverting blood from poorly to better ventilated areas of the lung. In such situations, impairment of HPV may consequently lead to insufficient oxygenation. However, HPV may be detrimental in global hypoxia, such as at rapid ascent to high altitude. HPV may then increase pulmonary artery pressure and PVR, and contribute to the development of pulmonary oedema, as well as induce right heart failure (Bartsch *et al.* 2005, Maggiorini *et al.* 2006, Preston 2007). When wanting to treat global acute hypoxia, the primary choice of treatment is the removal of the cause of hypoxia and to increase systemic oxygen supply via ventilation or extra-corporal membrane oxygenation. In the present study, though, we evaluated whether sGC stimulation alone, or in combination with dual ET-receptor blockade, may be a means to alleviate acute hypoxia-induced pulmonary vasoconstriction. This is of importance for developing new treatment strategies for PH of various aetiology, and potentially for developing alternative clinical strategies to infusion of organic nitrates, such as nitroglycerin, as the latter may be associated with the development of tolerance, protein nitrosation and oxidative stress (Warnholtz *et al.* 2002).

The present study indeed showed, in our porcine model, a marked acute HPV response to hypoxia at $\text{F}_i\text{O}_2 \sim 0.10$. This was illustrated by the marked increase in MPAP and PVR. The concomitant increase in MRAP with hypoxia may thus reflect an acute increase in load on the right heart owing to pulmonary vasoconstriction. Our hypoxia control protocol (protocol 1), where no treatment was given, furthermore showed that the HPV response remained stable during sustained hypoxia for 82.5 min, because MPAP and PVR remained at a stable elevated level for this period of time (Fig. 1).

Stimulation of sGC with BAY 41-8543 totally reverses HPV

Stimulation of sGC, by right atrial infusion of BAY 41-8543, was in the present study found to potently and dose-dependently attenuate, and in fact totally reverse, the pulmonary vasoconstrictor response to acute hypoxia, by reducing MPAP and PVR to values even slightly below those in normoxia. MRAP was also decreased to normoxic levels and if a similar lowering of MRAP occurred in sustained chronic hypoxia, right heart failure could potentially be prohibited. PCWP remained unaltered, suggesting that the increase in PCWP upon the induction of hypoxia was

not a direct result of a ventricular interdependence owing to the PH-induced increased load on the right side of the heart. Whether sGC stimulation additionally could be a means of prohibiting hypoxia-induced pulmonary oedema remains, however, unresolved.

Our *in vivo* findings add important information to previous *in vitro* studies, where the sGC activator HMR1766 has been found to dose-dependently inhibit the pressor response to acute hypoxia in the isolated perfused mouse lung (Weissmann *et al.* 2009). HMR1766 has additionally been found to partially reverse the haemodynamic changes and structural remodelling of the lung vasculature and right ventricle occurring in chronic hypoxia (Weissmann *et al.* 2009). Both BAY 41-2272, which stimulates sGC directly and enhances the sensitivity of sGC to NO, and BAY 58-2667, which activates sGC even in its oxidized or haeme-free form, independently of NO, have been found to reverse the haemodynamic and structural changes associated with chronic hypoxia, as well as monocrotaline-induced PH in rats (Dumitrascu *et al.* 2006). sGC α 1 seems furthermore essential for the NO-mediated pulmonary, but not systemic, vasodilatation and limits chronic hypoxia-induced vascular remodelling by inhibiting smooth muscle cell proliferation (Vermeersch *et al.* 2007). sGC α 1 has, however, not been found to regulate baseline pulmonary vascular tone or the acute response to hypoxia (Vermeersch *et al.* 2007). Even though sGC α 1 did not seem to participate in mediating HPV (Vermeersch *et al.* 2007), we show that treatment with sGC stimulation may totally reverse HPV. Thus, early sGC stimulator treatment for patients with hypoxic PH may both reverse HPV and positively affect pulmonary vascular remodelling, as has been shown in rats (Dumitrascu *et al.* 2006). However, the extent of effect in chronic hypoxia, compared with in acute hypoxia cannot be determined from the present study.

Moreover, therapies that act in synergy with endogenous NO, such as NO- and haeme-independent sGC activators and NO-independent haeme-dependent sGC stimulators, may be of future importance in PH to optimize ventilation/perfusion matching and modulate pulmonary vascular tone (Mittendorf *et al.* 2009). Stimulators and activators of sGC may potentially also be used as a complement to for instance PDE5 inhibitors and ET-receptor blockers, in the treatment for PAH (Evgenov *et al.* 2006). Additionally, it is of great interest that sGC stimulators may be administered via inhalation to directly act in the target organ, as described by Evgenov *et al.* (2006). With such a route of administration, the systemic effects of sGC stimulation could potentially be minimized and, furthermore, with respect to hypoxia-induced PH, the drug would primarily act in well-ventilated areas of

the lung. On the other hand, inhalation often requires administration 4–8 times a day, which may be cumbersome for the patients.

Stimulation of sGC with BAY 41-8543 potentiates the effects of dual ET-receptor blockade in attenuating HPV

Endothelin-1 is a potent vasoconstrictor, synthesized by the endothelium, mediating its effects via ET_A- and/or ET_B-receptors on vascular smooth muscle- and endothelial cells (Sato *et al.* 1995, McCulloch *et al.* 1998). Hypoxia *per se* has been found to increase the amount of ET-1 mRNA in the lung and right atrium (Kourembanas *et al.* 1991, Elton *et al.* 1992), the ET-1 immunoreactivity in plasma, as well as stimulate preproendothelin-1 promoter activity (Aversa *et al.* 1997) and up-regulate the amount of ET-receptors (Soma *et al.* 1999). Although it is widely recognized that ET-1 plays an important role in the effects of chronic hypoxia, its role in acute HPV has been controversial. Studies on ET-receptor blockade, both *in vivo* and *in vitro*, in different species, have shown both attenuation (Bonvallet *et al.* 1994, Chen *et al.* 1995, 1997, DiCarlo *et al.* 1995, Oparil *et al.* 1995, Holm *et al.* 1996, 1998, Franco-Cereceda & Holm 1998, Sato *et al.* 2000, Goirand *et al.* 2003, Modesti *et al.* 2006, Petersen *et al.* 2008) and no attenuation (Douglas *et al.* 1993, Takeoka *et al.* 1995, Lazor *et al.* 1996, Hubloue *et al.* 2003, Biarent *et al.* 2006) of the HPV response.

We recently showed, in our porcine model, that dual ET-receptor blockade with tezosentan markedly attenuates the acute hypoxia-induced increase in pulmonary arterial pressure and completely attenuates the PVR increase to hypoxia (Hedelin *et al.* 2011). As dual ET-receptor blockade, for instance in the setting of PAH, is not always sufficient to fully normalize the increased pulmonary vascular tone, it was important in the present study to evaluate whether combination treatments, for instance with sGC stimulation and dual ET-receptor blockade, could be additionally effective. In the present study, we confirmed that dual ET-receptor blockade with tezosentan, during acute hypoxia, effectively attenuates the hypoxia-induced increase in MPAP, PVR and MRAP, by decreasing MPAP to values slightly higher than in normoxia and by decreasing PVR and MRAP to normoxic levels, whereas PCWP was unaltered. sGC stimulation on top of the dual ET-receptor blockade further decreased MPAP to 'low' normoxic values and PVR to values slightly below those in normoxia, whereas MRAP and PCWP remained unaltered. Thus, such a combination treatment seems feasible and effective in reversing acute hypoxia-induced PH. Furthermore, such a strategy may also be of importance in the

treatment for PH related to other causes, such as PAH, because both ET-receptor blockers and sGC stimulation may, besides their vasodilating effects, also attenuate pulmonary vascular remodelling.

Systemic responses to hypoxia and stimulation of sGC with BAY 41-8543 alone and on top of dual ET-receptor blockade

The present study showed that sGC stimulation, as well as dual ET-receptor blockade also exert potent systemic effects, however, without causing a circulatory 'collapse'. Hypoxia decreased SVR, increased CO and SV, but left MAP and HR unaltered. In protocol 2, sGC stimulation during hypoxia decreased MAP and further decreased SVR to levels below those in normoxia, whereas HR, CO, SV and blood-O₂ consumption remained unaltered. In protocol 3, dual ET-receptor blockade also decreased MAP and SVR to values below the normoxic levels, whereas HR, CO, SV and blood-O₂ consumption were unaltered. A major part of the drop in SVR and MAP induced by tezosentan seems, however, to be species-related, as the same pronounced effect has not been observed with tezosentan in rats (Tabrizchi & Ford 2003) and with bosentan in humans (Clozel *et al.* 1994). Subsequent sGC stimulation, on top of dual ET-receptor blockade, further decreased MAP and SVR. This confirms a potent systemic effect of sGC stimulation. A pronounced drop in MAP, as observed with BAY 41-8543 in the present study, may be harmful in critically ill patients and furthermore induce the release of vasoconstrictor substances. Therefore, if wanting to treat PH with BAY 41-8543, doses should be carefully titrated and systemic blood pressure be monitored. However, the systemic blood pressure decreases observed with BAY 41-8543 indicate that sGC stimulation could have potential implications in the treatment for systemic hypertension and hypertensive crisis.

Conclusion

The present study uniquely shows, in a porcine model, that sGC stimulation alone, during acute hypoxia, dose-dependently attenuates the hypoxia-induced pulmonary vasoconstrictor response by decreasing MPAP, PVR and MRAP to normoxic values. Furthermore, sGC stimulation on top of dual ET-receptor blockade during hypoxia decreases MPAP and MRAP to 'low' normoxic values and PVR to values slightly lower than in normoxia. sGC stimulation alone, or in combination with tezosentan, accomplished its attenuation of the HPV response simultaneously with a marked dose-dependent systemic vasodilator response, without

affecting blood arterial-O₂ saturation and blood-O₂ consumption. These findings suggest that sGC stimulation alone, or in combination with dual ET-receptor blockade, may provide a valuable means to attenuate the vasoconstrictor response to acute hypoxia, which, in turn, may ease the tension and excessive load on the right heart.

Conflict of interest

The authors have declared no conflict of interest.

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Paper II



Hemodynamic Characteristics Including Pulmonary Hypertension at Rest and During Exercise Before and After Heart Transplantation

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Background—Little is known about the hemodynamic response to exercise in heart failure patients at various ages before and after heart transplantation (HT). This information is important because postoperative hemodynamics may be a predictor of survival. To investigate the hemodynamic response to HT and exercise, we grouped our patients based on preoperative age and examined their hemodynamics at rest and during exercise before and after HT.

Methods and Results—Ninety-four patients were evaluated at rest prior to HT with right heart catheterization at our laboratory. Of these patients, 32 were evaluated during slight supine exercise before and 1 year after HT. Postoperative evaluations were performed at rest 1 week after HT and at rest and during exercise at 4 weeks, 3 months, 6 months, and 1 year after HT. The exercise patients were divided into 2 groups based on preoperative age of ≤ 50 or > 50 years. There were no age-dependent differences in the preoperative hemodynamic exercise responses. Hemodynamics markedly improved at rest and during exercise at 1 and 4 weeks, respectively, after HT; however, pulmonary and, in particular, ventricular filling pressures remained high during exercise at 1 year after HT, resulting in normalized pulmonary vascular resistance response but deranged total pulmonary vascular resistance response.

Conclusions—Our findings suggest that, (1) in patients with heart failure age ≤ 50 or > 50 years may not affect the hemodynamic response to exercise to the same extent as in healthy persons, and (2) total pulmonary vascular resistance may be more adequate than pulmonary vascular resistance for evaluating the exercise response after HT. (*J Am Heart Assoc.* 2015;4:e001787 doi: 10.1161/JAHA.115.001787)

Key Words: catheterization • exercise • heart failure • heart transplantation

Physical activity imposes prominent stress on the cardiovascular system because adequate delivery of oxygen and substrates is a necessity to sustain muscular activity.¹ The provision of sufficient oxygen delivery requires both ventilatory and cardiovascular adaptations, including increases in ventilation, heart rate, and cardiac output (CO), in parallel with increased vascular conductance in active muscles and increased vascular resistance in inactive tissue.² In severe heart failure (HF), both cardiac output and endothelial function are impaired.³ Consequently, in HF, both

central and peripheral circulation may compromise sufficient oxygen delivery to active muscles, limiting exercise capacity and daily life activities.

Knowledge of systemic and pulmonary hemodynamics is important for the treatment of HF. The normal hemodynamic magnitudes have previously been clearly defined at rest^{4,5}; however, there is a lack of understanding of the normal hemodynamic response to exercise, especially with regard to pulmonary circulation but also related to sex, body position, and different exercise intensities.^{6–8} This is further complicated by that the hemodynamic alterations due to age seem to be more prominent during exercise than at rest.^{7,8} Even though several invasive studies have investigated hemodynamics during exercise,^{9–13} the normal response has not been clearly defined. This lack of consensus resulted in abandoning of the “exercise criteria” in the definition of pulmonary hypertension at the World Symposium on Pulmonary Hypertension in 2008.⁴

Moreover, the mechanisms behind exaggerated exercise-induced increases in pulmonary artery pressures are poorly understood. For these reasons, it remains of value to further characterize hemodynamics during exercise before and after HT. Such characteristics could assist in defining an abnormal

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response to exercise and potentially aid in a new definition of exercise-induced pulmonary hypertension. This is important because pulmonary hypertension during exercise might be a better prognostic marker for disease severity than measurements at rest.^{14–19} Such a definition is also relevant because there is conflicting evidence regarding the preoperative magnitudes of different hemodynamic parameters at rest that may affect outcome after HT. This aspect illustrates the complexity in differentiating between patients with excessive vasoconstriction and those with vascular remodeling²⁰ and highlights the need for refined criteria for defining pulmonary hypertension. Improved insight into exercise hemodynamics may also provide guidance for medical treatment, for listing for HT, and for detection of pulmonary vascular abnormalities earlier in HF and after HT.

The aim of the present study was therefore to characterize the hemodynamics of patients with severe end-stage HF at rest and during exercise in relation to age before and after HT and to relate that information to data from healthy persons in previously published systematic reviews.^{7,8} Our purpose was to clarify how patients with severe HF respond to slight exercise and how hemodynamics recover at rest and during exercise after HT, as well as to highlight potential abnormalities in the post-transplant response to exercise. We hypothesized that hemodynamics at rest and during exercise improve early after HT but with significant abnormalities remaining during exercise at 1 year after HT. We also hypothesized that any age-dependent differences in the hemodynamic response to exercise would be attenuated in patients with severe HF.

Methods

Study Design and Population

This retrospective single-center study reviewed the 215 HT patients followed at Skåne University Hospital in Lund, Sweden, during 1988–2010. The study was performed with informed consent and approval by the ethics board in Lund (DNR 2014/92, Dnr 2011/777, Dnr 2011/368, Dnr 2010/114) and in accordance with the Declarations of Helsinki and Istanbul. A total of 219 HTs were included, of which 214 (98%) were first-time HTs, and 72 patients (33%) received a mechanical assist prior to HT.

The study focused on adult patients evaluated at our hemodynamic laboratory prior to HT. Patients referred from and investigated at other university hospitals (n=87), children aged <18 years (n=21), repeated HTs (n=5), and other patients with incomplete hemodynamic data prior to HT (n=12) were excluded. After exclusion, 94 adults (study population) remained for analysis, of which 24 were women (25.6%). The mean age was 51.6 years, and age ranged from 19 to

69 years. The most common indications for HT were dilated (52 patients) and ischemic (24 patients) cardiomyopathy.²¹

Data Collection and Analysis

Right heart catheterization (RHC) was performed at rest prior to HT and at 1 and 4 weeks, 3 and 6 months, and 1 year after HT. In all patients who were capable of exercise, RHC measurements were also performed during supine bicycle exercise prior to HT and at 4 weeks, 3 and 6 months, and 1 year after HT. Men exercised at 50 W, and women exercised at 30 W. Measurements were conducted at steady state \approx 5 minutes after initiation of exercise. There was no graded increase in workload.

Thirty-two of the 94 patients exercised prior to and 1 year after HT. These patients were subsequently characterized in 2 groups, 1 with patients aged \leq 50 years (n=12) and 1 with patients aged >50 years (n=20) at the time of HT (Table 1). This subclassification was performed to compare our findings with those presented for healthy persons published in recent systematic reviews.^{7,8} Overall, 21 of the 94 included HT patients were bridged to transplantation using a mechanical assist. Of these patients, 4 exercised prior to and 1 year after HT.

Right Heart Catheterization

RHC was predominantly performed via the right internal jugular vein, using a Swan Ganz catheter (Baxter Health Care Corp). If patients had >1 RHC prior to HT, the one closest to HT or assist implantation was analyzed. Mean pulmonary artery pressure (MPAP), pulmonary artery wedge pressure (PAWP), mean right atrial pressure (MRAP), and mean arterial pressure were recorded during RHC. Heart rate was recorded from ECG. CO was measured by thermodilution. Cardiac index, stroke volume (SV), SV index, left ventricular stroke work index (LVSWI), right ventricular stroke work index (RVSWI), transpulmonary gradient (TPG), pulmonary vascular resistance (PVR), PVR index, and total PVR (TPVR) were calculated using the following formulas: cardiac index=CO/body surface area; SV=CO/heart rate; SV index=SV/body surface area; LVSWI=(mean arterial pressure–PAWP) \times SV index; RVSWI=(MPAP–MRAP) \times SV index; TPG=MPAP–PAWP; PVR=TPG/CO; PVR index=TPG/cardiac index; and TPVR=MPAP/CO.

Statistics

A SigmaStat system (SigmaPlot 11.0) was used for statistical analysis. Parametric or nonparametric statistics were used depending on the distribution of data. One-way repeated-measures ANOVA (Tukey test) or One-way repeated-measures ANOVA on ranks (Tukey test) were used when multiple groups

Table 1. Characteristics of the Patients Who Exercised Prior to and 1 Year After HT, Grouped According to Age at the Time of HT

	Patients Aged ≤50 Years at the Time of HT			Patients Aged >50 Years at the Time of HT		
	Mean±SD	n	%	Mean±SD	n	%
Recipient characteristics						
Sex of recipient		12			20	
Female		6	50.0		4	20.0
Age of recipient, y	42.8±7.3	12		56.6±3.7	20	
≤10		0	0		0	0
11 to 20		0	0		0	0
21 to 30		1	8.3		0	0
31 to 40		2	16.7		0	0
41 to 50		9	75.0		0	0
51 to 60		0	0		16	80.0
≥61		0	0		4	20.0
Recipient length, cm	171.0±8.6	12		175.8±8.2	20	
Recipient weight, kg	78.1±20.3	12		81.0±15.6	20	
Recipient blood group		12			20	
O		3	25.0		7	35.0
A		8	66.7		9	45.0
B		0	0		4	20
AB		1	8.3		0	0
Time on waiting list, days	121.5±146.6	12		138.9±130.1	20	
Indication for HT		12			20	
DCM		8	66.7		12	60.0
HCM		0	0		1	5
Myocarditis		2	16.7		1	5
IHD		0	0		6	30.0
RCM		2	16.7		0	0
Donor characteristics						
Sex of donor		12			20	
Female		5	41.7		9	45.0
Age of donor, y	35.5±13.9	12		45.2±15.4	20	
Donor length, cm	175.7±9.1	12		176.3±10.3	18	
Donor weight, kg	76.2±18.8	12		82.5±13.4	20	
Ischemic time, min	163.3±63.5	12		182.7±56.5	20	
Recipient–donor matching						
Age difference, y	13.7±9.4	12		13.3±12.6	20	
Sex matching		12			20	
Sex matched		9	75.0		15	75.0
ABO matching		12			20	
Identical		9	75.0		19	95.0
Compatible		3	25.0		1	5.0
Incompatible		0	0		0	0

DCM indicates dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; HT, heart transplantation; IHD, ischemic heart disease; RCM, restrictive cardiomyopathy.

were compared. The Student *t* test and Mann-Whitney rank-sum test were used, respectively, when 2 groups were compared. Paired Student *t* test and Wilcoxon signed rank test were used, respectively, when comparing 1 group before and after an intervention. Pearson and Spearman correlations were used, respectively, when measuring the association between 2 variables. A *P* value <0.05 was considered statistically significant. All values are mean±SD.

Results

Hemodynamics at Rest and During Exercise Prior to Heart Transplantation

Baseline hemodynamics for all 94 included patients and for the subgroup of patients who exercised (n=32) are shown in Table 2.

During the preoperative assessment of the 32 patients who exercised during RHC, exercise increased MPAP (*P*<0.001), PAWP (*P*<0.001), TPG (*P*<0.001), MRAP (*P*<0.001), CO (*P*<0.001), and SV (*P*<0.01) compared with performance at rest, whereas PVR and TPVR remained unchanged (*P* value not

significant) (Table 2). There was no correlation between change in PAWP and MRAP (*R*²=0.02, *P* value not significant) (Figure 1).

Exercise in patients aged ≤50 years increased MPAP by 15.7±10.0 mm Hg (58.1%, *P*<0.001), PAWP by 10.3±8.2 mm Hg (53.8%, *P*<0.001), TPG by 5.4±5.9 mm Hg (68.6%, *P*<0.001), MRAP by 10.2±4.0 mm Hg (125.5%, *P*<0.001), CO by 2.2±0.7 L×min⁻¹ (54.2%, *P*<0.001), and SV by 6.3±7.8 mL×beat⁻¹ (11.3%, *P*<0.04), whereas PVR and TPVR remained unaltered (*P* value not significant) (Figures 2 and 3).

Similarly, in patients aged >50 years, exercise increased MPAP by 18.8±11.5 mm Hg (69.4%, *P*<0.001), PAWP by 13.5±11.3 mm Hg (77.2%, *P*<0.001), TPG by 5.4±4.2 mm Hg (55.3%, *P*<0.001), MRAP by 10.8±3.8 mm Hg (259.0%, *P*<0.001), and CO by 2.0±1.7 L×min⁻¹ (48.1%, *P*<0.001), whereas SV, PVR, and TPVR remained unaltered (*P* value not significant) (Figures 2 and 3).

With regard to pre-HT age-dependent differences, none of these parameters differed for patients aged ≤50 or >50 years (*P* value not significant) at rest and during exercise. The percentage change did neither differ between the groups (*P* value not significant) (Figures 2 and 3).

Table 2. Preoperative Haemodynamic Characteristics of All 94 Patients and the 32 Patients Who Exercised Prior to, and 1 Year After, HT

Parameter	Entire Study Population (at Rest)	Patients Who Exercised (at Rest)	Patients Who Exercised (During Exercise)	Patients ≤50 Years (at Rest)	Patients >50 Years (at Rest)	Significance
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
Patients, n	94	32	32	12	20	
MAP, mm Hg	74.1±13.6	75.4±15.8	87.9±19.7*	73.5±10.9	77.1±18.1	*
MPAP, mm Hg	30.1±8.8	27.2±9.8	44.4±10.5*	29.1±9.7	27.9±10.1	*
PAWP, mm Hg	20.5±8.2	18.3±8.8	30.5±9.5*	18.2±9.3	18.4±8.7	*
TPG, mm Hg	9.6±3.5	8.9±3.1	14.0±5.3*	7.9±2.3	9.5±3.3	*
MRAP, mm Hg	7.9±6.1	6.1±5.0	16.3±7.4*	7.6±5.5	5.3±4.7 [†]	*, [†]
HR, beat × min ⁻¹	82.6±16.8	82.2±15.0	111.9±19.6*	77.5±16.1	84.8±14.1	*
CO, L × min ⁻¹	3.7±1.0	4.3±0.9 [†]	6.4±1.7*	4.3±1.1	4.3±0.9 [†]	*, [†]
SV, mL × beat ⁻¹	46.7±17.1	53.1±15.3 [†]	60.2±21.6*	57.4±19.5	50.7±12.4	*, [†]
PVR (WU)	2.8±1.3	2.2±0.7 [†]	2.2±0.8	2.0±0.6 [†]	2.3±0.8	[†]
TPVR (WU)	9.1±4.3	6.7±2.8 [†]	7.4±2.6	6.5±3.1 [†]	6.8±2.7 [†]	[†]
LVSWI, mm Hg × mL × m ⁻²	1395.0±730.1	1615.9±717.8	1894.7±1174.1	1680.5±689.9	1580.4±747.8	
RVSWI, mm Hg × mL × m ⁻²	546.2±271.1	594.6±333.2	814.8±404.4*	563.2±333.8	611.9±340.2	*

CO indicates cardiac output; HR, heart rate; HT, heart transplantation; LVSWI, left ventricular stroke work index; mean atrial pressure; MPAP, mean pulmonary artery pressure; MRAP, mean atrial pressure; PAWP, pulmonary artery wedge pressure; PVR, pulmonary vascular resistance; RVSWI, right ventricular stroke work index; SV, stroke volume; TPG, transpulmonary gradient; TPVR, total pulmonary vascular resistance; WU, Wood units.

*Indicates significance (*P*<0.05) between rest and exercise in patients who exercised.

[†]Indicates a significance (*P*<0.05) compared to the entire study population.

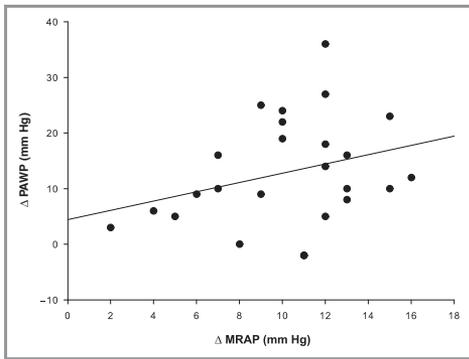


Figure 1. Correlation between preoperative changes to exercise in PAWP and MRAP. MRAP indicates mean right atrial pressure; PAWP, pulmonary artery wedge pressure.

Hemodynamic Improvement After Heart Transplantation

The hemodynamic response and improvement in relation to HT are shown in Figures 4 and 5 for all 94 patients at rest and for 32 patients during slight supine exercise. For the 94 patients at rest 1 week after HT, there was a decrease in MPAP ($P<0.001$), PAWP ($P<0.001$), PVR ($P<0.001$), and TPVR ($P<0.001$) along with an increase in mean arterial pressure ($P<0.001$), CO ($P<0.001$), and SV ($P<0.001$) compared with performance at rest prior to HT (Figures 4 and 5). TPG and MRAP were unaltered (P value not significant) (Figures 4 and 5).

Of these parameters, PVR remained constant (P value not significant) throughout the first year after HT, whereas MPAP further decreased ($P<0.009$) and mean arterial pressure further increased ($P<0.02$) at 4 weeks versus 1 week after HT and remained constant (P value not significant) thereafter. MRAP also decreased ($P<0.001$) at 4 weeks as compared to both prior to HT and one week after HT, and then temporarily decreased ($P<0.04$) at 6 months compared with 4 weeks after HT. SV further increased ($P<0.01$) at 3 months compared with 1 week after HT and remained constant (P value not significant) thereafter. Finally, PAWP and TPVR further decreased ($P<0.001$ and $P<0.02$, respectively) and CO further increased ($P<0.03$) at 6 months compared with 1 week after HT and remained constant (P value not significant) thereafter.

Compared with performance during exercise prior to HT, at 4 weeks after HT, there was a decrease in MPAP ($P<0.001$), PAWP ($P<0.001$), PVR ($P<0.003$), and TPVR ($P<0.001$) along with an increase in mean arterial pressure ($P<0.001$), CO ($P<0.001$), and SV ($P<0.001$). TPG and MRAP were unaltered (P value not significant) (Figures 4 and 5). All of these parameters remained stable (P value not significant) through-

out the first year after HT except for CO, which further increased ($P<0.004$), and for MRAP and PVR, which decreased ($P<0.005$ and $P<0.03$, respectively), at 1 year after HT (Figures 4 and 5).

Hemodynamic Response to Exercise at 1 Year After Heart Transplantation

For the 32 patients who performed RHC at rest and during exercise 1 year after HT, exercise increased ($P<0.001$ for all parameters) MPAP by 17.8 ± 6.1 mm Hg (114.6%), PAWP by 14.1 ± 5.5 mm Hg (193.1%), TPG by 3.7 ± 3.1 mm Hg (44.4%), MRAP by 9.8 ± 4.3 mm Hg (371.4%), CO by 5.4 ± 2.4 $L\times\text{min}^{-1}$ (88.1%), SV by 28.5 ± 15.6 $\text{mL}\times\text{beat}^{-1}$ (39.8%), and TPVR by 0.4 ± 0.7 Wood units (WU; 15.4%) compared with performance at rest, whereas PVR decreased by 0.3 ± 0.3 WU (22.1%, $P<0.001$) (Figures 2 and 3). Change in MPAP from rest to exercise correlated to change in PAWP ($R^2=0.75$, $P<0.001$) (Figure 6).

Exercise in patients aged ≤ 50 years increased MPAP by 14.8 ± 4.6 mm Hg (104.1%, $P<0.001$), PAWP by 12.1 ± 4.3 mm Hg (190.8%, $P<0.001$), TPG by 2.8 ± 3.3 mm Hg (34.7%, $P<0.016$), MRAP by 8.6 ± 5.3 mm Hg (412.0%, $P<0.001$), CO by 5.8 ± 3.4 $L\times\text{min}^{-1}$ (88.7%, $P<0.001$), SV by 30.9 ± 19.3 $\text{mL}\times\text{beat}^{-1}$ (41.7%, $P<0.001$), and TPVR by 0.2 ± 0.4 WU (11.1%, $P<0.05$), whereas PVR decreased by 0.3 ± 0.3 WU (26.7%, $P<0.004$) (Figures 2 and 3).

Similarly, exercise in patients aged >50 years increased MPAP by 19.6 ± 6.4 mm Hg (118.5%, $P<0.001$), PAWP by 15.3 ± 6.1 mm Hg (194.3%, $P<0.001$), TPG by 4.3 ± 2.8 mm Hg (49.7%, $P<0.001$), MRAP by 10.5 ± 4.0 mm Hg (354.2%, $P<0.001$), CO by 5.2 ± 1.6 $L\times\text{min}^{-1}$ (87.7%, $P<0.001$), SV by 27.1 ± 16.0 $\text{mL}\times\text{beat}^{-1}$ (38.6%, $P<0.001$), and TPVR by 0.5 ± 0.8 WU (17.5%, $P<0.008$), whereas PVR decreased by 0.3 ± 0.3 WU (19.7%, $P<0.001$) (Figures 2 and 3).

Resting TPVR was generally higher in patients aged >50 years compared with patients aged ≤ 50 years ($P<0.03$). In contrast, during exercise, not only TPVR but also MPAP and PVR were higher ($P<0.05$) in patients aged >50 years compared with patients aged ≤ 50 years; however, the percentage change in these parameters did not differ between groups (Figures 2 and 3).

Discussion

The present study evaluated the hemodynamics at rest and during slight supine exercise in patients with severe HF before and after HT. Our results showed that already at 1 week after HT, hemodynamics markedly improved at rest and remained constant or further improved during the first year of follow-up

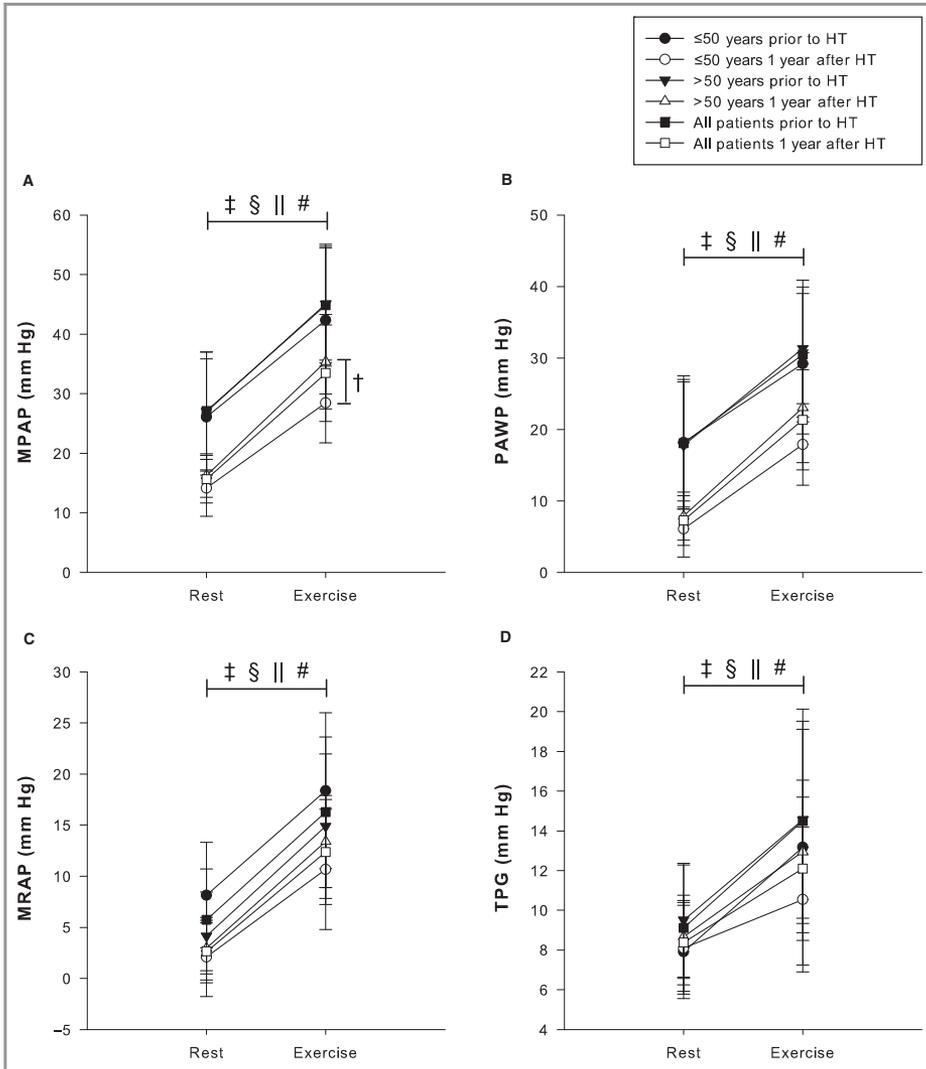


Figure 2. Hemodynamic response to exercise with regard to pulmonary and intracardiac pressures prior to and 1 year after HT. ● Patients aged ≤50 years prior to HT. ○ Patients aged ≤50 years 1 year after HT. ▼ Patients aged >50 years prior to HT; △ Patients aged >50 years 1 year after HT. ■ All 32 patients prior to HT. □ All 32 patients 1 year after HT. A, MPAP. B, PAWP. C, MRAP. D, TPG. †A statistically significant difference for MPAP during exercise between patients aged ≤50 and >50 years. ‡A statistically significant difference for MPAP, PAWP, MRAP, and TPG between rest and exercise prior to HT for patients aged ≤50 years. §A statistically significant difference for MPAP, PAWP, MRAP, and TPG between rest and exercise prior to HT for patients aged >50 years. ||A statistically significant difference for MPAP, PAWP, MRAP, and TPG between rest and exercise at 1 year after HT for patients aged ≤50 years. #A statistically significant difference for MPAP, PAWP, MRAP, and TPG between rest and exercise at 1 year after HT for patients aged >50 years. HT indicates heart transplantation; MPAP, mean pulmonary artery pressure; MRAP, mean right atrial pressure; PAWP, pulmonary artery wedge pressure; TPG, transpulmonary gradient.

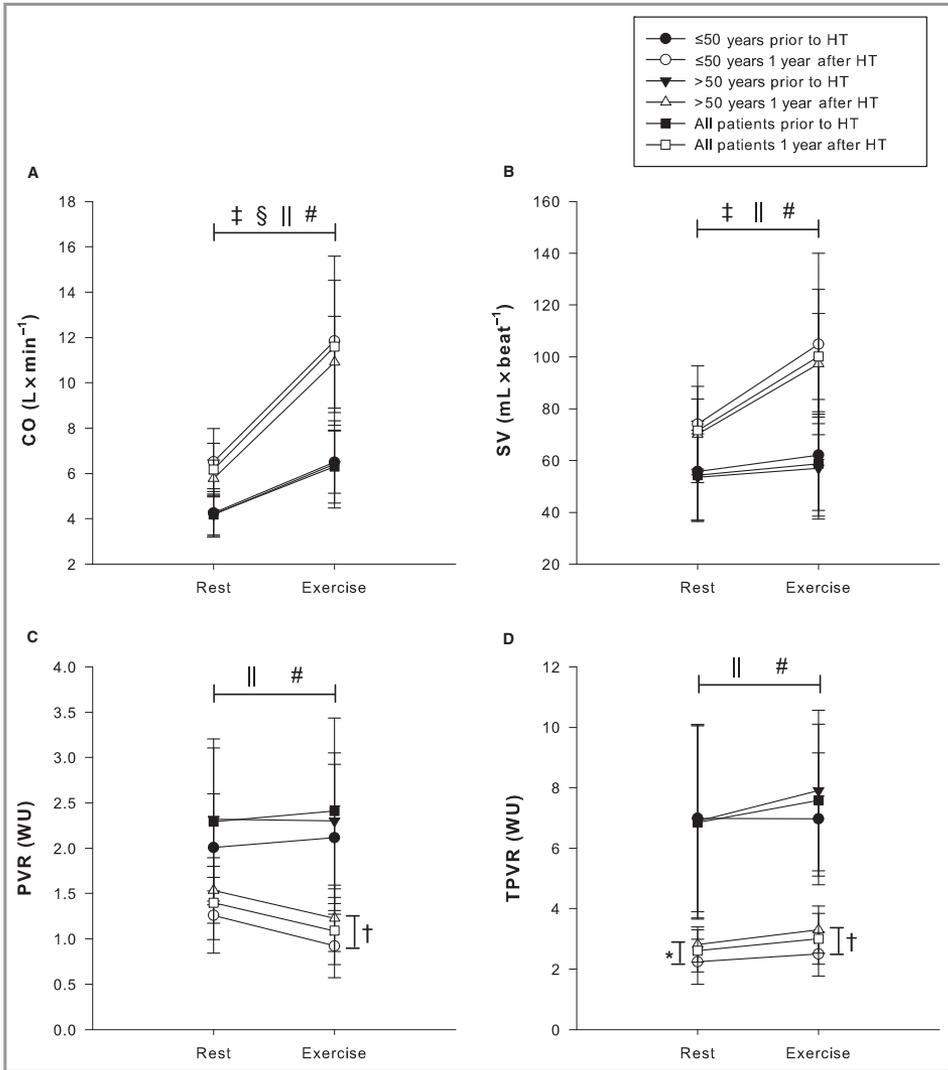


Figure 3. Hemodynamic response to exercise with regard to cardiac output, stroke volume, and pulmonary vascular resistances prior to and 1 year after HT. ● Patients aged ≤50 years prior to HT. ○ Patients aged ≤50 years 1 year after HT. ▼ Patients aged >50 years prior to HT. △ Patients aged >50 years 1 year after HT. ■ All 32 patients prior to HT. □ All 32 patients 1 year after HT. A, CO. B, SV. C, PVR. D, TPVR. *A statistically significant difference for TPVR at rest between patients aged ≤50 and >50 years 1 year after HT. †A statistically significant difference for PVR and TPVR during exercise between patients aged ≤50 and >50 years. ‡A statistically significant difference for CO and SV between rest and exercise prior to HT for patients aged ≤50 years. §A statistically significant difference for CO between rest and exercise prior to HT for patients aged >50 years. ¶A statistically significant difference for CO, SV, PVR, and TPVR between rest and exercise at 1 year after HT for patients aged ≤50 years. #A statistically significant difference for CO, SV, PVR, and TPVR between rest and exercise at 1 year after HT for patients aged >50 years. CO indicates cardiac output; HT, heart transplantation; PVR, pulmonary vascular resistance; SV, stroke volume; TPVR, total pulmonary vascular resistance; WU, Wood units.

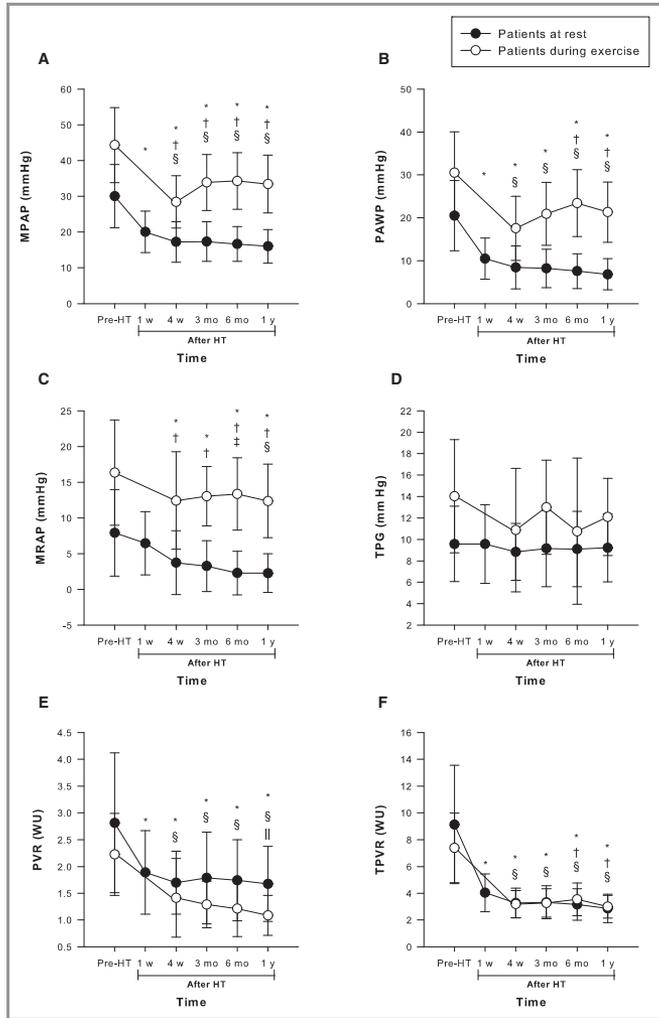


Figure 4. Hemodynamic characteristics with regard to pulmonary and intracardiac pressures in the 94 patients at rest prior to and after HT. ● Patients at rest. ○ Patients during exercise. Graphs show (A) MPAP, (B) PAWP, (C) MRAP (D) TPG, (E) PVR, and (F) TPVR at rest and during exercise before and after HT. *A statistically significant difference at rest compared with prior to HT. †A statistically significant difference at rest compared with 4 weeks after HT. ‡A statistically significant difference at rest compared with 4 weeks after HT. §A statistically significant difference during exercise compared with prior to HT. ‖A statistically significant difference during exercise compared with 4 weeks after HT. Of the 32 patients who exercised prior to HT, 16 exercised at 4 weeks, 22 at 3 months, 22 at 6 months, and 32 at 1 year after HT. HT indicates heart transplantation; MPAP, mean pulmonary artery pressure; MRAP, mean right atrial pressure; PAWP, pulmonary artery wedge pressure; PVR, pulmonary vascular resistance; TPG, transpulmonary gradient; TPVR, total pulmonary vascular resistance; WU, Wood units.

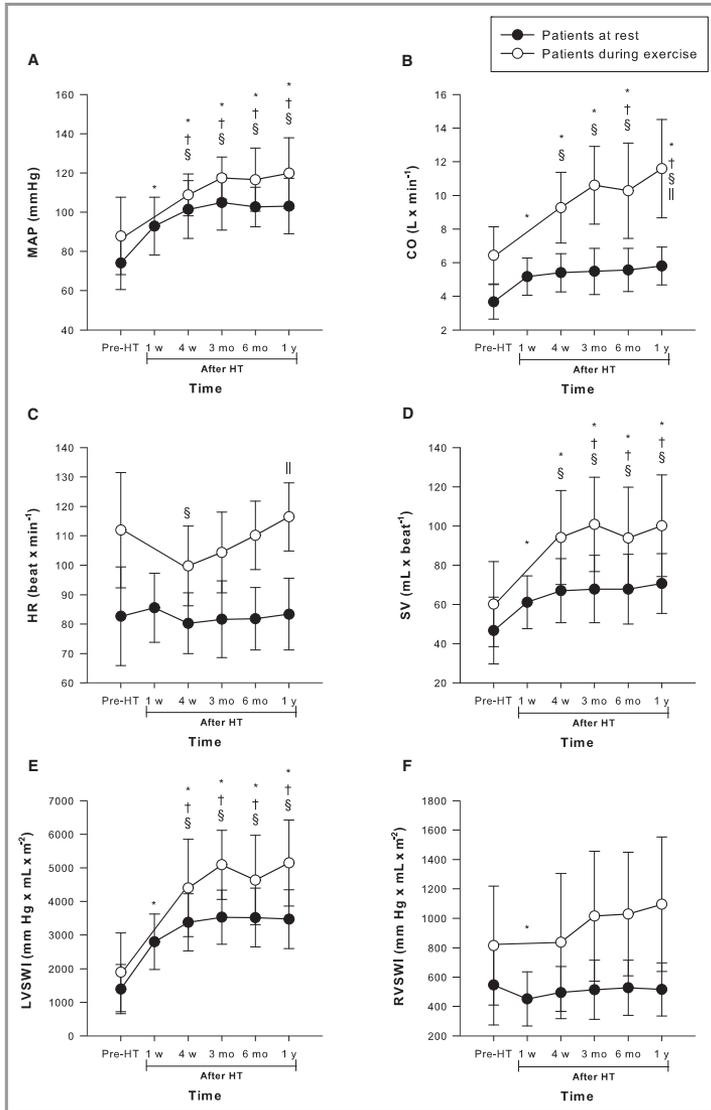


Figure 5. Hemodynamic characteristics with regard to heart rate, arterial pressure cardiac output, and stroke volume in the 94 patients at rest prior to and after HT. ● Patients at rest. ○ Patients during exercise. Graphs show (A) MAP, (B) CO, (C) HR, (D) SV, (E) LVSWI, and (F) RVSWI at rest and during exercise before and after HT. *A statistically significant difference compared with examination prior to HT. †A statistically significant difference compared with 1 week after HT. ‡A statistically significant difference during exercise compared to prior to HT. §A statistically significant difference during exercise compared with 4 weeks after HT. CO indicates cardiac output; HR, heart rate; HT, heart transplantation; LVSWI, left ventricular stroke work index; MAP, mean arterial pressure; RVSWI, right ventricular stroke work index; SV, stroke volume; WU, Wood units.

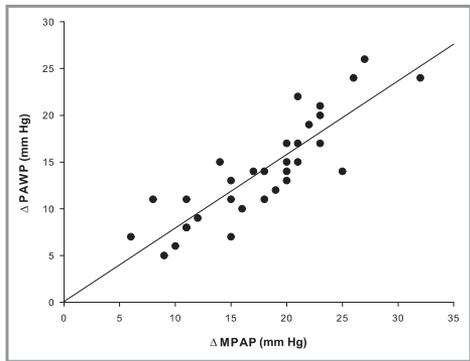


Figure 6. Correlation between changes to exercise in PAWP and MPAP at 1 year after HT. HT indicates heart transplantation; PAWP, pulmonary artery wedge pressure; MPAP, mean pulmonary artery pressure.

(Figures 4 and 5). The functional response to exercise was also substantially improved after HT, represented by an adequate increase in CO and SV (Figure 3); however, MPAP and particularly PAWP and MRAP remained high (Figures 2 and 5). The absolute response to exercise of these pressures was similar before and after HT, resulting in a higher relative response to exercise after HT (Figure 2). Moreover, the age-dependent differences in the hemodynamic response to exercise previously reported in healthy persons^{7,8} were not observed in our patients with severe end-stage HF prior to HT. Together, these findings suggest that in patients with severe HF, age may not be as dominant in affecting hemodynamic response to exercise as in healthy persons. The findings also indicate that despite normalized hemodynamics at rest, the response to even slight exercise after HT differed compared with the normal innervated heart.

Exercise Hemodynamics Prior to Heart Transplantation

The patients in our study who exercised prior to HT generally responded with a marked increase in PAWP and MRAP, suggesting enhanced biventricular failure on exertion (Table 2). Previous reports in healthy volunteers found a close relationship between exercise-induced increases in PAWP and MRAP.^{22,23} Such a correlation was not observed in our patients (Figure 1), suggesting that the increased load on the right ventricle is not solely due to increased left ventricular filling pressures and subsequent pulmonary venous hypertension or ventricular interdependence but also is due to an exaggerated increase in MPAP. Indeed, the TPG in our patients, which increased by 5.4 mm Hg, is in line with the findings by Lewis

and colleagues, who investigated 60 patients with stable New York Heart Association class II to IV during slight upright exercise.¹⁹ In contrast to the findings by Lewis et al¹⁹ and others^{24,25} investigating slight upright exercise in HF patients, in our study, PVR did not significantly decrease during slight supine exercise. This finding is also in contrast to the findings in healthy persons reported previously⁸ and further supports an exaggerated increase in MPAP compared with the increase in PAWP and CO (Table 2). The increase in MPAP could be due to several factors. One such factor that may explain a part of the increase is hypoxic pulmonary vasoconstriction²⁶ related to decreased mixed venous P_{O_2} . In support of this hypothesis, the mixed venous saturation decreased from 67% at rest to 26% during exercise in the 9 patients for whom these data were available (data not shown).

It is well known that during exercise in healthy persons, there is great interindividual variation in TPVR.⁶ Despite this knowledge, exercise-induced pulmonary hypertension was recently suggested to be defined as TPVR >3 WU.^{27,28} With a mean TPVR of 7.4 WU in our patients, the preoperative exercise TPVR was higher than in healthy persons^{27,28} but in line with previous results in patients with HF.¹⁹ This finding, together with the increase in TPG, suggests that although our study exclusively included patients referred for HT, the results with regard to hemodynamics are representative for patients with severe HF.

Hemodynamic Response to Heart Transplantation

Previous investigations of exercise after HT have used mainly noninvasive methods and focused primarily on exercise capacity and/or reinnervation of the transplanted heart^{29–38}; however, a few studies have evaluated hemodynamics obtained from RHC during exercise after HT.^{26,39–44} In 1980, Pope and colleagues presented a report on the hemodynamic and catecholamine response to exercise in 9 long-term HT survivors. They demonstrated that in denervated hearts, CO is increased by augmented preload and the Frank Starling mechanism early in exercise and later by the effects of increased levels of catecholamines.⁴⁰ This report⁴⁰ did not examine patients early after HT; however, this work was performed in a later study of 20 patients.²⁶ The authors then found that MPAP and PAWP decreased early after HT. In contrast to these reports, we illustrated the complete hemodynamic picture, at rest and during exercise prior to HT, in which the same patients were followed repeatedly during the first year after HT, enabling the study of hemodynamic changes over time.

In contrast to exercise hemodynamics after HT, several studies have evaluated hemodynamics at rest early and late after HT.^{41–43,45–56} In line with those reports, we showed that even at 1 week after HT, resting hemodynamics improved

dramatically and were maintained throughout the first year after HT. Pulmonary artery pressures as well as CO, SV, TPVR, and LVSWI continued to improve during the first year after HT (Figures 4 and 5). In contrast to what is expected in healthy persons based on previous systematic reviews,^{7,8} the pulmonary artery pressure, PAWP, and MRAP responses to exercise were exaggerated (Figures 2 and 4). The absolute increase was, in fact, similar to that seen prior to HT (Figure 2), resulting in a greater percentage increase after transplantation. Moreover, there was a correlation between the increase in MPAP and PAWP (Figure 6), suggesting that the MPAP response during exercise is caused, at least in part, by the elevated PAWP. This exaggerated increase in ventricular filling pressures has been described previously.^{42,44} The reason for similar pressure increases during exercise after versus before HT, despite lower resting values, needs further investigation. It could be hypothesized that, in our population, these findings were due to a prominent decrease in diastolic compliance in denervated hearts that reaches its maximum during slight to moderate exercise.⁵⁷ This is supported in that the denervated heart, due to lack of sympathetic stimulation, is more dependent on the Frank Starling mechanism than is the innervated heart.^{40,58,59} Factors such as lack of heart rate reserve and Bainbridge reflex, rejections, fluid retention, hypertension, myocardial ischemia, and diastolic dysfunction have also been suggested to account for post-transplant abnormalities.^{42–44,47,48} These studies, however, show diverging results. The ultimate cause remains unclear and possibly is multifactorial.⁴² In 1994, Kao and colleagues performed invasive hemodynamic measurements during upright graded exercise in 30 HT patients with normal left ventricular ejection fraction at 3 to 16 months after HT and compared their findings with healthy controls.⁴⁴ They showed that in their population, heart rate reserve and SV index were impaired after HT, suggesting that during maximal exercise, chronotropic insufficiency and diastolic dysfunction explained the increased filling pressures.⁴⁴ These findings, however, differ from ours. Based on what has been described previously in healthy persons,⁸ the patients in our study at 1 year after HT responded during slight supine exercise with adequate increase in heart rate and SV. In fact, the SV in our patients was in line with that of the healthy persons in the study by Kao et al during submaximal upright exercise.⁴⁴ Moreover, heart rate increased to 116.4 ± 11.6 beats \times min⁻¹, which is in the upper limit of what is expected during slight exercise in healthy persons⁸ and in line with other previous reports in HT patients.⁴⁰ The difference between our findings and those presented by Kao et al could potentially be explained by the difference in body positions in the studies; however, they may also, at least in part, be due to less healthy patients in the study by Kao et al, with low maximum oxygen consumption (12.3 ± 4.0 mL \times Kg⁻¹ \times min⁻¹) despite normal ejection

fraction.⁴⁴ Moreover, in contrast to all previous reports, in the present study, we evaluated the age-dependent differences in exercise response after HT and investigated the impact of the elevated filling pressures on pulmonary resistance.

Age-Dependent Exercise Response

It has been shown previously that the hemodynamic response to exercise differs for healthy persons aged ≤ 50 versus > 50 years.^{7,8} In the most recent of these reports, Kovacs and colleagues analyzed the hemodynamic response to exercise in 222 healthy persons from 24 different studies in a systematic review and stratified the participants according to age.⁸ The results confirmed earlier reports and showed that slight supine exercise results in an increase in CO by 85% in participants aged ≤ 50 years and is accompanied by an increase in MPAP by 41% and a decrease in PVR and TPVR by 12% and 25%, respectively. In contrast, in participants aged > 50 years, an increase in CO by 71% is accompanied by an increase in MPAP by 66% and a decrease in PVR by 19%, whereas TPVR remained virtually unchanged.⁸ These findings differ from those in our HF population both before and 1 year after HT. The MPAP response prior to HT was exaggerated in both groups when related to the corresponding increase in CO, whereas the PVR and TPVR responses were attenuated (Figures 2 and 3). After HT, the CO response to exercise was adequate, but the MPAP response was still increased when related to the increase in CO (Figures 2 and 3). Because the exercise increase in PAWP was even further exaggerated, PVR decreased in patients aged ≤ 50 and > 50 years, comparable to expectations for healthy persons.^{7,8} TPVR, which is not directly affected by PAWP, was still deranged at 1 year after HT and increased during exercise in patients aged ≤ 50 and > 50 years (Figures 2 and 3).

In contrast to previous findings in healthy persons,^{7,8} MPAP, PVR, and TPVR of our patients prior to HT did not differ significantly between patients aged ≤ 50 and > 50 years at rest (Figures 2 and 3). Furthermore, no difference was noted between patients aged ≤ 50 and > 50 years during exercise prior to HT (Figures 2 and 3). Considering the low number of patients in our trial, these findings have to be interpreted with caution. Indeed, MRAP tended to be higher in patients aged ≤ 50 years at rest prior to HT and could indicate that these patients had more pronounced biventricular heart failure, which may affect the results. PAWP, CO, and SV were however similar in both groups, and TPG and PVR tended to be even lower in the younger patients. Consequently, differences in disease severity between patients aged ≤ 50 and > 50 years are unlikely to play a major part in hemodynamic response. In contrast, at 1 year after transplantation, TPVR at rest and MPAP, PVR, and TPVR during exercise were higher among patients aged > 50 years (Figures 2 and 3).

Although the absolute and percentage responses did not differ significantly for any of the parameters, there was a tendency toward greater changes in pressures in the patients aged >50 years. Together, these observations suggest that in patients with end-stage HF, the age-dependent response to exercise may be attenuated due to the severity of the disease, and that parts of this response may be restored after HT. Because MPAP and particularly PAWP remained deranged at 1 year after HT, the findings also suggest that although the PVR response to exercise is normalized, PVR may not adequately reflect pulmonary circulation after HT. In this setting, TPVR may be a better marker because it reflects the increase in MPAP in relation to CO, independent of PAWP.

Limitations

Although a retrospective study such as the present has disadvantages compared with a prospective ditto, and that the long time span of our study could influence the results, the findings are of interest. This is supported by the fact that our study population was representative with regard to similar demographic parameters and survival compared with our entire HT population.²¹ However, the small sample size could mask age-dependent differences in the response to exercise; therefore, these results must be interpreted with caution. This is particularly true after HT, where patients aged >50 years seem to have a greater increase in pulmonary and ventricular filling pressures, whereas the response to exercise prior to HT seems to be similar between the groups, despite higher resting MRAP in the younger patients. When discussing the normal response to exercise, one must also keep in mind that we did not study healthy controls in the present report. Instead, we evaluated our results based on previously published systematic reviews of healthy persons. Although this approach may not be optimal, given the ethical issues of performing heart catheterizations on healthy persons, we find our approach to be a valid alternative. Furthermore, our study included only patients evaluated for HT; therefore, the findings prior to HT may not be applicable to all patients with severe HF. Nonetheless, considering that the TPG and TPVR responses to exercise in our patients were comparable to previous results in patients with HF,¹⁹ we believe that our observations are representative with regard to hemodynamics for patients with severe HF. Finally, previous studies have shown that rejections,^{43,44,48,60} ischemic time of the donor heart,^{43,44,48,60} and systemic hypertension^{44,60} do not account for the hemodynamic abnormalities seen after HT. It is possible, however, that other donor characteristics and donor–recipient mismatches as well as patients' medical therapies may influence the findings. This possibility was not investigated in the present study, apart from what is shown in Table 1, and further research is encouraged.

Conclusion

The present study illustrates the hemodynamic response to exercise in patients with severe end-stage HF prior to and after HT. Our findings reveal that resting hemodynamics recover within the first week after HT and are maintained or even improved throughout the first year after transplantation. Moreover, the age-dependent differences in response to exercise in healthy persons were not observed in our patients with severe end-stage HF prior to HT. This suggests that the hemodynamic response to exercise may be less age dependent in HF patients than in the normal population. Finally, although the hemodynamics at rest and during exercise were greatly improved after HT, and the functional response to exercise with regard to CO and SV is adequate at 1 year after HT, there is an exaggerated increase in MPAP, PAWP, and MRAP in response to exercise. This is evident for patients aged ≤50 and >50 years and, at least in part, is likely due to increased PAWP. As a result of the exaggerated increase in PAWP, the PVR response to exercise is normalized after HT, masking the increased MPAP. Consequently, TPVR, which is independent of PAWP, may be a better marker than PVR for hemodynamic evaluations after HT. Larger studies are encouraged to confirm these findings and to define the normal hemodynamic magnitudes during exercise prior to and after HT to explain the exaggerated increase in PAWP to exercise.

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Paper III



ORIGINAL ARTICLE

Preoperative pulmonary hypertension and its impact on survival after heart transplantation

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Abstract

Objectives. Pulmonary hypertension (PH) due to left heart disease may impair outcome after heart transplantation (HT). To evaluate to what extent previous, and present, haemodynamic criteria discriminate the impact of pre-operative-PH on survival, we characterized the PH in our HT-patients according to ESC's guidelines, ISHLT's summary statement and ISHLT's relative contraindications and criteria for early risk of death after HT. **Design.** Records from the 215 HT-patients in Lund during 1988–2010 were reviewed. Subsequent analysis included adults ($n = 94$) evaluated with right-heart-catheterization at our lab, at rest before HT. End of follow-up was 30th of June 2012. **Results.** Survival (mean, n) did not differ ($p = ns$) for the 94 HT-patients; without (13.0 years, $n = 28$) or with (13.9 years, $n = 66$) PH, passive (13.8 years, $n = 50$) or reactive (12.2 years, $n = 13$) post-capillary-PH, “modified” passive (13.1 years, $n = 40$), mixed (16.6 years, $n = 23$), “modified” reactive (12.6 years, $n = 7$) or non-reactive (12.2 years, $n = 8$) post-capillary-PH; or for ISHLT's relative contraindications (12.0 years, $n = 22$) or increased risk of right-heart-failure and early death (16.5 years, $n = 23$) after HT. **Conclusions.** As previous and present haemodynamic criteria did not sufficiently discriminate the impact of pre-operative-PH for survival after HT at our centre, larger multi-centre studies are encouraged to redefine criteria that may influence outcome.

Key words: heart failure, heart transplantation, haemodynamic, pulmonary hypertension, survival

Introduction

Pulmonary hypertension (PH) is defined by a mean pulmonary artery pressure (MPAP) ≥ 25 mmHg, and sub-categorized by the magnitude of pulmonary capillary wedge pressure (PCWP), cardiac output (CO) and trans-pulmonary gradient (TPG) (Table Ia) (1). Major efforts have focused on clarifying the cause of, and treatments for, pulmonary arterial hypertension (PAH), leading to improved survival in PAH, which from diagnosis, if untreated, was as low as ~ 1 – 2.8 years (2). In contrast, the precise pathophysiology behind PH due to left heart disease remains, however, unclear. Furthermore, there is no specific therapy for the pulmonary component of PH due to left heart disease, other than optimizing left heart function. This is important as left heart disease is the most common cause of PH, where PH may be a marker of disease severity (3)

and a predictor of early mortality after heart transplantation (HT) (4).

The threshold magnitude for haemodynamic parameters, including pulmonary vascular resistance (PVR) and TPG, that increase the risk of right heart failure and early mortality after HT, furthermore remains unclear. Several characterizations of PH severity and its impact on outcome have therefore been suggested (Table Ia, b) (1,3–13). Systolic pulmonary artery pressure (SPAP) > 60 mmHg and either $PVR > 5$ WU or $PVRI > 6$ WU·m⁻² or $TPG > 16$ – 20 mmHg have been proposed as risk criteria for developing right heart failure and early death after HT (Table Ia) (4). A $PVR > 5$ WU or $PVRI > 6$ WU·m⁻² or $TPG > 16$ – 20 mmHg have consequently been defined as relative contraindications for HT (4). In addition, irreversible $PVR > 2.5$ WU has been suggested to increase the risk for early

Table Ia. Definitions of PH in present ESC guidelines, ISHLT state of art summary statement and ISHLT's guidelines.

Definition/Author	Characterization
PH- definitions according to ESC-guidelines. Galie et al. (1)	<ol style="list-style-type: none"> 1. No PH: MPAP < 25 2. Pre-capillary PH: MPAP ≥ 25 and PCWP ≤ 15 and CO normal or reduced 3. Post-capillary PH: MPAP ≥ 25 and PCWP > 15 and CO normal or reduced <ol style="list-style-type: none"> 3a. Passive: TPG ≤ 12 3b. Reactive: TPG > 12
ISHLT's State of art summary statement. Fang et al. (3)	<ol style="list-style-type: none"> 1. No PH: MPAP < 25 2. Passive PH: MPAP ≥ 25 and PCWP > 15 and TPG ≤ 15 or PVR ≤ 3.0 3. Mixed PH: MPAP ≥ 25 and PCWP > 15 and TPG > 15 or PVR > 3.0 <ol style="list-style-type: none"> 3a. Reactive PH (with vasodilatation): TPG ≤ 15 or PVR ≤ 3.0 3b. Non-reactive PH (with vasodilatation): TPG > 15 or PVR > 3.0
ISHLT-guidelines for performing vasodilation during RHC. Mehra et al. (4)	<ol style="list-style-type: none"> 1. SPAP ≥ 50 and either TPG ≥ 15 or PVR > 3.0, while maintaining SAP > 85 with vasodilatation
ISHLT's defined relative contraindications for HT. Mehra et al. (4)	<ol style="list-style-type: none"> 1. PVR > 5 or PVRI > 6 or TPG > 16–20
ISHLT's defined increased risk of right heart failure and early death after HT. Mehra et al. (4).	<ol style="list-style-type: none"> 1. SPAP > 60 and either PVR > 5 or PVRI > 6 or TPG > 16–20 2. PVR > 2.5 with vasodilatation 3. PVR < 2.5 but SAP falls to < 85 with vasodilatation

Definition/Author, Definitions and the first author of the publication; Characterization, Haemodynamic characterization in the related publications; SAP, systolic artery pressure; SPAP, systolic pulmonary artery pressure; MPAP, mean pulmonary artery pressure; PCWP, pulmonary capillary wedge pressure; TPG, transpulmonary gradient; CO, cardiac output; PVR, pulmonary vascular resistance; PVRI, pulmonary vascular resistance index; RHC, right heart catheterization.

mortality after HT (4). On the other hand, reversible PH prior to HT has been found to be associated with an even worse outcome (14).

Recent studies, however, question the predictive value of PVR and to what extent and at which magnitude it influences mortality (15,16). TPG (6) and pulmonary vascular resistance index (PVRI) (8,11) have been suggested as better predictors of outcome than PVR. Which parameters that best predict outcome is further complicated by the different cut-off values that have been used to characterize the PH severity (Table Ib) (1,3–13). Moreover, as TPG may be sensitive to changes in CO and left atrial pressure (17), an increase in the diastolic pulmonary vascular pressure gradient (DPG), defined as the difference between diastolic pulmonary artery pressure (DPAP) and PCWP, has been proposed to better prognosticate pulmonary vascular disease and death in PH (17,18) if equal to, or higher than, 7 mmHg (18).

Our aim was, therefore, to evaluate the role and impact of preoperative PH for survival after HT, using previous haemodynamic criteria (5–13), ESC's guidelines (1), ISHLT's State of art summary statement (3), and ISHLT's relative contraindications, and criteria for increased risk of right heart failure and early death after HT (4) (Table Ia, b). We hypothesized that previous and present haemodynamic criteria do not sufficiently discriminate the impact of PH for survival after HT.

Materials and methods

Study design and population

This retrospective, single-centre, study reviewed the 215 HT patients followed at Skåne University

Hospital in Lund, Sweden, during 1988–2010. The study was performed with informed consent and approval by the ethics board in Lund (Dnr 2011/777, Dnr 2011/368, Dnr 2010/114). The investigation adheres with the principles outlined in the Declaration of Helsinki. A total of 219 HTs were included, out of which 214 (98%) were first-time HTs. Five (2%) were re-HTs; three within 7 days, one within 175 days and one 18 years after HT. Of the HTs, 218 were performed in Lund. One paediatric first time HT was performed abroad in 2006. Of the patients, 72 (33%) received a mechanical assist prior to HT.

Demographic data, indications for HT, donor characteristics and recipient–donor matching are shown in Table II. Our study focus on the 94 adults (Group 1), evaluated at our lab, at rest, prior to HT. Children under the age of 18 ($n = 21$), re-HTs ($n = 5$) and patients referred from, and evaluated at, other hospitals ($n = 83$) and/or with incomplete data prior to HT ($n = 12$) were excluded.

Right heart catheterization

Right heart catheterization prior to HT, was predominantly performed via the right internal jugular vein, using a Swan Ganz catheter (Baxter Health Care Corp, Santa Ana, CA). If more than one catheterization was performed, the one closest prior to HT or assist was analyzed. During right heart catheterization, SPAP, MPAP, DPAP, PCWP, mean right atrial pressure (MRAP), systolic arterial pressure (SAP) and mean arterial pressure (MAP), were recorded. Heart rate (HR) was recorded from ECG. CO was measured by thermodilution. Cardiac index (CI), stroke volume

Table Ib. Characterization of HT-patients according to PH in previous publications.

Author/Period	Characterization	Patients	Investigation aim	Results
Costard-Jackle and Fowler (5) 1980–1988	1. “Low risk”: PVR \leq 2.5 or PVR \leq 2.5 and SAP \geq 85 with vasodilatation	218	3-month mortality	– Increased 3-month mortality in high-risk group
	2. “High risk”: PVR \geq 2.5 or PVR \leq 2.5 but SAP \leq 85 with vasodilatation	72		
Murali et al. (6) 1980–1991	1. TPG < 15 and PVR < 5	332	0–2, 3–7 and	– Increased 0–2-day mortality in severe PH pre-HT (group 4)
	2. TPG < 15 and PVR \geq 5	15	8–30 days	
	3. TPG \geq 15 and PVR < 5	46	mortality	– Increased 30-day mortality with TPG \geq 15
	4. TPG \geq 15 and PVR \geq 5	32		
Lindelov et al. (7) 1988–1990	1. “Low PVR”: PVR \leq 3	44	Short-, intermediate- and long-term outcome	– No difference in mortality between groups
	2. “High PVR”: PVR \leq 3 and MAP \geq 70 with vasodilatation	36		
Delgado et al. (8) 1991–1996	1. No PH: TPG \leq 12 and PVR \leq 2.5	71	30-day mortality	– Increased 30-day mortality with PH
	2. PH: TPG > 12 and/or PVR > 2.5	41		
Klotz et al. (9) 1998–2001	1. No PH: TPG < 12 and PVR < 2.5	90	1-year mortality	– No difference in 1-year mortality between patients without PH vs. reversible PH
	2. PH: TPG \geq 12 and/or PVR \geq 2.5	61		
Chang et al. (10) 1983–2000	1. No PH: PVR < 2.5	101	Short- and long-term outcome	– Increased 1-, 3- and 6-month survival with mild/moderate PH
	2. Mild/moderate PH: PVR 2.5–4.9	55		
	3. Severe PH: PVR \geq 5	16		– Increased 1-year mortality with severe PH, otherwise unchanged
Goland et al. (11) 1989–2004	1. No PH: PVR < 3, TPG < 10	266	Short- and long-term outcome	– No difference in mortality with pre-HT PH
	2. Mild–Moderate PH: PVR 3–6, TPG 10–20	112		– Increased long-term mortality with sustained PH after HT
	3. Severe PH: PVR > 6, TPG > 20	32		
Klotz et al. (12) 1996–2005	1. No PH: TPG \leq 12 and PVR \leq 2.5	168	Short- and long-term outcome	– No difference in mortality between groups
	2. Rev PH: TPG \leq 12 and PVR \leq 2.5 with vasodilatation	49		
Gude et al. (13) 1983–2007	1. No PH: 2–3 of: SPAP \leq 50, TPG \leq 10 and PVR \leq 2.5	365	Long-term outcome	– No difference in long-term mortality with pre-HT PH
	2. PH: 2–3 of: SPAP \leq 50, TPG \leq 10 and PVR \leq 2.5 with vasodilatation	135		– Increased long-term mortality with sustained PH after HT

Author/Period, First author and years of investigation in the related publication; Characterization, PH-characterization in the related publications; Patients, Number of patients investigated in each group in the related publications; Investigation aim, The outcome investigated in the related publications; Results, Summary of findings in the related publications; SAP, systolic artery pressure; SPAP, systolic pulmonary artery pressure; MPAP, mean pulmonary artery pressure; PCWP, pulmonary capillary wedge pressure; TPG, transpulmonary gradient; CO, cardiac output; PVR, pulmonary vascular resistance.

(SV), stroke volume index (SVI), left ventricular stroke work index, (LVSWI), right ventricular stroke work index (RVSWI), PVR, PVRI, TPG, DPG and systemic vascular resistance (SVR) were calculated by the following formulas: $CI = CO/\text{body surface area (BSA)}$, $SV = CO/HR$, $SVI = SV/BSA$, $LVSWI = (MAP-PCWP) \cdot SVI \cdot 0.0136$, where 0.0136 is a conversion factor, $PVR = TPG/CO$, $PVRI = TPG/CI$, $TPG = MPAP - PCWP$, $DPG = DPAP - PCWP$ and $SVR = (MAP - MRAP)/CO$.

Data analysis and characterization

Our HT patients were characterized according to i) previous haemodynamic risk characterizations

(5–13), ii) ESC’s guidelines (1), iii) ISHLT’s State of art summary statement (3), iv) ISHLT’s relative contraindications and v) ISHLT’s criteria for increased risk of right heart failure and early death after HT (4) (Table Ia, b). To separate the patients into groups according to ISHLT’s State of art summary statement (Table Ia) (3), the criteria for passive and reactive mixed PH were modified. “Modified” passive PH was redefined as TPG \leq 15 and PVR \leq 3 instead of TPG \leq 15 or PVR \leq 3. “Modified” reactive mixed PH was redefined as TPG \leq 15 and PVR \leq 3 with vasodilatation instead of TPG \leq 15 or PVR \leq 3 with vasodilatation.

The 94 patients in Group 1 were furthermore characterized according to whether or not they were re-evaluated prior to HT at rest with vasodilatation

Table II. Characteristics for the entire HT- and study population at Skåne University Hospital in Lund during 1988–2010.

Risk factor category and group*	Entire population characteristics				Study population characteristics			
	Mean	SD ±	N	%	Mean	SD ±	N	%
<i>Recipient characteristics</i>								
Gender of recipient			215				94	
Female			68	31.6			24	25.6
Age of recipient (years)	44.6	17.2	215		51.6	9.9	94	
≤10			9	4.2			0	0
11–20			24	11.1			1	1.1
21–30			8	3.7			4	4.3
31–40			17	7.9			6	6.4
41–50			42	19.5			21	22.3
51–60			85	39.5			45	47.9
61≤			30	14.0			17	18.1
Recipient's height (cm)	167.3	25.3	197		173.4	7.9	93	
Recipient's weight (kg)	68.4	18.9	198		75.3	15.0	93	
Recipient's bloodgroup			215				94	
0			71	33.0			31	33.0
A			109	50.7			46	48.9
B			19	8.8			8	8.5
AB			16	7.4			9	9.6
Time on waiting list (days)	151.3	200.4	219		148.6	179.6	94	
Indication for HT			215				94	
ARVC			5	2.3			0	0
Cardiac Tumour			1	0.5			0	0
CHD			11	5.1			1	1.1
CVR			1	0.5			0	0
DCM			110	51.2			52	55.3
HCM			10	4.7			8	8.5
Myocarditis			13	6.0			6	6.4
IHD			58	27.0			24	25.6
RCM			6	2.8			3	3.2
<i>Donor characteristics</i>								
Gender of donor			219				94	
Female			81	37.0			36	38.3
Age of donor (years)	40.0	15.7	219		43.9	13.7	94	
Donor's height (cm)	172.4	18.7	210		176.3	9.5	92	
Donor's weight (kg)	74.1	18.5	211		79.4	13.5	93	
Ischaemic time (min)	184.7	63.2	201		182.6	61.5	86	
<i>Recipient–donor matching</i>								
Age difference (years)	12.5	10.8	219		12.7	10.6	94	
Sex matching			219				94	
Sex-matched			160	73.1			71	75.5
ABO-matching			219				94	
Identic			184	84.0			82	87.2
Compatible			32	14.6			12	12.8
Incompatible			3	1.4			0	0

ARVC, Arrhythmogenic right ventricular cardiomyopathy; CHD, congenital heart disease; CVR, Chronic vascular rejection; DCM, Dilated cardiomyopathy; HCM, Hypertrophic cardiomyopathy; IHD, Ischaemic heart disease; RCM, Restrictive cardiomyopathy.

*Risk factor category is marked in bold text and corresponding group is marked in normal text.

(Group 2, $n = 23$) or mechanical assist (Group 3b, $n = 12$), the latter performed 77 ± 47 days after implantation (Table III). Survival was compared for the different PH groups and characterizations.

Drugs and devices

Acute and sustained vaso-reactivity was assessed, according to ISHLT's guidelines (4), by a vasodilator

test or mechanical assist, respectively. Twenty-three patients were exposed to a vasodilator, either intravenous nitroprusside (Nitropress, Hospira Inc, Lake Forest, USA) or nitric oxide (NO, INOmax, INO Therapeutics AB, Lidingö, Sweden) inhalation (INOvent, INO Therapeutics AB, Lidingö, Sweden). The doses for nitroprusside were $\sim 0.5, 1, 2, 4$ and $8 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ and for NO were 20 or 40 parts per million (ppm). Of the 21 patients in Group 1 that

Table III. Haemodynamic characteristics of our HT study population without or with vasodilation or mechanical assist.

Parameter	Group 1		Group 2		Group 2 after vasodilatation		Group 3a		Group 3b		Group 3b with mechanical assist		Sign
	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	
Patients	94		23		23		21		12		12		
SAP (mmHg)	99.3	16.9	102.8	15.8	92.3 [§]	17.8	92.4	19.7	92.5	24.9	–	–	§
MAP (mmHg)	74.1	13.6	75.7	12.3	67.7 [§]	15.6	71.4	16.5	70.5	20.7	–	–	§
SPAP (mmHg)	45.4	13.3	52.4*	15.0	47.9	14.3	46.2	11.4	43.3	11.3	24.3 [#]	7.7	*, #
DPAP (mmHg)	19.3	8.0	22.4	8.9	18.9 [§]	8.5	20.8	8.2	19.5	9.5	7.1 [#]	6.7	§, #
MPAP (mmHg)	30.1	8.8	34.6*	9.6	30.0 [§]	10.8	31.3	7.6	29.3	7.8	14.7 [#]	6.3	*, §, #
PCWP (mmHg)	20.5	8.2	22.6	9.1	20.7	12.7	23.2	7.1	21.8	8.3	5.1 [#]	6.4	#
TPG (mmHg)	9.6	3.5	12.0*	3.6	9.4 [§]	4.5	8.1*	3.9	7.5*	2.8	9.6	3.1	*, §
DPG (mmHg)	–1.2	4.6	–0.1	4.2	–2.6	6.9	–2.3	4.7	–2.3	5.4	2.0 [#]	4.2	#
MRAP (mmHg)	7.9	6.1	9.5	6.6	7.7	7.9	7.6	6.3	6.7	7.7	2.8 [#]	4.3	#
HR (beat·min ^{–1})	82.6	16.8	79.7	15.7	81.3	17.3	93.1*	17.4	88.3	14.8	82.5	10.7	*
CO (L·min ^{–1})	3.7	1.0	3.0*	0.7	3.8	1.5	3.5	0.8	3.4	0.7	4.3 [#]	1.1	*, #
CI (L·min ^{–1} ·m ^{–2})	1.9	0.5	1.7*	0.4	2.1	0.8	1.8	0.4	1.8	0.4	2.3 [#]	0.6	*, #
SV (mL·beat ^{–1})	46.7	17.1	39.4	11.1	47.4	18.8	38.7	11.3	39.5	9.5	55.7 [#]	13.5	#
SVI (mL·beat ^{–1} ·m ^{–2})	24.7	8.7	21.6	6.0	26.3	10.1	20.5	6.1	21.1	5.8	29.7 [#]	8.0	#
SVR (WU)	19.0	6.5	22.8*	7.2	19.6	11.4	17.8	5.0	18.0	5.9	–	–	*
PVR (WU)	2.8	1.3	4.2*	1.6	2.6 [§]	0.9	2.4	1.1	2.2	0.7	2.2	0.9	*, §
PVRI (WU·m ^{–2})	5.2	2.3	7.6*	2.7	4.7 [§]	1.6	4.5*	2.1	4.2*	1.3	4.4	1.6	*
LVSWI (g·beat ^{–1} ·m ^{–2})	19.0	9.9	15.7	6.1	16.3	5.9	14.7	7.6	15.1	8.5	33.6 [#]	16.4	#
RVSWI (g·beat ^{–1} ·m ^{–2})	7.4	3.7	7.5	3.7	7.7	3.3	6.7	2.8	6.6	2.3	4.5 [#]	1.6	#
Survival (years)	13.7		13.8				13.7		13.2				

SAP, systolic arterial pressure; MAP, mean arterial pressure; SPAP, systolic pulmonary artery pressure; DPAP, diastolic pulmonary artery pressure; MPAP, mean pulmonary artery pressure; PCWP, pulmonary capillary wedge pressure; TPG, transpulmonary gradient; DPG, diastolic pulmonary vascular pressure gradient; MRAP, mean atrial pressure; HR, heart rate; CO, cardiac output; CI, cardiac index; SV, stroke volume; SVI, stroke volume index; SVR, systemic vascular resistance; PVR, pulmonary vascular resistance; PVRI, pulmonary vascular resistance index; LVSWI, left ventricular stroke work index; RVSWI, right ventricular stroke work index. SAP, MAP and SVR are not reported in patients with mechanical assist due to use of both pulsatile- and continuous-flow devices; Group 1, HT study population; Group 2, patients who performed vasodilatation test during RHC; Group 3a, patients bridged to HT with mechanical assist; Group 3b, patients bridged to HT with mechanical assist who were re-evaluated whilst on mechanical assist.

*Indicates a statistical significance ($p < 0.05$) compared with Group 1 (study population).

§Indicates statistical significance ($p < 0.05$) in Group 2 after vasodilation as compared with Group 2 prior to vasodilatation.

#Indicates statistical significance ($p < 0.05$) in Group 3b with mechanical assist as compared with Group 3b without mechanical assist.

received a mechanical assist (Group 3a), five were HeartMate IP (Thoratec, Pleasanton, CA, USA), five HeartMate VE (Thoratec, Pleasanton, CA, USA), three Jarvik 2000 (Jarvik Heart Inc., New York, NY, USA), one Abiomed (Abiomed Inc, Danvers, MA, USA) and seven HeartMate II (Thoratec, Pleasanton, CA, USA). Of the 12 patients with assist (Group 3b) who were haemodynamically re-evaluated prior to HT, three had a HeartMate IP, four HeartMate VE, two Jarvik 2000 and three HeartMate II.

Statistics

A SigmaStat System (SigmaPlot 11.0) was used for statistical analysis. Parametric or non-parametric statistics were used depending on the distribution of data; that is paired t-test or Wilcoxon signed rank test, respectively, when comparing one group prior to- and after an intervention, and a t-test or a Mann–Whitney rank sum test when two groups were compared. Survival curves were plotted using the Kaplan–Meier method. The Log-Rank test was performed when

comparing two or more groups. Last day of follow-up was 30th of June 2012. A $p < 0.05$ was considered significant. All values are mean \pm SD.

Results

Haemodynamic characterization at baseline with vasodilatation or mechanical assist

The haemodynamic measurements in Group 1 ($n = 94$), prior to HT, are shown in Table III. The 23 patients exposed to vasodilatation showed a decrease in MPAP ($p < 0.048$), PVR ($p < 0.001$) and PVRI ($p < 0.001$), by 4.6 ± 10.4 mmHg, 1.6 ± 1.3 WU and 2.9 ± 2.3 WU·m^{–2}, respectively, at the cost of SAP ($p < 0.015$) and MAP ($p < 0.013$), which decreased by 10.5 ± 16.8 and 8.0 ± 14.0 mmHg, respectively (Table III). PVR did not decrease to < 2.5 WU in 12 patients. In four patients where PVR decreased to < 2.5 WU, SAP decreased to < 85 mmHg, identifying 16 patients with an increased risk of right heart failure and early death after HT.

In the patients re-evaluated 77 ± 47 days after assist-implantation (Group 3b), MPAP, PCWP and MRAP decreased by 14.7 ± 5.4 ($p < 0.001$), 16.8 ± 6.5 ($p < 0.001$) and 3.8 ± 5.1 ($p < 0.027$) mmHg, respectively, to normal magnitudes (Table III). CI, SVI and DPG increased by 0.5 ± 0.4 L·min⁻¹·m⁻² ($p < 0.003$), 8.1 ± 4.7 mL·m⁻² ($p < 0.001$) and 4.3 ± 3.8 mmHg ($p < 0.002$) after implantation, respectively. PVR, PVRI and TPG remained unchanged ($p = \text{ns}$). Five of the 12 patients with assist, that were re-evaluated, still exhibited an irreversible $\text{PVR} > 2.5$ WU prior to HT, resulting together with the 16 patients remaining at increased risk of right heart failure and early death after acute vasodilatation (as mentioned above) in 21 patients with an increased risk of right heart failure and early death. If including the two patients with $\text{SPAP} > 60$ mmHg and either, $\text{PVR} > 5$ WU or $\text{PVRI} > 6$ WU·m⁻² or $\text{TPG} > 16\text{--}20$ mmHg, a total of 23 patients were considered at increased risk of right heart failure and early death after HT (Table IV, Supplementary Table I to be found online at <http://informahealthcare.com/doi/abs/10.3109/14017431.2013.877153>).

Furthermore, 66 of the 94 patients in Group 1 showed PH prior to HT. Of these, 63 had a post-capillary PH due to left heart disease (Table IV, Supplementary Table II to be found online at <http://informahealthcare.com/doi/abs/10.3109/14017431.2013.877153>) and three had a pre-capillary PH according to present definitions. Forty patients with post-capillary PH exhibited a "modified" passive PH and 23 exhibited a mixed PH (Table IV, Supplementary Table III to be found online at <http://informahealthcare.com/doi/abs/10.3109/14017431.2013.877153>).

Fifteen of the 23 patients with mixed PH were assessed for acute vaso-reactivity. Of these, eight patients had a non-reactive PH prior to HT (Table IV, Supplementary Table III to be found online at <http://informahealthcare.com/doi/abs/10.3109/14017431.2013.877153>). DPG equal to or above the cut-off value of 7 mmHg was only present in three patients.

Survival after HT

Mean survival of the 94 versus all 215 HT patients was 13.7 versus 15.1 years, respectively; 1-, 5- and 10-year survival was 92.6%, 81.1% and 71.1%, versus 91.3%, 82.6% and 71.3%, respectively, not significantly different from each other (Figure 1a). Mean follow-up of the 94 versus all 215 HT patients was 8.2 ± 5.8 and 8.2 ± 5.6 years, respectively ($p = \text{ns}$). Survival did neither differ between groups, with sufficient number of patients, characterized depending on the magnitude of SPAP, MPAP, PCWP,

TPG and PVR (Table IV). At the end of follow-up two of the three patients with $\text{DPG} \geq 7$ mmHg were still alive with a mean follow-up of 3.2 ± 1.9 years. The third patient died 6.7 years after HT due to acute pulmonary embolism.

Moreover, survival (mean, n) of the 94 HT patients (Group 1) did not differ ($p = \text{ns}$); for those without (13.0 years, $n = 28$) or with (13.9 years, $n = 66$) PH (Figure 1b), passive (13.8 years, $n = 50$) or reactive (12.2 years, $n = 13$) post-capillary PH (Table IV, Supplementary Table II to be found online at <http://informahealthcare.com/doi/abs/10.3109/14017431.2013.877153>, Figure 1c); "modified" passive (13.1 years, $n = 40$) or mixed (16.6 years, $n = 23$) post-capillary PH (Table IV, Supplementary Table II to be found online at <http://informahealthcare.com/doi/abs/10.3109/14017431.2013.877153>, Figure 1d); "modified" reactive (12.6 years, $n = 7$) or non-reactive (12.2 years, $n = 8$) PH (Table IV, Supplementary Table III to be found online at <http://informahealthcare.com/doi/abs/10.3109/14017431.2013.877153>); or for those with ISHLT's relative contraindications (12.0 years, $n = 22$) and criteria for increased risk of right heart failure and early death (9.1 years, $n = 7$) after HT, or for those assessed with vasodilator or assist that remained in the high-risk group (16.2 years, $n = 21$) (Table IV, Supplementary Table I to be found online at <http://informahealthcare.com/doi/abs/10.3109/14017431.2013.877153>, Figure 1e). Combining the latter two groups, 23 patients were considered at increased risk of right-heart-failure and early death after HT. Their survival (16.5 years, $n = 23$) did, however, not differ ($p = \text{ns}$) from the rest.

In addition, the survival of the patients with baseline PH who received LVAD ($n = 18$) versus those patients with baseline PH who did not receive LVAD ($n = 48$) did not differ ($p = \text{ns}$). Their mean survival was similar ($p = \text{ns}$); for example 14.0 years for patients with LVAD ($n = 18$) versus 13.6 years for patients without LVAD ($n = 48$).

With regards to early mortality, three of the 94 patients in the study population died within 24 h after HT, another two within 30 days, one after 330 days and one after 332 days; resulting in seven deaths within the first year after HT. Four of these patients had post-capillary PH prior to HT (three passive and one "modified" reactive) and three did not have PH. None of these patients had a $\text{DPG} \geq 7$ mmHg. The causes of death were vasoplegia in two cases, cerebrovascular insult in two and primary organ failure, cardiac fibrosis and liver cirrhosis in one case each.

Table IV. Characterization of our HT-study population according to PH-characterization in previous publications and current guidelines.

Author	Characterization	Patients (n)	SPAP (mmHg)	MPAP (mmHg)	PCWPP (mmHg)	TPG (mmHg)	PVR (WU)	Mean survival (Years)
Costard-Jäckle and Fowler (5)	1. "Low risk": PVR ≤ 2.5 or PVR ≤ 2.5 and SAP ≥ 85 with vasodilatation 2. "High risk": PVR ≥ 2.5 or PVR ≥ 2.5 but SAP ≤ 85 with vasodilatation	55	42.3 \pm 13.0	28.0 \pm 9.2	20.3 \pm 9.5	7.8 \pm 2.3	2.0 \pm 0.4	12.4
		39	48.2 \pm 13.0	31.9 \pm 7.9	21.5 \pm 9.1	10.4 \pm 4.1	3.1 \pm 0.7	14.6
		79	42.6 \pm 12.1	28.5 \pm 8.6	20.0 \pm 8.6	8.5 \pm 2.4	2.4 \pm 0.7	14.0
Murahi et al. (6)	1. TPG < 15 and PVR < 5 2. TPG < 15 and PVR ≥ 5 3. TPG ≥ 15 and PVR < 5 4. TPG ≥ 15 and PVR ≥ 5	5	56.4 \pm 11.8	36.2 \pm 3.3	23.4 \pm 4.7	12.8 \pm 1.8	6.3 \pm 0.8	10.3
		7	57.3 \pm 10.9	37.1 \pm 5.8	21.1 \pm 6.4	16.0 \pm 1.2	4.0 \pm 0.7	8.4
		3	67.3 \pm 7.2	43.3 \pm 3.8	25.7 \pm 3.8	17.7 \pm 2.9	5.5 \pm 0.5	10.9
		64	42.8 \pm 12.6	28.3 \pm 8.8	20.2 \pm 8.7	8.1 \pm 2.5	2.1 \pm 0.5	12.7*
Lindelow et al. (7)	1. "Low PVR": PVR ≤ 3 2. "High PVR": PVR ≥ 3 and MAP ≥ 70 with vasodilatation 3. Excluded:	3	46.0 \pm 17.1	32.7 \pm 9.7	25.3 \pm 11.0	7.3 \pm 1.5	2.6 \pm 0.3	1.4
		27	51.8 \pm 13.2	34.3 \pm 7.7	21.8 \pm 7.2	12.5 \pm 3.3	4.2 \pm 1.2	16.4*
		51	41.0 \pm 12.5	27.1 \pm 8.9	19.4 \pm 8.8	7.8 \pm 2.1	1.9 \pm 0.4	12.8
Delgado et al. (8)	1. No PH: TPG ≤ 12 and PVR ≤ 2.5 2. PH: TPG > 12 and/or PVR > 2.5	43	50.4 \pm 12.5	33.5 \pm 7.5	21.8 \pm 7.3	11.7 \pm 3.7	3.8 \pm 1.3	14.6
		46	40.3 \pm 11.6	26.6 \pm 8.3	19.2 \pm 8.4	7.4 \pm 2.2	1.9 \pm 0.4	13.3
Klotz et al. (9)	1. No PH: TPG < 12 and PVR < 2.5 2. PH: TPG ≥ 12 and/or PVR ≥ 2.5	48	50.0 \pm 13.2	33.3 \pm 8.2	21.7 \pm 7.9	11.6 \pm 3.3	3.7 \pm 1.3	14.1
		47	40.6 \pm 11.6	27.0 \pm 8.6	19.5 \pm 8.5	7.5 \pm 2.3	1.9 \pm 0.4	12.5
Chang et al. (10)	1. No PH: PVR < 2.5 2. Mild/moderate PH: PVR 2.5–4.9 3. Severe PH: PVR ≥ 5	39	47.7 \pm 12.7	31.9 \pm 8.2	20.9 \pm 8.3	11.0 \pm 3.1	3.2 \pm 0.6	14.1
		8	60.5 \pm 11.3	38.9 \pm 4.9	24.3 \pm 4.3	14.6 \pm 3.2	6.0 \pm 0.8	12.2
		44	40.6 \pm 11.7	27.6 \pm 8.5	20.7 \pm 8.4	6.9 \pm 1.8	2.0 \pm 0.5	12.5
Goland et al. (11)	1. No PH: PVR < 3, TPG < 10 2. Mild-Moderate PH: PVR 3–6, TPG 10–20 3. Severe PH: PVR > 6, TPG > 20	46	48.3 \pm 12.9	31.7 \pm 8.7	20.1 \pm 8.3	11.6 \pm 2.6	3.3 \pm 1.0	14.3
		52	40.9 \pm 12.4	27.1 \pm 8.8	19.4 \pm 8.8	7.7 \pm 2.2	1.9 \pm 0.4	12.4
Klotz et al. (12)	1. No PH: TPG ≤ 12 and PVR ≤ 2.5 2. Rev PH: TPG ≤ 12 and PVR ≤ 2.5 with vasodilatation 3. Excluded.	6	53.5 \pm 12.1	37.0 \pm 7.9	30.7 \pm 9.2	6.3 \pm 1.8	1.8 \pm 0.6	7.0
		36	49.8 \pm 12.4	32.9 \pm 7.3	21.0 \pm 7.0	11.9 \pm 3.4	3.8 \pm 1.3	15.6
		52	39.1 \pm 10.8	26.4 \pm 8.1	19.0 \pm 8.3	7.4 \pm 2.0	2.0 \pm 0.5	12.6
Gude et al. (13)	1. No PH: 2–3 of: SPAP ≤ 50 , TPG ≤ 10 and PVR ≤ 2.5 2. PH: 2–3 of: SPAP ≤ 50 , TPG ≤ 10 and PVR ≤ 2.5 with vasodilatation	42	52.5 \pm 12.3	34.4 \pm 7.7	22.3 \pm 7.8	12.2 \pm 3.1	3.8 \pm 1.3	14.5
		28	31.3 \pm 6.3	19.2 \pm 3.5	11.0 \pm 3.9	8.2 \pm 2.4	2.2 \pm 0.7	13.0
PH-definition according to ESC-guidelines by Galie et al. (1)	1. No PH: 2. Pre-capillary PH: 3. Post-capillary PH: 3a Passive; 3b Reactive:	3	42.3 \pm 6.1	27.3 \pm 1.5	12.7 \pm 2.1	14.7 \pm 1.2	3.2 \pm 0.8	XX
		63	51.5 \pm 11.1	34.7 \pm 6.2	24.8 \pm 5.8	9.9 \pm 3.7	3.1 \pm 1.4	14.0
		50	49.7 \pm 10.6	33.7 \pm 6.2	25.3 \pm 6.0	8.4 \pm 2.2	2.6 \pm 1.0	13.8
13	58.1 \pm 9.9	38.4 \pm 4.7	22.7 \pm 4.1	15.7 \pm 2.1	4.9 \pm 1.2	12.2		

(Continued)

Table IV (Continued)

Author	Suggested PH-definition according to Fang et al. (3)	Characterization	Patients (n)	SPAP (mmHg)	MPAP (mmHg)	PCWP (mmHg)	TPG (mmHg)	PVR (WU)	Mean survival (Years)
	1. No PH		28	31.3 ± 6.3	19.2 ± 3.5	11.0 ± 3.9	8.2 ± 2.4	2.2 ± 0.7	13.0
	2. Pre-capillary PH:		3	42.3 ± 6.1	27.3 ± 1.5	12.7 ± 2.1	14.7 ± 1.2	3.2 ± 0.8	XX
	3. "Modified" Passive PH:		39	49.2 ± 10.5	33.4 ± 6.4	25.3 ± 6.0	8.1 ± 2.5	2.2 ± 0.5	13.8
	4. Mixed PH:		23	55.2 ± 10.9	36.8 ± 5.3	23.9 ± 5.2	13.0 ± 3.5	4.6 ± 1.2	16.6
	4a "Modified" Reactive:		7	49.1 ± 12.1	33.3 ± 8.3	25.6 ± 10.2	7.7 ± 2.4	2.2 ± 0.4	12.6
	4b Non-reactive:		8	58.0 ± 10.7	37.3 ± 4.3	22.8 ± 4.2	14.5 ± 3.3	5.7 ± 1.3	12.2
	4c Untested:		8	52.3 ± 10.3	35.0 ± 5.2	23.1 ± 3.4	11.9 ± 3.4	3.9 ± 0.5	YY

Characterization, PH-characterization in the related publications; Patients, Number of our HT study patients investigated in each group when characterized in accordance to the related publication; Excluded, Patients in whom the haemodynamics were too deranged to include them in the corresponding study; SPAP, systolic pulmonary artery pressure; MPAP, mean pulmonary artery pressure; PCWP, pulmonary capillary wedge pressure; TPG, transpulmonary gradient; PVR, pulmonary vascular gradient; Mean survival, mean survival in years in each group.

*Indicates significance ($p < 0.05$) in survival compared with the "high-risk" group, however only with three patients.

XX indicates that no mean survival could be calculated.

YY indicates that no mean survival could be calculated as all patients lived at the end of follow up (mean follow up in this group was 6.7 ± 4.1 years).

"Modified" Passive = $TPG \geq 15$ and $PVR \leq 3$ instead of $TPG \geq 15$ or $PVR \leq 3$, in order to enable grouping of the patients to either a passive or mixed group.

"Modified" Reactive = $TPG \leq 15$ and $PVR \leq 3$ after vasodilatation instead of $TPG \leq 15$ or $PVR \leq 3$ after vasodilatation, in order to enable grouping of the patients to either a reactive or non-reactive group.

Discussion

The present study shows that short- and long-term survival was similar after HT, no matter whether or not the patients had preoperative post-capillary PH, irrespective of type and PH characterization used (Table IV, Supplementary Tables I–III to be found online at <http://informahealthcare.com/doi/abs/10.3109/14017431.2013.877153>), Figure 1b–e). Thus, preoperative PH was, as defined previously and at present (1,3–13), at our centre not as decisive for outcome as previously postulated (Figure 1b–e). Consequently, even though we still consider preoperative, post-capillary, PH to be a risk factor for right heart failure and early death after HT, previous and present, haemodynamic criteria (Tables I and IV, Supplementary Tables I–III to be found online at <http://informahealthcare.com/doi/abs/10.3109/14017431.2013.877153>) (1,3–13), do not sufficiently discriminate the impact of PH for survival after HT. Consequently, new haemodynamic risk criteria are urged for.

Previous studies have proposed different thresholds, of various haemodynamic parameters that may predict outcome after HT (Table I) (1,3–13). Of our 94 patients evaluated before HT, 28 did not have and 66 had PH (1). However, survival at our centre was not impaired in the HT patients with preoperative post-capillary PH, irrespective of severity and haemodynamic criterion analyzed (Table IV, Supplementary Table I–III to be found online at <http://informahealthcare.com/doi/abs/10.3109/14017431.2013.877153>), Figure 1b–e). Thus, in support of recent findings (11,13,15,16), the precise magnitude of preoperative elevation of PVR and TPG, or other haemodynamic parameters that may influence outcome after HT remains unclear. $DPG \geq 7$ mmHg, which recently was suggested to indicate severe pulmonary vascular disease and increased mortality in patients with PH due to left heart disease (18), was only observed in three of our 94 patients prior to HT. If $DPG \geq 7$ is assumed as a new risk criterion also for outcome after HT, the low DPG in our study could be a reason for the good outcome in our PH patients. The low DPG could furthermore also suggest that the PH in our population was mainly due to passive congestion, rather than pulmonary vascular remodelling.

Our study furthermore confirms that mechanical assist prior to HT improves haemodynamics, making more patients eligible for HT (15,16,19–21). Of interest, a $RVSWI < 5$ g·beat⁻¹·m⁻² in HT candidates, or a $RVSWI < 399$ mmHg·mL·m⁻² prior to mechanical assist implantation, has been described as risk factor for complications (22,23). In our material, four of twelve patients re-evaluated on mechanical assist had a $RVSWI$ below the levels thought to

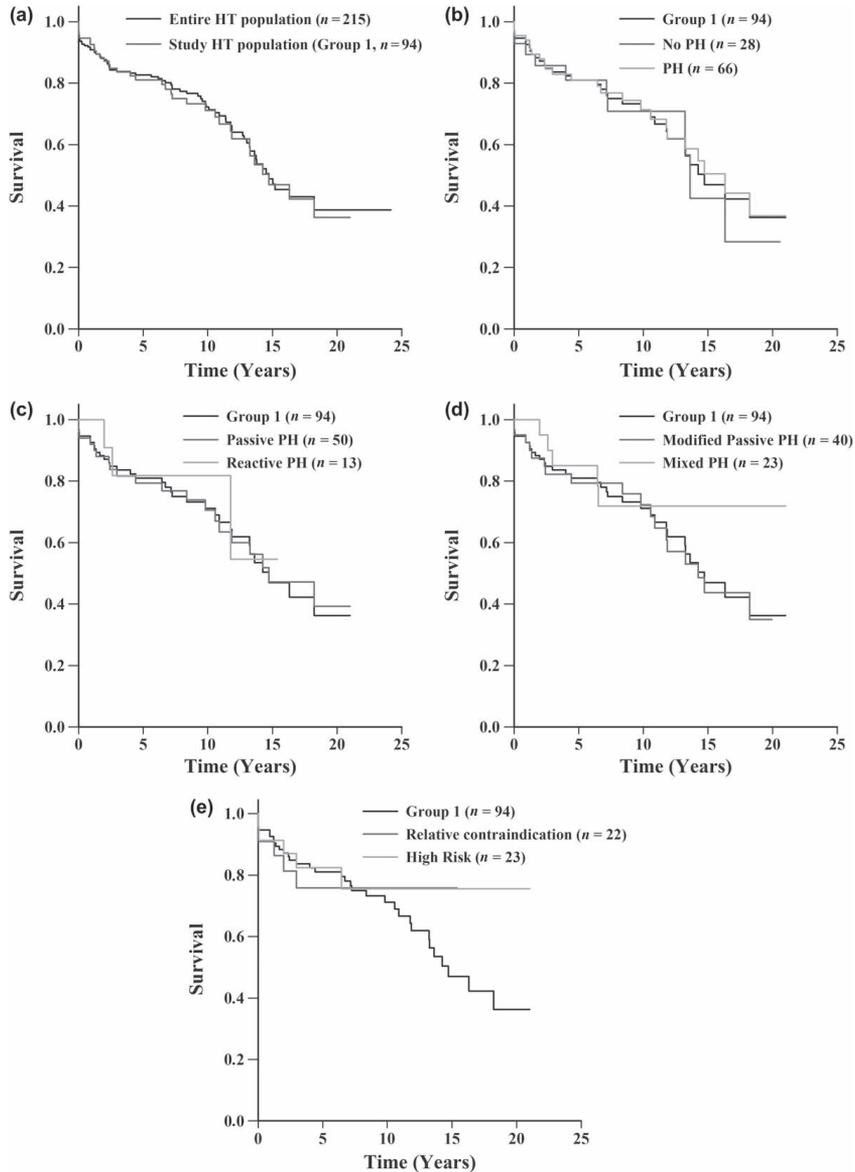


Figure 1. Survival curves for the entire HT population, HT study population and patients characterized with different types of PH at Skåne University Hospital in Lund during 1988–2010. Survival was not different ($p = ns$) for our HT study population (Group 1, $n = 94$) as compared with; (a) the entire population of HT patients ($n = 215$), (b) patients without ($n = 28$) or with ($n = 66$) PH (1,3), (c) with passive ($n = 50$) or reactive ($n = 13$) post-capillary PH, defined according to ESC guidelines (1), or for (d) patients with “modified” passive ($n = 40$) or mixed ($n = 23$) post-capillary PH, defined according to ISHLT’s state of art summary statement (3), (e) Survival was neither different ($p = ns$) for the patients with ISHLT’s defined relative contraindications for HT ($n = 22$) and criteria for increased risk of right heart failure and early death after HT ($n = 23$) (4) as compared with our HT study population (Group 1, $n = 94$).

increase the risk for complications, as defined above. RVSWI further decreased after assist implantation, however, without influencing survival after HT. Moreover, none of the 21 patients who received an assist died the first year after HT, and their mean survival did not differ from the rest of the population. These findings suggest that LVAD therapy improve haemodynamics as well as survival, to levels similar to the general HT-population. Therefore, patients should not be disqualified for HT based purely on PH prior to LVAD implantation.

Another explanation for the good outcome in our PH patients may be the pre-, intra- and postoperative treatment at our thoracic intensive care unit, focusing on keeping filling pressures low, maintaining adequate perfusion pressure and, by aggressive diuretic therapy, preventing patients from accumulating fluids. The treatment has included drugs such as dobutamin, milrinone, inhaled NO and prostacyclines, as well as levosimendan since year 2000. Venò-arterial extracorporeal membrane oxygenation (ECMO) has furthermore been used, when necessary. Transthoracic- and/or transesophageal echocardiography has routinely been utilized intra- and post-operatively since 1989 till 1990. Future, complimentary clinical trials are encouraged to evaluate whether novel PAH-targeted therapies may exert additional beneficial effects in specific cases of PH due to left heart disease, as previous studies have shown diverging results (24–30).

Finally, even though a retrospective study like the present may have disadvantages compared with a prospective study, and that the relative small sample size and long timespan of the present study could influence the results, our findings are of interest. This is supported by that our study population was representative with regards to similar demographic parameters (Table II) and survival, as compared with our entire HT-population (Figure 1a). Survival was furthermore similar during the entire period of transplantations. However, due to the small number of patients in some groups, these results have to be interpreted with caution, illustrating the value of larger, future, multi-centre, studies.

In conclusion, the present study showed that at our centre survival did not differ between patients with or without preoperative, post-capillary, PH (Figure 1b–e), defined according to present ESC's guidelines (1) and ISHLT's state of art summary statement (3). Survival did neither differ for previous haemodynamic criteria (5–13), nor for ISHLT's relative contraindications and criteria for increased risk of right heart failure and early death after HT (4) (Table IV, Supplementary Table I–III to be found online at <http://informahealthcare.com/doi/abs/10.3109/14017431.2013.877153>).

Our findings therefore suggest that preoperative PH prior to HT, as defined according to the previous and present haemodynamic criteria, does not have to be associated with worse outcome. Thus, larger multi-centre studies are encouraged to find the best haemodynamic predictors, and their magnitudes, for outcome after HT, thereby providing better guidance for whom to list for HT. In this context, DPG could be a new interesting marker, but its impact needs further evaluation.

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Dr. Rådegran is, and has been, primary- or co-investigator in; clinical PAH trials for Glaxo-

SmithKline, Actelion Pharmaceuticals Sweden AB, Pfizer, Bayer and United Therapeutics, and in clinical HT immuno-suppression trials for Novartis.

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Supplementary material available online

Supplementary Supplementary Table I–III.

Paper IV





Impact of postoperative pulmonary hypertension on outcome after heart transplantation

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ABSTRACT

Objectives: We wanted to investigate the effects of postoperative pulmonary hypertension (PH_{postop}: mean pulmonary artery pressure [MPAP] \geq 25 mmHg), diastolic pressure gradient (DPG), pulmonary vascular resistance (PVR), and repeated hemodynamic measurements on long-term survival after heart transplantation (HT).

Design: Eighty-nine patients who underwent HT at Skåne University Hospital in Lund in the period 1988–2010 and who were evaluated with right-heart-catheterization at rest, prior to HT and repeatedly during the first postoperative year, were grouped based on their MPAP, DPG, and PVR.

Results: One year after HT, survival was lower in patients with PH_{postop} than in those without, in patients with DPG \geq 7 mmHg than in those with DPG $<$ 7 mmHg, and in patients with PVR $>$ 3 WU than in those with PVR \leq 3 WU. Moreover, compared to patients with no PH_{postop} or with PH_{postop} at one evaluation during the first year after HT, PH_{postop} at repeated evaluations was associated with higher mortality (hazard ratio 3.4, 95% CI 1.4–8.0). There was no significant difference in acute cellular rejection between patients with and without PH_{postop} but postoperative kidney function was worse in patients with repeated PH_{postop}.

Conclusions: When defined according to present guidelines, PH one year after HT may emerge as a prognostic marker for long-term outcome after HT. Moreover, PH_{postop} at repeated evaluations during the first year after HT had stronger prognostic value than PH_{postop} at a single examination, illustrating a means of identifying a high-risk population. However, confirmation in larger multi-center studies is warranted.

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Introduction

Pulmonary hypertension (PH) due to left heart disease is a negative prognostic marker in left heart failure (LHF) [1]. Early in its course, the PH is caused by passive post-capillary congestion due to elevated left ventricular filling pressures [2]. If the congestion is sustained, the elevated pressures may cause endothelial damage and dysfunction, leading to an imbalance between vasoactive substances and resulting in excessive pulmonary vasoconstriction. In some patients, this vasoconstriction may be further complicated by vascular remodeling of the pulmonary vessels [3]. With previous definitions of PH, it has, however, been difficult to differentiate between patients with excessive vasoconstriction who do or do not have remodeling of the pulmonary arteries [3]. In this context, it has been suggested that the diastolic pressure gradient (DPG) may differentiate between vasoconstriction and remodeling of the pulmonary arteries. DPG has therefore been assumed to be a better marker for subdefining post-capillary PH than previously used parameters, such as the transpulmonary gradient (TPG), as DPG is less dependent of volume

and flow [4]. DPG \geq 7 mmHg was also recently shown to be associated with pulmonary vascular abnormalities and impaired outcome in patients with left heart disease [5]. Thus, despite some conflicting results [6,7], DPG in combination with pulmonary vascular resistance (PVR) \geq 3 WU has been included in the new PH guidelines to subdefine PH due to left heart disease [2].

Despite PH being associated with poor prognosis in LHF, there is conflicting evidence regarding its effect on survival after heart transplantation (HT). Whereas some studies have shown that preoperative PH and elevated TPG or PVR may influence postoperative outcome [8,9], other studies have found no difference in postoperative survival based on preoperative hemodynamic data [6,10–12]. A few studies have instead suggested that elevated mean pulmonary artery pressure (MPAP) and PVR one year after HT affect outcome [10,11]. These studies have, however, not used the recommended definition of PH (MPAP \geq 25 mmHg) [2] when investigating survival, so their clinical implications remain uncertain. Moreover, very little is known about the impact of postoperative DPG on outcome after HT.

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Furthermore, besides LHF and vascular abnormalities, pulmonary artery pressure and PVR may be temporarily affected by other external factors such as physiological stress and hypoxia. Post-capillary PH could potentially also be caused by an increased volume load and congestion of the pulmonary circuit related to kidney dysfunction, and impaired ventricular function related to episodes of acute cellular rejection (ACR) post-HT. PH at a single examination may thus not reflect permanent vascular changes. It has therefore been suggested that repeated hemodynamic evaluations should be performed in PH due to LHF to adequately subdefine these patients [13]. However, the role of repeated hemodynamic measurements after HT in defining a high risk population has not been investigated. Such studies are therefore of great interest to determine the effect of repeated postoperative PH on long-term survival after HT.

For these reasons, we investigated the extent to which PH (MPAP ≥ 25 mmHg) and/or DPG ≥ 7 mmHg influenced outcome after HT in our population. We also investigated the association between preoperative and postoperative hemodynamics, and the relationship between postoperative PH and both kidney function and occurrence of ACR.

Patients and methods

Study design and population

This retrospective, single-center study was based on the 215 patients who underwent HT at Skåne University Hospital in Lund in the period 1988–2010. Demographics of the 215 patients have previously been described [12]. The study was performed with approval from the ethics board in Lund (approval nos. 2014/92, 2011/777, 2011/368, and 2010/114) and in accordance with the Declarations of Helsinki and Istanbul.

The study focused on the adult patients who were evaluated at our hemodynamics laboratory prior to HT. Patients referred from and investigated at other university hospitals ($n=87$), children under the age of 18 ($n=21$), re-transplanted patients ($n=1$), and patients with incomplete hemodynamic data prior to HT ($n=12$) were excluded. After exclusion, 94 adult patients remained. Their preoperative demographics and hemodynamic profiles have also previously been described [12]. Eighty-nine of the 94 patients who were postoperatively evaluated with routine right heart catheterization (RHC) at our laboratory were included in the present analysis (Table 1). No additional clinically indicated, hemodynamic evaluations were included in the study. The five patients who did not perform RHC after HT died early after transplantation: three within one day, one after 14 days, and one after 25 days. All were men, three had preoperative PH, and their baseline hemodynamics did not differ significantly from those of the 89 surviving patients.

Right heart catheterization

Hemodynamic measurements with RHC were routinely performed at rest prior to HT, as well as one and four weeks,

three and six months, and one year after HT. However, for natural reasons and because of the real-life clinical setting, all patients did not have all postoperative evaluations performed. Preoperative RHC was performed 143.1 ± 113.3 days before HT, primarily via the right internal jugular vein, using a Swan-Ganz catheter (Baxter Health Care Corp, Santa Ana, CA), with the zero level at the mid-thoracic position. If patients had more than one RHC prior to HT, the one closest to HT-or assist implantation-was chosen for inclusion. During examination, complete hemodynamics were recorded. In the present study, we focused on mean arterial pressure (MAP), MPAP, pulmonary artery wedge pressure (PAWP), mean right atrial pressure (MRAP), cardiac output (CO), TPG, DPG, and PVR. MAP, MPAP, PAWP, and MRAP were recorded. CO was measured by thermodilution. TPG, DPG, and PVR were calculated using the following equations: $TPG = MPAP - PAWP$, $DPG = \text{diastolic pulmonary artery pressure} - PAWP$, and $PVR = TPG/CO$.

Acute cellular rejection and renal function

To investigate other possible contributors to postoperative PH, data on acute cellular rejection (ACR) and renal function were also evaluated.

Table 1. Characteristics of the 89 patients included in the present study.

	Mean	\pm SD	n	%
Recipient characteristics				
Recipient gender			89	
Female			23	26
Recipient age, years	51	10	89	
18–20			1	1
21–30			4	4
31–40			6	7
41–50			20	22
51–60			42	47
≥ 61			16	18
Recipient height, cm	173	8	88	
Recipient weight, kg	75	14	88	
Time on waiting list, days	150	184	89	
Indication for HT			89	
CHD			1	1
DCM			49	55
HCM			8	9
Myocarditis			6	7
IHD			22	25
RCM			3	3
Ischaemic time, min	180	62	86	
Pulmonary Hypertension			63 [87]	72
Hypertension			1 [89]	1
Diabetes			6 [67]	7
LVAD			21 [89]	24
RVAD			0 [89]	
BIVAD			0 [89]	
ECMO			0 [89]	
Creatinine, $\mu\text{mol}\cdot\text{L}^{-1}$	99	33	85	
Donor characteristics				
Donor gender			89	
Female			35	39
Donor age, years	43	14	89	
Donor height, cm	176	10	87	
Donor weight, kg	79	14	88	

CHD: congenital heart disease; DCM: dilated cardiomyopathy; HCM: hypertrophic cardiomyopathy; IHD: ischemic heart disease; RCM: restrictive cardiomyopathy; diabetes: based on HbA1c-levels at the pre-HT evaluation; LVAD: left ventricular assist device; RVAD: right ventricular assist device; BIVAD: biventricular assist device; ECMO: extracorporeal membrane oxygenation; [X] indicates number of patients with the specific parameter available.

The occurrence of ACR was monitored with endomyocardial biopsies (EMBs), which were performed on a routine basis on 14 occasions during the first postoperative year (week 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, 24, 32, 40, and 52). Thus, some of the EMBs were performed in connection with the RHCs previously described. Additional EMBs were also performed where there were symptoms, or previous episodes, of ACR. From 2005 onwards, EMBs were graded according to both the 1990 and the 2004 International Society for Heart and Lung Transplantation (ISHLT) grading systems [14,15]. Before then, EMBs were solely graded according to the 1990 system. Therefore, all EMBs in the present study were histopathologically analyzed and graded according to the 1990 ISHLT working formulation [14].

With regard to renal function, glomerular filtration rate (GFR) was measured at one year post-HT using the iohexol clearance method. During the measurements, patients were given a dose-adjusted solution of iohexol (Omnipaque; GE Healthcare), after which plasma sampling was performed in relation to patient morphometrics. High-performance liquid chromatography was used to determine the iohexol concentrations. In a few cases where iohexol clearance measurements were missing, the creatinine-based CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation, e.g. the currently recommended GFR estimating equation by KDIGO [16], was used to increase the amount of data available for analysis [16,17].

Statistics

SigmaStat/SigmaPlot version 11.0 (Systat Software Inc, San Jose, CA) was used for statistical analysis. Parametric or non-parametric statistics were used, depending on the distribution of data: i.e. t-test or Mann-Whitney rank sum test, respectively, when differences between two groups were being compared and Pearson- or Spearman correlation, respectively, when measuring the association between two variables. Survival curves were plotted using the Kaplan-Meier method and hazard ratios (HRs) for death were estimated with Cox proportional hazards regression analysis and adjusted for gender, age at the time of HT, and kidney function one year after HT. The last day of follow-up was June 30, 2012. Mean follow-up was 8.2 ± 5.8 years. Any p-value of $< .05$ was considered to be statistically significant. All values are presented as mean \pm SD. Estimated survival, when presented, is based on unadjusted data.

Results

Preoperative pulmonary hypertension and elevated diastolic pressure gradient

Sixty-three patients had preoperative PH. Of these 63 patients, 53 (84%) performed RHC at one week after HT. In 37 (70%), the PH was reversed and in 16 (30%) it persisted one week after HT. With regard to DPG, only three of the patients had DPG ≥ 7 mmHg prior to HT. In all three, DPG was normalized one week after HT.

Postoperative pulmonary hypertension and its effect on long-term survival

Survival, based on hemodynamics at the different evaluations, are shown in Figure 1(a–e). Long-term survival was lower in patients with PH at three months than in those without (estimated survival 10.1 vs. 15.7 years, $p < .006$), and also at one year (estimated survival 7.0 vs. 15.1 years, $p < .001$). There was, however, no significant difference in survival whether or not the patients had PH at one or four weeks after HT-or at six months (Figure 1(a–e)). Of the eight patients with PH at one year after HT, four had precapillary PH, one had combined precapillary and postcapillary PH, and three had isolated postcapillary PH. When adjusted for gender and age at the time of transplantation, MPAP at one year after HT was associated with a higher risk of death in the Cox regression model (HR 1.2, 95% CI 1.1–2.3). The hemodynamic characteristics of the patients with postoperative PH are (for each examination) shown in Supplemental Table 1.

Diastolic pulmonary gradient, pulmonary vascular resistance, and transpulmonary gradient one year after heart transplantation

One year after HT, DPG, PVR and TPG data were available from 67, 78, and 78 patients (75%, 88%, and 88%), respectively. Seven patients had DPG ≥ 7 mmHg. Their survival was significantly lower than in those with DPG < 7 mmHg (estimated survival 5.0 vs. 14.2 years, $p < .001$). Moreover, survival was significantly lower in patients with PVR > 3 WU ($n = 4$) than in those with PVR ≤ 3 WU ($n = 74$; estimated survival 3.1 vs. 14.5 years, $p < .006$). In contrast, there was no significant difference in survival between the patients with TPG ≥ 12 mmHg ($n = 9$) and in those with TPG < 12 mmHg ($n = 69$).

Effect of postoperative pulmonary hypertension during repeated measurements after heart transplantation

Survival in patients without postoperative PH and those with PH at one or repeated evaluations is shown in Figure 2(a,b). Twenty-nine patients (33%) exhibited PH at least once during the first year after HT, and 60 (67%) did not have PH at any of the postoperative evaluations. There was no significant difference in survival between these two groups (estimated survival 12.4 vs. 15.7 years) (Figure 2(a)). Of the 29 patients with postoperative PH, 18 had PH at a single examination and 11 had PH at two or more examinations. Sixteen of the patients with PH at one measurement, and 10 of the patients with PH at two or more measurements, also had PH preoperatively. Of the 18 patients who exhibited PH at a single examination, nine had PH at one week, three at four weeks, two at three months, one at six months, and three at one year after HT. The survival was not significantly different in the 60 patients who did not exhibit PH and in the 18 patients who exhibited PH at one measurement (estimated survival 15.7 and 16.1 years, respectively), whereas survival was significantly lower in the

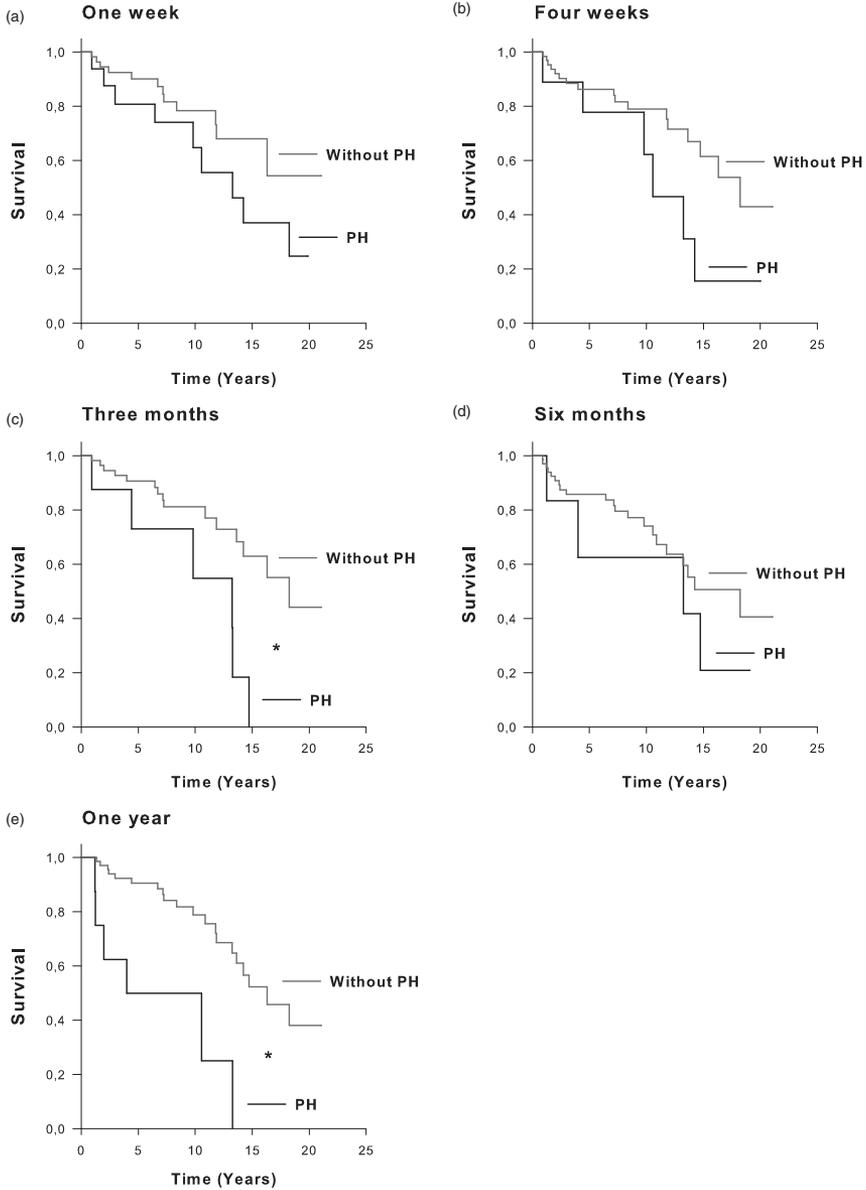


Figure 1. Survival in patients with and without PH after HT. (a) One week, (b) four weeks, (c) three months, (d) six months, and (e) one year after HT. *indicates statistical significance between patients with PH and patients without PH.

11 patients who exhibited PH at two or more examinations (estimated survival 7.8 years, $p < .001$) (Figure 2(b)). In a multivariate Cox regression model adjusted for gender and age at the time of HT, PH at repeated measurements was associated with a higher risk of death (HR 4.4, 95% CI

2.0–9.8) than no PH and PH at a single examination after HT.

There was no significant difference in the number of postoperative catheterizations performed in patients without postoperative PH, in patients with PH at one postoperative

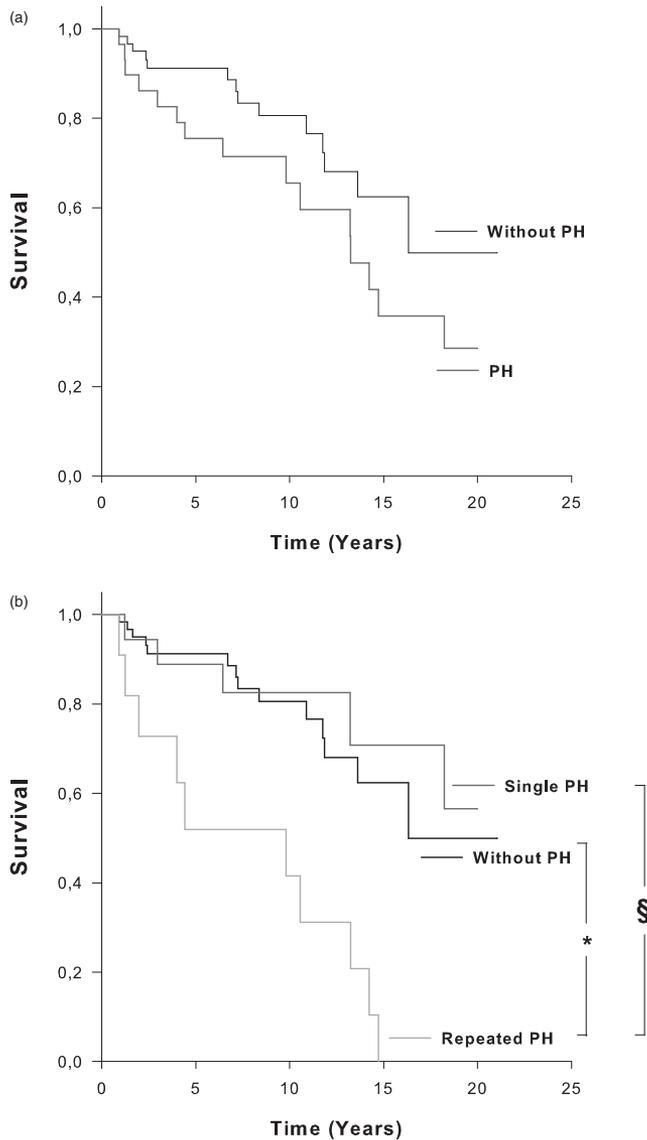


Figure 2. Survival in patients with no PH, with PH at one examination, and with PH at several examinations after HT. Comparison between (a) patients with no PH and with PH after HT, and: (b) patients without PH, with PH at a single examination, and with PH at repeated examinations after HT. *indicates statistical significance between patients without PH and patients with PH at repeated examinations. § indicates statistical significance between patients with PH at one examination and patients with PH at repeated examinations.

evaluation, and in patients with PH at repeated evaluations ($n = 4.0$, $n = 3.9$, and $n = 4.3$, respectively).

Preoperative hemodynamic comparisons based on postoperative pulmonary hypertension

Preoperative hemodynamics were compared for patients who, after HT: (1) did not exhibit PH, (2) exhibited PH at

one examination, or (3) exhibited PH at several examinations (Table 2). Compared to patients without postoperative PH, preoperative MPAP, PAWP, MRAP, and PVR were significantly higher ($p < .005$, $p < .02$, $p < .004$, and $p < .02$, respectively) in patients with PH at one examination, and preoperative MPAP and PAWP were significantly higher ($p < .008$ and $p < .04$, respectively) in patients with PH at

repeated examinations (Table 2). Moreover, CO was significantly lower ($p < .02$) in patients with PH at one examination than in patients with PH at repeated examinations. Finally, there was a weak positive correlation between preoperative MPAP and MPAP at all postoperative examinations, except at the six-month evaluation. This association decreased with time. The R^2 -values were 0.39 ($p < .001$) at one week, 0.22 ($p < .001$) at four weeks, 0.19 ($p < .001$) at three months, and 0.06 ($p < .03$) one year after HT.

Acute cellular rejection and renal function

The proportions of EMBs with ACR grade ≥ 2 or $\geq 3A/3B$ in patients with and without postoperative PH are shown in Table 3. A total of 1,376 EMBs were performed during the first postoperative year. There was no significant difference in the proportion of EMBs with ACR grade ≥ 2 or $\geq 3A/3B$ in patients with and without PH at one or repeated postoperative evaluations. Nor was there any significant difference in the proportion of EMBs with ACR grade ≥ 2 or $3A/3B$ in patients with PH at one or repeated postoperative evaluations (Table 3).

Kidney function one year after HT in patients with and without postoperative PH is shown in Figure 3. One year after HT, measurements of renal function were available

from 57 of the 60 patients (95%) without postoperative PH, in 17 of the 18 patients (94%) with PH at a single evaluation, and in 10 of the 11 patients (91%) with PH at repeated evaluations. There was no significant difference in renal function between patients without postoperative PH and those with PH at a single postoperative evaluation. In contrast, renal function was lower in patients with repeated PH, both when compared to patients without postoperative PH ($p < .003$) and when compared to those with PH at one postoperative evaluation ($p < .04$) (Figure 3). However, when kidney function was included in the multivariate regression, PH at repeated evaluations remained an independent predictor of mortality (HR 3.4, 95% CI 1.4–8.0), compared to no PH and to PH at a single evaluation.

Kidney function and corresponding MPAP were available from 77 patients at one year after HT, and a negative correlation was observed between the parameters ($R^2 = .11$, $p < .004$).

Cause of death

The causes of death in the different patient groups are given in Table 4. Forty-six of the 60 patients (77%) without postoperative PH, 13 of the 18 patients (72%) with PH at one postoperative examination, and one of the 11 patients (9%)

Table 2. Preoperative hemodynamic characteristics of patients with PH and without PH after HT.

Parameter	All patients		No PH post-HT		PH at one examination post-HT		PH at repeated examinations post-HT		Sign
	Mean \pm SD		Mean \pm SD		Mean \pm SD		Mean \pm SD		
Patients	89		60		18		11		
MAP, mmHg	73.9	13.6	74.7	15.4	71.2	8.5	74.1	10.2	
MPAP, mmHg	30.3	8.8	27.9	8.6	34.6*	7.0	35.8 [§]	8.4	* [§]
PAWP, mmHg	20.6	8.1	18.8	7.8	24.1*	7.7	24.4 [§]	8.0	* [§]
TPG, mmHg	9.7	3.5	9.1	3.4	10.4	3.1	11.5	4.3	
DPG, mmHg	-1.2	4.4	-1.1	4.5	-1.8	4.6	0.0	3.5	
MRAP, mmHg	7.9	6.0	6.6	5.3	12.0*	6.9	8.2	5.0	*
CO, L·min ⁻¹	3.7	1.1	3.7	1.0	3.2	1.0	4.2 [#]	1.0	#
PVR, WU	2.8	1.3	2.6	1.0	3.7*	1.8	2.8	1.3	*

Mean: mean values from the preoperative hemodynamic evaluation; *indicates preoperative statistical significance between patients with no PH post-HT and those with PH at one examination; [§]indicates preoperative statistical significance between patients with no PH post-HT and those with PH at repeated examinations; [#]indicates preoperative statistical significance between patients with PH at one examination post-HT and those with PH at repeated examinations; MAP: mean arterial pressure; MPAP: mean pulmonary artery pressure; PAWP: pulmonary artery wedge pressure; TPG: transpulmonary gradient; DPG: diastolic pressure gradient; MRAP: mean right atrial pressure; CO: cardiac output; PVR: pulmonary vascular resistance.

Table 3. Pulmonary hypertension in relation to acute cellular rejection.

				<i>P</i>
% of EMBs with ACR \geq grade 2 or 3A/3B in patients without PH ($n = 60$)				
• ≥ 2	8.5%	(79/933)		
• $\geq 3A/3B$	5.0%	(47/933)		
% of EMBs with ACR \geq grade 2 or 3A/3B in patients with PH at one RHC ($n = 18$)				
• ≥ 2	5.9%	(16/270)		0.217
• $\geq 3A/3B$	3.0%	(8/270)		0.203
vs. % of EMBs with ACR \geq grade 2 or 3A/3B in patients with PH at repeated RHCs ($n = 11$)				
• ≥ 2	8.7%	(15/173)		0.952
• $\geq 3A/3B$	4.0%	(7/173)		0.716
vs. % of EMBs with ACR \geq grade 2 or 3A/3B in patients with PH at repeated RHCs ($n = 11$)				
• ≥ 2	8.7%	(15/173)		0.361
• $\geq 3A/3B$	4.0%	(7/173)		0.730

PH: pulmonary hypertension; ACR: acute cellular rejection; EMB: endomyocardial biopsy; RHC: right heart catheterization.

with PH at repeated postoperative evaluations were alive at the end of follow-up. Of the 29 patients who died, the most common cause of death was rejection ($n = 7$, 24%) – including cellular and humoral rejections, as well as coronary allograft vasculopathy – followed by malignancy ($n = 6$, 21%) and infection ($n = 5$, 17%).

Discussion

The present study defined the characteristics of hemodynamics in HT patients during repeated measurements in the first year after HT, and investigated the effects of postoperative PH and elevated DPG on survival. Our results indicate that persistent PH after HT may influence outcome. Moreover, DPG of ≥ 7 mmHg and PVR > 3 WU one year after HT was also associated with a lower long-term survival, whereas TPG > 12 mmHg was not. Furthermore, survival was similar for patients with PH at a single postoperative evaluation and in patients without any postoperative PH. In

contrast, survival was markedly lower in those who had PH at two or more examinations during the first postoperative year, with only one of 11 patients being alive at the end of follow-up. This illustrates the importance of early and repeated catheterizations after HT, to identify patients with persistent PH who may have impaired survival.

With the same cohort of patients, we recently showed that pre-HT PH, defined according to previous and present definitions, does not have to be associated with impaired survival after HT [12]. As shown in our previous study, patients selected for HT showed reversibility of PVR in acute vasodilatation tests. Consequently, the patients included in both the previous study and the present study probably did not show extensive vascular remodeling, but instead passive congestion and excessive vasoconstriction. In agreement with the lack of effect of preoperative PH in the previous report, Gude and colleagues did not observe any difference in survival in a cohort of 500 HT patients whether or not they had preoperative PH [11]. Similarly, Goland and colleagues did not observe any difference in survival in a cohort of 427 HT patients, based on their preoperative hemodynamics. Instead, they found that MPAP > 20 mmHg six months and one year after HT [11], and PVR ≥ 3 WU one year after HT [10], were associated with lower long-term outcome. These reports did not, however, use the current definitions of PH when defining a high-risk population, so the impact of the current ESC guidelines in this regard remains uncertain. Consequently, the present study is the first to use the current definition of PH [2] when evaluating the influence of post-HT PH on survival. Despite using a different definition [2], our findings are in line with a few previous reports [10,11] emphasizing that persistent PH one year after HT is associated with reduced long-term survival. This is further supported by the finding that MPAP at one year after HT in our Cox regression model was associated with a higher risk of death, indicating that PH may emerge as a prognostic marker for outcome following HT.

Moreover, to the best of our knowledge, this is the first time that findings from repeated measurements during the

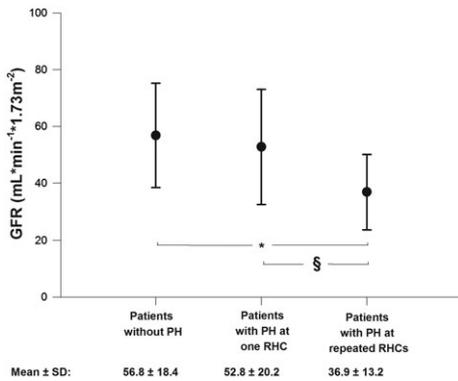


Figure 3. GFR one year after HT in relation to postoperative PH. GFR, glomerular filtration rate; PH, pulmonary hypertension; RHC, right heart catheterization. *indicates statistical significance between patients without PH and patients with PH at repeated examinations. §indicates statistical significance between patients with PH at one examination and patients with PH at repeated examinations.

Table 4. Causes of death in the different patient groups.

Cause of death	All patients		No PH post-HT		PH at one examination post-HT		PH at repeated examinations post-HT	
	N	%	N	%	N	%	N	%
Patients	89		60		18		11	
Deaths	29		14		5		10	
Infection	5	17	2	14	0	–	3	30
Kidney Failure	1	3	1	7	0	–	0	–
Malignancy	6	21	3	21	1	20	2	20
Rejection	7	24	4	29	3	60	0	–
Intracranial bleeding	3	10	2	14	0	–	1	10
Ruptured aortic aneurysm	1	3	0	–	0	–	1	10
Circulatory failure	1	3	0	–	0	–	1	10
Acute coronary syndrome	1	3	1	7	0	–	0	–
Amyloidosis	1	3	0	–	1	20	0	–
Multi organ failure	1	3	0	–	0	–	1	10
Pulmonary embolism	1	3	1	7	0	–	0	–
Unknown	1	3	0	–	0	–	1	10

%: percent of the total number of deaths in the different groups; Unknown: one patient died under unclear circumstances when abroad, therefore the cause of death is unclear.

first year after HT have been reported. As already stated, survival was significantly reduced in patients with PH at three months and one year after HT, but not at six months. The findings at six months contrast with those reported by Gude [11], and may have been due to the low number of patients with PH ($n=6$) at the six-month evaluation in our study. Another possible explanation may be that PH exhibited at a single examination may be caused by temporary factors such as hypoxic pulmonary vasoconstriction, postoperative stress, and therapies that may influence pulmonary vascular tone. The latter argument is supported by the fact that the patients in our study who exhibited PH at one examination had an estimated survival similar to that of patients who did not exhibit postoperative PH at all (16.1 and 15.7 years, respectively), whereas the survival of patients with PH at repeated examinations was markedly reduced when adjusted for age and gender (7.8 years, HR 4.4). In fact, only 9% of the patients with repeated PH were alive at the end of follow-up, as compared to 77% of the patients with no postoperative PH and 72% of the patients with PH at one postoperative examination. Furthermore, despite the difference in survival, there was no difference in preoperative MPAP, PAWP, TPG, MRAP, PVR, or DPG between patients exhibiting PH at one examination or repeated examinations after HT. If anything, patients with PH at one evaluation showed signs of more pronounced biventricular failure, with lower CO and a trend of higher MRAP. These findings illustrate the complexity in identifying patients with combined pre-capillary and post-capillary PH based on a single evaluation. This emphasizes the importance of close hemodynamic monitoring before and after HT to identify a high-risk population at an early stage. Such a population could be included in future randomized controlled clinical trials with vasoactive therapies, in order to determine whether these patients may benefit from such treatments [3,18]. Finally, in the present study we did not histologically examine the pulmonary vessels of the patients who died, so it is uncertain whether the repeated PH represented permanent pulmonary vascular changes. Instead, repeated PH could represent other persistent complications that may affect long-term survival. This is supported by the fact that most deaths in patients with repeated PH were non-cardiovascular and that MPAP was only slightly elevated. We therefore also investigated the occurrence of ACR and reduced kidney function, which are both thought to affect hemodynamics and outcome [19,20] after HT. Based on our findings, there did not appear to be a close relationship between ACR and PH early after HT. In contrast, kidney function one year after HT was markedly reduced in patients with PH at repeated evaluations, both compared to patients without PH and to those with PH at a single evaluation. Furthermore, there was a weak negative correlation between kidney function and MPAP one year after HT, suggesting a connection between pulmonary hemodynamics and kidney function already during the first year after HT. Nonetheless, in our multivariate regression, adjusted for age, gender, and kidney function, repeated PH (in contrast to PH at a single

evaluation) did affect survival, which suggests that repeated PH is a negative prognostic marker, independent of kidney function. Consequently, our findings illustrate the importance of repeated hemodynamic measurements to identify high-risk patients who are predisposed to early mortality.

Despite $PVR >3$ WU and $DPG \geq 7$ mmHg one year after HT being associated with a lower long-term survival, definite conclusions on the role of these parameters are difficult to make, as very few patients showed elevated values. Furthermore, only three of our patients had $DPG \geq 7$ mmHg prior to HT [12]. None of these patients had elevated DPG after HT; nor did they show PH at repeated measurements. These findings argue against recent reports [5,21] suggesting that DPG is a prognostic marker associated with pulmonary vascular disease in patients with LHF. However, they are in line with a recent registry-based study that did not find any difference in post-transplant survival based on preoperative DPG levels in patients with elevated PVR or TPG [6]. In light of the findings from the present study, indicating that repeated hemodynamic measurements are of value for identification of patients with impaired outcome, the patients with high preoperative DPG may have had other external factors that falsely increased their preoperative DPG-levels. Moreover, the heart rate of patients with $DPG \geq 7$ mmHg was higher than in patients with $DPG < 7$ mmHg (data not shown), highlighting one of the main shortcomings of DPG, which is affected by the heart rate. This further suggests that the high DPG levels at one year were due to a generally worse condition, rather than to pulmonary vascular disease. The risk of relying on a single hemodynamic evaluation was recently also highlighted in an editorial comment [13] after DPG had been shown not to affect survival in LHF [7]. Thus, although there is a strong theoretical rationale for using DPG to sub-define post-capillary PH [4] and despite the fact that DPG has been included in the new definition of post-capillary PH [2], the sub-definition (due to these conflicting reports) is still a matter of debate [13,18,21–23].

Although the retrospective design of our study had disadvantages compared to a prospective design, and although the relatively small sample size and the long duration of our study could have influenced the results, the findings are of interest in relation to the new ESC/ERS PH guidelines [2]. This is supported by the fact that our study population was representative with regard to similar demographic parameters and survival when compared to our entire HT population [12]. The measurements were all performed routinely, based on our follow-up program after HT, and they were also performed in the same manner, by mostly the same physicians—minimizing the risk of bias despite the long study period. The pulmonary vessels of the patients who died were not histologically examined, however, so it cannot be determined whether repeat PH represented pulmonary vascular disease. Moreover, we did not investigate whether repeat PH was a stronger prognostic factor than PH one year after HT, or whether their predictive values were similar. Finally, due to the very small numbers of patients in some groups, especially those with $DPG \geq 7$ mmHg and $PVR >3$ WU, some findings must be interpreted with caution.

Conclusions

In conclusion, our study suggests that persistent PH, defined according to present guidelines, at one year after HT is related to impaired long-term survival. Moreover, the findings indicate that PH at repeated measurements during the first postoperative year is a negative prognostic marker, associated with worse outcome than PH at a single examination. This highlights that repeated and early hemodynamic measurements after HT could be used to identify patients with persistent PH who may exhibit impaired survival. Whether repeated PH is a stronger prognosticator of death than PH at one year after HT needs investigation in future trials. Further studies in larger patient cohorts are also encouraged to confirm our findings and to clarify whether patients who exhibit PH at repeated examinations after HT have signs of pulmonary vascular changes or whether the findings represent a generally worse condition.

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Disclosure statement

Dr Lundgren reports receiving personal lecture fees from Actelion Pharmaceuticals Sweden AB and GlaxoSmithKline for services outwith the work submitted. Dr Söderlund reports receiving personal lecture fees from Sandoz/Novartis for services outwith the work submitted. Dr Rådegran reports receiving personal lecture fees from Actelion Pharmaceuticals Sweden AB, Bayer Healthcare, GlaxoSmithKline, NordiciInfu Care, and Sandoz/Novartis for services outwith the work submitted.

Dr. Rådegran is, and has been, primary investigator or co-investigator in clinical PAH trials for GlaxoSmithKline, Actelion Pharmaceuticals Sweden AB, Pfizer, Bayer, and United Therapeutics, and in clinical heart transplantation immunosuppression trials for Novartis.

The companies had no role in the collection, analysis, and interpretation of data relating to this article and had no right in disapproving of the manuscript.

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Paper V





Alterations in plasma L-arginine and methylarginines in heart failure and after heart transplantation

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ABSTRACT

Objective. Endothelial function, including the nitric oxide (NO)-pathway, has previously been extensively investigated in heart failure (HF). In contrast, studies are lacking on the NO pathway after heart transplantation (HT). We therefore investigated substances in the NO pathway prior to and after HT in relation to hemodynamic parameters. **Design.** 12 patients (median age 50.0 yrs, 2 females), heart transplanted between June 2012 and February 2014, evaluated at our hemodynamic lab, at rest, prior to HT, as well as four weeks and six months after HT were included. All patients had normal left ventricular function post-operatively and none had post-operative pulmonary hypertension or acute cellular rejection requiring therapy at the evaluations. Plasma concentrations of ADMA, SDMA, L-Arginine, L-Ornithine and L-Citrulline were analyzed at each evaluation. **Results.** In comparison to controls, the plasma L-Arginine concentration was low and ADMA high in HF patients, resulting in low L-Arginine/ADMA-ratio pre-HT. Already four weeks after HT L-Arginine was normalized whereas ADMA remained high. Consequently the L-Arginine/ADMA-ratio improved, but did not normalize. The biomarkers remained unchanged at the six-month evaluation and the L-Arginine/ADMA-ratio correlated inversely to pulmonary vascular resistance (PVR) six months post-HT. **Conclusions.** Plasma L-Arginine concentrations normalize after HT. However, as ADMA is unchanged, the L-Arginine/ADMA-ratio remained low and correlated inversely to PVR. Together these findings suggest that (i) the L-Arginine/ADMA-ratio may be an indicator of pulmonary vascular tone after HT, and that (ii) NO-dependent endothelial function is partly restored after HT. Considering the good postoperative outcome, the biomarker levels may be considered "normal" after HT.

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Introduction

Heart failure (HF) is common and despite continuous improvement in HF therapy, the condition carries a poor prognosis, where heart transplantation (HT) resides as the ultimate treatment. Pulmonary hypertension (PH) is a complicating factor in HF, associated with worse prognosis [1]. With the present methods and definitions [2,3], it is difficult to differentiate between isolated post-capillary PH (Ipc-PH), caused by passive congestion of the pulmonary vasculature and combined pre-capillary and post-capillary PH (Cpc-PH), complicated by endothelial dysfunction, excessive vasoconstriction and, in some cases, vascular remodelling [2]. Adequately sub-defining post-capillary PH is, however, of major importance in HT where Cpc-PH with vascular remodelling may increase the risk for acute right heart failure and early mortality after HT.

Endothelial dysfunction is central in PH, but is also present in HF. This dysfunction is, at least in part, caused by

impaired nitric oxide (NO) production secondary to endothelial damage [4]. The L-Arginine/NO pathway is a complex system with effects on vasomotor tone as well as on cellular adhesion, platelet aggregation, vascular proliferation and angiogenesis [5]. NO is produced from L-Arginine, which can form either NO and citrulline or urea and ornithine, respectively. The former reaction is catalysed by the nitric oxide synthase (NOS) family and the latter by arginase [6,7].

Moreover, the competitive NOS inhibitor asymmetric dimethylarginine (ADMA) has been shown to be elevated in plasma and serum in various cardiovascular conditions [8,9] as well as to be an independent predictor of outcome in acutely decompensated chronic HF [10]. ADMA is primarily metabolized by dimethylarginine dimethylaminohydrolase-1 (DDAH-1) and to a lesser extent excreted by the kidneys [5]. DDAH-1 is primarily expressed in the kidneys and liver [11] and its activity is reduced by oxidative stress and impaired in the above-mentioned conditions. In contrast,

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 Supplemental data for this article can be accessed [here](#).

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Arginase activity has been shown to be elevated in HF [12]. This, together, leads to decreased NO synthesis and increased production of urea, which in turn results in lower L-Arginine available for NO production [12].

In contrast to in HF, there is scarce knowledge on the NO pathway in relation to HT. One study has shown that NO production is impaired after HT, primarily due to increased inflammation [13]. Another study has shown that the immunosuppressive compound sirolimus, but not mycophenolate mofetil, decrease ADMA in HT patients [14]. However, little is known about the levels of various substances of the nitric oxide pathway, their development over time and their correlation to hemodynamics after HT. We therefore evaluated substances related to the NO pathway in severe HF patients prior to and after HT and related the findings to commonly used hemodynamic parameters. The aim was to define the “normal” post-HT plasma levels of substances in the NO pathway, as well as to identify potential biomarkers and targets for future medical therapies.

Materials and methods

Study design and population

Between June 2012 and February 2014, 43 patients were heart transplanted in Lund and included in a prospective cohort study. To identify a “normal” post-HT population, the 12 patients (median age 50.0 yrs, 2 females) who were hemodynamically evaluated at our lab, at rest, prior to HT, as well as four weeks and six months after HT, prior to August 2014, and who, at the four week and six month evaluations, (i) did not have post-operative rejections requiring specific therapy; (ii) did not have postoperative PH and; (iii) had left ventricular ejection fraction $\geq 50\%$, were included in the present study. For comparison, we included 12 healthy, age-matched, non-smokers without drug treatment and no symptoms or signs of common cold.

The study was performed with informed consent and approval by the ethics board in Lund and Uppsala (Approval nos. 2010/114, 2010/343, 2010/442, 2011/368, 2011/777, 2015/270) and in accordance with the Declarations of Helsinki and Istanbul.

Right heart catheterization

Right heart catheterization (RHC) were performed at rest prior to HT, as well as four weeks and six months after HT. RHC was performed via the right internal jugular vein, using a Swan Ganz catheter (Baxter Health Care Corp, Santa Ana, CA). Complete pulmonary hemodynamics were recorded. In the present study we focused on mean pulmonary artery pressure (MPAP), pulmonary artery wedge pressure (PAWP), mean right atrial pressure (MRAP), cardiac output (CO), transpulmonary gradient (TPG), diastolic pressure gradient (DPG) and pulmonary vascular resistance (PVR). MPAP, PAWP and MRAP were recorded. CO was measured by thermodilution. TPG, DPG and PVR were calculated using the formulas: $TPG = MPAP - PAWP$,

$DPG = \text{diastolic pulmonary artery pressure} - PAWP$ and $PVR = TPG/CO$.

Biomarker analysis

Plasma samples from patients, collected from the pulmonary arteries during RHC, were stored at -80°C in the Lund Cardio Pulmonary Register (LCPR) cohort of Region Skåne’s biobank. The samples were analyzed for plasma concentrations of ADMA, symmetric dimethylarginine (SDMA), L-Arginine, L-Ornithine and L-Citrulline with liquid chromatography – tandem mass spectrometry (LC-MS/MS), at the Swedish National Veterinary Institute in Uppsala, Sweden, as described previously [15]. The L-Arginine/ADMA-ratio as well as the L-Arginine/L-Ornithine-ratio were evaluated and the global arginine bioavailability ratio (GABR) calculated using the formula: $GABR = (L\text{-Arginine} / (L\text{-Ornithine} + L\text{-Citrulline}))$. Control samples were collected at Uppsala University Hospital and analyzed for L-Arginine, ADMA and SDMA as described previously [16]. The patients and controls were analyzed at different time points. The methods used were the same. However, at the time of analysis of the samples from controls, the L-Ornithine and L-Citrulline analyzes were not available so these markers are lacking in controls.

To monitor acute cellular rejections (ACR), endomyocardial biopsies were routinely performed from the right interventricular septum, and graded according to the 2005 International Society for Heart and Lung Transplantation working formulation [17].

Statistics

A SigmaStat/SigmaPlot version 11.0 (Systat Software Inc, San Jose, CA) was used for statistical analysis. Parametric or non-parametric statistics were used depending on the distribution of data: i.e. One Way Repeated Measures ANOVA (Tukey-test) or One Way Repeated Measures ANOVA on Ranks (Tukey-test), when the same group was compared over time; t-test or Mann-Whitney Rank Sum test, respectively, when two groups were compared and Pearson- or Spearman Correlation, respectively, when measuring the association between two variables. A p value $< .05$ was considered statistically significant. All values are presented as median and interquartile range.

Results

Study population

Baseline demographical data are shown in Table 1. Nine of the 12 patients had pre-operative PH (MPAP ≥ 25 mmHg). Of these patients, three had combined pre-capillary and post-capillary PH (Cpc-PH, DPG ≥ 7 mmHg and/or PVR > 3 WU) and five had isolated post-capillary PH (Ipc-PH, DPG < 7 mmHg and/or PVR ≤ 3 WU). One patient had very severe orthopnea at the RHC when blood samples were collected and adequate PAWP measurements could not be

Table 1. Baseline characteristics of patients and healthy controls.

Parameter	Patients		Healthy subjects	
	n	median (IQR)	n	median (IQR)
Clinical features				
Sex, male/female	12	10/2	12	5/7
Age, years	12	50 (45–60)	12	51 (46–60)
Weight, kg	12	80 (71–92)		N/A
Length, cm	12	179 (176–180)		N/A
BSA, m ²	12	2.0 (1.9–2.1)		N/A
NYHA class, IIIb/IV		3/9		N/A
Etiology, DCM/HCM/IHD	12	8/1/3		N/A
Diabetes mellitus, yes/no	12	0/12	12	0/12
Hypertension, yes/no	12	0/12	12	0/12
AF, yes/no	12	2/10	12	0/12
History of smoking, yes/no	12	3/9	12	0/12
Medical history				
Betablockers, %	12	12 (100)		–
ACEi/ARB, %	12	8/4 (100)		–
MRA, %	12	8 (67)		–
Anticoagulants, %	12	10 (83)		–
Statins, %	12	3 (25)		–
Diuretics, %	12	12 (100)		–
Biochemical indicators				
L-Arginine, μM	12	56 (48–60)	12	86 (78–92)*
ADMA, μM	12	0.57 (0.47–0.71)	12	0.39 (0.32–0.42)*
SDMA, μM	12	0.85 (0.68–1.15)	12	0.41 (0.36–0.45)*
L-Arginine/ADMA	12	86 (68–128)	12	225 (203–273)*
P-Urea, mmol·L ⁻¹	12	9 (8–13)		N/A
P-Homocysteine, μM·L ⁻¹	12	16 (13–27)		N/A
P-NT-proBNP, ng·L ⁻¹	12	4058 (3348–7408)		N/A
P-Creatinine, μM·L ⁻¹	12	106 (95–131)		N/A
eGFR, mL·min ⁻¹ ·1.73 m ⁻²	12	61 (44–78)		N/A

IQR: interquartile range; n: individuals where the specific parameter was available; BSA: body surface area; NYHA: New York Heart Association; DCM: dilated cardiomyopathy; HCM: hypertrophic cardiomyopathy; IHD: ischemic heart disease; AF: atrial fibrillation; ACEi: angiotensin converting enzyme inhibitor; ARB: angiotensin II receptor blocker; MRA: mineralocorticoid receptor antagonist; ADMA: asymmetric dimethylarginine; SDMA: symmetric dimethylarginine; N/A: not assessed.

*Indicates statistical significant difference ($p < .05$) between patients and controls.

performed, wherefore PH-subgrouping is lacking in this patient. However, the extensive pre-HT evaluation revealed no signs of pulmonary vascular disease and in a second RHC, MPAP and PAWP were 30 mmHg and 24 mmHg, respectively, with a PVR of 1.5 WU and a DPG of 0 mmHg. Prior to the second RHC the patient had been medically optimized with intravenous furosemide and levosimendan and was subsequently accepted for HT.

Follow-up data, with regards to clinical features and medications, are shown in Table 2. Four weeks after HT three patients exhibited myocardial biopsies with rejection grade 1R and six months after HT two patients had rejection grade 1R. None needed specific rejection treatment.

Substances in the nitric oxide pathway and their characteristics in relation to heart transplantation

Prior to HT, the plasma concentration of L-Arginine was 56 (48–60) μM, L-Ornithine 93 (71–119) μM, L-Citrulline 26 (19–33) μM, ADMA 0.57 (0.47–0.71) μM, SDMA 0.85 (0.68–1.15) μM, L-Arginine/ADMA-ratio 86 (68–128), L-Arginine/L-Ornithine-ratio 0.57 (0.46–0.74) and GABR 0.43 (0.37–0.55) (Figures 1 and 2). L-Arginine was lower ($p < .001$) in patients than in controls, whereas ADMA and SDMA were higher ($p < .003$ and $p < .001$, respectively), resulting in a markedly lower L-Arginine/ADMA-ratio in patients ($p < .001$).

There was a trend ($p = ns$) towards higher plasma concentration of SDMA, as well as lower L-Arginine/ADMA-ratio, GABR and L-Arginine/Ornithine-ratio in patients with, compared to those without, pre-operative PH (Table 3).

Four weeks after HT there was an increase in the plasma concentration of; L-Arginine to 83 (65–101) μM ($p < .008$), L-Arginine/ADMA-ratio to 164 (105–189) ($p < .006$), L-Arginine/L-Ornithine-ratio to 0.89 (0.82–1.08) ($p < .05$) and GABR to 0.75 (0.65–0.85) ($p < .05$), compared to prior to HT. All concentrations remained stable ($p = ns$) at the six month evaluation. After HT, L-Arginine was in line with healthy controls ($p = ns$), whereas L-Arginine/ADMA, although improved, remained decreased ($p < .001$). There was no change in ADMA, SDMA, L-Ornithine and L-Citrulline levels after HT ($p = ns$, Figures 1 and 2).

Hemodynamic characteristics in relation to heart transplantation

Hemodynamics prior to HT, as well as four weeks and six months thereafter, are shown in Table 4. Prior to HT, MPAP was 35 (27–42) mmHg, PAWP 25 (20–30) mmHg, TPG 9 (6–12) mmHg, DPG 1.0 (–0.8–4.8) mmHg, MRAP 15 (12–19) mmHg, CO 3.4 (2.6–4.3) L·min⁻¹ and PVR 1.9 (1.9–3.0) WU.

Table 2. Characteristics of the patients four weeks and six months after HT.

Parameter	Four weeks		Six months	
	n	median (IQR)	n	median (IQR)
Clinical features				
Weight, kg	12	80 (71–92)	12	79 (67–93)
BSA, m ²	12	2.0 (1.9–2.1)	12	2.0 (1.8–2.1)
LVEF, low/normal	12	0/12	11	0/11
Pulmonary hypertension, yes/no	12	0/12	12	0/12
Acute cellular rejection, yes/no	12	3/9	12	2/10
ACR = grade 1A/1B		3		2
ACR ≥ grade 2		0		0
Biochemical indicators				
S-Urea, mmol·L ⁻¹	12	13 (9–16)	12	8 (7–11)
P-Creatinine, μM·L ⁻¹	12	115 (97–148)	12	102 (94–160)
eGFR, mL·min ⁻¹ ·1.73 m ⁻²	12	39 (27–58)	12	N/A
Medications				
		n (%)		n (%)
Betablockers	12	4 (33)	12	7 (58)
ACEI/ARB	12	2 (17)	12	0/4 (33)
MRA	12	2 (17)	12	–
Anticoagulants	12	11 (92)	12	8 (67)
Statins	12	5 (42)	12	5 (42)
Diuretics	12	10 (83)	12	6 (50)
Insulin	12	4 (33)	12	5 (42)
Steroids	12	12 (100)	12	12 (100)
Tacrolimus	12	10 (83)	12	10 (83)
Cyclosporine	12	2 (17)	12	2 (17)
Mycophenolate mofetil	12	12 (100)	12	10 (83)
Mycophenolate sodium	12	–	12	2 (17)
Everolimus	12	–	12	1 (8)

IQR: interquartile range; n: individuals where the specific parameter was available; BSA: body surface area; LVEF: left ventricular ejection fraction; ACR: acute cellular rejection; ACEI: angiotensin converting enzyme inhibitor; ARB: angiotensin II receptor blocker; MRA: mineralocorticoid receptor antagonist; N/A: not assessed.

Four weeks after HT there was an increase in CO to 6.0 (5.6–6.6) L·min⁻¹ ($p < .001$), as well as a decrease in MPAP to 17 (12–21) mmHg ($p < .05$), PAWP to 8 (5–11) mmHg ($p < .001$) and PVR to 1.3 (1.1–1.5) WU ($p < .05$), compared to prior to HT, remaining stable ($p = ns$) at the six month evaluation. Six months after HT MRAP decreased to 1.5 (0.0–4.0) mmHg ($p < .05$), as compared to prior to HT. There was no change ($p = ns$) in DPG and TPG after, compared to prior to, HT. None of the patients had post-operative PH (Tables 2 and 4).

In a univariate analysis prior to HT there was a modest correlation between GABR and creatinine ($R^2: 0.37, p < .04$). Six months after HT, there was an inverse correlation between PVR and the L-Arginine/ADMA-ratio ($R^2: 0.39, p < .03$) as well as GABR ($R^2: 0.40, p < .03$) (Figure 3(a,b)). There were no other significant correlations, neither at the pre-operative nor post-operative evaluations, between the L-Arginine/ADMA-ratio or GABR and the hemodynamic parameters, NT-proBNP, creatinine or urea (Supplemental table 1). Moreover, neither the plasma levels of L-Arginine nor ADMA correlated to PVR, or any of the other investigated hemodynamic parameters, after HT ($p = ns$). In contrast, there was a strong correlation between the L-Arginine/ADMA ratio and aspartate aminotransferase (ASAT) and glutamyl transferase (GT), as well as between GABR and ASAT, GT and alanine aminotransferase (ALAT) (Supplemental table 1). However, these correlations were primarily due to the correlation between L-Arginine and the transferases, whereas no correlation was observed between ADMA and these markers.

Discussion

In the present study, we investigated the plasma concentrations of substances related to the NO pathway in HF-patients prior to and after HT, without post-operative PH and rejections above 1R, as well as with normal left heart function after HT. In support of previous findings, we found impaired L-Arginine, ADMA and L-Arginine/ADMA-ratio prior to HT. Whereas L-Arginine greatly improved, to levels comparable to healthy individuals, already four weeks after HT, ADMA, L-Citrulline and L-Ornithine remained unchanged. Consequently, the L-Arginine/ADMA-ratio was improved, but not fully normalized. The L-Arginine/ADMA-ratio was furthermore inversely correlated to PVR, suggesting a partly restored NO-dependent endothelial function. Based on the good post-operative outcome, without severe complications, our findings indicate the “normal” plasma concentrations of these biomarkers after HT.

The 12 patients included in the present study had severe HF with low CO and high filling pressures as well as severely deranged biomarkers prior to HT. They presented with significantly lower plasma concentrations of L-Arginine but higher ADMA and SDMA compared to healthy controls, as well as compared to patients with mild to moderate HF, recently reported by our group [15]. In fact, the plasma levels of the investigated biomarkers related to the NO pathway were, prior to HT, in line with those of patients with PAH, in our previous report by Sandqvist et al. [15]. These findings may represent the progressive nature of HF, with worsening endothelial function and congestion of the

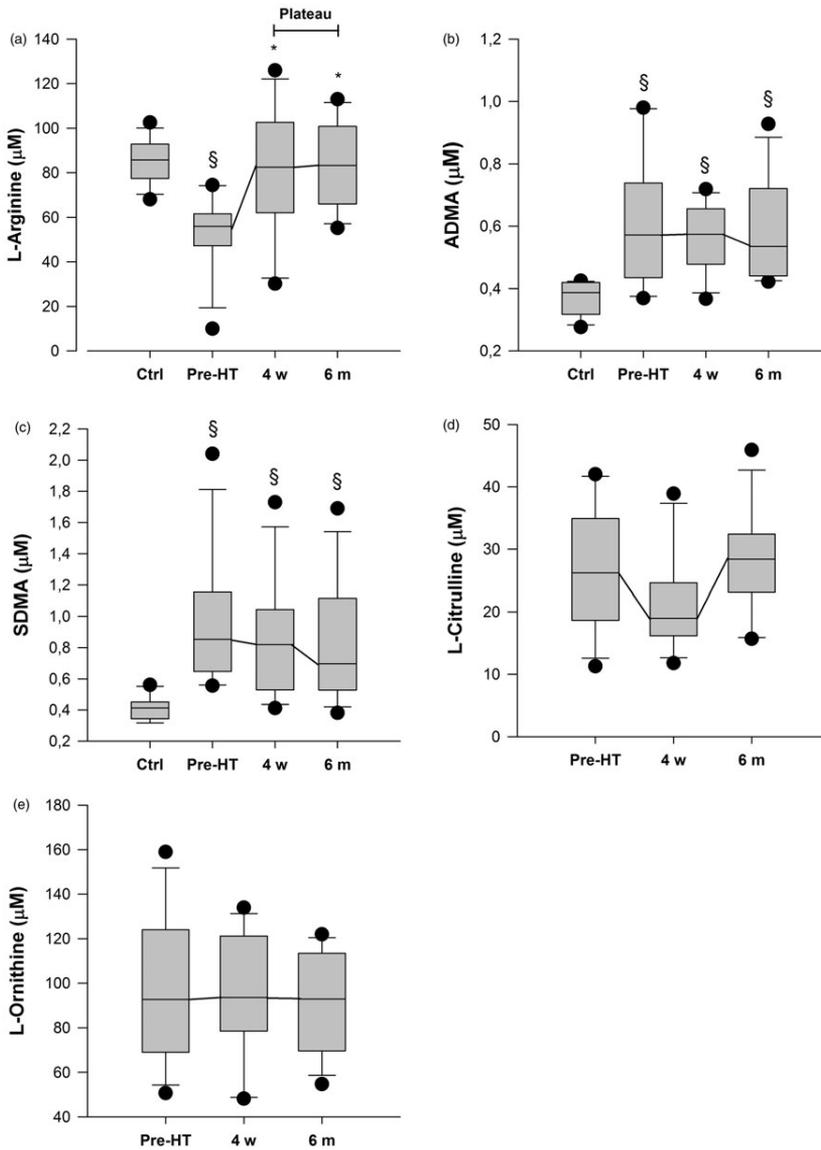


Figure 1. Characteristics of biomarkers related to the nitric oxide pathway prior to and after HT. (a) L-Arginine, (b) ADMA = asymmetric dimethylarginine, (c) SDMA = symmetric dimethylarginine, (d) L-Citrulline, and (e) L-Ornithine. *indicates statistical significance compared to prior to HT. §indicates statistical significance compared to controls.

pulmonary circulation. Similar results have been observed in other studies, where ADMA has been shown to correlate to NYHA functional class [8,9,18] and pulmonary pressures [19]. Of the patients included in our study, nine had PH. There was furthermore a non-significant trend

towards a worse level of several biomarkers related to the NO pathway in these patients, compared to those without PH. Larger studies are encouraged to define whether the biomarkers in fact differ between HF patients with and without PH.

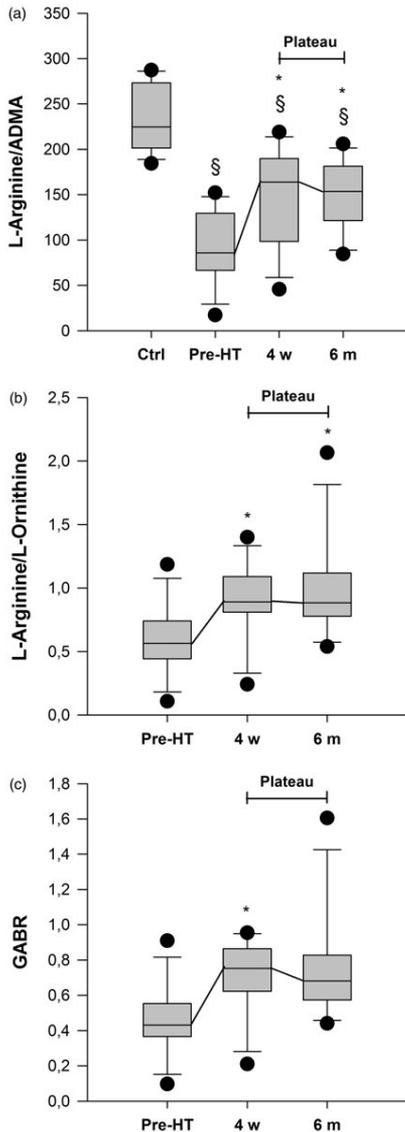


Figure 2. Characteristics of biomarker-ratios related to the nitric oxide pathway prior to and after HT. (a) L-Arginine/ADMA-ratio, (b) L-Arginine/L-Ornithine-ratio, and (c) GABR = global arginine bioavailability-ratio. *indicates statistical significance compared to prior to HT. §indicates statistical significance compared to controls.

It is well established that the bioavailability of NOS is limited by the availability of L-Arginine, despite L-Arginine in plasma being manifold above K_m for NO generation by NOS. This contradiction is referred to as the arginine

Table 3. Biomarker-levels of patients with and without PH at baseline.

Biomarker	PH pre-HT		No PH pre-HT	
	n	median (IQR)	n	median (IQR)
L-Arginine, μM	9	56 (47–59)	3	49 (47–68)
ADMA, μM	9	0.58 (0.50–0.82)	3	0.57 (0.43–0.63)
SDMA, μM	9	1.03 (0.77–1.19)	3	0.61 (0.58–0.85)
L-Citrulline, μM	9	26 (19–32)	3	27 (18–38)
L-Ornithine, μM	9	92 (66–131)	3	94 (79–98)
L-Arginine/ADMA	9	77 (63–128)	3	126 (86–129)
L-Arginine/L-Ornithine	9	0.52 (0.41–0.76)	3	0.66 (0.54–0.72)
GABR	9	0.40 (0.34–0.57)	3	0.52 (0.42–0.54)

IQR: interquartile range; n: individuals where the specific parameter was available; ADMA: asymmetric dimethylarginine; SDMA: symmetric dimethylarginine; GABR: global arginine bioavailability ratio.

paradox [20] and has been attributed to ADMA, which competes with L-Arginine for NOS [20]. Therefore, the activity of NOS and NO production increases with higher concentrations of the substrate [21]. The L-Arginine/ADMA-ratio has consequently been suggested to give a more correct insight into NOS activity, NO production and endothelial function, than L-Arginine and ADMA separately, as it reflects the relationship between the substrate and inhibitor [22]. The L-Arginine/ADMA-ratio has indeed been shown to be associated with disease severity [18] as well as mortality [23] in HF. Moreover, low GABR, representing the relationship between substrate and products, was recently found to be associated with impaired outcome in chronic HF [24]. In the present study, we found a marked improvement in the plasma concentration of L-Arginine after HT whereas ADMA remained high. Consequently, the L-Arginine/ADMA-ratio remained low, suggesting that endothelial function, although improved, is still partly impaired during the first six months after HT. The fact that the endothelial function is only partly restored after HT is also supported by that the L-Arginine/ADMA-ratio as well as the GABR shows a moderate inverse correlation with PVR six months after HT. This observation suggests that these ratios may reflect pulmonary vascular tone after HT. In contrast, neither L-Arginine nor ADMA correlated to post-operative hemodynamics and does not seem to reflect vascular tone after HT. Furthermore, as L-Arginine is converted to NO and L-Citrulline, altered NO production may be reflected in changes in L-Citrulline levels. In the present study no alterations in plasma L-Citrulline levels were observed after HT and as NO and cGMP-levels were not measured, we do not provide a clear explanation to these findings. However, circulating L-Citrulline is primarily released from the intestines and not from the endothelium. It is possible that a potential increase in the release of L-Citrulline from the endothelium is not sufficient to significantly alter plasma L-Citrulline levels. Also, with improved cardiac output and possibly reduced inflammation after HT it is a reasonable assumption that the sensitivity for NO in the vasculature is increased, resulting in partly restored NO mediated vasodilation irrespective of NO production.

A possible explanation for the improved plasma levels of L-Arginine after HT is decreased arginase activity. Arginase catalyses the formation of urea and ornithine from L-Arginine and has been shown to be elevated in HF [12].

Table 4. Selected hemodynamic parameters at baseline and during follow-up.

Hemodynamic parameter	Pre-HT		Four weeks post-HT		Six months post-HT	
	n	median (IQR)	n	median (IQR)	n	median (IQR)
MPAP, mmHg	12	35 (27–42)	12	17 (12–21)*	12	15 (12–18)*
DPG, mmHg	11	1.0 (–0.8–4.8)	11	2.0 (0.3–5.0)	12	3.0 (0.0–5.0)
TPG, mmHg	11	9 (6–12)	12	7.5 (6.0–9.0)	12	8.0 (6.0–9.5)
PAWP, mmHg	11	25 (20–30)	12	8 (5–11)*	12	5.5 (4.0–8.5)*
MRAP, mmHg	12	15 (12–19)	12	4.0 (3.0–6.0)	12	1.5 (0.0–4.0)*
CO, L·min ⁻¹	12	3.4 (2.6–4.3)	12	6.0 (5.6–6.6)*	12	5.6 (5.1–5.8)*
PVR, WU	11	2.4 (1.9–3.0)	12	1.3 (1.1–1.5)*	12	1.4 (1.1–1.7)

IQR: interquartile range; n: individuals where the specific parameter was available; MPAP: mean pulmonary artery pressure; DPG: diastolic pressure gradient; TPG: transpulmonary gradient; PAWP: pulmonary artery wedge pressure; MRAP: mean right atrial pressure; CO: cardiac output; PVR: pulmonary vascular resistance.

*indicates statistical significant difference ($p < .05$) compared to prior to HT.

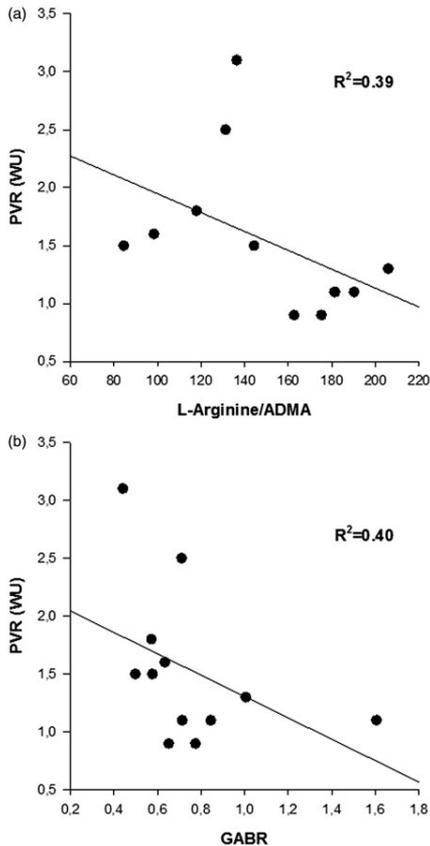


Figure 3. (a) Correlation between the L-Arginine/ADMA-ratio and PVR six months after HT. (b) Correlation between GABR and PVR six months after HT. GABR: global arginine bioavailability ratio; PVR: pulmonary vascular resistance.

Arginase increases during oxidative stress and infections [25] and as it competes with NOS for L-Arginine it can cause “uncoupling” of NOS, leading, in sequence, to increased superoxide production, diminished NO production

and endothelial dysfunction [25]. In our material, the L-Arginine/Ornithine-ratio, which can be used to assess arginase activity, increased after HT. However, the increase was solely due to increase in plasma concentration of L-Arginine and it is unlikely that arginase, to a greater extent, is affected by HT. The present study does moreover not provide a solid explanation to the persistently elevated ADMA levels after HT. Considering the low plasma concentrations of L-Arginine prior to HT, L-Arginine is an appealing therapy in HF. However, despite the strong rationale for such treatment, long-term investigations have been inconclusive [26,27]. It is possible that the lack of effect in these trials is due to that L-Arginine levels do not represent endothelial function. This is supported by the present study, in which correlations between post-operative hemodynamics and L-Arginine were lacking. Based on our findings, with low pre-operative plasma levels of L-Arginine, in combination with the high plasma levels of ADMA and decreased L-Arginine/ADMA-ratio throughout the study, where the L-Arginine/ADMA-ratio indeed reflected post-HT pulmonary vascular tone, it is likely that NO production is reduced both prior to and after HT. Consequently, drugs targeting the NO pathway independent of NO, such as soluble guanylate cyclase (sGC) stimulators, may be more beneficial than L-Arginine as well as NO dependent substances. Indeed, in patients with systolic HF, a phase II trial with the novel sGC-stimulator vericiguat recently demonstrated positive effects on NT-proBNP levels in a dose-dependent manner [28], supporting this hypothesis. Future studies may elucidate the potential of such a therapeutic approach.

Limitations

A major limitation of the present study is the small sample size. However, the size is in line with previous pilot studies in HT which has stimulated the initiation of larger trials. Moreover, in previous studies [29] we have, in larger HT-populations from our hospital, shown similar pre-operative and post-operative hemodynamics as in the present group, suggesting that the current findings can be applied in larger populations. Furthermore, we did not measure NO and cGMP levels so we cannot be certain that the production is decreased. However, the findings of decreased L-Arginine/ADMA-ratio throughout our study, in combination with the

fact that the ratio reflect pulmonary vascular tone, points in the direction of NO dependent endothelial dysfunction prior to and after HT. Finally, medical therapies prior to and after HT were individually adjusted. As some of these medications, such as betablockers [24], ACEi and ARB [30] have been shown to decrease circulating ADMA levels, diminished use of these medications after HT could, in theory, increase plasma levels of ADMA and the results in the present study must consequently be interpreted with this as a precaution. However, the use of these drugs represent a cornerstone in heart failure therapy and a true clinical situation so our findings are relevant to generate hypotheses for future, larger, trials.

Conclusions

The present study shows that plasma concentrations of L-Arginine, by an unknown mechanism, normalizes after HT. However, as ADMA plasma levels are unaltered, the L-Arginine/ADMA-ratio remains low and correlates inversely to PVR. This suggests that the L-Arginine/ADMA-ratio reflects pulmonary vascular tone after HT and that NO-dependent endothelial function is post-operatively improved, yet not normalized. Moreover, considering the good post-operative outcome without complications, our results could represent normal plasma magnitudes of the investigated substances after HT and the plasma derived L-Arginine/ADMA-ratio may therefore be used to assess pulmonary vascular tone after HT. Finally, based on the low L-Arginine/ADMA-ratio prior to and after HT, it is reasonable to conclude that, if targeting the NO pathway in HF, treatment with NO independent vasoactive substances may be more beneficial than NO dependent drugs. Larger trials on this subject are therefore encouraged.

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Dr. Wikström is, and has been primary-, or co-, investigator in; clinical PAH trials for GlaxoSmithKline, Actelion Pharmaceuticals Sweden AB, Pfizer, Bayer Healthcare and United Therapeutics, and in clinical heart failure trials for Novartis. Dr. Rådegran is, and has been primary-, or co-, investigator in; clinical PAH trials for GlaxoSmithKline, Actelion Pharmaceuticals Sweden AB, Pfizer, Bayer Healthcare and United Therapeutics, and in clinical heart transplantation immuno-suppression trials for Novartis.

Adjunct prof. Hedeland and prof. Bondesson report no conflicts of interest.

Since the completion of the study and initial submission of the manuscript, PhD Sandqvist has started working at Actelion Pharmaceuticals Sweden AB. The company has no role in data collection, analysis, interpretation of data or publishing of the manuscript.

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REVIEW

Pathophysiology and potential treatments of pulmonary hypertension due to systolic left heart failure**J. Lundgren^{1,2} and G. Rådegran^{1,2}**¹ The Haemodynamic Laboratory, The Clinic for Heart Failure and Valvular Disease, Skåne University Hospital, Lund, Sweden² Department of Cardiology, Clinical Sciences, Lund University, Lund, Sweden

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Abstract

Pulmonary hypertension (PH) due to left heart failure is becoming increasingly prevalent and is associated with poor outcome. The precise pathophysiological mechanisms behind PH due to left heart failure are, however, still unclear. In its early course, PH is caused by increased left ventricular filling pressures, without pulmonary vessel abnormalities. Conventional treatment for heart failure may partly reverse such passive PH by optimizing left ventricular function. However, if increased pulmonary pressures persist, endothelial damage, excessive vasoconstriction and structural changes in the pulmonary vasculature may occur. There is, at present, no recommended medical treatment for this active component of PH due to left heart failure. However, as the vascular changes in PH due to left heart failure may be similar to those in pulmonary arterial hypertension (PAH), a selected group of these patients may benefit from PAH treatment targeting the endothelin, nitric oxide or prostacyclin pathways. Such potent pulmonary vasodilators could, however, be detrimental in patients with left heart failure without pulmonary vascular pathology, as selective pulmonary vasodilatation may lead to further congestion in the pulmonary circuit, resulting in pulmonary oedema. The use of PAH therapies is therefore currently not recommended and would require the selection of suitable patients based on the underlying causes of the disease and careful monitoring of their progress. The present review focuses on the following: (i) the pathophysiology behind PH resulting from systolic left heart failure, and (ii) the current evidence for medical treatment of this condition, especially the role of PAH-targeted therapies in systolic left heart failure.

Keywords left heart failure, pulmonary arterial hypertension, pulmonary hypertension, therapy.

According to the classification presented at the Fifth World Symposium on PH (WSPH) in Nice 2013, pulmonary hypertension (PH) is divided into five groups: (i) pulmonary arterial hypertension (PAH), (ii) PH due to left heart disease, (iii) PH due to lung disease and/or hypoxaemia, (iv) chronic thromboembolic pulmonary hypertension and (v) PH with unclear and/

or multifactorial mechanisms. Group 1 also includes the subgroups 1'. pulmonary veno-occlusive disease and/or pulmonary capillary haemangiomatosis and 1''. persistent PH of the newborn (Simonneau *et al.* 2013). Group 2 is defined as post-capillary PH, while the others are defined as pre-capillary PH, depending on the location of the pathological changes (Galie *et al.* 2009).

Left heart disease is the most common cause of PH and is mainly caused by left heart systolic or diastolic dysfunction (Ghio *et al.* 2001, Lam *et al.* 2009) but also, to a lesser extent, by valvular heart disease (Simonneau *et al.* 2013). The pathophysiology behind PH due to left heart disease is not completely understood. Furthermore, there are no specific therapies for the pulmonary component of PH due to left heart disease, other than optimizing left ventricular and valvular function (Galie *et al.* 2009). This is of importance as PH is a negative prognostic marker in left heart disease (Lucas *et al.* 2000, Gheorghide *et al.* 2006).

Pulmonary hypertension is diagnosed by right heart catheterization and defined by a mean pulmonary artery pressure (MPAP) ≥ 25 mmHg. It is subcategorized into pre- or post-capillary PH depending on whether the pulmonary artery wedge pressure (PAWP), previously also called pulmonary capillary wedge pressure or pulmonary artery occlusion pressure, is ≤ 15 or > 15 mmHg, respectively, at normal or reduced cardiac output (Galie *et al.* 2009) (Fig. 1a). Depending on the definition used, post-capillary PH is further subcategorized according to the levels of the transpulmonary gradient (TPG) (Galie *et al.* 2009) (Fig. 1a) and/or pulmonary vascular resistance (PVR) (Fang *et al.* 2012) (Fig. 1b). According to the ESC guidelines, post-capillary PH may then be divided into passive or reactive post-capillary PH (Fig. 1a) (Galie *et al.* 2009) or, according to the International Society for Heart and Lung Transplantation (ISHLT) state-of-the-art summary statement, into passive or mixed post-capillary PH (Fig. 1b) (Fang *et al.* 2012). The mixed group may furthermore be subclassified into reactive or non-reactive post-capillary PH, based on the response to a vasodilatation test.

Recently, the diastolic pressure difference (DPD), calculated by subtracting PAWP from the diastolic pulmonary artery pressure, was introduced as a new means of subclassifying post-capillary PH. DPD has been suggested to identify pulmonary vascular disease in left heart failure (LHF) more correctly than TPG, as diastolic pulmonary artery pressure is less flow dependent than MPAP (Naeije *et al.* 2013). In support of this, Gerges *et al.* found that a $DPD \geq 7$ mmHg indicated severe pulmonary vascular disease and increased mortality in patients with PH due to LHF (Fig. 1c) (Gerges *et al.* 2013). DPD has consequently been suggested as a new important parameter in the definition of PH, based on the discussions at the Fifth WSPH (Fig. 1d) (Vachieri *et al.* 2013).

Concerning the lack of therapies for PH due to LHF, it has been debated whether PAH-targeted therapies could be used to treat this condition. However, selective dilation of the pulmonary vessels in patients with impaired left ventricular function, without simultaneous

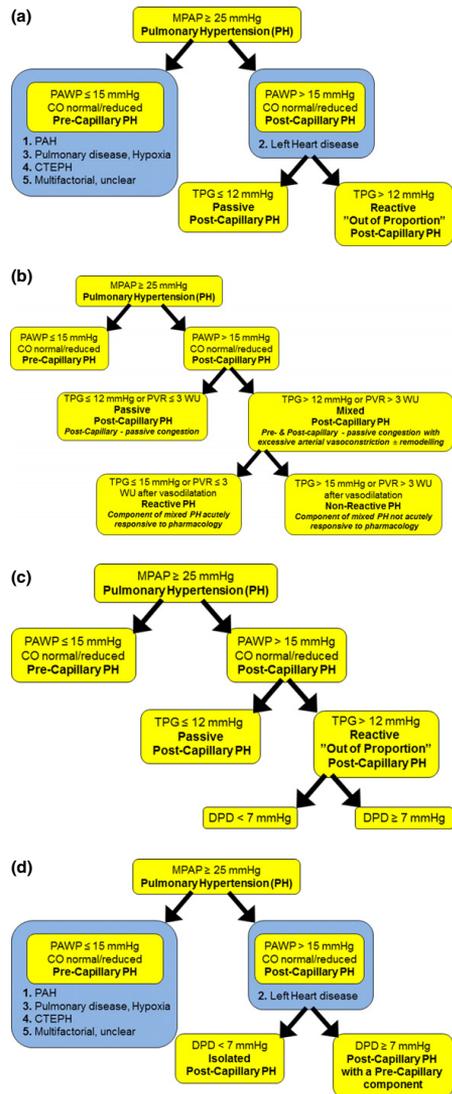


Figure 1 (a) Haemodynamic definition of pulmonary hypertension (PH) according to the ESC guidelines, (b) Haemodynamic definition of pulmonary hypertension (PH) according to the ISHLT state-of-the-art summary statement, (c) Haemodynamic definition of PH according to Gerges *et al.*, (d) Haemodynamic definition suggested after the Fifth World Symposium on PH. MPAP, mean pulmonary artery pressure; PAWP, pulmonary artery wedge pressure; CO, cardiac output; TPG, transpulmonary gradient; PAH, pulmonary arterial hypertension; CTEPH, chronic thromboembolic pulmonary hypertension; DPD, diastolic pressure difference; PVR, pulmonary vascular resistance; LV, left ventricular.

unloading of the left ventricle, could be detrimental, causing further congestion of the pulmonary veins (Haywood *et al.* 1992, Loh *et al.* 1994, Michelakis *et al.* 2002). This congestion may, in turn, lead to pulmonary oedema and rapid worsening of the patient's condition (Packer *et al.* 2005). As a consequence, PAH-targeted therapies have not been recommended for the treatment of patients with LHF, with or without PH. However, as it has been suggested that the changes observed in the pulmonary vessels, in persisting PH due to LHF, may be similar to those in PAH, selected patients with excessive vasoconstriction with or without secondary vascular remodelling could, potentially, benefit from PAH-targeted therapies.

The present review focuses on the pathophysiology and pathogenesis of PH due to LHF related to systolic dysfunction, as well as the existing knowledge on and potential role of PAH-targeted therapies for this patient population.

Pathophysiology of pulmonary hypertension

Pulmonary arterial hypertension

During the past thirty years, considerable effort has been devoted to clarifying the cause of PAH. PAH is a rare, but complex and serious disease, and if not treated, the survival has been reported to be as low as approx. 1–2.8 years, depending on its underlying cause (D'Alonzo *et al.* 1991). It has been suggested that PAH is triggered by an imbalance between vasodilative and vasoconstrictive factors, causing excessive pulmonary arteriolar vasoconstriction (Schermuly *et al.* 2011). This initial vasoconstriction, in combination with other, unknown, factors, is thought to lead to vascular remodelling of the vessel layers of the pulmonary arteries (Schermuly *et al.* 2011). Smooth muscle cell proliferation, inflammation and the development of plexiform lesions are additional hallmarks of PAH (Schermuly *et al.* 2011). Together with *in situ* thrombosis, this remodelling results in a further reduction in the size of the arterial lumen (Schermuly *et al.* 2011). As PAH progresses, the influence of vascular remodelling seems to become the most prominent feature of the disease, leading to a further increase in PVR and increased load on the right ventricle. This, in turn, leads to right ventricular hypertrophy. However, at a certain threshold, the right ventricle fails to cope with the increasing pressure and begins to dilate, leading to syncope and, ultimately, death (Rubin 1997).

Pulmonary hypertension due to left heart disease

The precise pathophysiology of PH due to LHF is unclear and is probably multifactorial. In the acute

setting, LHF leads to increased pressures in the pulmonary veins and capillaries. This venous PH is due to passive congestion in the pulmonary vessels, caused by the increased left ventricular filling pressures (Drazner *et al.* 1999). The increased pressures in the pulmonary capillaries result in increased stress on the alveolar capillary wall, potentially causing acute vascular wall damage and subsequent oedema (Kurdak *et al.* 1995, West & Mathieu-Costello 1995). Initially, the damage is reversible, but if it persists, it may lead to irreversible, structural changes and remodelling, causing the walls of the alveolar capillary membrane to thicken, resulting in reduced diffusion capacity of the lungs (Guazzi 2008).

In addition to affecting the pulmonary capillaries, LHF may also lead to secondary increased pressure in the pulmonary arteries. Such passive PH is caused by persisting, increased left ventricular filling pressures and is seen as increased pulmonary artery, and wedge pressures. At this stage, however, TPG and PVR remain low (Galie *et al.* 2009, Fang *et al.* 2012) (Fig. 2). Moreover, if the increased pulmonary artery pressures persist over a long period, this may lead to endothelial damage, suggested to be the result of impaired Ca^{2+} signalling and cytoskeletal reorganization (Kerem *et al.* 2010). The endothelial damage in turn causes an imbalance between vasoactive substances, resulting in impaired smooth muscle relaxation and, consequently, vasoconstriction (Cooper *et al.* 1998, Ooi *et al.* 2002). In LHF, as in PAH, nitric oxide (NO)-dependent vasodilation is impaired, and endothelin-1 levels may be increased (Cody *et al.* 1992, Tsutamoto *et al.* 1994, Cooper *et al.* 1998, Ooi *et al.* 2002). Such active PH is illustrated by increased TPG and/or PVR, in addition to the increase in pulmonary artery and wedge pressures (Fig. 2). The ESC guidelines refer to this as reactive PH (Galie *et al.* 2009) (Fig. 1a), whereas ISHLT state-of-the-art summary statement (Fang *et al.* 2012) refers to it as mixed PH (Fig. 1b). Such excessive vasoconstriction may initially be reversible with an acute vasodilatation test, but long-standing vasoconstriction may become so-called fixed, that is, not acutely reversible, due to changes in the pulmonary vasculature (Rich & Rabinovitch 2008) (Fig. 2). The vascular changes that occur include intimal thickening and fibrosis as well as medial hypertrophy and may be similar to, but not precisely the same as, those seen in PAH (Rich & Rabinovitch 2008) (Galie *et al.* 2009). For instance, plexiform lesions are generally not seen in PH due to LHF.

The reasons for the lack of knowledge on the pathophysiology of, and the lack of therapy for, PH due to systolic LHF are many. One may be the lack of good animal models for chronic heart failure with

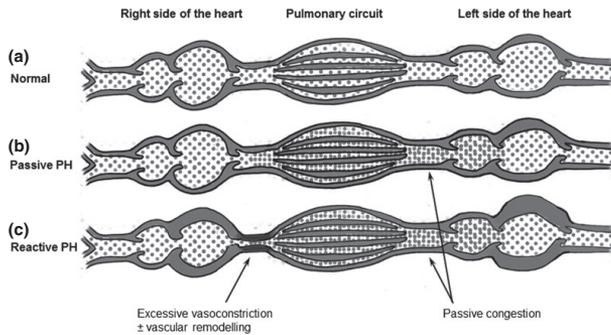


Figure 2 The progression of disease in PH due to LHF: (a) normal condition. (b) Passive pulmonary hypertension – Passive congestion of the pulmonary circuit due to increased left ventricular filling pressures, rendering high pulmonary artery and wedge pressures. (c) Reactive pulmonary hypertension – Passive congestion with excessive vasoconstriction of the pulmonary arteries with or without vascular remodelling, rendering high pulmonary artery and wedge pressures in combination with high transpulmonary gradient and/or pulmonary vascular resistance. The passive congestion in (b) and (c) is illustrated by an increase in the density of the dots denoting blood cells. The excessive vasoconstriction and remodelling in (c) is illustrated by a narrower vascular lumen and darker vascular walls.

secondary PH. Another may be the complex and diffuse symptomatology of the disease, as well as the lack of non-invasive diagnostic options for PH, together leading to delayed diagnosis and a failure to follow the development of the disease. Therefore, to improve our understanding of PH due to systolic LHF and to devise suitable therapies, new animal models and non-invasive diagnostic tools are of importance.

Recently, a $\text{DPD} \geq 7$ mmHg in patients with reactive PH was suggested to indicate severe pulmonary vascular disease and increased mortality (Fig. 1c) (Gerges *et al.* 2013). Furthermore, the authors found that medial hypertrophy was more common in reactive PH with a $\text{DPD} \geq 7$ mmHg than in patients with passive PH as well as in patients with reactive PH with a $\text{DPD} < 7$ mmHg. This suggests that an increased $\text{DPD} \geq 7$ mmHg in left heart disease indicates pulmonary vascular disease, which has led to the suggestion that backward pressure transmission in PH due to left heart disease may be central in triggering myofibroblast proliferation (Gerges *et al.* 2013). This new insight into pulmonary vascular disease in left heart disease has resulted in DPD being included in the new definition of PH due to left heart disease. Based on the discussions at the Fifth WSPH, Vachieri *et al.* recently published a paper in which they urged that the term out of proportion PH should be abandoned (Vachieri *et al.* 2013). Instead, they suggested that PH due to left heart disease should be subdefined into isolated post-capillary PH and post-capillary PH with a pre-capillary component, depending on whether the DPD is < 7 mmHg or ≥ 7 mmHg (Fig. 1d) (Vachieri *et al.* 2013).

PAH-targeted therapies

Increased understanding of the mechanisms behind PAH has led to the development of several drugs targeting the endothelin, NO and prostacyclin pathways (Humbert *et al.* 2004). The available drugs include dual and single endothelin receptor antagonists (ERAs), such as bosentan and ambrisentan, respectively, phosphodiesterase-5 inhibitors (PDE5is), such as sildenafil and tadalafil, and prostacyclin analogues, such as flolan and treprostinil (Fig. 3). Soluble guanylate cyclase (sGC) stimulators are another group of drugs targeting the NO system, which has shown promising results (Ghofrani *et al.* 2013a,b) and will soon be available for PAH treatment (Fig. 3). A new, and more potent, dual ERA, with better tissue penetration and higher receptor affinity, macitentan (Pulido *et al.* 2013), will furthermore soon also be available for use. Finally, the oral prostacyclin analogue selexipag is being evaluated for use in treating PAH in the GRIPHON phase III trial (Clinicaltrials.gov NCT01106014). It has also been found that PAH-targeted therapies that dilate pulmonary arteries improve survival compared with untreated patients (D'Alonzo *et al.* 1991). These drugs may slow the progress of the disease and vascular remodelling, but none of them can stop the vicious circle of PAH which, sooner or later, leads to right heart failure and ultimately death due to increased pulmonary arterial pressures (Rubin 1997). Therefore, until new drugs are developed that can inhibit or restore the vascular remodelling resulting from PAH, lung transplantation will remain the ultimate therapy of choice.

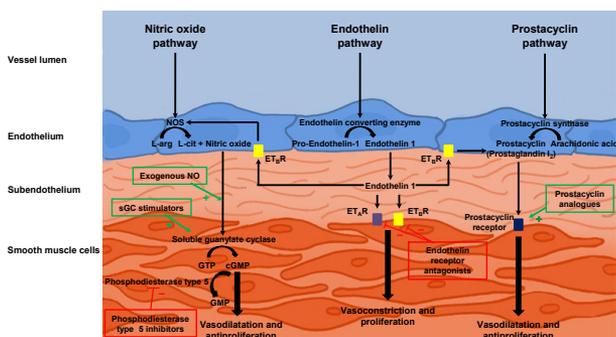


Figure 3 Therapeutic pathways in PAH. NOS, nitric oxide synthase; L-arg, L-arginin; L-cit, L-citrulline; NO, nitric oxide; sGC, soluble guanylate cyclase; ET_A R, endothelin receptor A; ET_B R, endothelin receptor B; GTP, guanosine triphosphate; cGMP, cyclic guanosine monophosphate.

The treatment of PH due to LHF

Although PH is common in LHF, and some vascular changes seen in this condition may be similar to those in PAH, there is no specific therapy for the pulmonary component of PH due to LHF, other than optimizing the function of the left heart (Galie *et al.* 2009). Conventional medical therapy for systolic LHF, including beta-blockers, angiotensin-converting enzyme inhibitors and/or angiotensin receptor blockers, mineralocorticoid receptor antagonists, I_f (funny current) inhibitor (ivabradine), digoxin and diuretics (McMurray *et al.* 2012), may reduce the PH caused by passive congestion, by optimizing the ventricular function of the heart. However, they are not thought to affect the excessive vasoconstriction or remodelling of the pulmonary vasculature seen in reactive PH. Therefore, it is of great importance to develop and evaluate new therapies for PH due to LHF, directly related to the pulmonary vascular component. This is further strengthened by the fact that PH in LHF is a marker of disease severity and a suggested risk factor for early mortality after heart transplantation (Mehra *et al.* 2006). However, in a recent study, our group found that with improved medical therapies and mechanical assist devices, the pre-operative magnitudes of the haemodynamic parameters that best predict outcome after heart transplantation remained unclear (Lundgren *et al.* 2014). Although pre-operative PH may be associated with poorer prognosis after heart transplantation, it appears that, with the diagnostic methods currently used, and when PH is defined at current magnitudes, it is difficult to differentiate between patients with excessive vasoconstriction without vascular remodelling and those with remodelling. Therefore, it is difficult to decide which patients are eligible for heart transplantation. If the assumptions regarding DPD are correct, and that $DPD \geq 7$ mmHg indeed indicates severe pulmonary vascular disease in patients with PH due to LHF, this parameter could possibly

help in the future selection of patients suitable for heart transplantation.

It has been suggested that PAH-targeted therapies could be of value in selected cases of PH due to LHF. However, as previously mentioned, selective pulmonary vasodilation, in patients with LHF, may lead to increased left ventricular filling pressures (Haywood *et al.* 1992, Loh *et al.* 1994, Michelakis *et al.* 2002) and the worsening of heart failure. For this reason, and the lack of appropriate studies, the use of such therapies has not been recommended. It is, however, possible that PAH-targeted therapies could prove valuable in PH patients with excessive vasoconstriction of the pulmonary arteries, with or without secondary vascular remodelling.

Left ventricular assist device

Patients with heart failure can remain stable for a relatively long time due to improvements in medical therapy (McMurray *et al.* 2012). Consequently, the number of patients suffering from end-stage heart failure and secondary PH has increased, illustrating the need for therapies that also target the pulmonary component of the disease. Left ventricular assist devices (LVADs) may be useful in this context. In addition to improving LHF, unloading of the left ventricle with a LVAD for a few months has also been shown to reverse passive and reactive PH (John *et al.* 2010, Nair *et al.* 2010, Kutty *et al.* 2013). These findings were recently confirmed by our group, in patients who received a LVAD as a bridge to heart transplantation (Lundgren *et al.* 2014). We found that the use of a LVAD (mean 77 days) decreased MPAP, PAWP and mean right atrial pressure and increased cardiac output, resulting in normal values (Fig. 4) (Lundgren *et al.* 2014). Long-term use of LVADs may, furthermore, reverse PH in patients who are not responsive to vasodilatation in the acute phase (Gallagher *et al.* 1991, Smedira *et al.* 1996). However, LVAD implan-

tation is an invasive procedure associated with potential complications related to surgery, driveline infections, gastrointestinal bleeding and thrombosis (Felix *et al.* 2012). Therefore, it is also important to develop new drugs directly targeting the PH component of LHF. Furthermore, in the 12 patients investigated in our study, we did not observe a decrease in PVR as a result of LVAD therapy (Fig. 4). In contrast, a reduction in PVR was observed in 23 patients following acute vasodilatation (Fig. 5) (Lundgren *et al.* 2014), suggesting that medical vasodilatation therapy, alone or in combination with a LVAD, could prove useful in treating PH due to LHF. Several studies have, in fact, investigated the effects of PDE5i and ERAs in left heart disease without (Sutsch *et al.* 1998, Katz *et al.* 2000, Cotter *et al.* 2001, Louis *et al.* 2001, Schalcher *et al.* 2001, Torre-Amione *et al.* 2001a,b, Coletta *et al.* 2002, Luscher *et al.* 2002, Schofield *et al.* 2003, Anand *et al.* 2004, Guazzi *et al.* 2004, 2007, 2009, 2010, 2011, Hirata *et al.* 2005, Packer *et al.* 2005,

Lewis *et al.* 2007a, McMurray *et al.* 2007, Behling *et al.* 2008, De Santo *et al.* 2008, Boffini *et al.* 2009, Amin *et al.* 2013) and with (Angel Gomez-Sanchez *et al.* 2004, Gomez-Moreno *et al.* 2005, Jabbour *et al.* 2007, Lewis *et al.* 2007b, Maruszewski *et al.* 2007, Zakliczynski *et al.* 2007, Kaluski *et al.* 2008, Tedford *et al.* 2008, De Santo *et al.* 2012, Guazzi *et al.* 2012, Hefke *et al.* 2012, Pons *et al.* 2012, Potter *et al.* 2012, Imamura *et al.* 2013b, Perez-Villa *et al.* 2013, Reichenbach *et al.* 2013) secondary PH. These studies are discussed and illustrated in the text and tables below (Tables 1–3).

Phosphodiesterase-5 inhibition

Studies with the PDE5i sildenafil have generally shown positive results in patients with LHF, with or without PH. The effects seem to be short term (Schofield *et al.* 2003, Angel Gomez-Sanchez *et al.* 2004, Guazzi *et al.* 2004, Hirata *et al.* 2005, Lepore *et al.* 2005, Lewis

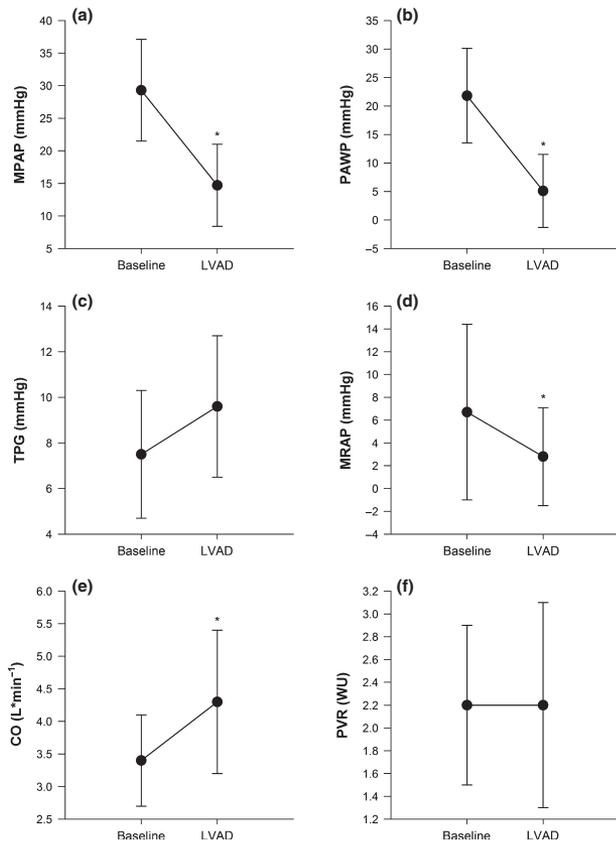


Figure 4 Haemodynamic response to LVAD therapy with regards to: (a) MPAP, mean pulmonary artery pressure; (b) PAWP, pulmonary artery wedge pressure; (c) TPG, transpulmonary gradient; (d) MRAP, mean right atrial pressure; (e) CO, cardiac output; (f) PVR, pulmonary vascular resistance; LVAD, left ventricular assist device. *indicates a statistically significant difference compared with baseline measurements.

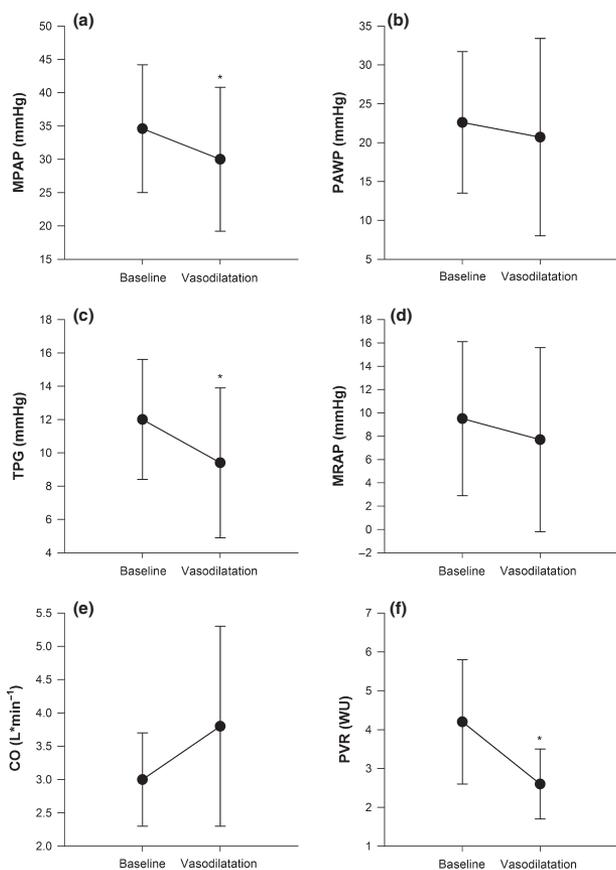


Figure 5 Haemodynamic response to acute vasodilatation during right heart catheterization with regards to: (a) MPAP, mean pulmonary artery pressure; (b) PAWP, pulmonary artery wedge pressure; (c) TPG, transpulmonary gradient; (d) MRAP, mean right atrial pressure; (e) CO, cardiac output; (f) PVR, pulmonary vascular resistance. *indicates a statistically significant difference compared with baseline measurements.

et al. 2007a, Freitas *et al.* 2012, Imamura *et al.* 2013a), as well as long term (Gomez-Moreno *et al.* 2005, Guazzi *et al.* 2007, 2009, 2010, 2011, 2012, Jabbour *et al.* 2007, Lewis *et al.* 2007b, Maruszewski *et al.* 2007, Zakliczynski *et al.* 2007, Behling *et al.* 2008, De Santo *et al.* 2008, 2012, Tedford *et al.* 2008, Boffini *et al.* 2009, Pons *et al.* 2012, Potter *et al.* 2012, Amin *et al.* 2013, Reichenbach *et al.* 2013), and systemic as well as pulmonary (Table 1a, b). Furthermore, PDE5 inhibition does not seem to cause any major adverse events (Katz *et al.* 2000, Schofield *et al.* 2003, Angel Gomez-Sanchez *et al.* 2004, Guazzi *et al.* 2004, 2007, 2009, 2010, 2011, 2012, Gomez-Moreno *et al.* 2005, Hirata *et al.* 2005, Lepore *et al.* 2005, Mogollon *et al.* 2006, Jabbour *et al.* 2007, Lewis *et al.* 2007a,b, Maruszewski *et al.* 2007, Zakliczynski *et al.* 2007, Behling *et al.* 2008, De Santo *et al.* 2008, 2012, Tedford *et al.* 2008, Boffini *et al.* 2009, Perez-Villa *et al.* 2010, Al-Hiti *et al.* 2011, Freitas *et al.* 2012, Pons *et al.* 2012,

Potter *et al.* 2012, Amin *et al.* 2013, Imamura *et al.* 2013a, Reichenbach *et al.* 2013). Initial studies involving sildenafil investigated the short-term effects of a single dose of sildenafil in patients with LHF, but not necessarily PH (Katz *et al.* 2000, Guazzi *et al.* 2004). Sildenafil was found to improve brachial flow-mediated vasodilatation, pulmonary and systemic haemodynamics, diffusion capacity, peak oxygen uptake and work capacity, among other parameters. Moreover, sildenafil therapy was not associated with any serious adverse events. Later studies have included the long-term effects of sildenafil in patients with PH due to LHF (Jabbour *et al.* 2007, Lewis *et al.* 2007b, Zakliczynski *et al.* 2007), confirming the positive results obtained by the earlier studies. In 2008, Tedford and colleagues presented a study in which patients with persisting PVR > 3 WU after LVAD implantation were given sildenafil for 15 weeks (Tedford *et al.* 2008). PVR and right ventricular function were improved, making 19 of 26 patients eli-

Table 1 (a) Previous studies (2000–2007) with sildenafil in patients with LHF with or without PH; (b) Previous studies (2008–2013) with sildenafil in patients with LHF with or without PH

Design, author and year	Patient population	PH inclusion criteria	Outcome
(a)			
Randomized 12.5–50 mg single dose: Katz <i>et al.</i> 2000	Stable NYHA II-III for 3 months ($n = 48$) LVEF<40% Optimized HF therapy for 4 weeks	No	25 and 50 mg of sildenafil increased brachial flow-mediated vasodilatation and arterial occlusion No cases of hypotension.
Observational 50 mg single dose: Schofield <i>et al.</i> 2003	HT ($n = 15$ male, mean 38 months post-HT) Hypertension therapy	No	Improved BP, aortic augmentation index and LV stress. No case of hypotension.
Randomized 50 mg single dose: Guazzi <i>et al.</i> 2004	NYHA II-III ($n = 16$ male) LVEF<40% FEV ₁ /FVC>70%	No	Improved MPAP, PVR, DL _{CO} , D _M , peak VO ₂ , recovery tau, brachial reactive hyperaemia, Δ VO ₂ / Δ WR, exercise capacity and ventilation efficiency. The effects did not remain after 24 h.
Observational 100 mg single sublingual dose: Gómez-Sánchez <i>et al.</i> 2004	NYHA III-IV ($n = 7$) Optimal HF therapy	Yes; TPG>12 mmHg and PVR>2.5 WU	Decreased MPAP, TPG and PVR.
Case report 25 mg t.i.d. (4 months): Gomez-Moreno <i>et al.</i> 2005	NYHA III-IV ($n = 1$) LVEF<15%	Yes; MPAP 32 mmHg, PVR 5 WU	PH was partly reversed and the patient accepted for HT.
Randomized 50 mg single dose: Hirata <i>et al.</i> 2005	NYHA II-III ($n = 20$) LVEF<35%	No	Acutely improved cardiac performance.
Randomized 50 mg b.i.d. (6 months): Guazzi <i>et al.</i> 2007	NYHA class II-III ($n = 46$ male<65 years) LVEF<45% FEV ₁ /FVC>70% sAP 110–140	No	Improved sPAP, ergoreflex effect on ventilation, brachial artery flow-mediated dilatation, exercise performance, ventilation efficiency and QoL after 3 months. No further improvement after 6 months.
Randomized 25–75 mg t.i.d. (12 week): Lewis <i>et al.</i> 2007	NYHA II-IV ($n = 34$) LVEF<40% Standard HF therapy.	Yes; MPAP>25 mmHg	Improved peak VO ₂ , 6MWD, QoL, PVR, PVR/SVR, RVEF, CO and SV. Fewer hospitalizations.
Observational 25–50 mg t.i.d. (68 ± 58 days): Jabbour <i>et al.</i> 2007	Severe DCM ($n = 6$).	Yes; Irreversible TPG>15 mmHg	Improved TPG, PAWP, PVR and CO. 4 patients listed for HT. Three patients had undergone successful HT at the end of follow-up.
Observational 50 mg b.i.d. (1 month): Zakliczynski <i>et al.</i> 2007	NYHA III-IV ($n = 6$ male) LVEF<25%	Yes; Irreversible TPG>12 mmHg and/or PVR>2.5 WU	Normal, or reversible, TPG and PVR in 5 patients, subsequently listed for HT.
Observational 50 mg single dose: Lewis <i>et al.</i> 2007	Stable NYHA III ($n = 13$; 3 months) LVEF<35%	No	Improved exercise capacity, ventilation efficiency, MPAP, CI, PVR, RVEF and PVR/SVR in PH patients ($n = 7$). No improvement in patients without PH ($n = 6$).
Observational 25–50 mg t.i.d.: Maruszewski <i>et al.</i> 2007	HT ($n = 6$)	Yes; TPG>12 and/or PVR>2.5	4 of 6 patients survived the early post-operative period.
(b)			
Observational 3 mg·kg ⁻¹ – 250 mg·day ⁻¹ (1 month): De Santo <i>et al.</i> 2008	Acute RVD after HT ($n = 13$)	No	RVD resolved within 72 h in all patients. 12 patients survived the post-operative period.
Observational 25–75 mg t.i.d. (15 weeks): Tedford <i>et al.</i> 2008	Persistent PH 7–14 days after LVAD ($n = 26$) Normalized PAWP	Yes; PVR>3 WU	Improved PVR and RV function. Nineteen patients considered eligible for HT. Ten underwent HT at the end of follow-up. 1 developed acute RVE.

Table I Continued

Design, author and year	Patient population	PH inclusion criteria	Outcome
Randomized 50 mg t.i.d. (4 weeks): Behling <i>et al.</i> 2008	LVEF \leq 40% ($n = 19$) Standard HF therapy	No	Improved functional capacity, ventilator efficiency, oxygen uptake and sPAP.
Observational 25 mg t.i.d. (6 months): Guazzi <i>et al.</i> 2009	HF ($n = 40$ male) Optimal medical therapy	No	Improved heart rate recovery.
Observational 12.5 mg b.i.d. – 50 mg t.i.d. (approx. 1 months): Boffini <i>et al.</i> 2009	RVD after HT ($n = 10$)	No	Improved sPAP 1 month after HT. Other haemodynamic parameters did not differ with sildenafil therapy.
Observational 50 mg t.i.d. (3 months): Guazzi <i>et al.</i> 2010	HF ($n = 40$ male) Optimal medical therapy	No	Improved sPAP, PVR, exercise performance, ventilation efficiency and QoL.
Randomized 50 mg t.i.d. (1 year): Guazzi <i>et al.</i> 2011	Stable NYHA II-III ($n = 45$ male; 6 months) LVEF $<$ 40% FEV ₁ /FVC $>$ 70% LV diastolic dysfunction	No	Improved LAVI, LVEDV, LVMI, LVEF, E', exercise performance, ventilation efficiency, NT-proBNP and QoL after 6 months. No further improvement after 1 year of therapy.
Randomized 50 mg t.i.d. (1 year): Guazzi <i>et al.</i> 2012	NYHA III-IV ($n = 32$ male) Exercise oscillatory breathing	Yes; MPAP 25–35 mmHg	Reversed EOB in 15 of 16 patients. Improved functional performance, exercise ventilation efficiency, QoL, MPAP, PAWP and PVR.
Observational 20–60 mg t.i.d. (prior to, and after, HT): Pons <i>et al.</i> 2012	High risk prior to HT ($n = 15$)	Yes; irreversible PVR $>$ 2.5 WU and/or TPG $>$ 12 mmHg	All patients underwent successful HT. Survival was similar to patients without PH ($n = 104$).
Observational 102.5 \pm 54 mg·day ⁻¹ (mean 260 days): Potter <i>et al.</i> 2012	On HT waiting list ($n = 16$)	Yes; significant PH reversibility	Improved sPAP and CI. Eight patients underwent successful HT. Two patients were removed from HT waiting list due to improvement.
Observational 20–60 mg t.i.d.: Reichenbach <i>et al.</i> 2012	Advanced HF ($n = 47$) LVEF $<$ 40%	Yes; irreversible TPG $>$ 15 mmHg	Improved PVR, TPG, CO and NYHA class. Maintained bodyweight and improved 30-day survival after HT.
Observational 25–75 mg t.i.d. (12 weeks): De Santo <i>et al.</i> 2012	Stable NYHA III ($n = 32$; 3 months) LVEF $<$ 35% Maximal medical therapy	Yes; irreversible PVR $>$ 6 WU	Improved MPAP, PAWP, TPG and PVR. 31 patients underwent HT (some still at high risk according to ISHLT guidelines). 3 developed acute RHF. 1-year mortality was 6.5%.
Randomized 25 mg b.i.d. – 50 mg t.i.d. (12 weeks): Amin <i>et al.</i> 2013	Stable NYHA II- III ($n = 106$; 3 months) LVEF $<$ 35% on optimal HF therapy Primary endpoint: Change in MAP.	No	MAP was maintained. No improvement in 6MWD or hospitalization.

b.i.d., twice a day; BP, blood pressure; CI, cardiac index; CO, cardiac output; DCM, dilated cardiomyopathy; DL_{CO}, carbon monoxide diffusion capacity; D_M, alveolar capillary membrane conductance; E', mitral annulus early velocity; EOB, exercise oscillatory breathing; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; HF, heart failure; HT, heart transplantation; ISHLT, International Society of Heart and Lung Transplantation; LAVI, left atrial volume index; LVAD, left ventricular assist device; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; MAP, mean artery pressure; MPAP, mean pulmonary artery pressure; NYHA, New York Heart Association; PAWP, pulmonary artery wedge pressure; PH, pulmonary hypertension; PVR, pulmonary vascular resistance; QoL, quality of life; RVD, right ventricular dysfunction; RVF, right ventricular failure; recovery tau, recovery time constant for VO₂; RVEF, right ventricular ejection fraction; sAP, systolic artery pressure; sPAP, systolic pulmonary artery pressure; SV, stroke volume; SVR, systemic vascular resistance; t.i.d., three times a day; TPG, transpulmonary gradient; VO₂, oxygen uptake; WR, work rate; 6MWD, six-minute walking distance.

Table 2 (a) Previous studies on patients with LHF with or without PH, using bosentan; (b) Previous studies on patients with LHF with or without PH, using tezosentan or darusentan

Design, author and year	Patient population	PH inclusion criteria	Outcome
(a)			
Randomized 1 g b.i.d. (2 weeks): Sutsch <i>et al.</i> 1998	Stable NYHA III ($n = 36$ male) LVEF<30% Optimal conventional therapy PAWP \geq 15 mmHg and/or CI \leq 2.5 L \cdot min $^{-1}\cdot$ m $^{-2}$	No	Improved CO, MPAP, PAWP, MRAP, PVR. Reduced SVR. One case of symptomatic hypotension resulting in discontinuation of therapy.
REACH-1 500 mg b.i.d. target (6 months): 1998	NYHA IIIb-IV ($n = 370$) LVEF<35% Diuretic/ACEi therapy and Hospitalization for HF within the previous 12 months/ 6MWD<375 m. Primary endpoint: clinical status of the patients after 6 months.	No	Stopped prematurely due to increased hepatic transaminases. Less adverse events after 3 months, in patients who completed the study.
ENABLE 125 mg b.i.d. (18 months): 2002	NYHA IIIb-IV ($n = 1613$) LVEF<35% Optimal conventional therapy Primary endpoint: All-cause mortality or hospitalization.	No	No improvement in primary endpoint. Increased risk of worsening HF, likely due to fluid retention.
Randomized 8–125 mg b.i.d. (20 weeks): Kaluski <i>et al.</i> 2008	NYHA IIIb-IV ($n = 90$) LVEF<35% Hospitalized for worsening HF within 6 months prior to study. Primary endpoint: change in ECHO-measured sPAP.	Yes; sPAP \geq 40 mmHg	No difference in primary endpoint. Increased risk of adverse events.
Case report 125 once daily (3 months): Imamura <i>et al.</i> 2012	Progressing HF ($n = 1$ male) LVAD	Yes; persistent PH 1 month after LVAD implantation	PH was reversed.
Observational 125 mg b.i.d. target: Hefke <i>et al.</i> 2012	On HT waiting list ($n = 84$)	Yes; MPAP>35, TPG>15 and/or PVR>240 dyn \cdot s \cdot cm $^{-5}$	Improved HD and 1-year survival on HT waiting list. Independent predictor of reduced mortality on HT waiting list. Decreased haematocrit and in 3 patients ASAT and/or ALAT increased.
Observational 125 mg b.i.d. (4 months–1 year): Perez-Villa <i>et al.</i> 2013	HT candidate ($n = 24$)	Yes; irreversible PVR>2.5 WU	PH reversed in 13 patients after 4 months and in 19 patients after 12 months. 14 patients underwent HT. 1-year survival was 93% compared with 83% in 56 patients with PVR \leq 2.5 WU during the same period.
(b)			
Randomized tezosentan 5–100 mg h $^{-1}$: Cotter <i>et al.</i> 2001	NYHA III ($n = 38$) LVEF \leq 35% PAWP \geq 15 mmHg CI \leq 2.7 L \cdot min $^{-1}\cdot$ m $^{-2}$	No	Dose-dependent improvement in PAWP, PVR, cardiac power index and SVR index. No decrease in MAP.
Randomized 5, 20, 50 or 100 mg h $^{-1}$ (6 h): Torre-Amione <i>et al.</i> 2001	NYHA III-IV ($n = 61$, 19–70 years) LVEF<35% CI<2.5 L \cdot min $^{-1}\cdot$ m $^{-2}$ PAWP>18 mmHg	No	Dose-dependent improvement in MPAP, PAWP, CI, PVR and SVR. No case of symptomatic hypotension.
Randomized tezosentan 20 mg h $^{-1}$ or 50 mg h $^{-1}$ (48 h): Torre-Amione <i>et al.</i> 2001	NYHA III-IV ($n = 14$) LVEF<35%.	No	Improved ECHO sPAP, RAP, PAWP, CI and SV. No serious adverse events.

Table 2 Continued

Design, author and year	Patient population	PH inclusion criteria	Outcome
RITZ-2 tezosentan 50 or 100 mg·h ⁻¹ ; 2001	NYHA class III-IV (<i>n</i> = 184) CI < 2.5 L·min ⁻¹ ·m ⁻² PAWP > 15 mmHg	No	Improved CI, PAWP and dyspnea score. Less side effects with 50 mg·h ⁻¹ than 100 mg·h ⁻¹ .
Randomized tezosentan 5–100 mg·h ⁻¹ ; Schalcher <i>et al.</i> 2001	NYHA III (<i>n</i> = 38) LVEF < 35% PAWP > 15 mmHg CI < 2.7 L·min ⁻¹ ·m ⁻²		Dose-dependent increase in CI and stroke index. Decrease in PVR and SVR. No decrease in MAP. No serious adverse events.
VERITAS tezosentan 5 mg·h ⁻¹ for 30 min, followed by 1 mg·h ⁻¹ for 24–72 h; 2007	Hospitalized due to acute HF within the previous 24 h (<i>n</i> = 1448) Persistent dyspnea (RR > 24/min) Primary endpoints: change in dyspnea over 24 h and incidence of death or worsening of HF at 7 days.	No	Improved MPAP, MRAP, PAWP, PVR and SVR. No difference in primary endpoints. More adverse events, mainly hypotension.
HEAT darusentan 30, 100 or 300 mg·day ⁻¹ (3 weeks); 2002	NYHA class III (<i>n</i> = 157) for at least 3 months LVEF < 35% PAWP ≥ 12 mmHg CI < 2.6 L·min ⁻¹ ·m ⁻² Primary endpoint: 3-week change in CI and PAWP.	No	Increased CI, most pronounced in the 100 mg·day ⁻¹ group, and decreased SVR. More adverse events.
EARTH darusentan 10, 25, 50, 100 or 300 mg·day ⁻¹ (24 weeks); 2004	NYHA II-IV (<i>n</i> = 642) LVEF < 35%.	No	No improvement in primary endpoint. No beneficial effects on clinical outcomes.

b.i.d., twice a day; CI, cardiac index; CO, cardiac output; HD, haemodynamic; HF, heart failure; HT, heart transplantation; LVAD, left ventricular assist device; LVEF, left ventricular ejection fraction; MAP, mean arterial pressure; MPAP, mean pulmonary artery pressure; MRAP, mean right atrial pressure; NYHA, New York Heart Association; PAWP, pulmonary artery wedge pressure; PH, pulmonary hypertension; PVR, pulmonary vascular resistance; RAP, right atrial pressure; RR, respiratory rate; sPAP, systolic pulmonary artery pressure; SV, stroke volume; SVR, systemic vascular resistance; TPG, transpulmonary gradient; 6MWD, six-minute walking distance.

gible for heart transplantation (Tedford *et al.* 2008). However, these results must be interpreted with caution, as sildenafil therapy was initiated only 7–14 days after LVAD implantation. In the light of the current knowledge on LVAD therapy, it is likely that a substantial part of the PH was reversed by left ventricle unloading by the LVAD (Gallagher *et al.* 1991, Smedira *et al.* 1996). Finally, in 2012, three studies on patients with irreversible PH were published (De Santo *et al.* 2012, Pons *et al.* 2012, Reichenbach *et al.* 2013). Two of these showed that sildenafil was effective in reversing PH previously defined as irreversible (De Santo *et al.* 2012, Reichenbach *et al.* 2013), and all three showed that heart transplantation could be performed without an increased risk of acute right heart failure or early mortality.

It could be hypothesized that the positive results observed with sildenafil in patients with LHF, without and with PH, are due to the dilatation of pulmonary

as well as systemic vessels. Such vasodilation would result in unloading of both the left and right ventricle, decreasing the risk of further pulmonary congestion. PDE5is could thus provide a new, promising therapy in patients with LHF, without or with, PH. It is notable that no further haemodynamic improvement was observed in any of the published studies beyond 3 months of therapy, resulting in a plateau phase thereafter. Finally, as no large, multicentre, placebo-controlled trials involving the treatment of LHF using sildenafil have been published, further research is necessary.

Dual endothelin receptor blockade

The dual ERA bosentan has been found to improve pulmonary as well as systemic haemodynamics in patients with LHF (Sutsch *et al.* 1998). However, the two randomized, multicentre, placebo-controlled trials

Table 3 Previous studies on patients with LHF with or without PH, using multiple drugs

Design, author and year	Patient population	PH inclusion criteria	Outcome
Case report sildenafil 75 mg·day ⁻¹ (12 months) and bosentan 62.5 mg·day ⁻¹ (6 months): Mogollón <i>et al.</i> 2006	Dilated, alcohol-induced cardiomyopathy (<i>n</i> = 1 male) LVEF<20%	Yes; irreversible MPAP 59 mmHg and PVR 6.4 WU	No HD improvement after 6 months of sildenafil therapy. Normalized MPAP, PAWP, TPG, CO and PVR after 6 months of combination therapy. Underwent HT without RHF.
Observational sildenafil 20–80 mg t.i.d. or bosentan 62.5–125 mg b.i.d. (16 weeks): Perez-Villa <i>et al.</i> 2010	Ineligible for HT (<i>n</i> = 18)	Yes; irreversible PVR>2.5 WU	Greater reduction in PVR with bosentan. Improved sPAP, dPAP and TPG with bosentan. 12 patients (4 sildenafil, 8 bosentan) underwent successful HT.
Observational sildenafil 100 mg single dose or nitroprusside 1–2 µg·kg ⁻¹ ·min ⁻¹ : Freitas <i>et al.</i> 2012	NYHA III-IV (<i>n</i> = 29) LVEF<45% Formal indication of HT	No	Reduced PH, maintained MAP and improved left and right cardiac functions, pO ₂ and SvO ₂ with sildenafil. Improved left and right cardiac functions and decreased MAP, pO ₂ and SvO ₂ with nitroprusside. No improvement in PH.
Observational NO 5–30 ppm or iloprost 50 µg: Sablotzki <i>et al.</i> 2002	Dilated or ischaemic DCM (<i>n</i> = 14 male)	Yes; PVR≥180 dyn·s ⁻¹ ·cm ⁻⁵	Decreased MPAP and PVR with both drugs. Increased CI and SVI with iloprost. Greater decrease in MPAP with iloprost.
Observational NO 5–40 ppm or eprostenol 1–12 ng·kg ⁻¹ ·min ⁻¹ : Mogollón Jimenez <i>et al.</i> 2008,	Pre-HT evaluation (<i>n</i> = 54)	Yes; MPAP>35 mmHg or PVR>4 WU	Improved MPAP, PVR and CO with eprostenol. NO did not improve HD.
Randomized NO 40–80 ppm and PGE ₁ 0.05–0.5 µg·kg ⁻¹ ·min ⁻¹ (crossover): Radovancevic <i>et al.</i> 2005	Potential HT candidates (<i>n</i> = 19) Optimal medical therapy	Yes; sPAP>60 mmHg, TPG>12 mmHg or PVR>4 WU	TPG and PVR decreased with both drugs. MAP decreased with PGE ₁ . PH was reversed in 6 patients with both drugs, in 4 with PGE ₁ only and in 4 with NO only.
Observational sildenafil 40 mg or PGE ₁ 200 ng·kg ⁻¹ ·min ⁻¹ : Al-Hiti <i>et al.</i> 2011	Potential HT candidates (<i>n</i> = 13)	Yes; TPG>15 mmHg or PVR>3 WU	Greater decrease in TPG and PVR with sildenafil. Similar decrease in MAP, MPAP and PAWP and similar increase in CI with both drugs. Decreased PVR/SVR ratio with sildenafil. More patients with PVR acceptable for HT with sildenafil.
Observational sildenafil 50 mg single dose and NO 80 ppm: Lepore <i>et al.</i> 2005	NYHA III-IV (<i>n</i> = 11) LVEF<40%	Yes; MPAP>25 mmHg	Improved CI, PVR, SVR and PVR/SVR ratio with combination therapy compared with single therapy with either agent. No case of hypotension.
Case report sildenafil 20 mg single dose or NO 20 ppm for 10 min: Imamura <i>et al.</i> 2013	DCM (<i>n</i> = 1) LVEF<28%	Yes; MPAP 44 mmHg and PVR 5.96 WU	Decreased PVR with both drugs. Decreased PAWP with sildenafil, increased PAWP with NO.

b.i.d., twice a day; CI, cardiac index; CO, cardiac output; DCM, dilated cardiomyopathy; dPAP, diastolic pulmonary artery pressure; HD, haemodynamic; HT, heart transplantation; LVEF, left ventricular ejection fraction; MAP, mean arterial pressure; MPAP, mean pulmonary artery pressure; NO, nitric oxide; NYHA, New York Heart Association; PAWP, pulmonary artery wedge pressure; PH, pulmonary hypertension; pO₂, oxygen partial pressure; PVR, pulmonary vascular resistance; RHF, right heart failure; sPAP, systolic pulmonary arterial pressure, SVI, stroke volume index; SvO₂, venous oxygen saturation; SVR, systemic vascular resistance; t.i.d., three times a day; TPG, transpulmonary gradient.

REACH-1 (Packer *et al.* 2005) and ENABLE (Coletta *et al.* 2002) failed to meet their primary endpoints: clinical status after 6 months and all-cause mortality or hospitalization respectively. Bosentan was instead found to increase the risk of adverse events such as hepatic transaminase elevation and fluid retention

(Coletta *et al.* 2002, Packer *et al.* 2005). In fact, the REACH-1 trial was terminated early due to increased hepatic transaminases in the patients given bosentan (Packer *et al.* 2005). However, both these trials were performed in patients with LHF, but not necessarily with secondary PH (Table 2a). Similar findings have

been reported on the effects of tezosentan and darusentan in patients with LHF (Cotter *et al.* 2001, Louis *et al.* 2001, Schalcher *et al.* 2001, Torre-Amione *et al.* 2001a,b, Luscher *et al.* 2002, Anand *et al.* 2004, McMurray *et al.* 2007) (Table 2b).

A few studies on the use of bosentan in patients with PH due to LHF have on the other hand shown promising long-term effects (Hefke *et al.* 2012, Imamura *et al.* 2013b, Perez-Villa *et al.* 2013). Hefke and colleagues reported that bosentan was an independent predictor of reduced mortality in a group of 84 patients on the waiting list for heart transplantation (Hefke *et al.* 2012). Perez-Villa *et al.* reported that 12 months of bosentan therapy reversed previously defined irreversible PVR in 19 of 26 patients, making them eligible for heart transplantation (Perez-Villa *et al.* 2013). They also observed that one-year survival among the 14 patients who underwent heart transplantation was similar to that in the 56 patients who did not have increased PVR prior to heart transplantation (Perez-Villa *et al.* 2013) (Table 2a). One study in which PH was based on echocardiographic measurements and defined as sPAP \geq 40 mmHg is the only study in PH due to left heart disease not reporting positive results following bosentan therapy (Kaluski *et al.* 2008) (Table 2a). However, echocardiographic measurements of the tricuspid regurgitation and the gradient between the right ventricle and right atrium may be influenced by the echo-signal detected as well as changes in cardiac output. Moreover, the positive effects seen with bosentan in both small and large studies have been shown to increase with the length of therapy (Packer *et al.* 2005, Perez-Villa *et al.* 2013). Consequently, a 'plateau' similar to the one seen with sildenafil does not seem to occur with bosentan. Interestingly, sildenafil and bosentan have been compared in one case report (Mogollon *et al.* 2006) and a small study with 18 subjects (Perez-Villa *et al.* 2010). The results of both studies suggested that bosentan was more effective than sildenafil in reversing PH and in making patients eligible for heart transplantation (Mogollon *et al.* 2006, Perez-Villa *et al.* 2010) (Table 3).

The reason for the diverging results in the studies on bosentan therapy could be the pathophysiology of the patients' disease in combination with the pulmonary selectivity of bosentan. In the studies showing negative results, either PH was not used as an inclusion criterion, or the patients were included based on their pulmonary artery pressures, that is, passive PH. In contrast, the studies in which positive results were found all included patients with reactive PH, with increased PVR and/or TPG (Mogollon *et al.* 2006, Perez-Villa *et al.* 2010, 2013, Hefke *et al.* 2012, Imamura *et al.* 2013b). Based on these findings, it can

be hypothesized that if the PH is worsened by an active component, it may be partly reversed by selective pulmonary vasodilation, leading to unloading of the right ventricle. There is, however, also a risk of congestion in these patients, and they should thus be closely monitored.

Prostaglandins

Early studies were performed in the 1980s and 1990s on patients with LHF using prostacyclin and the prostacyclin analogue epoprostenol in patients with LHF. Yui *et al.* found improved short-term haemodynamics with prostacyclin (Yui *et al.* 1982), while Sueta *et al.* reported improved 6-min walking distance and left ventricular ejection fraction (LVEF) in a 12-week trial using epoprostenol (Sueta *et al.* 1995). The FIRST study using epoprostenol, in patients with LHF but not necessarily with PH, was, however, terminated early due to a strong tendency towards increased mortality in the group given the drug (Califf *et al.* 1997). Since then, only a few small studies have been performed on the effects of prostacyclin analogues during right heart catheterization (Weston *et al.* 2001, Sablotzki *et al.* 2002a,b, Mogollon Jimenez *et al.* 2008) (Tables 3 and 4). All of these studies demonstrated improvement in pulmonary haemodynamics together with an increase in cardiac output or cardiac index, depending on the parameter observed.

A few studies have also been carried out on the effects of the prostaglandin PGE₁ in PH due to LHF. In three of these studies, the short-term effects of PGE₁ infusion were investigated and found to improve the haemodynamics (Radovancevic *et al.* 2005, von Scheidt *et al.* 2006, Al-Hiti *et al.* 2011). However, in one study in which PGE₁ was compared with a single dose of sildenafil, it was found that sildenafil was more potent in reversing the PH, as well as being more pulmonary specific than PGE₁ (Al-Hiti *et al.* 2011). In 2011, Serra and colleagues presented a study on PGE₁ therapy in patients with severe LHF and increased sPAP based on echocardiographic measurements (Serra *et al.* 2011). PGE₁ was administered for three consecutive days each month for 36 months and was found to improve the New York Heart Association (NYHA) classification of heart failure and LVEF. Furthermore, the 36-month mortality was 27% in the PGE₁ group, compared with 44% in the controls (Serra *et al.* 2011). However, in this study, the PH was diagnosed by echocardiography and 25% of the patients were reported to have pre-capillary PH, and these results must thus be interpreted with caution. Considering the positive findings from these early studies, it would be interesting to perform similar

Table 4 Previous studies on patients with LHF with or without PH using prostaglandins

Design, Author and Year	Patient population	PH inclusion criteria	Outcome
Observational prostacyclin 22 ± 11 ng·kg ⁻¹ ·min ⁻¹ ; Yui <i>et al.</i> 1982	NYHA IV due to CAD (<i>n</i> = 9) Conventional medical therapy	No	Decreased MAP, PAWP, SVR and PVR. Increased HR, CI, SVI and plasma epinephrine. No serious adverse events.
Randomized epoprostenol 8.8 ± 3.5 ng·kg ⁻¹ ·min ⁻¹ (12 weeks); Sueta <i>et al.</i> 1995	NYHA III-IV (<i>n</i> = 33) LVEF ≤ 30% Conventional medical therapy	No	Improved 6MWD with epoprostenol compared with control. Improved LVEF. 3 patients withdrawn due to serious adverse events. 2 deaths in epoprostenol group and 8 in controls.
FIRST Epoprostenol 4 ng·kg ⁻¹ ·min ⁻¹ ; 1997	NYHA class IIIb-IV (<i>n</i> = 471) Low LVEF	No	Terminated early due to a strong trend towards increased mortality.
Observational iloprost 580 µg (mean) single dose; Weston <i>et al.</i> 2001	Pre-HT evaluation (<i>n</i> = 6)	Yes; irreversible sPAP > 50 mmHg, TPG > 15 mmHg or PVR > 3 WU	Improved sPAP, MPAP, PAWP, TPG, CO and PVR/SVR ratio. 4 patients underwent successful HT.
Observational iloprost inhal 50 µg; Sablotzki <i>et al.</i> 2002	HT candidates (<i>n</i> = 30)	Yes; increased PVR (206 ± 94 dyn·s ⁻¹ ·cm ⁻⁵)	Improved MPAP, PAWP, CI and PVRI.
PROPHET PGE ₁ 173 ± 115 ng·kg ⁻¹ ·min ⁻¹ ; 2006	Potential HT candidates (<i>n</i> = 92)	Yes; PVR > 2.5 WU or TPG > 12 mmHg	Decreased MAP, MPAP, PAWP, SVR and PVR. Increased CO. No case of symptomatic hypotension. 90 patients were accepted for HT (PVR < 4 WU).
Observational PGE ₁ 10 ng·kg ⁻¹ ·min ⁻¹ (mean) (3 consecutive days per month for 36 months); Serra <i>et al.</i> 2011	NYHA III-IV (<i>n</i> = 22) LVEF < 35%	Yes; ECHO- sPAP > 3 m·s ⁻¹	Improved sPAP, NYHA class and LVEF. One case of severe hypotension. 36-month mortality was 27% in PGE ₁ group vs. 44% in controls.

CAD, coronary artery disease; CI, cardiac index; CO, cardiac output; HR, heart rate; HT, heart transplantation; LVEF, left ventricular ejection fraction; MAP, mean arterial pressure; MPAP, mean pulmonary artery pressure; NYHA, New York Heart Association; PAWP, pulmonary artery wedge pressure; PH, pulmonary hypertension; PVR, pulmonary vascular resistance; sPAP, systolic pulmonary arterial pressure; SVI, stroke volume index; SVR, systemic vascular resistance; TPG, transpulmonary gradient; 6MWD, six-minute walking distance.

investigations in patients with post-capillary PH diagnosed by right heart catheterization.

Soluble guanylate cyclase stimulators

The novel sGC stimulator riociguat was recently evaluated in a phase IIb study in patients with PH and LHF, but not necessarily with increased filling pressures (Bonderman *et al.* 2013) (Table 5). The primary endpoint, that is, change in MPAP, was not met. However, several other parameters such as cardiac index, stroke volume index, PVR and the Minnesota Living with Heart Failure score improved as a result of sGC stimulation. Further studies on the effects of sGC stimulators in patients with PH due to LHF and increased left ventricular filling pressures, with more precise endpoints than MPAP, should be performed to further investigate the clinical implications of these

findings. This is of interest as sGC stimulators, in contrast to PDE5 inhibitors, work in both the presence and absence of NO. Therefore, therapy with sGC stimulators may prove more valuable in patients with impaired NO synthesis and signalling.

Future therapies

Recently, two randomized, placebo-controlled, multi-centre, trials were initiated in patients with PH due to LHF. The drugs planned for investigation were the PDE5s sildenafil (Cooper *et al.* 2013) and tadalafil (Clinicaltrials.gov NCT01910389), both acting on the NO pathway. The tadalafil-trial was recently terminated by the funding agency. However, the sildenafil-trial is still ongoing and if it is successful it could herald a new era in treating PH due to LHF (Table 6). In addition, a trial with a novel sGC stimulator

Table 5 Previous study on patients with PH due to LHF using sGC stimulation

Design, author and year	Patient population	PH inclusion criteria	Outcome
LEPHT riociguat 0.5–2 mg t.i.d. (16 weeks): 2013	LVEF \leq 40% ($n = 201$) Optimal medical therapy Primary endpoint: change in MPAP.	Yes; MPAP \geq 25 mmHg	No change in primary endpoint. Improved CI, SVI, PVR and Minnesota Living With Heart Failure score. No serious adverse events.

CI, cardiac index; LVEF, left ventricular ejection fraction; MPAP, mean pulmonary artery pressure; PH, pulmonary hypertension; PVR, pulmonary vascular resistance; SVI, stroke volume index; t.i.d., three times daily.

Table 6 Ongoing, or planned, trials with PAH targeted therapies in patients with LHF without or with PH

Drug	Study acronym	Patient population	PH as inclusion criteria
Sildenafil 40 mg t.i.d. (6 mo)	SilHF	NYHA II-III ($n = 210$) LVEF \leq 40% 6MWD < 400 m Optimal medical therapy Primary endpoint: Patient global assessment and 6MWD	Yes; sPAP \geq 40 mmHg
BAY1021189 (12 wk)	SOCRATES-REDUCED	LVEF \leq 45% ($n = 410$) Worsening chronic HF requiring hospitalization (or iv. diuretics) Primary endpoint: NT-proBNP	No
Macitentan 10 mg once daily (12 wk)	MELODY-1	Left ventricular dysfunction ($n \approx 60$) Optimized diuretic therapy Primary endpoint: Safety and tolerability	Yes; MPAP \geq 25 mmHg, PAWP > 15 mmHg and DPD > 7 mmHg

HF, heart failure; LVEF, left ventricular ejection fraction; mo, month; NYHA, New York heart association; PH, pulmonary hypertension; sPAP, systolic pulmonary artery pressure; wk, week; 6MWD, six minute walking distance.

(BAY1021189) is planned in patients with systolic LHF (Clinicaltrials.gov NCT01951625) (Table 6). It is also of interest to perform a similar study with the new, more potent ERA, macitentan, as smaller studies with bosentan have shown promising results in patients with reactive PH. In fact, a phase II trial with Macitentan, aiming to evaluate the safety and tolerability of the drug in patients with combined pre- and post-capillary PH (Vachery *et al.* 2013), is currently being initiated (Clinicaltrials.gov NCT02070991) (Table 6).

Conclusions

Pulmonary hypertension is common in LHF, and is a marker of disease severity. Early in its course, PH is caused by passive congestion in the pulmonary vessels due to elevated left ventricular filling pressures. If these elevated pulmonary pressures persist, endothelial damage may occur, leading to an active PH component. In its early stages, active PH is characterized by excessive vasoconstriction and may be reversible. However, long-standing PH, may not be acutely

reversible due to remodelling of the pulmonary vasculature. To date, there is no specific recommended therapy for the pulmonary component of PH due to LHF. However, studies suggest that some PAH-specific drugs could be beneficial. PDE5is in particular have shown encouraging results on studies in LHF patients with and without PH. Ongoing trials with PDE5i in PH due to LHF may clarify the potential of drugs targeting the NO pathway, and could pave the way for novel forms of treating PH due to LHF. In this light, it would also be of interest to evaluate the implications of findings from previous studies on sGC stimulation in a PH population with excessive vasoconstriction with or without vascular remodelling.

Recent studies also suggest that ERAs may be useful in patients with reactive PH, and complimentary clinical trials with ERAs would, therefore, be of interest in this specific patient population. Finally, although some PAH specific drugs may prove valuable in selected cases of PH due to LHF, it should be borne in mind that selective pulmonary vasodilation without simultaneous unloading of the left ventricle can lead to pulmonary oedema and worsening of heart failure.

Due to these potentially detrimental effects, careful patient selection and monitoring are required if selected LHF patients are to be treated with PAH-targeted therapies. At present, PAH-targeted therapies are therefore generally not recommended in PH due to LHF. However, further investigations are warranted in well controlled clinical settings and trials.

Conflict of interest

Mr. Lundgren reports an unrestricted research grant from The Swedish Society of Pulmonary Hypertension, and Dr. Rådegran reports unrestricted research grants from Anna-Lisa and Sven-Erik Lundgren's, Maggie Stephen's, ALF's, Skåne University Hospital's Foundations and Actelion Pharmaceuticals Sweden AB, during the conduct of the study.

Mr. Lundgren reports personal lecture fees from Actelion Pharmaceuticals Sweden AB and GlaxoSmithKline outside the submitted work. Dr. Rådegran reports personal lecture fees from Actelion Pharmaceuticals Sweden AB, GlaxoSmithKline and Sandoz/Novartis outside the submitted work.

Dr Rådegran is and has been primary-, or co-, investigator in clinical PAH trials for GlaxoSmithKline, Actelion Pharmaceuticals Sweden AB, Pfizer, Bayer and United Therapeutics, and in clinical heart transplantation immunosuppression trials for Novartis.

The companies have no role in the data collection, analysis and interpretation and have no right in disapproving of the manuscript.

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