

**Pulmonary Hypertension associated with
Congenital Systemic to Pulmonary shunts –
Aspects of Disease Monitoring**

PhD Thesis

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List of Papers

Henrik Brun, Erik Thaulow, Per Morten Fredriksen, Henrik Holmstrøm. Treatment of patients with Eisenmenger's syndrome with bosentan. *Cardiol Young* 2007; 17: 288-294.

Henrik Brun, Henrik Holmstrøm, Erik Thaulow, Jan Kristian Damås, Arne Yndestad, Pål Aukrust and Thor Ueland. Patients with pulmonary hypertension related to congenital systemic-to-pulmonary shunts are characterized by inflammation involving endothelial cell activation and platelet-mediated inflammation. *Congenit Heart Dis.* 2009;4:153-159.

Henrik Brun, Thor Ueland, Erik Thaulow, Jan K Damas, Arne Yndestad, Pal Aukrust and Henrik Holmstrøm. No Inflammatory response related to pulmonary hemodynamics in children with systemic to pulmonary shunts. *Congenit Heart Dis.* 2011;6:338-346.

Henrik Brun, Thomas Moller, Per M Fredriksen, Erik Thaulow, Are Hugo Pripp, Henrik Holmstrom. Mechanisms of exercise induced pulmonary hypertension in patients with cardiac septal defects *Pediatr Cardiol* 2012 Epub ahead of print DOI 10.1007/s00246-012-0216-9

Abbreviations

6MWT	Six minute walk test
ASD	atrial septal defect
AVSD	atrioventricular septal defect
A' peak	peak diastolic tissue Doppler velocity at atrial contraction
CHD-PAH	pulmonary hypertension associated with congenital heart disease
CRP	C-reactive protein
CSPS	Congenital Systemic to Pulmonary Shunt
E' peak	peak early diastolic tissue Doppler velocity
IPAH	idiopathic pulmonary arterial hypertension
MCP-1	macrophage chemoattractant protein 1
NTproBNP	N-terminal pro type B natriuretic peptide
NYHA	New York Heart Association
PAH	pulmonary arterial hypertension
PAH-CSPS	pulmonary arterial hypertension related to congenital systemic to pulmonary shunt
PAPVC	partial anomalous pulmonary venous connection
PDA	persistent ductus arteriosus
Qp/Qs	ratio between pulmonary and systemic blood flow
RANKL	receptor activator of nuclear factor kappa B ligand
Rp/Rs	ratio between pulmonary and systemic total vascular resistance
sCD40L	soluble CD 40 ligand
S' peak	peak systolic tissue Doppler velocity
SpO ₂	transcutaneous oxygen saturation by pulse oximetry
sTNFR I	soluble tumor necrosis factor receptor I
TAPSE	tricuspid annulus plane systolic excursion
TAPVC	totally anomalous pulmonary venous connection
TRAIL	tumor necrosis factor receptor activator inducing ligand
VSD	ventricular septal defect

WHO world health organization
vWf von Willebrand factor

1. Introduction

1.1 Sudden death during change of treatment of pulmonary arterial hypertension – the case history that started the project

During my pediatric cardiology trainee period, I was engaged in the treatment of a baby girl with a large primum ASD, presenting at 8 months, with crying spells leading to near syncope. She had poor feeding and weight gain, increased sweating, reduced activity and pulmonary artery pressures at systemic level. Eight weeks of oral sildenafil brought some symptomatic improvement but no reduction of tricuspid regurgitation peak jet velocity. Intravenous epoprostenol was started, replacing the insufficient sildenafil treatment. Lack of experience with combination therapy made this an unpredictable option. She was continuously monitored by ECG, SpO₂ plus frequent registration of vital signs. During the uptitrating of epoprostenol, she became more irritable and had slight desaturation during crying, accompanied twice by short bradycardias. She died suddenly on day four, when awake, just after the observing nurse had commented that her vitality was improving. There was no response to advanced heart-lung resuscitation. An exact death mechanism could not be established. The question was what mechanism of hemodynamic deterioration that had occurred without being detected by our monitoring tools. Based on history, blood tests, radiograms and autopsy findings, we concluded lung congestion with microatelectasis could have induced ventilation-perfusion mismatch following epoprostenol infusion, producing desaturations that she did not tolerate in a situation with low cardiac output. Rebound PAH crisis due to the termination of oral sildenafil was considered less probable, as it had been replaced by a very potent drug, although with a different mechanism of action. Reviewing possible mechanisms of sudden, unexpected death during change of pulmonary vasodilator drugs in this infant with ASD primum and out of proportion pulmonary hypertension, we concluded that improved monitoring options in patients with severe PAH-CSPS are required(1). This sad case history increased my respect for, and interest in the PAH diseases, and it also draws a thematic line through the present thesis:

The search for monitoring parameters and disease markers in acute and chronic PAH-CSPS

Valid and reliable biomarkers and endpoints in studies of pediatric PAH are highly requested(2). The European Society of Cardiology list of gaps of PH evidence mentions disease assessment as one of the main topics (www.escardio.org). The present thesis provides a review of current knowledge about PAH-CSPS monitoring tools, integrating the experiences from the five published papers.

1.2 The pulmonary circulation in pediatric cardiology

The normal, four-chambered heart serves two vascular circuits that normally become separate when the fetal shunts close after birth. When the neonatal adaptation is complete, these two vascular beds have striking differences regarding the nature of their pumping chambers, the pressure levels and resistances, vasoregulatory mechanisms, and the drugs that modify these. In adult cardiology, much attention has been directed to the systemic circulation and the left ventricle, in conditions like atherosclerosis and systemic arterial hypertension. In pediatric cardiology, on the contrary, pulmonary hemodynamics is an important part of the background for decision making, in a wide range of clinical situations. Postnatal pulmonary vascular adaptation is an important issue in almost all newborns with congenital heart disease.

1.2.1 The significance of pulmonary vascular disease in children with congenital systemic to pulmonary shunts

The most common congenital heart defects, the systemic to pulmonary shunts, such as, VSD, PDA and also ASD may produce pulmonary arterial hypertension if they are not repaired in time. Patients with these diagnoses are the focus of this thesis. Based on hemodynamic characteristics, these shunts can be divided into pretricuspid (ie ASD and P/TAPVC) and post-tricuspid (VSD, PDA, AP-window). The blood flow and pressure transmitted through such a defect, stretch the pulmonary vessel walls. These stretch stimuli induce vasoconstriction and subsequently, vascular wall thickening and lumen narrowing

(3;4). (Further details in chapter 1.3.1) At first, these are reversible changes, but a continued stimulus leads to irreversible PAH with time in many patients, but not in all. Genetic susceptibility for vascular changes probably plays a role, but predisposing mutations with effect on cell growth control have only been documented in a few patients(5). Granton et al found that 50 % of patients with a large VSD, 10 % of patients with ASD, as compared to 100 % of patients with truncus arteriosus will develop PAH if left untreated (6). In some patients with small or pre-tricuspid shunts, as in paper I, the increased flow stimulus is only a trigger of disease development, while PAH development seems to be out of proportion and driven by other mechanisms (7;8). Children with Down syndrome are, for reasons incompletely understood, predisposed to faster and more frequent development of irreversible PAH than non-Down patients(9). Without repair, the majority of patients with non-restrictive VSD develop increasing pulmonary vascular resistance due to wall changes, and finally reversal of the shunt. This condition is called Eisenmenger's syndrome. Patients with Eisenmenger's syndrome and simple cardiac lesions, such as ASD, VSD, PDA have a life expectancy reduction of about 20 years(10). Life quality is also affected by severely reduced physical performance. Serious complications occur frequently, like brain abscess or thromboembolic disease. (10;11). Only 15 years ago, patients with CSPS were generally operated at a higher age than today. Consequently, pulmonary vascular disease was established at the time of operation in many cases, and postoperative pulmonary hypertensive crises were not infrequent. With further development of surgery, anesthesia and heart-lung-machine technology, operations could be performed earlier, leaving less time for preoperative pulmonary vascular injury. Further, the introduction of inhaled nitric oxide has made patients with acute postoperative PH easier to handle, making death from PH rare in this setting. A few studies on the reversibility of vascular changes and timing of operation have set the standards for the current policy of timing for defect closure(12;13). The current clinical standard is to repair large, non-restrictive defects between 3 and 12 months of age, depending on control of heart failure (14). Earlier operations are undertaken in children with failure to thrive despite maximal drug treatment and in patients with risk factors, such as Down syndrome. The risk of permanent pulmonary vascular injury increases with age at operation. Closure of a non-

restrictive posttricuspid defect after 1 year of age, carries a higher risk of postoperative acute pulmonary hypertensive crises and irreversible PAH. The point of no return (irreversible disease), at which surgery is detrimental, probably differs with genetic predisposition. Some patients tolerate pulmonary vascular wall stress for a longer time. This difference is incompletely investigated.

So – is PAH of any significance in modern pediatric cardiology? Yes, the PAH-CSPS patients still occur, and some data indicate a higher prevalence than previously recognized at long term follow up(15). Even more frequent subclinical disease has been suggested(16).

1.2.2 Definitions

Pulmonary vascular (obstructive) disease is a histopathological term, and denotes any degree and permanency of thickening of the pulmonary vascular walls, distorted vascular structure and reduced number of small vessels, leading to intermittently or permanently increased pulmonary vascular resistance. It is a useful term in congenital heart disease, because some patients have no pulmonary pumping chamber (Fontan circulation) and low pulmonary artery pressures, but still a too high pulmonary vascular resistance.

Pulmonary hypertension is a hemodynamic term, defined by pulmonary artery pressure above 25 mmHg at rest, irrespective of the cause and vascular resistance. Thus, both pulmonary arteriolar obstruction, venous obstruction and increased flow and pressure through large post-tricuspid shunts can produce pulmonary hypertension. Earlier guidelines also included mean pulmonary artery pressure above 30 mmHg during exercise(17). This was excluded from the 2009 version, due to its lack of discrimination between health and disease(18).

Pulmonary arterial hypertension is a group of rare, usually progressive conditions, hemodynamically defined by a mean pulmonary artery pressure above 25 mmHg with a left atrial pressure/pulmonary wedge pressure below 15 mmHg. A pulmonary vascular resistance > 3 Wood units is also used as a criterion, but less emphasized in the presence of intracardiac shunts as these lesions make pulmonary vascular resistance measurements less reliable. In these situations, ratios of systemic to pulmonary flow and resistance

ratios (Q_p/Q_s and R_p/R_s) are often used, with R_p/R_s above 0.3-0.4 as a commonly applied cutoff.

Eisenmenger's syndrome is a clinical condition, first related to congenital heart disease in 1897(19). Its relation to pulmonary vascular resistance was described later by Paul Wood (20;21). Strictly, it is PAH due to a nonrestrictive congenital post-tricuspid shunt with reversed shunt flow, cyanosis and secondary erythrocytosis. Patients with surgically created shunts and univentricular conditions may develop similar pathophysiology. Most authors also include pre-tricuspid shunts, despite hemodynamic differences. Shunts between these low pressure chambers primarily relates to diastolic ventricular pressures.

Eisenmenger physiology is a hemodynamic term, which, in addition to classical Eisenmenger's syndrome, often includes patients with normal saturations at rest. These patients may desaturate during exercise only, and represent different points at a disease continuum between less advanced PAH-CSPS and Eisenmenger's syndrome.

1.2.3 WHO classification of pulmonary hypertension

The first WHO classification (1974) of PH with two main categories (primary and secondary) was completely restructured into five categories at the second world conference in Evian in 1998. Further revisions were accomplished in 2003 (17) and at Dana Point 2008, introducing a sixth group, pulmonary venoocclusive disease/capillary hemangiomatosis (see table below). This classification serves as the framework for the updated treatment guidelines from ERS/ESC(18) ACCF/AHA(22). Although supported by AEPC, the classification primarily holds an adult cardiology perspective and still has shortcomings with respect to the great variety of causes of pediatric pulmonary vascular disease. The PAH-CSPS subclassification has become more detailed (below), a problem being that the defect size cutoffs are not indexed for BSA. Recognizing that pediatric PH often is more complex in presentation and diagnosis than adult disease (23), a pure pediatric classification system was suggested by the Pulmonary Vascular Research Institute in 2011. This has ten main categories, listed according to their supposed importance (24).

WHO/ESC PH main groups (Dana Point -09)*

1. Pulmonary arterial hypertension (PAH)

PAH related to CSPA is one of several PAH subgroups

- 1' Pulmonary venoocclusive disease and pulmonary capillary hemangiomatosis
- 2. PH owing to left heart disease
- 3. PH owing to lung disease and/or chronic hypoxia
- 4. Chronic thromboembolic PH
- 5. PH from unclear multifactorial mechanisms

Subclassification of PAH related to CSPA

A. Eisenmenger's syndrome

Large defects with left to right shunt that has led to pulmonary vascular resistance increase. Patients have resting cyanosis, erythrocytosis and multiple organ disease. Cyanotic, large ASD included.

B. PAH associated with S-P shunts.

Moderate to large defects with mild to moderately increased pulmonary vascular resistance. Left to right shunt still largely present. No cyanosis at rest. ASDs included.

C. PAH with small defects.

VSD < 1 and ASD < 2 cm (applies for adults only) Clinical picture similar to IPAH

D. PAH after corrective surgery

No residual defect. PAH either present directly after surgery or recurred after years.

* Reference (17)

1.2.4 Epidemiology

A recent review estimated that worldwide, 3 million children are at risk of developing PAH related to CHD, the majority having a repairable heart defect such as ASD or VSD(25). Only 2-15 % of all patients with significant shunt lesions receive curative treatment, leaving CHD as one of the main contributors to PAH prevalence in the developing world (26). This means that the most effective strategy to reduce pediatric PAH incidence, would be to increase the availability of congenital heart defect repair for children in developing countries. Representing developed countries, a Dutch retrospective registry study found a 4.2 % prevalence of PAH and 1 % Eisenmenger's syndrome among 5970 adult CHD patients. Within the subgroup with septal defects (n=1824), 6,1 % had PAH. An underestimation was assumed, and a 5-10 % prevalence is considered realistic(27;28)

Two large registries of pediatric pulmonary hypertension have recently been established. TOPP is a pediatric registry that was started in 2008, with 571 patients included as of february 2012. 60 % of these are female. Data from the first patients shows that the largest subgroup was PAH (88%), whereof associated (secondary) pulmonary arterial hypertension was 43%, and 85% (115) of these were CHD-PAH. Trisomy 21 was reported in 13% of all PH patients (29).

REVEAL is a large, multicenter, US-based PAH registry with completed enrolment. The 3500 included patients, ages 3 months and up, will be followed for five years from 2009. A recent study of risk factors, analyzed 216 patients <18 yrs , 30 % being CHD-PAH (30). Five-year survival was 74 +/- 6%. Surprisingly, no difference was found between IPAH and CHD-PAH (more unrepaired than repaired). Age at diagnosis was the only significant risk factor. These registries may have a survival bias, but are representative of clinical cohorts, and will provide unique insight into important prognostic factors, improving the evaluation of treatment effects.

Although these estimates are variable, it seems reasonable to state that, as the number of CHD patients that survive into adulthood may be increasing, PAH-CSPS will persist as a problem, even in the developed and wealthy parts of the world. This means that prevention, but also tools for detection, treatment decisions and monitoring of PAH-CSPS will be of importance for an increasing number of patients.

1.3 Pathophysiology of PAH development in congenital systemic to pulmonary shunts

1.3.1 PAH-CSPS disease mechanisms in general

Flow and pressure induced mechanical forces act on the pulmonary endothelium as the first hit. Continuously increased blood flow mediates increased shear stress. In the case of a post-tricuspid, nonrestrictive defect (e.g. large VSD) there is additional cyclic, pulsatile stress on the vascular walls. This is probably aggravated by increased pulmonary pulse pressure, as in a large PDA or with the coexistence of significant pulmonary regurgitation. The exact link between wall stress and early wall change is unclear. Experimentally, and supported by human data, mechano-chemical transducers in the endothelium can stimulate the release of smooth muscle cell growth factors. Another possible pathway is leak of serum factors through an overstretched endothelial cell layer, into the subintimal layers, triggering proteases that partly act through inflammatory mechanisms, starting proliferative and antiapoptotic signaling. Distal migration of smooth muscle cells to normally unmuscularized arterioles, has been shown to be driven by gradients of matrix molecules such as fibronectin (31;32)

The histopathological results of these processes are:

- Smooth muscle cell phenotypic change, proliferation, migration, hypertrophy and sustained vasoconstriction

- Endothelial cell phenotypic change and proliferation, neointima formation
- Fibrous tissue deposition
- Necrosis, calcification and loss of arterioles
- Dysfunctional neovascularization and plexiform lesions

There is evidence that even in advanced disease, mechanisms differ between idiopathic/hereditary PAH and PAH associated with diseases such as CHD. Plexiform lesions look similar on a light microscopic level but contain monoclonal cells in IPAH and are polyclonal in associated PAH (33). Similarly, the TGF beta-1 pathway was found to be involved in the pulmonary vascular responses in IPAH, but not in Eisenmenger's syndrome (34).

However, three important signaling systems are brought out of homeostasis in all PAH subgroups. These pathways represent the three main categories of drug of the current treatment armamentarium:

1. Endothelin pathway
2. Prostacyclin/Thromboxane-cAMP pathway
3. NO-cGMP pathway

The status of these regulatory pathways contribute to pulmonary arteriolar vasomotor tonus and the degree of proliferation and apoptosis of endothelial and smooth muscle cells in the pulmonary vascular walls.

1.3.2 Inflammation in pediatric heart failure related to left to right shunts

Inflammatory mechanisms have become an established part of the understanding of chronic heart failure in adults, possibly contributing to symptoms such as cachexia (35;36). However, the role of inflammation in heart failure due to CSPS is unclear, as well as its role in the very early and reversible stages of the pulmonary vascular process

resulting in PAH-CSPS. The only relevant study (37) demonstrated elevated cytokine levels in 15 infants with heart failure from left to right shunts, as compared to a group of cyanotic patients with Tetralogy of Fallot. This represents a potential type 1 error (incorrectly rejecting the null hypothesis), because comparing with a “hemodynamically opposite” patient group may have exaggerated the group differences. Study III of the present thesis was designed to investigate the presence of inflammatory responses in the period of pulmonary vascular stress due to overcirculation, long time before irreversible vascular wall damage has become established.

1.3.3 Inflammation in PAH-CSPS

Inflammatory mechanisms have an established role in the pathophysiology of IPAH and several subclasses of adult associated PAH plus other PH groups (38-48)

Looking at the subgroup PAH-CSPS only, the literature is more scarce, with a few papers indicating the significance of inflammatory mechanisms.

Levy et al studied lung biopsies from children with PAH-CSPS (Down syndrome excluded), sampled at time of repair (49). Patients were considered having reversible or irreversible PAH-CSPS, based on invasive measurements one year postoperatively. Irreversible PAH-CSPS was strongly associated with impaired apoptosis, induced by perivascular inflammatory cells, leading to intimal proliferation. The antiapoptotic protein Bcl-2 was highly expressed in all cases of irreversible, but not in reversible PH. The reverse was seen for proapoptotic proteins p53 and caspase-3. These findings are supported by in vitro data (50). This suggests that early apoptosis in reversible PAH is followed by the inflammation driven development of apoptosis resistant endothelial cells and intimal proliferation, denoting irreversible disease. In further support of inflammation as part of PAH-CSPS development, Pinto et al analyzed 26 lung biopsies from patients with PAH-CSPS compared to healthy controls and found a predominance of recently recruited macrophages infiltrating peripheral pulmonary artery walls, related to intimal proliferation, together with decreased numbers of regulatory T-lymphocytes, possibly reflecting a deviant immune response (51).

Geiger et al, demonstrated increased VEGF expression in the plexiform lesions of CHD-pulmonary arterial hypertension (52), and Grosjean has pointed out a possible role for the inflammatory regulator NF- kappa B in VEGF-signalling, determining endothelial cell survival which points to a possible inflammatory pathway in advanced disease(53).

In sum, the presence of inflammation in PAH-CSPS is indicated in late and irreversible stages, but the links between inflammation and dysfunctional cell growth in PAH-CSPS are far from clarified. Animal models may provide important data on the early phase of disease development (54;55).

1.4 Treatment of pulmonary hypertension related to congenital systemic to pulmonary shunts

Treatment with the three subclasses of PAH drugs (prostanoids, endothelin receptor antagonists and phosphodiesterase inhibitors) in pediatric pulmonary arterial hypertension was almost undescribed at the initiation of study I in 2001. This included subgroups such as PAH-CSPS. Pediatric data had been published for prostacyclin only (56). Small, open label studies were published during the study period.

Later developments:

At present (but not at the time of the study I of this thesis) bosentan and sildenafil tablets are officially approved in Norway for PAH-CSPS, functional class II and III. The BREATHE 5 trial (57) with 54 patients with Eisenmenger's syndrome, was the study that led to this approval. It has been followed up by an open label extension study (58), showing continued effects on functional status. Less specifically designed studies have confirmed these findings (59-61). The pivotal sildenafil study (SUPER-1), had less focus on CHD(62), but in a recently published pediatric PAH study sildenafil improved peakVO₂, functional class and hemodynamics at medium and high dosage. However, importantly, increased mortality was seen at the high dose level in the open extension study (63). A recently published retrospective analysis of 229 patients with Eisenmenger's syndrome concludes with a surprisingly much lower death risk (unadjusted HR 0.21, adjusted 0.10) for those receiving new vasodilator drugs, as

compared to patients receiving conservative treatment. These data should be interpreted with caution until they are reproduced, as it is a retrospective, single tertiary center study. However, results as these, rapidly get an impact in clinical decision making. The increasing use of the new PAH drugs, has not been followed by a development of exact and clinically valid monitoring and decision making tools relevant for PAH-CSPS. The importance of treatment monitoring is underscored by the fact that the drugs may have significant adverse effects, and even lead to increased mortality (64-66).

1.5 Disease assessment and monitoring in PAH-CSPS

PAH is a concealed disease, the site of primary pathology being the small arterioles in the lungs, not (yet) accessible for precise invasive or noninvasive functional assessment, nor for in vivo imaging techniques. Techniques for direct, in vivo assessment of the pulmonary vasculature are considered a major evidence gap in the latest ESC guidelines along with the lack of PH disease markers in general(<http://www.escardio.org/guidelines-surveys/esc-guidelines/GuidelinesDocuments/Essential-Messages-PH.pdf>). Further, the hemodynamic consequence of arteriolar disease, total pulmonary vascular resistance elevation, can be difficult to measure reliably, especially in the presence of an open systemic to pulmonary shunt (67). Exact methods for Qp/Qs and thus, Rp/Rs measurement are needed. Vasodilator test protocols and cutoff values are still debated, and other markers of reversibility are just being explored. Hence, markers of disease progress constitute an important research field, in which most contributions so far comes from the adult PAH area. Children pose specific challenges with respect to assessment methods that require cooperation, such as exercise tests. Treadmill tests are reliable from around age 8 and 6MWT has been applied from age 4. Symptoms are often reported through parent interviews. A review on the challenges of assessing pediatric PAH was published (68) concluding that it is a specialized PAH centre task evolving from invasive gold standards towards non-invasive assessments.

1.6 Exercise induced PH in CSPS

Previous versions of the WHO definition of PAH included both a resting and an exercise mean pulmonary artery pressure cutoff value, as alternative diagnostic criteria. The exercise definition (30 mmHg) was excluded from the Dana Point classification, due to the high exercise pressures reported in apparently healthy individuals, such as endurance athletes(18). ESC publications list exercise responses as the number one gap in PH evidence (<http://www.escardio.org/guidelines-surveys/esc-guidelines/GuidelinesDocuments/Essential-Messages-PH.pdf>). Measuring pulmonary artery pressure during exercise can be done invasively, but is also reliably estimated by echocardiography(69;70). This is performed in many centers as part of the evaluation of unexplained exertional dyspnea. Studying patients with systemic sclerosis with normal resting pressures invasively, Saggar described four categories of pulmonary vascular response to exercise: normal response, pure precapillary exercise PH, venous exercise PH and out of proportion precapillary reaction to increased pulmonary venous pressures (the so called Kitajev reflex)(71). Around 150 invasive exercise studies per year are performed at Massachusetts General Hospital, including wedge measurements during exercise. Among the diagnoses are exercise induced heart failure with preserved ejection fraction (exercise induced diastolic dysfunction) (72) (www.phaonlineuniv.org). In systemic sclerosis, pure precapillary exercise induced pulmonary hypertension was found in 37% of cases with normal resting pulmonary artery pressure, representing subclinical disease. (71) Asymptomatic family members of patients with IPAH who carry BMPR2 mutations, show abnormal exercise response, possibly indicating the presence of subclinical disease. In a recent thesis from our centre, the presence of increased right ventricular systolic pressure during supine bicycling in patients with cardiac septal defects was studied, and found to be of surprisingly high prevalence(16). However, whether this represents subclinical increased pulmonary arteriolar resistance (reversible or irreversible) or exercise induced heart failure with preserved ejection fraction was not explored. The relation between exercise induced pulmonary hypertension and exercise capacity (VO₂) was described in a large invasive study by Tolle (73), but this association was not present in the ASD/VSD population studied at our centre(74).

2. Aims of the studies

A general aim for all studies in this thesis was to investigate potential monitoring parameters in patients with pulmonary arterial hypertension related to congenital heart defects. The specific aims of the four papers of the thesis were:

2.1 Paper I

The primary hypothesis was that a positive symptomatic effect of bosentan in patients with Eisenmenger's physiology would be associated with improvement in 24-hour oxygen saturation measurements. Further explorative hypotheses were that change of peak VO₂ during treadmill testing, and selected blood tests would be associated with changes in PAH symptoms score.

2.2 Paper II

This prospective follow-up study was part of the previous study. The aim was to describe circulating markers of inflammation and endothelial activation in patients with CHD-PAH, as compared to healthy controls and as changes by treatment with bosentan. Patients with Down syndrome were analyzed separately because of their known susceptibility to develop pulmonary arterial hypertension.

2.3 Paper III

In this cross-sectional study of patients with systemic to pulmonary shunts, the aim was to explore the associations between the degree of pulmonary hemodynamic load and the levels of circulating markers of inflammation and endothelial activation. Again, the significance of Down syndrome diagnosis was studied separately.

2.4 Paper IV

This RCT examined exercise induced pulmonary hypertension in patients with cardiac septal defects. The primary hypothesis was that pulmonary vasoconstriction was present and would be responsive to sildenafil, accompanied by increased peak VO₂.

At an explorative level, left ventricular diastolic reserve and its association with right ventricular systolic pressure during exercise was studied. Effects of sildenafil on indicators of right and left ventricular systolic function were also described. Lastly (unpublished data) alveolocapillary membrane area size was estimated by diffusion capacity for carbon monoxide during exercise with and without sildenafil.

3. Methodological considerations

3.1 General considerations

OUS (Rikshospitalet) was the only surgical CHD centre in Norway during this study period. Thus, the consecutively recruited patients in paper III can be regarded as a population based sample. All studies were performed in accordance with the Helsinki declaration of 1964 including later amendments (www.wma.net). Written informed consent was acquired. Children above 12 years read and signed a specially written assent form in addition to the one read and signed by the parents. All studies were accepted by the regional ethics committee and study IV was registered at www.clinicaltrials.gov as appropriate for drug studies. At the initiation of study I, this was not generally required, and the registration service was not established.

3.2 Recruitment and selection of patients and controls

3.2.1 Patient group, paper I

Patients with classical Eisenmenger's syndrome, L-R shunt at rest but desaturating during exercise and some with "greyzone" pulmonary vascular resistance index were included. Thus, the use of the term Eisenmenger's syndrome in the title is a simplification from a period of less precise term definition. According to today's nomenclature, Eisenmenger's physiology may be preferable. However, these patients were all in development of PAH that would end with Eisenmenger's syndrome. A list of patients waiting for trials of new pulmonary vasodilator drugs had been accumulated and kept for some time in the department, and these patients/families were contacted. All families accepted participation. New patients that were admitted for evaluation during the study period were also asked for participation.

3.2.2 Systemic to pulmonary shunt group (paper III)

Oslo University Hospital is a tertiary level hospital which at the time of the study had achieved nationwide responsibility for neonatal pediatric cardiac surgery and also performed the great majority of catheter interventions in CHD. Patients admitted for definitive treatment of ASD or PDA (n=55) were enrolled during the years 2002-4. Patients with VSD and AVSD (n= 19) were recruited during the second half of the study period, following an amendment based on a desire to include a wider spectrum of pulmonary vascular hemodynamic load. The parents of patients scheduled for interventional catheterizations were prospectively asked for participation. Demographic or other characteristics of the limited number of patients who declined participation were not registered. Exclusion criteria were those that would preclude the child to anesthesia, i.e. active infectious disease, as evaluated by clinical examination, chest radiogram and CRP level.

3.2.3 Exercise PH group (paper IV)

Patients with known exe-PH and VSD from the previously mentioned study from our centre were asked, and nine accepted participation. Further ten patients from south

eastern Norway previously operated for VSD, were screened for exercise induced pulmonary hypertension by reclined bicycle echocardiography, resulting in six more patients filling inclusion criteria, of whom five accepted participation.

3.2.4 Control group – CSPS and PAH-CSPS study (paper II and III)

As healthy controls, we included otherwise healthy children admitted to the skin department for laser treatment of capillary hemangiomas, some children of hospital staff. All were screened clinically and by CRP levels for intercurrent infectious disease.

3.3 *Clinical examination and symptoms scores*

Clinical assessment

A standard pediatric history taking and clinical examination was performed in all patients, including heart and lung auscultation, measurement of the extension of the liver below the costal margin, palpation of peripheral pulses, standardized supine resting blood pressure measurement, assessment of respiratory rate and peripheral microcirculation. Systolic blood pressure was measured as the average of two measurements at the time of echocardiography, by standard automated sphygmomanometry (Dinamap, GE Healthcare, WI, USA)

Symptoms scores

In effort to standardize description of symptoms and their change, specific PAH and pediatric heart failure symptom scores were sought in the literature. The Ross score of infant heart failure and the pediatric PAH score used by Bowyer et al were the ones identified as applicable at the time (75;76).

Heart failure symptoms score

The scoring system for pediatric heart failure published by Ross (75), was applied in study III. To extend its applicability into higher age groups, we replaced the item “feeding” with reported activity level for patients above 12 months of age.

Ross score table (modified)

Score	0	1	2
Respiration	Normal	Abnormal/exertional dyspnea	---
Respiratory pattern	Normal	Abnormal	---
Peripheral perfusion	Normal	Decreased	---
HR/min	<160/normal	160-170	>170
Feeding volumes	>100ml	70-100	<70ml
Feeding time	< 40 min	> 40 min	---
Activity level (replacing feeding time after 12 months age)	Normal	Moderately decreased	Severely decreased
Liver edge	<2cm	2-3 cm	>3 cm
S3/diastolic rumble	Absent	Present	

Total score: 0-2: no heart failure, 3-6: mild heart failure, 7-9: mod heart failure, 10-12: severe heart failure(74).

WHO/NYHA class was assigned to all PAH patients in study I, at all checkups, based on the history. Further, a pediatric PAH symptoms score, interviewing patients and caregivers about five specific areas of functional status was applied (76). A high score denotes good functional status.

PAH symptoms score*

	0	1	2	3	4
School participation	none	<50%	50-100% or special school	Full school, Sport limited	Full school, Full sport
Walking flat	Breathless at rest	Breathless on minimal exertion	30-50 m slow, OK	100-400 m slow, OK	3 km slowly, OK
Running/jogging	Never	Few paces only	20 m gently, OK	100 m jogging, OK	Normal speed 100 m
Walking stairs	Never tries	1 flight difficult	1 flight OK, 2 is difficult	2 flights OK at average speed	normal
Tiredness	always	Very quickly tired each day after school	Frequently tired	Sometimes after long day	normal

* Reference (75).

3.4 Radiography (paper III)

Standard front and lateral views were reviewed for pulmonary vascular markings and heart size by an expert pediatric radiologist, unaware of the clinical data. Both issues were assigned a score of 0-3 points, denoting no, mild, moderately and severely increased, respectively. This was combined with echocardiographic measurements to categorize patients into the low or high pulmonary blood flow groups (see next section

for details). The assessment of chest radiograms was performed subjectively, as per clinical practice. In the PAH study (study I), chest radiograms were not used as follow-up parameter.

3.5 Echocardiography

All the patients in paper I-IV had a standard clinical pediatric echocardiogram performed to confirm the diagnosis, and to rule out additional cardiac pathology that would lead to exclusion, such as right ventricular outflow tract obstruction, pulmonary artery branch stenosis or impaired ventricular function.

3.5.1 Echocardiography in the CSPS study (paper III)

The measurement methods in paper III are all part of standard clinical echocardiographic evaluation of congenital heart disease, as recommended in past and current clinical guidelines.

Pulmonary artery pressure

In patients that were not catheterized, pulmonary artery pressure estimation and categorization were based on echocardiographic estimates using the modified Bernoulli equation. Right ventricular outflow tract obstruction was ruled out by echocardiography in all patients. Shunt flow velocity and tricuspid valvar regurgitant flow velocity tracings were registered from all standard views in order to obtain the optimal continuous wave Doppler beam direction and maximal velocity. Based on the VSD or PDA peak systolic flow velocity or tricuspid regurgitant flow peak velocity, patients were allocated either to the high (pulmonary artery systolic pressure above 50 % of systolic systemic blood pressure) or low (PASP below 50 % of SBP) pressure group.

Pulmonary artery flow

In all patients, left ventricular end diastolic diameter Z-score by parasternal short axis m-mode, left atrium/Aortic root ratio by parasternal long-axis m-mode and indexed atrial areas from the apical four chamber view were registered, in order to quantify the degree of excessive volume load. All echocardiographic measurements were done offline, using the Echopac software (GE, Horten Norway), en bloc and performed in duplicate or as the average of three if the first two differed more than 10 %. In the case of a VSD or PDA, the left atrium and left ventricle indexed dimensions were used, and in an ASD, indexed right atrial area was used for evaluation of volume load, comparing the right and left atrial area tracings. These measurements were done blinded to the other clinical and hemodynamic data and to the inflammatory marker levels. Based on these measurements, patients were decided to have mild, moderate or severely increased pulmonary blood flow. Held together with the radiographic findings, patients were then allocated to high or low flow group as follows. Patients with mild or inconsistent (mild/moderate) echo and radiographic findings indicative of pulmonary hyperflow, were allocated to the Qp/Qs < 2,5 group. Those with consistently moderate to severe echocardiographic dilatation or heart size/vascular markings were allocated to the Qp/Qs > 2,5 group. Estimated Qp/Qs by pulsed wave Doppler tracing areas and aortic/pulmonary valve annulus diameter demands a high image quality and were only used for classification of a few patients with good quality tracings and consistent findings.

As an indicator of coherence between methods, the combined echocardiography and chest radiogram based Qp/Qs categorization method was compared to the invasive measurements in patients who had been evaluated by both methods. Significant Spearman's correlations were found between all individual noninvasive indicators and the Qp/Qs estimated by the Fick method.

3.5.2 Echocardiography in the PAH-CSPS (bosentan) study (paper I)

At all check-ups, standard clinical echocardiograms were recorded, evaluating qualitatively biventricular systolic function and valvar function for safety purposes. Shunt flow velocity, eccentricity index and tricuspid and pulmonary valve regurgitation

velocities were measured as applicable for the cardiac defect. Pulsed wave measurements of shunt flow velocity and direction (77) were recorded as a possible outcome indicator of change in the Rp/Rs ratio. No consistent change of echocardiographic measurements could be registered and these data were not statistically analyzed or reported.

3.5.3 Exercise echocardiography (paper IV)

Exercise echocardiography, both for right ventricular systolic pressure estimates and tissue Doppler based ventricular function, are research methods, with an undefined position as clinical tools. We used stress echocardiography to:

1. Assess changes by sildenafil in pulmonary vascular resistance during exercise indirectly, by measuring right ventricular pressure. Doppler measurement of pulmonary blood flow during exercise was evaluated as too inaccurate.
2. Describe changes in systolic and diastolic function of the left and right ventricle with increasing exercise level.

The stress echocardiography method, measuring i.v. saline enhanced TR jet peak velocities by CW Doppler during reclined bicycling has been validated against invasive measurements(69). It has been described with respect to its feasibility without saline enhancement (78), and the intra- and interobserver reproducibility of the method as applied in the present protocol was documented in a previous study performed at our centre(79), providing a detailed description. Echocardiographic images during exercise were registered by one experienced cardiologist (T.M.) with patients cycling constantly at 60 rev/min. Digitally stored images of TR jet velocities were analyzed offline, en bloc by one observer, registering the average of peak values from two good quality tracings for each stage, accepting only studies with evaluable tracings from at least the second last exercise stage (all studies accepted). The study of left ventricular diastolic function during exercise by tissue Doppler echocardiography has been described (80). This method has obvious limitations of reliability as a clinical decision making tool, but these are of less importance as we used the patient as her own control in an acute study. Mitral flow velocities for the calculation of E/E' ratios were not recorded in this study. Again, paired comparisons makes this less important. Apical four chamber TDI images

(minimum three cardiac cycles) were recorded at all stages. All tissue Doppler images were analyzed offline, en bloc by one observer (HB), using the Echopac software (GE, Horten Norway). Manual, frame by frame myocardial tracking was applied as necessary to reduce curve disruption from respiratory movements. Peak systolic velocity was defined as the highest positive velocity measured after the onset of QRS and before the aortic valve closure time point, indicated by the software. Peak diastolic velocity (during exercise) was, in the absence of clearly defined separate A' and E' waves, defined as the highest negative velocity between aortic valve closure and onset of QRS. With a right ventricular flow outflow tract Vmax above 2m/s at 100 W as exclusion criterion, no patients were excluded due to dynamic obstruction. Further details are provided in the methods section of paper IV.

3.6 Cardiac catheterization and blood sampling protocol

In adult patients and the absence of a S-P shunt, right heart catheterization with thermodilution based cardiac output measurement and pulmonary vascular resistance estimation is regarded as the gold standard for describing changes in the pulmonary vasculature. This, already, implies simplifications, such as the application of Ohms law when calculating pulmonary vascular resistance. In children with cardiac shunts, invasive data are less precise, because intubated anesthesia is required. Unavoidable changes in pCO₂, oxygenation, stressors, sedation and anesthetics during the procedure change hemodynamics, and may create big errors in the Qp/Qs and Rp/Rs data. Missing measurements of pulmonary venous saturation may lead to pulmonary flow overestimation. Further, catheterization in general anesthesia has been considered a high risk, especially in highly symptomatic PAH patients which makes it less useful as regular follow-up tool. Thus, no patients in the present studies were catheterized for scientific purposes alone.

A standard clinical catheterization protocol was followed for the evaluation and treatment of patients with ASD and PDA in study III. In study I, catheter data were collected retrospectively from clinically indicated catheterizations. All studies were performed with

the patient in general anesthesia, breathing room air, in a situation as hemodynamically stable as possible in this setting. In patients with ASD, both pulmonary artery pressures and pulmonary to systemic flow and resistance ratios were calculated, based on the Fick principle. Oxygen consumption was not measured, and the vascular resistances thus were expressed as a pulmonary to systemic ratio. Due to incomplete mixing of blood at the sampling site in patients with PDA, oximetry based shunt flow estimate is unreliable. Thus, only pulmonary artery pressures were reported from the invasive data in patients with PDA. In patients with elevated pulmonary artery pressure, left atrial or pulmonary wedge pressure was measured. The values for wedge pressure measurements were within normal values in the catheterized patients (not reported in papers). Vasoreactivity testing was not part of the scientific protocol.

Blood samples were drawn from the inferior caval vein, away from the renal veins, and from the left atrium or femoral artery. Plasma level values from the central venous blood samples were subtracted from the arterial sample values to calculate transpulmonary gradients. This was studied as a measurement of net release or uptake of the analyzed inflammatory markers in the pulmonary vascular bed.

Catheterized patients had blood samples drawn from the antecubital vein on the day before the procedure to compare with the venous samples drawn during anesthesia in effort to control for any effects on the inflammatory markers induced by anesthesia.

3.7 Cardiopulmonary exercise testing

In PAH assessment, exercise testing holds a central position, as loss of exercise tolerance is one of the main symptoms of the disease. Cardiopulmonary exercise testing as a maximal exercise test (treadmill or bicycle) with breath to breath gas analysis, has been extensively used in assessment of adult PAH and can usually be performed in children from the age seven (81) (82). Traditionally, however, the six minute walk test has been used in most adult PAH treatment studies, although being a submaximal exercise test, correlating with hemodynamics and outcome (83). With a 6MW result above 300 m, a

cardiopulmonary exercise test has been recommended for a more detailed description of exercise ability, and most of the participants in study I were NYHA class II. At the time of inclusion in study I, there was little data published on the use of 6MW in children, and large variation due to motivational factors was assumed. Cardiopulmonary exercise test is reliable and suitable for serial measurements during follow up(81). Peak VO₂ in children with PAH has been shown to correlate with pulmonary vascular resistance index ($r = -0,6, p= 0,006$)(84). Thus, for the assessment of exercise ability in study I, cardiopulmonary exercise test on treadmill was chosen, due to the experience in the group (85), although this resulted in some participants not being able to perform these studies. Some of these, in retrospect, could have performed 6MW tests, However, the PAH symptom score (above) covers similar information on functional capacity by interview. In study IV, cardiopulmonary exercise test was performed as standard treadmill testing at inclusion. Further, VO₂ was measured during the reclined bicycling studies for intra-patient comparison. All treadmill exercise tests in paper II and IV were performed by the same experienced physiotherapist (PMF), accompanied by a cardiologist (HB).

3.8 Twenty-four hour oxygen saturation measurement (paper I)

The principal idea of study no I was to explore and improve non-invasive parameters, avoiding the use of unreliable and hazardous invasive hemodynamic assessments. Arterial blood gas is the gold standard for oxygen saturation, but is painful and thus not feasible for the follow up of children. Transcutaneous oxygen saturation is widely used in hypoxemic patients. Acknowledging the large spontaneous variation in oxygen saturation values, in individual patients, over short periods of time, we chose a long measuring interval as way of enhancing the data representativity for the of the patients' situation. AS hypoxemia is a key finding in Eisenmenger's syndrome, the need for valid SpO₂ measurements is undebatable. However, its value as treatment monitoring parameter had received little attention. Clinical assessments normally include measurements for a couple of minutes, after a few minutes rest on a chair/bench. It has been shown that oxygen saturation in patients with Eisenmenger's syndrome varies with body position,

probably due to ventilation perfusion distribution phenomena and/or diffusion abnormalities(86). We therefore assumed that longer periods of measurement would improve the validity of the SpO₂ values. Thus, we registered during rest and activity, day and night, as an analogy to 24 hour ambulatory BP measurements in systemic hypertension. The mean value of 24 hour oxygen saturation correlated well with hematocrit and hemoglobin values at baseline as shown in the figures below, indicating that these long term measurements provide a valid indicator of the patients' tissue oxygenation over time (see figures below).

The pulse oximetry data in study I were collected in a standardized setting, during a 24 hour hospital admission, thus minimizing variation in activity type and duration between the time points. All alarm functions were turned off during measurement and no patient received extra oxygen. Oxygen saturation and heart rate was measured transcutaneously, with a sampling frequency of one dataset every two seconds. Self-adhesive sensors for one time use were applied. Sensors were placed at the less used left or right handed index finger, unless the lesion involved a PDA, making foot measurement necessary. The data sets were cleaned for periods of low signal quality (automated software function, followed by manual surveillance, excluding periods with obviously deviant HR and/or SpO₂ curves), and analyzed with the Download 2001 software (Stowood inc, UK).

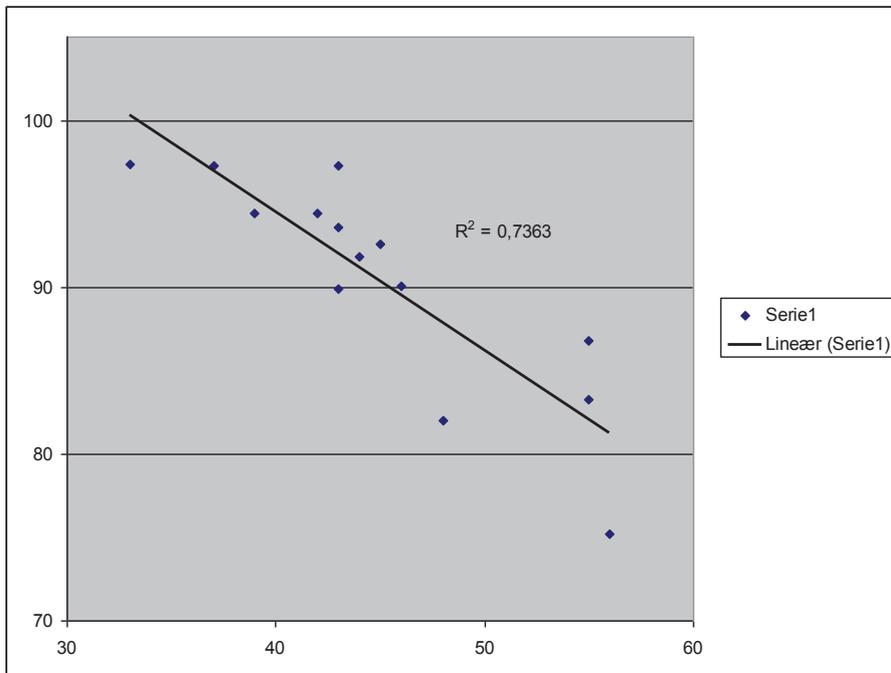


Figure Hematocrit (%) vs mean of 24 hour oxygen saturation (%) at baseline.

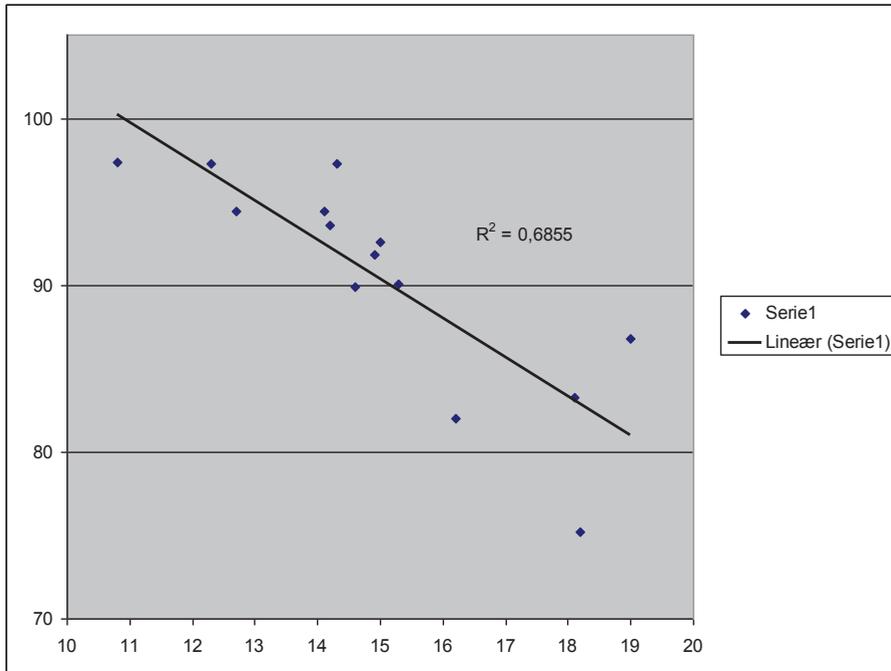


Figure Hemoglobin (g/L) vs mean of 24 hour oxygen saturation at baseline

3.9 Pulmonary function tests

In study I and IV, participants were examined by standard spirometry (ATS guidelines Eu Resp J 2005, Miller MR) at all time points. Spirometry at baseline was performed to exclude overt lung pathology (restrictive or subclinical obstructive disease) that could be relevant for the development of PAH. As no significant changes or deviations from normal were seen in the spirometry data, these were not considered of importance for the conclusions in the papers or thesis. Coherently, no association between FVC and abnormal right ventricular pressure response during exercise was found in our previous ASD and VSD study (87). In study IV, DLco measurements were performed at rest and during exercise, as DLco has been used as a surrogate marker for available pulmonary

capillary bed area, expecting that if sildenafil lowered pulmonary vascular resistance, DLco would increase.

3.10 Biochemical analyses – circulating biomarkers

A simple blood test that tells the doctor and patient about the disease status and whether to intensify treatment does not exist for PAH-CSPS.

NTproBNP values may be difficult to interpret in the presence of S-P shunts, as the shunt flow initially imposes neurohumoral activation and cardiac peptide elevation due to volume overload of the right and or left ventricles. With time, as pulmonary vascular resistance increases, NTproBNP will decrease with the reduction of the shunt flow, until a new increase occurs as result of pressure load and gradually developing right ventricular failure. Thus a normal peptide value in this condition can be present with high pulmonary vascular resistance but good right ventricle function.

At the initiation of study I, cardiac peptides were emerging as markers of heart failure, also in pediatric literature (88). However NTproBNP replaced ANP as routine analysis at our centre during the study, implying that complete datasets are not available for all patients in study I. In study III, however, The NTproBNP analyses were performed systematically and outside clinical routine, ensuring completeness and maximal reliability of data. NT proBNP was also not described as an assessment parameter in pediatric PAH at the time of start study I, but proANP (later NTproBNP) was measured as part of the safety protocol, in the case treatment with bosentan should be so effective that volume overload resumed.

Uric Acid had a demonstrated prognostic value in IPAH(89), and was thus included as a possible marker of symptomatic improvement in the present PAH patients.

Hemoglobin and hematocrit have specific roles in monitoring Eisenmenger's patients, together with iron status, as very high Hct levels, above the 0.70 range, lead to a dramatical increase of total pulmonary vascular resistance, often accompanied by iron deficiency with further increased viscosity by poorly deformable microcytes. Further, a

decline in Hgb/Hct was expected in the case SpO₂ values should increase by bosentan treatment.

Inflammation marker analysis

Although inflammation, as represented by various established circulating markers, had gained a position in the pathophysiology of PAH in general, circulating markers such as CRP, had not and still have not become established as biomarkers. For the presented studies, we chose a range of established markers studied in both pulmonary and systemic arterial diseases and in adult heart failure, in effort to find a marker that either correlated well with A. signs and symptoms of heart failure (study III) or B. PAH symptoms or change in symptoms with drug treatment (study II) .

Biomarker assays

Enzyme immunoassays from R&D systems (Minneapolis, MN, USA), Bender Medsystems GmbH (Vienna, Austria), Peprtech (London, UK) and DakoCytomation (Glostrup, Denmark) were used for the inflammatory markers and von Willebrand factor in papers II and III. All assays were carried out in the laboratory at the Research Institute for Internal Medicine, under the supervision of Thor Ueland.

ELISA method: For the EIAs, standard 96 well polystyrene microtiter plates were coated with primary antibodies, immobilising the desired antigen to the surface, applying second (detecting) antibodies with enzyme, then adding substrate creating the color reaction. Automated detergent washing and color density reading was used. Analyses were performed in duplicate and blinded to the clinical data. Inter- and intra- coefficients of variation was <10% for all EIAs. To minimize run-to-run variability, serial samples from one individual were analyzed on the same plate.

NTproBNP (paper III) was analyzed at the OUS clinical laboratory. All samples were run en bloc, with the instrument Modular E and commercial sandwich based chemiluminescence kits from Roche Diagnostics (Mannheim, Germany). Uric acid and Hemoglobin (paper I) were measured as routine analyses at the accredited OUS clinical laboratory.

3.11 Bosentan treatment protocol (paper I)

After the baseline evaluation, patients were started at oral bosentan 1 mg/kg BID, increasing to the target dose of 2 mg/kg BID after two weeks, continuing treatment for 12 months with monthly liver enzyme measurements and study visits every 3 months. Pediatric dosage recommendations were published later during the study period confirming the adequacy of the chosen regimen(90).

3.12 Sildenafil test protocol (paper IV)

All participants received capsules with either sildenafil 50 mg or placebo on two consecutive study days. Capsules were administered with a glass of water, 90 minutes before the study start, in a randomized, doubly blinded fashion, with at least 24 hours washout between the two testing time points. The applied dose is large as compared to the standard 20 mg dose applied in PAH treatment. A single oral dose of 50 mg has been effectively used in acute studies of hemodynamic effects in adults (91).

3.13 Statistical methods (SPSS versions 15.0-18.0)

Paper I: 0-12 month comparisons were analyzed by single sample t-test.

Relationship between symptoms change and SpO₂ change was analyzed with linear regression.

Paper II: Skewedness of data was present, and Mann-Whitney U test was used for comparison of patients and controls. Treatment effects were analyzed by Wilcoxon matched pairs test and correlations were analyzed by Spearman's rank test.

Paper III: Group comparisons: Mann-Whitney U test.

Compartment differences: paired t-test

Univariate analysis: Spearman rank test

The main analysis was done with multiple linear regression, log transforming explanatory and dependent variables as necessary for model fit.

Paper IV: Mixed models analysis for repeated measurements with random intercept and slope was performed by medical statistician (AHP). Normal distribution of data was examined by residual plots and histograms. Linear or square curvilinear relation of hemodynamic parameters with time and exercise intensity were applied as needed for the best model fit.

4. Summary of results

4.1 Paper I

Among 14 patients with PAH-CSPS treated with bosentan for 12 months, all patients reported improvement or stability of symptoms, apart from one patient with side effects that required cessation of treatment. Mean of 24 hour oxygen saturation showed a small decline, in parallel with a lowered diastolic blood pressure. Peak VO₂ (n=6) declined with mean 8 ml with a trend towards decreased ventilatory efficiency. Large, individual day to day variations of mean 30 minute supine SpO₂ was demonstrated.

4.2 Paper II

Patients with PAH-CSPS were characterized by increased plasma levels of von Willebrand factor (endothelial cell activation) CRP (systemic inflammation), CD40 ligand (platelet mediated inflammation) and osteoprotegerin (vascular inflammation, possibly involving calcium metabolism). Within the study group, NT-proBNP levels correlated with vWf and CRP levels. 12 months treatment with bosentan reduced MCP-1 levels in those with improvement of symptoms and RANKL in the group as a whole. Patients with Down syndrome (average age 118 months) had higher s TNFR1 and MCP-1 levels than non-DS patients and controls.

4.3 Paper III

In 74 patients with CSPPS association between hemodynamic stress and systemic inflammatory markers could be demonstrated. Furthermore, no net production or uptake of inflammatory markers through the pulmonary vasculature was found. As in the previous paper, Down syndrome was an independent risk factor for increased inflammatory activity. Children with CHD and Down syndrome have a different inflammatory profile when comparing with age matched CHD patients, irrespective of their hemodynamic characteristics.

4.4 Paper IV

In 14 patients with cardiac septal defects and known exercise induced pulmonary hypertension, we found no effect of sildenafil on right ventricular pressures or peak VO₂ during reclined bicycling. However, exercise induced right ventricular systolic pressure increase was associated with left ventricle diastolic reserve as measured by change in left ventricular lateral wall E' velocity. Lastly, right ventricular systolic function improved with sildenafil, according to the tissue Doppler velocities and TAPSE measurements.

5. Discussion PAH-CSPPS - mechanisms and monitoring

PAH-CSPPS including Eisenmenger's syndrome have been regarded as clinically and hemodynamic stable conditions even at long-term, when comparing to the rapidly progressive IPAH. This view is currently being challenged in adult cardiology, by studies demonstrating functional deterioration over short periods of time (92), and from survival data indicating a standardized mortality rate of 3.8 (2-7), and concluding that PAH-CSPPS is not a stable disease (10). An ideal disease monitoring parameter should describe whether the disease process is stable, improving due to treatment or in progression. It should predict mortality and be reliable, simple to perform and safe. Monitoring options

in PAH-CSPS is far from perfect, and no significant new concepts have been implemented into clinical practice since the start of study I. Therefore, the current clinical monitoring concept, as in all PAH treatment, is to make a qualified but subjective evaluation of all available parameters together(18).

Currently applied outcome parameters in trials of new drugs in PAH-CSPS and Eisenmenger's syndrome are:

- NYHA functional class assessment and other clinical scores
- Six minute walk test or cardiopulmonary exercise test
- Circulating biomarkers, such as NTproBNP
- SpO₂ (in patients with open shunts)
- If invasive study: pulmonary artery pressures and resistance

In the following, monitoring tools are discussed, with an emphasis on those studied in this thesis.

5.1 Functional status assessment and scoring systems

The NYHA PH functional class assessment was developed for adult patients, and has obvious limitations for use in infants and pre-school children. With a median age of 10 years in paper I, we applied a combination of adult-like functional class assessment and a symptom score that had been used in a previous PAH-CSPS study(76). The majority of patients were in class II, thus NYHA functional status was not considered a sensitive monitoring tool for this group. The pediatric PAH symptoms score, however, did improve with treatment. In an uncontrolled study, obviously, placebo effects and the parents' expectations may account for some of this, but effects were consistent over 12 months. However, no correlation was found between the biomarkers and the symptoms score. Similarly, in adult PAH studies, lack of consistency between clinical improvement and e.g. hemodynamic parameters are not infrequent. Holding the patient and caregivers experience of improved clinical condition as the treatment effect reference of this study, this indicates that the chosen biomarkers may not be sufficient alone in the monitoring of

this treatment effect. A correlation with six minute walk distance could perhaps have been demonstrated, but was not examined, due to the poor standardization of this test for children at the time. This discussion is continued below. Functional status assessment (NYHA classification) in pediatric PAH was recently reviewed by Lammers et al (93). An age-specific classification system was proposed, creating possibilities for more valid and reliable outcome parameters in future treatment studies. More recent pediatric PAH studies include quality of life and global functioning assessments (63). These may provide important additional information about a chronic disabling disease which has no available cure.

5.2 Exercise testing in PAH-CSPS

Tests of exercise capacity play a critical role in the evaluation of PAH patients. The main symptom of the disease is limitation of exercise ability and changes provide immediate information about worsening or symptom relief. Exercise assessments can be divided into submaximal tests, such as the 6MW and maximal tests, as treadmill or bicycle cardiopulmonary exercise test with peak VO₂ measurement. The Borg scale is frequently used to assess the patients' experience of exhaustion. The prognostic value of peak VO₂, 6MWT and response to treatment in 6MWT distance is documented in adult PAH (83;94). In pediatric care, the choice of test method depends both on motor skills and functional class. Highly symptomatic children are validly evaluated by submaximal tests, whereas early treatment studies should apply cardiopulmonary exercise testing (82). With mainly NYHA II patients in study I, we chose cardiopulmonary exercise testing, although this was found to be unfeasible in some patients. However, effects of treatment may be difficult to measure by cardiopulmonary exercise testing with peak VO₂ measurement, perhaps because the new pulmonary vasodilator drugs tend to improve submaximal exercise capacity more than peak capacity (63). Submaximal exercise testing in children has been extensively studied in recent years. The feasibility and normal values for the 6 minute walk test in European children from 4-11 was described by Lammers et al (95). This study included SpO₂ data, in which 96-99 % was found to be the normal area. SpO₂

measurement during 6MWT is recommended in PAH-CSPS with open shunts. Geiger studied ages 3-18 and found normal median values plateauing in girls at 11 years in the 660 meter range(96). The male values increased further with age to 730 meters. Normative values may be population specific (97). Chinese normative pediatric values were published by Li (98). Importantly, an improvement significance cutoff of 68 m was found in a pediatric validation study, while 54 m has been accepted in the adult literature(99). Mean 6MWT improvement in drug studies is often in the 10-15 % range, indicating that in clinical practice, many patients will have treatment effects that cannot be assessed by 6MWT alone. Being a submaximal test 6MWT would probably have had a low discriminative value in our NYHA 2 patients.

Pulmonary vascular resistance and right ventricular function are among factors with impact on exercise capacity. Pulmonary vascular resistance is a flow dependent variable. Flow, or cardiac output, is difficult to assess directly in this patient group. Vasodilator PAH drugs have other hemodynamic properties than the assumed selective pulmonary vascular resistance reduction. This may include inotropic effects that could enhance right ventricular function(100). Signs of increased systolic ventricular function were seen in the sildenafil study (paper IV). In study I, an endothelin receptor antagonist was used. Endothelin-1 has complex effects on the myocardium that may differ with hemodynamic situation. One could speculate that positive inotropic effects could have contributed to an improvement of cardiac output and exercise capacity at submaximal levels, without improving the maximal cardiac output and peak VO₂.

In conclusion, both submaximal and maximal exercise tests hold key positions in PAH assessment, the choice of method depending on NYHA class.

5.3 Pulse oximetry for the assessment of patients with Eisenmenger physiology

A principal idea of study no I was that pulse oximetry might provide enough information about response to therapy, thereby avoiding the use of invasive hemodynamic assessment. The gold standard for oxygen saturation measurement is arterial blood gas analysis. Finger-tip oximetry values in a later study of patients with Eisenmenger's were

found to be mean five per cent higher than arterial blood gas values (101). There are no published guidelines for the measurement of SpO₂ in patients with Eisenmenger or PAH-CSPS. However, there is an increasing awareness of the need for standardization of measurements, which may include elements such as hydration status, resting time prior to measurement, body position and duration of measurement. As demonstrated in figure 3, paper I, large day to day variations of SpO₂ in patients with Eisenmenger's syndrome are seen, even with standardized supine measurement for 30 minutes. As demonstrated in the figure below, oxygen saturation also changed significantly within the 30 minute periods from start to end, possibly reflecting the altered ventilation perfusion matching when lying down. Increased venous return from the legs in the supine position, leading to larger volumes of right to left shunting could be responsible for this desaturation, but the saturation dip seen in this specific patient appears after approximately 10 minutes, which seems late, if this were the mechanism.

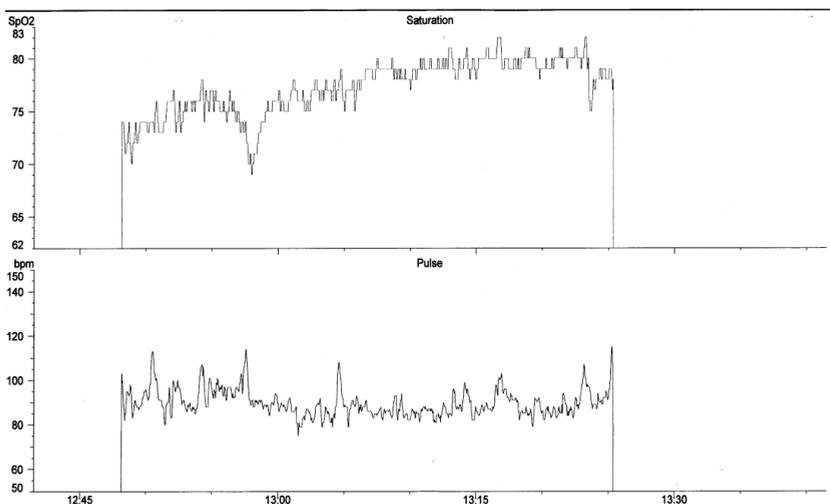


Figure: Heart rate and SpO₂ measurement in Eisenmenger patient during the first 40 minutes after lying down

Not all patients in study I were hypoxemic at rest. In accordance with the later Dana Point classification, only 8 of the 14 patients in the study had the classical Eisenmenger's syndrome. The rest would currently be classified as PAH-CSPS, subgroup B, desaturating with exercise. Correspondingly, three patients had hematocrit values in the normal range, and their exercise desaturations were too infrequent or mild to be detected by the 5th percentile SpO₂ value derived from the 24 hour SpO₂ analysis at baseline (Table 1, paper I). In these patients, desaturations were, with our methods, observed as 1-2 % of the measurement time period with saturations below 94%.

The simple hemodynamic concept of study I was that, in patients with an open shunt and some degree of desaturation, a drug induced, selective or relative reduction of pulmonary vascular resistance would be detectable, either as increased mean, or maximal, transcutaneous oxygen saturation. In Eisenmenger's physiology, decreased oxygen saturation is seen during muscle activity (due to lowered SVR) that is not accompanied by lowered pulmonary vascular resistance. It would be expected that in a hypoxemic Eisenmenger's patient, selective pulmonary vasodilation would lead to a higher oxygen saturation both at rest and during activity, and thus SpO₂ would be the ideal pulmonary vascular resistance marker. In patients with activity related desaturation only, less pronounced desaturations were predicted as the desirable treatment effect. However, other factors affect the measured SpO₂, such as the systemic venous oxygen saturation depending on cardiac output and peripheral oxygen extraction. Thus, in right to left shunts, less systemic arterial desaturation follows improved cardiac output, irrespective of the change in pulmonary vascular resistance. With reference to Sandoval's study of the impact of body position (86), we separately assessed 6 hours of sleep, representing the supine position. However, in some patients, vasodilators produce nasal congestion, affecting sleep breathing pattern. Patient no 2 demonstrated this kind of decreased night time oxygen saturation. Reduced SpO₂ during sleep could also be an indicator of ventilation perfusion mismatch by nonselective, drug induced vasodilation that may be more significant in the supine position. Specific night time desaturations, however, were not a systematic finding in the patient group of study I. This indicates that mechanisms for the desaturation seen after bosentan treatment were independent of sleep induced respiratory disturbances. A possible explanation for the discrepancy between SpO₂

decrease and improvement of symptoms in paper I, could be that bosentan reduces both the systemic and pulmonary vascular resistances. This could lead to increased cardiac output at rest and at low exercise levels, and thus improve daily life functioning, but still reduce maximal exercise ability through mechanisms involving even lower oxygen saturation at higher exercise levels. The submaximal capacity may be the most relevant for daily life functioning, which should be reflected by the symptoms score applied in study I. Another explanation for the increased desaturation observed, could be that some patients may have deteriorated during the study period, despite treatment with bosentan. The patients with ASD in study I-II may be considered as having PAH out of proportion (PAH-CSPS, subgroup C) which is increasingly recognized as a more rapidly progressive disease, resembling idiopathic PAH. A placebo arm would have facilitated the interpretation of the results of study I significantly. And again, desaturation from systemically administered vasodilator therapy may be related to disturbed V/Q-relationship, recently demonstrated in patients with PH related to interstitial lung disease(102). This could also apply in PAH-CSPS patients.

Our data confirm that the measurement method was feasible, as 24 hour SpO₂ data could be retrieved in all 14 patients, including those who needed postductal sensor placement. Foot measurements produced more movement artifacts, and more periods of low signal quality had to be taken out of the analysis, but this should apply equally at baseline and follow-up.

The surprisingly large individual day to day SpO₂ variation calls for a validation study of pulse oximetry in patients with Eisenmenger's syndrome. We suggest comparing day to day standardized measurements, during sleep and awake rest (supine and upright) and during a standardized activity such as the 6MW test. This could help establishing a reproducible method for clinical oxygen saturation measurements and decision-making. Such measurements could be combined with continuous blood pressure measurements, serving as an indicator of SVR variation, and heart rate reflecting activity level.

In summary, SpO₂ measurements in patients with Eisenmenger physiology integrate many different hemodynamic and respiratory factors into one (SVR, pulmonary vascular resistance, cardiac output, pulmonary V-Q relationship, peripheral oxygen extraction). This means that additional data are needed for the interpretation of treatment induced

oxygen saturation changes. The prognostic importance of (resting) SpO₂ in Eisenmenger's syndrome has been documented (103;104). Standardization of measurement is absolutely necessary to allow comparison over time. A feasibility and reliability study of standardized SpO₂ measurements is warranted, and is planned at our centre.

5.4 Circulating biomarkers in CSPS and PAH-CSPS

5.4.1 Inflammatory biomarkers

The activity of a disease promoting pathway with its specific ligands and receptors may be difficult to assess by blood tests, and a valid and reliable biomarker may be found downstream to important disease mechanisms, but still be suitable for clinical use, due to its stability in a blood sample or precise measuring method. A potential confounder in studies of circulating inflammatory factors as in the present studies, is that both the lungs and the heart, and several other organs in the case of Eisenmenger's syndrome, are affected and may release the same biomarkers. Nevertheless, a biomarker that stems from multiple organs may still bring information about total disease progression. We sought to find new disease markers that could contribute to better understanding of the disease development from early through intermediate and to late stages.

Specific disease markers that describe the stage and degree of vascular remodeling on an individual level, could help tailoring therapies and improve selection of those patients in the hemodynamic "greyzone" who will benefit from shunt closure.

5.4.2 Hemodynamic classification (study III)

Of vital importance for the interpretation of paper III is that the hemodynamic groups are considered valid. Acknowledging that a true gold standard for Qp/Qs measurement does not exist, we considered both the invasive and noninvasive data equally accurate for grouping the patients into this simple model with only two categories of flow and

pressure load. Further, NTproBNP level has been well documented as a marker of Qp/Qs in CSPS (105;106), but did not correlate with left ventricular end diastolic pressures in patients with VSD(107). In our data, as predicted, the NTproBNP values were higher with increasing pulmonary artery pressure and flow (figure 2 paper III). A linear regression model for NTproBNP level could be fit for the whole group, with flow and pressure categories explaining 73 per cent of the NTproBNP variation ($p<0.001$). However, surprisingly, symptoms of heart failure did not correlate with NTproBNP levels. This may be attributed to the low prevalence of such symptoms in the group as a whole.

A further validation of the classification would have implied application of our model on a new set of patients to assess the prediction of Qp/Qs, which however was beyond the scope of this work. Pulmonary artery pressure estimate by echocardiography is an established part of standard clinical assessment, and were accepted as sufficiently precise and valid for the categorization without further validation(108). Obviously, some patients with borderline PA pressure may have been allocated to the wrong category, but this should apply both ways, with no special bias, as lack of Doppler signal from VSD and TR jet flow velocity creates opposing errors/bias. The strengths of paper III is that it includes healthy controls and has a good size study group without identified selection bias. A main methodological limitation is the combination of invasive and non-invasive measurements. This may challenge the reader, but can be defended. As an example, a patient with an isolated large VSD with a diameter close to, or larger than the aortic root, and laminar VSD flow at echocardiography, can without question be categorized as having a systolic pulmonary artery pressure above fifty per cent of systolic blood pressure. Further, in effort to bring the study closer to the primarily affected organ, transpulmonary gradients were calculated.

5.4.3 General inflammatory mechanisms

As in adult heart failure, a role for inflammatory mechanisms has been demonstrated in pediatric non-CSPS related heart failure, i.e cardiomyopathy related heart failure (109;110). Looking at paper II and III together, inflammatory markers seem to be of limited value in monitoring the early phase of disease, characterized by heart failure

induced by volume overload. Partly opposing this, in a recent study comparing cyanotic with acyanotic CHD and healthy controls, higher serum IL-6, ghrelin and $\text{tnf-}\alpha$ was found in the acyanotic group. However, this study was not designed to look specifically at factors related to pulmonary overcirculation and heart failure (111), but focused on growth failure as a consequence of heart failure.

On the contrary, based on the inflammatory characteristics of the PAH-CSPS group in paper II, circulating inflammation markers may be worthy of further investigation as monitoring parameters of manifest pulmonary vascular disease. Finding a marker that separates reversible from irreversible disease would be a great achievement. This could be realistic, looking at Levy's findings of periarteriolar inflammatory infiltrates with antiapoptotic properties and intimal proliferation in irreversible but not in reversible PAH-CSPS(49). Contrarily, Hall S (112) et al compared lung biopsies in IPAH, PAH-CSPS and normal lungs, and found that only the IPAH lung specimens had increased perivascular inflammatory cell aggregates. Summarized, although the elevated CRP levels in PAH-CSPS (paper II) may be epiphenomena in terms of pathophysiology, CRP measurement could be worth following up in new studies as a potential parameter of vascular inflammation, and with reference to Levy's findings, of reversibility in PAH-CSPS. In patients with PAH or thromboembolic PH, CRP level predicts outcome and response to therapy(113). CRP measurements were entered as an option in the TOPP registry, on our request, after paper II was published. With systematic use, this registry may provide data on CRP changes with PAH treatment in children.

5.4.4 Specific inflammatory markers

In paper III, the group with low flow and high pressure (i.e. increased pulmonary vascular resistance) could represent an intermediate stage between pulmonary overcirculation and Eisenmenger's syndrome. However, this group had few patients (n=5), significant characteristics could not be found, apart from a trend towards a higher vWf (147 ± 118 vs. 111 ± 60 in the low flow and low pressure group, $p=0.24$) This could indicate an activated pulmonary arterial endothelium. Von Willebrand factor increase could thus be an early marker of increased pulmonary vascular resistance. In patients

with Eisenmenger's syndrome, von Willebrand factor function is disturbed with increased dysfunctional high weight multimer composition (114;115)

With respect to study III, the possibility of a type two error (failing to reject a false null hypothesis) must be considered. Inflammatory mechanisms were expected to be most pronounced in the high flow and high pressure group. The number in this group was limited, but comparable to the sample in Buchhorn's study(37). The high flow and high pressure group size had 80% power to demonstrate 25% differences with an alpha of 0,05. Moreover, the combination of a group comparison with healthy controls for the patients with the most pronounced hemodynamic load, and a regression model including patients with all degrees of shunting, further should reduce the risk of a false negative conclusion. It should be mentioned that a recently published growing piglet study demonstrated activation of inflammatory mechanisms after six months of aortopulmonary shunt flow, primarily related to right ventricular expression of inflammation markers(116).

Osteoprotegerin has been shown to stimulate smooth muscle cell proliferation in adult IPAH (117). The elevated osteoprotegerin values in PAH-CSPS (paper II) could indicate that smooth muscle cell proliferation still is an active process in the patient population of paper II. This specific inflammatory mediator has emerged as a valid marker of vascular diseases, involved in matrix regulation and calcium deposition within the vascular lesions. Calcium deposition becomes more prominent with time in Eisenmenger's syndrome(118). This makes osteoprotegerin a possible candidate as PAH-CSPS assessment tool in advanced disease as well. It has been described, that in some patients evaluated as having irreversible PAH, pulmonary vascular resistance normalizes with vasodilator therapy(119;120). Smooth muscle cell phenotypic change and proliferation is probably more reversible than similar endothelial cell changes (121). With respect to circulating markers of vascular change in a study group similar to the one in paper IV, elevated circulating fibronectin – a matrix protein involved in migration of SMCs in PAH-CSPS, and thus connected to pulmonary vascular remodeling, was found in patients with septal defects and exercise induced pulmonary hypertension, possibly indicating ongoing vascular remodeling(16).

The limitations of studying potential disease mechanisms by samples taken distant from the diseased organ have been discussed, but may be considered less important when the factors are assessed as markers of patient deterioration or improvement.

Recently, studies of circulating vascular cells or progenitor cells have consistently been found to be associated with PAH-CSPS(122). Importantly, the number of circulating endothelial cells could discriminate reversible from irreversible PAH-CSPS, hypothesizing an increased shedding and turnover of endothelial cells in the pulmonary arterioles of reversible PAH-CSPS. However, analysis of circulating markers of endothelial activation, failed to provide a similar differentiation(123). Diller (124) found that circulating endothelial progenitor cells were lower in Eisenmenger's and IPAH, and even lower in patients with Down syndrome and Eisenmenger's(124). Circulating inflammatory mediators were increased in this study, supporting that inflammatory markers can be important in characterizing irreversible PH disease(124). Patients with Down syndrome and CSPS more often develop PAH (125;126).

In further support of inflammatory mechanisms in PAH development, Yeager et al found increased levels of circulating myeloid suppressor cells and fibrocytes in both idiopathic and associated pulmonary arterial hypertension including children with CSPS (127;128). Fibrocyte numbers were positively correlated with pulmonary hemodynamics. Hassoun has recently provided a comprehensive review of the inflammatory mechanisms potentially involved in pulmonary vascular remodeling(38).

5.4.5 Down syndrome

We could not demonstrate a relationship between hemodynamic parameters or functional status and the inflammatory markers. However, the group of patients with Down syndrome, both operable and inoperable, were characterized by an increase of inflammatory markers. We speculate that, in addition to lung hypoplasia, deviant immune responses, related to trisomy 21, may participate in increasing PAH risk in DS. This warrants further exploration. Studies of preoperatively administered drugs with anti-inflammatory properties, such as statins, could be of special benefit to patients with Down syndrome.

5.4.6 Other circulating biomarkers in PAH-CSPS

NTproBNP :

NTproBNP is established as a prognostic marker in adult PAH of various etiologies. The utility of BNP levels as management tool in pediatric PAH was described by Bernus, who found large variation in values between patients with similar functional or hemodynamic status, thus not being able to define cutoff values(129). However, serial measurements over time correlated well with hemodynamic changes. Van Albada studied 29 patients with PAH (10 with Eisenmenger's) median age 7, and found that NTproBNP correlated with functional data, 6MW and WHO class(130). NTproBNP decreased with treatment and was predictive of mortality. Remarkably, NTproBNP >1664 predicted the two year mortality with 100% sensitivity and 94% specificity(130). Lammers' pediatric data showed a good correlation between BNP and functional status but limited prediction of mortality or transplantation(131). Diller et al contrarily published that NTproBNP predicts survival and reflects therapy in adult Eisenmenger's syndrome (132). The different prognostic value of cardiac peptides in children and adults may be related to the phenomenon that right ventricular failure largely determines NTproBNP level, and takes many years to develop. NTproBNP also serves as a PAH screening marker in some high risk groups, such as patients with systemic sclerosis (133). In these situations NTproBNP has good specificity for PAH, but low sensitivity.

Uric acid:

Uric acid level is regarded as a marker of tissue oxygenation. Van Albada et al found that uric acid level correlated with invasive hemodynamic data (mean pulmonary artery pressure, pulmonary vascular resistance and cardiac index) in pediatric PH including patients with Eisenmenger's syndrome(130). Uric acid increased during the 12 months of our study (paper I) in keeping with a (relative to SVR) possible disease progression with decreased exercise capacity, decreased oxygen saturation and possibly increased pulmonary vascular resistance increase in our patient group. Some patients with small

defects (ASD) and PAH out of proportion were included, who are believed to have a worse prognosis than Eisenmenger patients. Again, a placebo arm would have improved result interpretation, but the small group size made this a less attractive choice.

Troponin I

Troponin I is a prognostic marker in IPAH (134;135). Troponin T served as a marker of prognosis and severity of disease in a mixed (non PAH-CSPS) adult group, probably due to leak of small amounts of protein from a pressure overloaded right ventricle.

Norepinephrine

Norepinephrine was predictive of mortality (ROC 0.84) in a pediatric PAH study (130). In conclusion, NTproBNP, Uric acid and possibly norepinephrine have a role in monitoring advanced PAH-CSPS and Eisenmenger's syndrome. These markers are not sufficiently validated in the early disease stages.

5.5 Echocardiography in PAH-CSPS

Due to the low reliability of Doppler based estimates of systemic to pulmonary flow ratios, especially in the presence of a PDA, little emphasis was put on the echocardiographic part of the monitoring protocol in paper I. No change could be demonstrated between follow-ups in technically robust parameters, such as post-tricuspid shunt flow velocity. This indicates that Rp/Rs changes may have been too small for detection by standard echocardiography. At this time, images were not captured in such a way that the new modalities, such as tissue Doppler or strain analysis could be utilized. In a future study, a more elaborate echo protocol is warranted, as developments have been made. Measurements such as TAPSE, 3D right ventricle volumes with ejection fraction (including novel 2D based 3D reconstruction methods(136)), tissue Doppler velocities (137) 2D based strain and strain rate of the right ventricle (138), Doppler indices beyond the Tei index(139) may provide markers of disease progression. Further, advanced echocardiographic methods such as capacitance measurements may bring new monitoring parameters in PAH-CSPS treatment (140). However, many initially promising methods fail to find their place in clinical use, often due to complexity and reproducibility problems (141). Increased stroke volume by echocardiography has been

demonstrated in a long term follow up study of patients PAH-CSPS treated with bosentan (142). In sum there is ongoing development in echocardiographic assessment of PAH, including PAH-CSPS, that makes this a central tool for future studies.

5.6 Exercise-induced pulmonary hypertension assessment

There are some PH related diagnoses, that exclusively can be made during exercise studies, such as exercise induced PAH, exercise induced heart failure with preserved ejection fraction and preload failure of left ventricle during exercise. As indicated by the revised WHO definition, increased right ventricular systolic pressure during exercise still is insufficiently understood. Exercise related pulmonary hypertension has been demonstrated in different populations, such as patients with systemic sclerosis(71), BMPR2 mutation heterozygotes(143), healthy elderly, endurance athletes (144) and more. It was recently abandoned as a clinically meaningful entity due to its overlapping occurrence in both health and disease, thus not being suitable for clinical decision-making. Nevertheless, many believe that with further development, the demonstration of exercise related pulmonary hypertension can be a useful tool for the early diagnosis of PAH. As treatment options improve, an early diagnosis becomes more important(145). Exercise echocardiography studies have the potential of screening risk groups non-invasively for preclinical disease. We have, like other groups, shown that pulmonary artery pressure can be estimated reliably during supine bicycling(78;79). One of the problems during noninvasive exercise induced pulmonary hypertension assessment, is separating increased precapillary and postcapillary resistance, due to difficulties measuring left ventricular filling pressures during exercise. Further, detection of an out of proportion pulmonary arteriolar constrictive response (Kitajev reflex) to exercise increased left atrium pressure is challenging. Invasive exercise studies, as those performed at the Massachusetts General Hospital, referred to at www.phaunivonline.org, seem to have overcome some of these issues. The prevalence of exercise related pulmonary hypertension among patients with septal defects was first reported in our previous paper (16). Elaborating further the understanding of exercise induced pulmonary hypertension mechanisms, paper IV indicate that in ASD and VSD, exercise related

pulmonary hypertension occurs at least partly as a consequence of increased left ventricular filling pressure. An exaggerated arteriolar constrictive component does not seem to be present, because this reflex would be expected to be blunted by sildenafil. Obviously, stiff pulmonary arteriolar walls with less dilative capacity and reduced total pulmonary vascular cross-sectional area may contribute in at least some of these patients, but this is difficult to measure. The size of the lungs may play a role in all PAH patients, and a pulmonary vascular bed area Z-score concept has been suggested. Patients with a subnormal area Z-score could be presenting with exercise induced pulmonary hypertension. Spirometry data were normal in the paper IV study group. Unpublished diffusion capacity data showed a trend towards reduced DLco with sildenafil. As left ventricular exercise diastolic dysfunction was suggested as responsible for RVSP increase, reduced DLco could be due to some degree of pulmonary congestion from the lowering of precapillary resistance by sildenafil, although not significantly affecting the RVSP, which is dependent on the achieved cardiac output.

The strengths of study IV are the randomized placebo controlled design without relevant selection bias. A central limitation is the lack of reliable cardiac output measurements and invasive hemodynamic data. A higher dose of 100 mg has been applied in some studies, but the dose applied in our study of 50 mg has a documented acute effect (91). There was no feasibility and reproducibility testing of the TDI measurements performed in this study, but the same method was tested in pediatric heart transplant recipients, with acceptable reliability, presented as an abstract at Euroecho 2010 by the author.

Whether exercise induced pulmonary hypertension in septal defects is a clinically relevant condition or will become so with time is still unknown. A cross-sectional study in a higher age group after VSD closure is ongoing at our centre in effort to answer some of these questions. To summarize, we believe that with further development of methods, exercise induced pulmonary hypertension will be reintroduced as a diagnostic entity in subsequent revisions of WHO guidelines, with a more precise definition, and with exercise echocardiography as a screening tool for monitoring high risk groups with predefined PH mechanisms. Further, there is a need for developing noninvasive diastolic parameters such as strain based diastolic function, for use during exercise tests.

5.7 Invasive hemodynamic data and operability

Heart catheterization with vasodilator testing was not part of the present thesis. However, it is a well defined part of the diagnostic work-up of pulmonary hypertension (18), and still is the key to evaluation of CSPA operability in the case of “greyzone” pulmonary vascular resistance as assessed by echocardiography screening (13). The INOP study emphasized the importance of vasoreactivity for operability assessment, applying $R_p/R_s < 0,33$ as operability criterion. Further studies have suggested guidelines (18;146;147). A baseline pulmonary vascular resistance index < 6 Wood Units/ m^2 with $R_p/R_s < 0.3$ or achieving similar numbers during vasodilator challenge, allows for biventricular repair. (148). Other authors argue that 4-8 Wood Units is a “grey zone”. With respect to the follow up of PAH-CSPA treatment, the place for invasive studies is less clear. There is little data on safety of invasive studies in pediatric PAH, but general anesthesia in severe PAH-CSPA is considered a risk. No deaths are reported during catheterization in the TOPP pediatric PAH registry until date. However, an underreporting would be expected, as death during diagnostic catheterization would exclude the patient from the registry. With respect to the validity, up to 13 % spontaneous variation of snapshot pulmonary vascular resistance measurements has been reported(67). Further, Ohms law and the total pulmonary vascular resistance index concept are simplifications, and may need supplements, such as capacitance measurement to improve prognostic value (149).

5.8 Conventional Radiology in PAH-CSPA

Chest radiograms in patients with PAH show PA contour and dimension. These may increase with time, and heart size increases with heart failure development. Peripheral pulmonary vascular markings gradually decrease with disease progression. Calcifications of pulmonary arteries may be seen in Eisenmenger’s syndrome(118). Cardiothoracic ratio has recently been shown to correlate well with survival in adult PAH (150). Standard

radiography is part of clinical assessment but offers little in detailed therapeutic monitoring of children.

5.9 Electrophysiology

Repeated ECG's may provide information about changes in right ventricular hypertrophy, and including Holter monitoring, is a part of standard clinical PAH assessment. However, pediatric data are missing (151;152) with respect to the value of ECG's in the assessment of disease severity and treatment effect.

5.10 Lung function tests

Lung volumes measurement are an important part of initial assessment of susceptibility factors such as small lung volumes, but provide little detailed information in further assessments of treatment effect. Ventilatory efficiency during exercise testing is a reliable parameter that may be related to pulmonary vascular resistance(153). DLco is a surrogate for number of functioning alveolocappillary units and may have a place in serial assessments, given that the measuring methods are reliable (154). Exhaled nitric oxide increased with bosentan treatment in a group consisting mainly of patients with IPAH, and could play a role in treatment monitoring(155).

5.11 Advanced radiological imaging

The non-invasive nature of MRI makes it an attractive monitoring tool. Right ventricular volumes and ejection fraction have become standard MRI techniques, applicable in IPAH patients for the assessment of right ventricle function and provide prognostic information(156). Right ventricular geometry correlates with invasive pressure measurements (157). However, the availability of MRI is low and smaller children require general anesthesia. MRI derived pulmonary artery flow data are feasible in

congenital heart disease and can provide detailed hemodynamic data together with invasive pressure measurements(158). MRI also allows for assessment of large pulmonary artery distensibility, which has been correlated with functional status and invasive pulmonary artery pressures(159). Pulmonary angiography: Irreversible change as in Heath Edwards III is seen as loss of arborization, tortuosity, narrowing and cutoff of small pulm arteries(160). The invasive nature of the technique makes it less attractive as follow-up parameter.

5.12 Lung Biopsy: the ultimate gold standard?

Obviously, lung biopsy is one way of looking directly at the PAH disease process and as such represents a sort of gold standard. However only regional data are sampled, and as illustrated by the present case history, these may be unrepresentative, even at autopsy. Multiple lung biopsies are still considered a high risk in patients with PAH. Histological classifications have to some degree succeeded at bringing borderline operable patients to corrective surgery with good outcome(161), but there is still a fear of false inoperability statements, due to the potential lack of representativity for the whole pulmonary vascular bed. A lung biopsy is due to its invasive nature and uncertain prognostic value, rarely performed, but may be indicated when there is a suspicion of such diseases as pulmonary veno-occlusive disease or capillary hemangiomatosis(161). In the case a clear-cut reversibility marker was achievable by biopsy, its use could theoretically be re-vitalized through less invasive thoracoscopic techniques, for shunt closure decision making in patients with “greyzone” pulmonary vascular resistance.

6. Future perspectives

Evaluating hemodynamic changes in patients with PAH-CSPS and especially Eisenmenger’s physiology is complex. Short term controlled trials of bosentan in Eisenmenger’s Syndrome with open extensions (57;58) have established the indication for treatment with bosentan. Similar studies of sildenafil are recently published (63).

Most studies demonstrate moderately increased 6MWT. Although closely related to the disease process, hemodynamic treatment effects are less consistent. Pulmonary vascular resistance index reductions may be minor and accompanied by simultaneously reduced SVRI(57). Cardiac output measurements are difficult in patients with Eisenmenger's physiology, but developing these may provide an important key to understanding how the new treatment strategies provide symptomatic relief and apparently improved survival in patients with PAH-CSPS(103). To understand the mechanisms behind symptomatic improvement in PAH-CSPS, a comprehensive evaluation is still needed, and should perhaps also include measurements of cardiac output, peripheral oxygen delivery and ventilation perfusion-relationship. A principle of enhancing the sensitivity and possibly also validity of monitoring parameters, is the assessment of acute responses to stress (e.g. exercise induced circulating biomarker change) or to treatment (e.g. acute vasodilator induced change in exercise ability or oxygen saturation).

Assessing vascular reactions during exercise addresses the very core of the patients problem: to increase pulmonary blood flow during exercise. A circulating marker that either measures vasodilation (endothelium in/dependent) or lack of vasodilation reserve could be a candidate for providing a more detailed and pathophysiology-oriented assessment of the pulmonary vascular bed.

Further – the heart is a monitoring target in PAH. right ventricle hypertrophy is an early finding, dilation and failure being late phenomena. The value of parameters reflecting RV failure, such as NTproBNP or myocardial leak of Troponins, have both been demonstrated, and they are natural candidates for further investigation of cardiac responses in PAH-CSPS, including acute stress studies.

7. Conclusions

The scientific and clinical consequences of the present thesis can be summarized as follows:

The monitoring of treatment effects in PAH-CSPS is still under development, and at present multimodal, including both noninvasive and invasive measurements.

The referred case report pointed to challenges in providing the critical therapeutic monitoring of patients with severe PAH-CSPS. Paper I emphasizes the utility of well standardized and longer term SpO₂ measurements in patients with PAH-CSPS.

Paper II identifies endothelial activation and inflammation involving platelet activation in patients with PAH-CSPS. This suggests future trials of drugs with anti-inflammatory properties such as statins, or immunomodulators such as imatinib, in patients with PAH-CSPS. Positive effects have already been documented in other PAH subclasses (162;163). Based on the finding of increased CRP in PAH-CSPS group, we suggest that CRP should be included in such treatment protocols in parallel to NTproBNP, possibly as an expression of inflammatory activity in the vascular lesions. Paper III indicates that inflammatory mechanisms are not activated in operable patients with CSPS, and thus that cytokines are not candidates for preoperative assessment of pulmonary vascular stress in operable patients with CSPS. Studies assessing inflammatory biomarkers in patients with CSPS and increased pulmonary vascular resistance, comparing operable and inoperable patients, are still warranted.

Paper IV identified left ventricular diastolic dysfunction during exercise as a possible mechanism of exercise induced pulmonary hypertension in patients with VSD, suggesting that this is related to left heart filling pressures, rather than pulmonary arterial wall changes. This raises questions for further studies: Are these diastolic characteristics related to left ventricle myocardial properties that follow the VSD phenotype, or to left ventricular myocardial remodeling due to shunt volume load? Does the timing and hemodynamic indication level for VSD closure affect the occurrence of exercise induced pulmonary hypertension? Lastly, the absence of effect on peak VO₂ by sildenafil indicates that the drug has no acute exercise capacity enhancing effect in this group, and lends support to its absence from the World Anti Doping Agency black list.

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Paper I – IV.....

Original Article

Treatment of patients with Eisenmenger's syndrome with Bosentan

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Abstract We treated prospectively 14 patients with Eisenmenger's syndrome, with a mean age of 10 years, ranging from 3 to 18 years. Treatment continued for 12 months, and demonstrated a lasting symptomatic improvement, but no improvement in terms of mean saturation of oxygen over 24 hours. Exercise capacity, as judged by peak uptake of oxygen, worsened in the six patients able to perform a treadmill test. The symptomatic benefit from dual blockage of endothelin receptors in these patients may be due to mechanisms other than selective pulmonary vasodilatation alone.

Keywords: Pulmonary hypertension; congenital heart disease; exercise testing

PULMONARY ARTERIAL HYPERTENSION REMAINS an important factor for morbidity and mortality in patients with congenital cardiac defects. Among the new medical therapies for pulmonary arterial hypertension, the oral dual endothelin receptor antagonist, Bosentan (Actelion Pharmaceuticals Ltd., Allschwil, Switzerland), has gained a central position. Long-term improvement of both symptoms and haemodynamic data,^{1–3} and improved survival,⁴ is documented in adults with idiopathic pulmonary arterial hypertension and some forms of secondary pulmonary arterial hypertension. In children, congenital systemic-to-pulmonary arterial shunts represent an important aetiology of pulmonary arterial hypertension. Eisenmenger's syndrome shares both clinical, histopathological, and pathophysiological properties with idiopathic pulmonary arterial hypertension.⁵ The open shunt, however, represents a significant haemodynamic difference, and the natural history is more favourable than for idiopathic pulmonary arterial hypertension.^{6–8} The role of new medical therapies against pulmonary arterial hypertension for patients with Eisenmenger's syndrome is not yet

established, although preliminary uncontrolled short term studies show some benefits.^{9–12} A short-term safety study in children not including patients with Eisenmenger's syndrome demonstrated haemodynamic improvement.¹³ A retrospective study including 24 children with unoperated cardiac defects demonstrated clinical and haemodynamic improvement from treatment with Bosentan at a mean of 14 months, but there was no separate analysis of the subgroup with Eisenmenger's syndrome.¹⁴ Our present study, therefore, aims to describe the effects of Bosentan over the intermediate term in children and adolescents with Eisenmenger physiology. Our primary hypothesis was that a selective reduction of pulmonary vascular resistance would decrease the degree of desaturation. Our secondary aim was to search for other non-invasive variables that might prove valuable in the monitoring of treatment in patients with Eisenmenger's syndrome.

Material and methods

We define Eisenmenger's syndrome as a systemic-to-pulmonary shunt permitting reversed or bidirectional flow due to high pulmonary vascular resistance. We included patients with both pre- and post-tricuspid lesions if they satisfied our definition. The indications for treatment ranged from the possibility for

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cure and corrective operation in the younger and less symptomatic patients, to relief of symptoms in those most affected. We identified 14 patients with the syndrome as thus defined referred for evaluation at a tertiary centre over the years 2003 and 2004, and all agreed to participate in the study. Informed consent was obtained from all patients or their providers of care. The patients were in a clinically stable condition at enrolment. No change had been made to their medical treatment over the preceding 12 months. Of the 14 patients, 11 had undergone catheterization of the right heart with vasodilator testing prior to treatment. The ratio of pulmonary to systemic vascular resistances at baseline ranged from 0.36 to 1.0, with a mean of 0.6, and was at the time considered a contraindication for surgical treatment. Demographic and clinical data at baseline are presented in Table 1.

Design of the study

The study was prospective, uncontrolled, single centre, open-label, and performed during the period from 2003 to 2005. After the baseline examinations, treatment was started with Bosentan at 1 milligram per kilogram twice daily, increasing to 2 milligrams per kilo twice daily after four weeks. Initiation and control of treatment was done in-hospital. In this way, physical activity was held at a controlled and low level. Patients were re-examined at one, three, six, nine and 12 months. At all visits, clinical examination, echocardiography, electrocardiogram, 24 hour pulse oximetry, blood tests and a symptom score were performed in all patients. In the 6 patients able to exercise, treadmill testing was performed. The study was approved by the local ethics committee

and conducted in agreement with the Helsinki declaration of 1975, revised in 1983.

Pulse oximetry

Peripheral transcutaneous saturations of oxygen were measured distal to the shunt for 24 hours, using Masimo SET® (USA) pulse oximeters. Saturation and pulse data were analysed with Download 2001® (UK) software. For each 24 hour measurement, the following parameters were calculated: mean oxygen saturation, median oxygen saturation with 5th and 95th percentiles and mean heart rate. The 5th percentiles were assumed to reflect activity related desaturations. The 95th percentile was assumed to represent maximal saturation. The range between 5th and 95th percentiles was registered as a parameter of variation in the saturations of oxygen. Similar values were calculated for 6 hour sleep samples from the same registration. Sleep was identified by a stable, low heart rate at night time.

Clinical assessment and laboratory values

In addition to a standard clinical examination, symptoms were registered according to a five issues score for symptoms of childhood pulmonary hypertension.¹⁵ The score of each issue ranges from one to four points. The lowest possible sum of five points represents serious disability, whereas the highest score of 20 points corresponds to normal exercise ability. Functional class was registered according to the World Health Organization adaptation of the New York Heart Association classification of heart failure. Haematological parameters, and a set of

Table 1. Baseline demographic and clinical data.

Patient number	Sex	Age at inclusion	Diagnoses	Weight (kg)	WHO class	24 hours oxygen saturation (median and 5 and 95 percentile)
1	m	7	DS, VSD, PAD	22	2	95 (91–97)
2	m	9	DS, AVSD	20	2	94 (91–97)
3	f	10	Large PAD	21	2	97 (96–99)
4	m	7	AVSD	20	3	83 (75–86)
5	f	17	Large PAD	55	2	86 (64–93)
6	f	18	Residual VSD	50	3	77 (63–83)
7	m	11	DS, large PAD		2	93 (87–97)
8	f	11	ASD	22	3	87 (81–92)
9	f	13	DS, VSD	51	2	98 (94–99)
10	f	6	ASD	15	2	98 (96–99)
11	f	13	AVSD	30	2	91 (84–93)
12	m	10	Residual VSD	29	2	92 (86–95)
13	m	7	DS, ASD, VSD, PAD	18	2	91 (86–95)
14	f	3	Large PAD	13,1	2	98 (96–99)

Abbreviations: DS: Down's syndrome; VSD: ventricular septal defect; PAD: persistent patency of arterial duct; AVSD: atrioventricular septal defect; ASD: atrial septal defect

standard blood tests including uric acid analysis and natriuretic peptides (Nt-proANP or Nt-proBNP), were performed at all controls. Liver enzymes were checked at monthly intervals as required for treatment with Bosentan.

Echocardiography

A standard echocardiographic assessment was done at all visits, including estimation of chamber areas and deviation of the ventricular septum where applicable. The maximal velocities of the jets from tricuspid and pulmonary regurgitation were registered, as well as the direction, timing, and velocity of flow across the shunt.

Exercise testing

We were able to perform a treadmill test in 6 patients, with measurement of peak uptake of oxygen. Young age, and presence of Down's syndrome, were the limitations in the remaining 8 patients. Testing was done by a specially trained physiotherapist and the same cardiologist at all assessments. The tests were conducted to volitional fatigue on treadmill (Technogym, Italy), using the Oslo-protocol for children.¹⁶ Uptake of oxygen was assessed by an oxygen analyzer (SensorMedics Vmax 229, USA). Electrocardiograms were recorded during the tests using Siemens Megachart (Germany).

Statistics

All values are reported as means plus or minus standard deviations. The differences between findings at baseline and after 12 months of treatment were analysed by use of single sample t-tests, with a level of significance of 0.05. The relationship between changes in symptoms and saturations of oxygen was investigated with linear regression. With respect to the limited number of participants, no statistical analyses were performed for the results at three, six and nine months. The values at six months are, however, included in the figures.

Results

Clinical data

Of the children, 6 were males, and five had Down's syndrome. Except for 3 patients with atrioventricular septal defects, all had simple defects, as described in Table 1. Surgery had been previously performed in 2 patients to close ventricular septal defects, but both patients had non-restrictive residual shunts. All patients survived to the last follow-up. At enrolment, one patient was receiving captopril, and two others digoxin. The fourth patient had hepatic

enlargement, but no other signs of right ventricular failure. The tenth patient received nebulized iloprost after nine months of treatment with Bosentan. No other change of supplementary medication was made during the period of study, and none received supplementary oxygen. No significant increment of hepatic enzymes was registered. The second patient reported persistent nasal congestion. He developed obstructive sleep apnoea and worsened general condition at nine months, with nocturnal desaturation. No improvement was seen with reduced doses, but the symptoms disappeared after cessation of treatment at 12 months. No other serious side effects were seen. Diastolic blood pressure declined by 10 plus or minus 11.2 millimetres of mercury (p is equal to 0.009) following treatment with Bosentan. There was no change of systolic blood pressure (p is equal to 0.9).

Symptoms

All patients but the second reported either improvement, in 8 cases, or stayed stable, as in the remaining 5 in regard to symptoms from baseline to 12 months follow up. Improvement was seen in 3 of the 5 children with Down's syndrome. The pulmonary hypertension symptom score increased from baseline to 12 months by 2.7 plus or minus 3.3 (p is equal to 0.009). The individual changes are shown in Figure 1, and the scores of the different issues of the questionnaire are presented in Table 2. Significant improvement was seen for the flat walking and tiredness subscores, and a positive trend was seen for walking stairs. The fourth

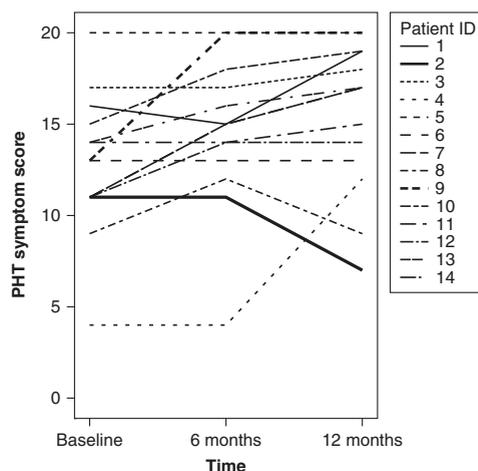


Figure 1. Individual changes of the total pulmonary hypertension symptom score at baseline, and after 6, and 12, months of treatment. A higher score signifies fewer symptoms.

Table 2. Reported performance before and after 12 months of therapy with Bosentan. Values represent symptom score as mean plus or minus standard deviation.

	Baseline	12 months	p-value
School participation	2,8 ($\pm 0,9$)	3,2 ($\pm 1,0$)	0,165
Walking flat surface	2,9 ($\pm 0,8$)	3,5 ($\pm 0,5$)	0,014
Running/jogging	2,0 ($\pm 0,9$)	2,4 ($\pm 1,4$)	0,315
Walking stairs	2,6 ($\pm 0,8$)	3,1 ($\pm 0,7$)	0,082
Tiredness	2,5 ($\pm 1,0$)	3,4 ($\pm 0,9$)	<0,001
Total score	12,8 ($\pm 3,8$)	15,5 ($\pm 4,1$)	0,009

patient improved from functional class 3 to 2 as graded using the system of the World Health Organization.

Exemplary cases

Our fourth patient was a boy, aged seven years, with uncorrected atrioventricular septal defect, severe hypoxaemia, and erythrocytosis. Before treatment, his mother carried him from ground to first floor at home due to dyspnoea. After treatment with Bosentan, he could run the same distance, and had abandoned the wheelchair for everyday use. Former headaches and large mood variations had disappeared. Previous attendance at school had been no more than half-time, but with Bosentan he could attend school throughout the day. During the same period, peak uptake of oxygen during treadmill testing declined from 83 to 66 millilitres per 0.67 kilo. His mean saturation over 24 hours remained unchanged during the period of study, but his mean saturation over six hours of sleep declined from 96% to 90%. Selective daytime increase could explain these findings.

Our ninth patient was a 13 old year girl with Down's syndrome and a non-restrictive ventricular septal defect. Prior to treatment with Bosentan, she was passive and difficult to mobilize. After 6 months follow-up, her parents reported a marked improvement in her mood and initiative. In contrast to her stopping multiple times with blue lips when climbing the small hill to their mountain cabin, she was now able to walk this distance rapidly with a pink-lipped smile. Measurement of mean saturations of oxygen over 24 hours declined from 97.3% to 94.7%, albeit that night time saturations improved from 85.3% to 88.1%.

Saturations of oxygen

Baseline mean levels ranged from 85% to 95%. Patients with normal baseline saturations at rest demonstrated significant desaturations during exercise. Six hours of sleep, including night time saturations, were analysed as a separate parameter. Group values before and after 12 months of treatment are presented in Table 3, and the individual values of the

Table 3. Group oxygen saturation variables before and after 12 months of Bosentan treatment. Scores are for the mean plus or minus standard deviations.

	Baseline	12 months	p-value
24 hours mean	90,7 ($\pm 6,8$)	89,0 ($\pm 6,0$)	0,031
5th centile 24 hours	85,1 ($\pm 11,0$)	83,3 ($\pm 7,9$)	0,27
95th centile 24 hours	94,6 ($\pm 4,9$)	93,3 ($\pm 5,2$)	0,003
6 hours night, mean	91,1 ($\pm 6,6$)	88,0 ($\pm 6,2$)	0,08

mean saturations over 24 hours are shown in Figure 2. The average of 24 hour mean heart rate at baseline was 84.2 plus or minus 12.2, and at 12 months was 80.8 plus or minus 12.0 beats per minute (p is equal to 0.12). The saturations of oxygen improved in one patient, were stable in seven, and declined in six. Changes were minor, except for the third and seventh patients. We could not identify any special characteristics in these two patients, and no relationship could be found between changes in saturation and symptoms. Even with the exclusion of the second patient, who developed nasal congestion, differences in saturations over 24 hours were significant (p is equal to 0.05).

The saturations showed large variations within each registration. The mean value of the difference between the 5th and 95th percentile from all recordings was 10.7% plus or minus 5.9. This marker of variation was similar at baseline and 12 months (p is equal to 0.7). In most patients, significant resting variation was observed. Figure 3 shows the variation in mean and median saturations from standardized 30 minutes resting measurements in our fourth patient during 8 consecutive days.

Exercise testing

We were able to complete treadmill testing in 6 patients. The individual values of peak uptake of oxygen are shown in Figure 4, indicating a gradual fall in exercise tolerance during the period of study. The mean reduction of peak uptake adjusted for age was 7.7 plus or minus 6.9 millilitres per 0.67 kilo (p is equal to 0.045). All treadmill tests were conducted to volitional fatigue, and we failed to register any significant falls in blood pressure, signs of ischaemia, or arrhythmias. Saturations of oxygen at end exercise declined to levels below the reliability of our equipment, and could not be compared. Submaximal exercise capacity also showed a trend to decline as represented by the uptake at 80% of maximal heart rate. Mean reduction was 15.3 plus or minus 15.7 millilitres per 0.67 kilo (p is equal to 0.06). Mean

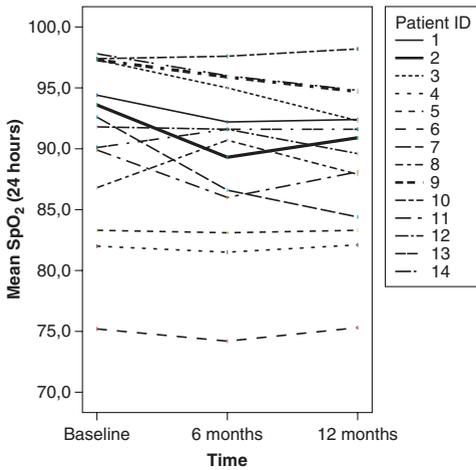


Figure 2. Individual values of mean 24 hour oxygen saturation at baseline, and after 6 and 12 months of treatment.

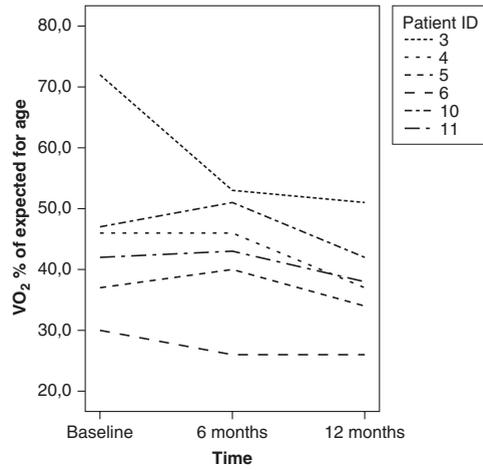


Figure 4. Individual values of peak oxygen uptake as percent of the average for age and gender.

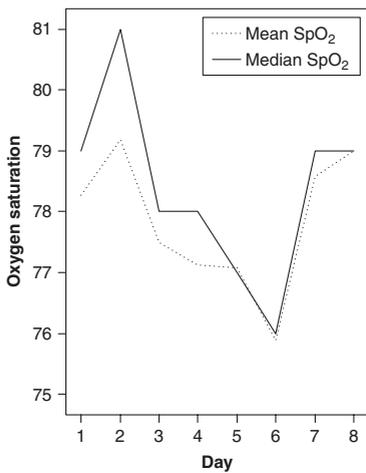


Figure 3. Example of standardized, supine 30 minute measurements in one patient at the same time of the day for eight consecutive days.

slope of minute ventilation plotted against uptake increased with 12.2 plus or minus 7.4 from above normal levels at baseline (p is equal to 0.01). A trend towards increased slope of minute ventilation to production of carbon dioxide was found. The mean increment was 7.1 plus or minus 7.4 (p is equal to 0.06).

Blood tests

All patients had blood tests performed at all assessments in addition to the monthly control of hepatic enzymes. No significant change was seen in levels of

haemoglobin or haematocrit from baseline to 12 months follow-up. A mean rise in serum uric acid of 25 plus or minus 24 units was seen during the same period (p is equal to 0.05). Values of Nt-proANP were normal in all but the second and eighth patients, who had moderately elevated values, ranging from 1800 to 2200 throughout the period of study.

Echocardiography

Because of the varying anatomy, no parameter was found suitable for comparison on a group level. No definite echocardiographic changes were registered in any individual patient.

Discussion

This is, to our knowledge, the first study over the intermediate term of treatment with Bosentan addressing specifically children and adolescents with the Eisenmenger syndrome. We registered a small but significant fall in saturations of oxygen, and reduced exercise tolerance in a subset. Despite this, the patients reported symptomatic relief.

With regard to saturations of oxygen, invasive measurements of pulmonary vascular resistance employing the Fick method may be imprecise, and the findings during general anaesthesia do not necessarily reflect the natural condition of the patient. Besides, cardiac catheterization is not without risks in this group of patients. We thus chose to use non-invasive endpoints, with saturation as the most important. The primary hypothesis was that, in the presence of a reversed systemic-to-pulmonary shunt,

a reduction of pulmonary vascular resistance should decrease the degree of desaturation, either at rest in hypoxaemic patients, or during physical activity in those who were normoxaemic. There is no gold standard for measurements in this situation. Traditionally, saturations have been registered in the supine position for a short period. As demonstrated in Figure 3, this may be imprecise and unreliable. In analogy with measurements of blood pressure taken over 24 hours, we believe that a long-time recording increases the representivity and validity of the findings. A major advantage with the chosen equipment is the possibility of computerized registration and calculation of data. Several mechanisms may account for the observed decline in saturations after 12 months of treatment with Bosentan. Symptomatic improvement resulting in increased physical activity could lead to more frequent or longer lasting desaturations. Registrations, however, were done in hospital at stable and low levels of activity, and the value of the fifth centile for saturation remained unchanged. In a recently published randomised controlled trial of Bosentan in adults with Eisenmenger's syndrome, a borderline significant reduction in systemic vascular resistance was observed.¹⁷ We suggest that systemic vasodilation is the most likely explanation for the small but statistically significant fall in saturation observed in our patients. This is supported by the reduction of diastolic blood pressure. Measurements taken at night time show a less pronounced decline than the values for the 95th centile. There were individual variations, but preservation of cerebrovascular saturation during sleep, and increased cardiac output, could be a mechanism of the significant reduction of tiredness.

With regard to symptoms, our study differs markedly from other studies in including patients almost exclusively in functional class 2 of the system of the New York Heart Association. The improved functioning reported by the patients and their parents may, of course, represent a placebo effect. Such a mechanism, however, would be expected to fade away with time, and in several cases, we registered a marked and long-lasting improvement. The relief of symptoms in spite of lower saturations of oxygen may be explained by improvement of cardiac output, following reduction of both systemic and pulmonary vascular resistances. Improved cardiac function and increased coronary arterial flow by antagonists of endothelin receptors are described in experimental models.¹⁸⁻²⁰ We did not include measurements of cardiac output in this study.

Exercise testing, with measurement of uptake of oxygen, is validated in children and regularly used as a core parameter of cardiopulmonary function in our department.¹⁶ It is also useful for the assessment of pulmonary hypertension.²¹ In our study, significant improvement of flat walking distance was reported,

which implies improved submaximal exercise tolerance. This is difficult to integrate with the decline in peak uptake of oxygen. One explanation may be that a reduced systemic vascular resistance improves cardiac output at submaximal exercise, while increased desaturation becomes a limiting factor at higher intensity levels. Increased ventilation perfusion mismatch during maximal exercise may also be a factor of importance.

Levels of uric acid in the serum correlate with severity and survival in idiopathic pulmonary hypertension, possibly reflecting peripheral oxygenation of the tissues.²² In our study, such levels increased, but values of haemoglobin and haematocrit were stable, supporting preserved oxygenation.

Our study has the general limitations of uncontrolled observational studies. Eisenmenger's syndrome is assumed to be a stable condition at this age. All patients were clinically stable during their last year before starting treatment. This makes the use of the patients as their own controls more reliable. Stable low values of natriuretic peptides strongly contradict development of heart failure. We cannot excluded completely, however, the possibility that deterioration may have been prevented by treatment with Bosentan. The outcome in our study may have become affected by a deviant treatment response in patients with genetic predisposition for development of pulmonary hypertension. This may apply particularly for our 2 patients with atrial septal defects, and for the 5 patients with Down's syndrome, who represent a large proportion of our cohort. The distribution, nonetheless, is probably representative for the occurrence of Eisenmenger's syndrome in childhood. The most important limitation of the study is our failure to measure cardiac output.

Other reports of treatment with Bosentan in pulmonary arterial hypertension are almost uniformly positive. Previous uncontrolled studies, including both patients with and without Eisenmenger's physiology, report positive clinical and hemodynamic effects at 16 weeks.^{9,12,14} Recent small-scale, uncontrolled trials in adults with Eisenmenger's syndrome demonstrated improvement in saturations of oxygen, and in echocardiographic, haemodynamic, and exercise parameters at 16 weeks.^{10,11} Our findings are also partly in conflict with the findings of Apostolopoulou *et al.* in younger patients,⁹ albeit that the different methods for measuring saturations of oxygen must be kept in mind. The first randomized trial of Bosentan in adults with Eisenmenger's syndrome demonstrated non-inferiority of the primary end point of saturations of oxygen, and a slight improvement of 6 minutes walk test after 16 weeks of treatment.¹⁷ Placebo-controlled short-term studies will, however, only answer some of the questions related to treatment of this complex group of patients, and

our protocol was designed to study the effects after one year. In contrast to other patients with pulmonary hypertension, patients with Eisenmenger's syndrome could be re-exposed to pulmonary hyperflow in case of therapeutic success. We emphasize, therefore, the need for studies of long-term outcome.

In conclusion, our data suggest that saturations of oxygen in mildly symptomatic children and adolescents with Eisenmenger's physiology are not improved by treatment with Bosentan as judged by follow-up at one year. In contrast to the original hypothesis, our findings indicate that giving Bosentan in children with Eisenmenger's syndrome does not cause a selective reduction in pulmonary vascular resistance. Our findings may indicate, nonetheless, that there is symptomatic improvement. A possible explanation may be improved cardiac output, following a fall also in the systemic vascular resistance. For a proper understanding of the complex haemodynamic alterations, it is necessary to address ventilation-perfusion mismatch and cardiac output, in addition to vascular resistances and clinical findings. Our study does not permit conclusions to be drawn regarding the long term therapeutic benefit from Bosentan as given to young patients with Eisenmenger's syndrome.

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Conflicts of interest

Henrik Brun has received travel grants from Actelion Pharmaceuticals for participation at three international meetings, and Henrik Holmstrøm has received one similar grant. Henrik Brun has also received a similar travel grant from Schering AG.

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