

University College London

**NON-INVASIVE EVALUATION, THERAPY AND TRANSPLANTATION IN
CHILDREN WITH PULMONARY ARTERIAL HYPERTENSION**

MD thesis

by

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I, Astrid Elisabeth Lammers confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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London, 10 September 2010

ABSTRACT

Pulmonary hypertension (PHT) is a progressive and ultimately fatal disease. The condition is poorly understood in children. This thesis evaluated non-invasive assessment techniques, experience with epoprostenol, atrial septostomy and lung transplantation in children. As therapy is guided by patients' functional capacity, studies focused on 6-minute walk test distance (6MWTd) and cardiopulmonary exercise testing (CPET). Age-related normal values of 6MWTd were determined, and the relationship between 6MWTd and CPET was assessed. Echocardiographic markers of impaired myocardial function were investigated using tissue Doppler (TDI) and potential risk-factors of mortality were sought using heart rate variability (HRV) and B-natriuretic peptide (BNP).

Parameters of CPET correlated with 6MWTd in highly compromised children but in less impaired children the 6MWT represented a sub-maximal test, indicating that a CPET is required when 6MWTd exceeds 300m. BNP and HRV were related to prognosis but sensitivity and specificity are limited. TDI demonstrated that biventricular function is impaired in children with PHT and that left ventricular impairment cannot be evaluated adequately using conventional echocardiographic techniques. Reviewing our experience with intravenous epoprostenol, it improved functional capacity, survival and could be used safely in children with acceptable morbidity. Atrial septostomy abolished syncope and improved right ventricular function, at the expense of mild systemic arterial oxygen desaturation. Septostomies, with and without a fenestrated device, can close spontaneously with time, stressing the need for a more reliable device. When medical therapies were exhausted, transplantation dramatically improved quality of life and survival. Listing criteria were shown to have been appropriate but more objective criteria are needed. These studies evaluating assessment techniques, prognosticators, and therapies have given further insight into the management of children with PHT. Future work should include assessment of non-invasive magnetic resonance imaging, exploring a combined prognosticator, combination therapy and improving classification of clinical status to optimise therapy and facilitate trials of new, urgently needed medicines.

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List of abbreviations

6MWT	Six-minute walk test
APH	associated pulmonary hypertension
BMPR-II	Bone morphogenetic protein receptor type II
BMPR2	Bone morphogenetic protein receptor II gene
BNP	B-type natriuretic peptide
CHD	congenital heart disease
CI	confidence interval
CO	cardiac output
CO ₂	carbon dioxide
ECG	electrocardiogram
ET-1	endothelin-1
HIV	Human immunodeficiency virus
HR	heart rate
HRV	heart rate variability
IPAH	idiopathic pulmonary arterial hypertension
MV	mitral valve
NO	nitric oxide
O ₂	oxygen
PA	pulmonary artery
PAH	pulmonary arterial hypertension
PAP	pulmonary artery pressure
PDE-5	phosphodiesterase-5
PHT	pulmonary hypertension
PR	pulmonary regurgitation
PV	pulmonary valve
PVR	pulmonary vascular resistance
PVRI	pulmonary vascular resistance index
RVH	right ventricular hypertrophy
SE	standard error

SD	standard deviation
SpO ₂	transcutaneous oxygen saturation
TR	tricuspid regurgitation
TV	tricuspid valve
v max	maximal velocity
WHO	World Health Organization

1. INTRODUCTION

The normal pulmonary vascular bed is a compliant system with a low pressure and low resistance (1,2). Acute or chronic injury to the pulmonary vasculature may lead to an elevation of pulmonary artery pressure (PAP) and pulmonary vascular resistance, known as pulmonary hypertension (PHT) (3). Severe PHT leads to a progressive reduction in exercise capacity, right heart failure and death. Survival prospects depend primarily on the aetiology of the disease but are generally poor. Without treatment, the median survival time in idiopathic pulmonary arterial hypertension (IPAH) has been reported to be only 2.8 years in adults (4) and less than 1 year in children (5-7). Unfortunately, symptoms are variable and non-specific. Therefore, even in the current era the average time from symptom onset to diagnosis is 2.8 years (8). Patients may present with increasing exercise intolerance, shortness of breath on exertion, tiredness or syncope. Children are often more sick at presentation than adults (9). Failure to thrive and fatigue may be noted but symptoms can be subtle. Chest pain and peripheral oedema are rare in children, but may occur in adults. In IPAH presentation with syncope appears to be more frequent in children (7).

The first description of the condition dates back to the 19th century when Julius Klob described the case of a 59-year prebendary in 1865, who died of right heart failure and pulmonary hypertension ('endarteriitis pulmonalis deformans') (10,11) and Ernst von Romberg the case of a 24-year old patient with a similar clinical course, 'vascular sclerosis' of the pulmonary artery and no evidence of associated heart- or lung disease in 1891 (12). Subsequently, in Argentina Arrillaga, labelled the combination of pulmonary hypertension, cyanosis and right heart failure as black heart disease ('cardiacos negros') or Ayerza's disease, a tribute to his teacher (13,14). He also suggested that the condition may be related to syphilitic pulmonary arteritis. This misconception was not undisputed (15) and was eventually disproved by Oscar Brenner examining autopsy files at the Massachusetts General Hospital and reviewing the available literature in 1935 (16). Further insights into the pathophysiology of the condition was provided by the physiological experiments of von Euler and Liljestrand (17,18) - demonstrating that acute hypoxia induced

pulmonary vasoconstriction - and the clinical work of Paul Wood and David Dresdale (19,20) showing a reduction in pulmonary arterial pressures after infusion of vasodilators (acetylcholine and tolazoline, respectively). These observations were made possible by advances in cardiac catheterization pioneered by Forssman, Cournard and Richards (21-23) who were jointly awarded The Nobel Prize in Physiology and Medicine in 1956 for their discoveries. It became apparent that vasodilators failed to significantly reduce pulmonary arterial pressures in many patients with advanced disease suggesting that vasoconstriction is not the only mechanism responsible (24). This interpretation was supported by the typical histological findings in patients with PHT such as medial hypertrophy, muscularisation of small arteries and plexiform lesions (25). Further research into the condition was stimulated in the late 1960 by an epidemic of PAH in some European countries and later the US (26-28) related to the use of appetite-suppressants such as aminorex and fenfluramine. In response to this epidemic the first WHO meeting on PHT took place in 1973 and subsequently a national registry was established in the US which provided important epidemiologic, clinical and prognostic information of the condition (4). In the past 30 years the understanding of PHT has increased exponentially with identification of genetic mutations co-responsible for the condition (29) and the identification of important paracrine factors inducing vasoconstriction and vascular remodelling (30). Above all, however, based on this understanding drugs have become available that have transformed the outcome of patients with PHT (31). The first available disease-targeting drug was epoprostenol, first successfully used in 1984 (32), followed by other prostacyclins, endothelin-receptor antagonists and phosphodiesterase-5 inhibitors (33,34).

Definition and Clinical Classification of Pulmonary Hypertension

In normal individuals the mean pulmonary arterial pressure at rest is 14.0 ± 3.3 mmHg (1). Assuming a normal distribution, this gives an upper 95% confidence interval (mean+2 standard deviations) limit of 20.5 mmHg. As a consequence, a mean PAP below 21 mmHg has been defined as normal at the 4th World Symposium on Pulmonary Hypertension held at Dana Point in 2008 (35). A mean PAP between 21 and 25 mmHg at rest is regarded as borderline PHT, while manifest PHT is defined as a mean PAP of more than 25 mmHg at rest (36,37). Because the pulmonary

arterial pressure can vary considerably during exercise in normal individuals (1) the previous definition of PHT as an elevated mean PAP of more than 30 mmHg during exercise (37) has been abandoned.

The first classification of PHT established at the first World Symposium in 1973 was based on an histological grading system (25) and classified PHT only into two categories, primary or secondary PHT, depending on the presence or absence of an identifiable causes or risk factor (38). Due to increased understanding of the condition, the insight that pathological features are shared between many forms of PHT and, first of all, the advent of disease-targeting therapies, subsequent classification systems attempt to create categories of PHT that share pathologic and clinical features as well as therapeutic options (39). The current Dana Point classification subdivides PHT into five categories: (1) Pulmonary arterial hypertension (PAH), (2) PHT owing to left heart disease, (3) PHT due to lung diseases and/or hypoxia, (4) chronic thromboembolic pulmonary hypertension (CTEPH) and (5) PHT with unclear or multifactorial mechanisms as shown in Table 1.1.

Table 1.1. Clinical classification of pulmonary hypertension (Dana Point, 2008) (39).

Clinical classification of pulmonary hypertension (Dana Point, 2008)

I. Pulmonary arterial hypertension (PAH)

- Idiopathic PAH
- Heritable PAH
- Drug- and toxin-induced
- PAH associated with
 - Connective tissue disease
 - HIV
 - Portal hypertension
 - Congenital systemic to pulmonary shunts
 - Schistosomiasis
 - Chronic haemolytic anaemia
 - Persistent pulmonary hypertension of the newborn
- Pulmonary veno-occlusive disease or pulmonary capillary haemangiomatosis

II. Pulmonary hypertension owing to left heart disease

III. Pulmonary hypertension owing to lung diseases and/or hypoxia

IV. Chronic thromboembolic pulmonary hypertension (CTEPH)

V. Pulmonary hypertension with unclear multifactorial mechanisms

Idiopathic pulmonary arterial hypertension accounts for approximately 28% of all paediatric cases with PHT. The most common aetiology in children is PHT secondary to congenital heart disease (approximately 48%, either pre- or postoperative, due to complex CHD or Eisenmenger physiology). Other aetiologies seen in children are pulmonary hypertension associated to lung disease (13%) or connective tissue disease (4%). Approximately 7% are miscellaneous forms of PHT (40). These numbers were derived from a cohort of children with PHT, who have been referred to the UK Pulmonary Hypertension Service for Children between 2001 and 2006 (40). Other forms such as chronic thromboembolic, drug or toxin-induced or HIV associated pulmonary arterial hypertension are only rarely seen in children.

Data reporting 63 patients seen in a national referral centre for paediatric PAH in the Netherlands, reported a proportion of 46% with IPAH/ or 'idiopathic like' PAH. In this registry, PAH associated to congenital heart disease accounts for 37% of all patients. PAH secondary to respiratory disease and/or hypoxaemia was noted in 12% and connective tissue disease 3% (41).

This thesis focused on children with PAH. The following paragraphs, therefore, provide details of the aetiology, pathophysiology and treatment of PAH. Other forms of PHT have been reviewed in detail recently by Hoeper et al. (42).

Epidemiology of PAH

The incidence of PAH in the general population is estimated to 2.4 – 7.6 cases per million per year (43,44). A recent study based on the Scottish Morbidity Record Scheme estimated a PAH prevalence of approximately 26 - 52 cases per million inhabitants (44). This compares with data from a French registry suggesting a prevalence of approximately 15 cases per million (43). The incidence of IPAH in children in the UK has been recently reported to be 0.48 cases per million per year, with a prevalence of 2.1 cases per million (7). Data on the incidence and prevalence of PAH in children is lacking, however, data from a Swiss registry suggested that IPAH accounts for approximately 1/3 of the paediatric PAH cases (45).

Pathophysiology and Genetic Factors

The aetiology of pulmonary arterial hypertension is multi-factorial. It is thought that different pathophysiologic mechanisms acting synergistically lead to the

development and maintenance of pulmonary vascular disease. Pulmonary vascular disease is characterized by muscularization of distal - normally non-muscular - arterioles, smooth muscle cell hypertrophy, obstructive intimal proliferation and the formation of plexiform lesions (25,46) (47). Recently, a number of genetic mutations have been linked to pulmonary vascular disease (48). The most important genetic mutations known to date are those affecting the regulation of the transforming growth factor- β family of receptors, the bone morphogenetic protein receptor type II gene (BMPR2) and the activin-receptor-like kinase 1 (ALK-1) gene (49). These receptors are functionally linked as they form heteromeric complexes after ligand binding by the type 2 receptor (for example BMPR2), resulting in activation of activin-receptor-like kinase 1 with subsequent phosphorylation of cytoplasmic proteins and gene transcription (50,51). However, genetic mutations alone are not sufficient to explain the development of pulmonary vascular disease. Instead it has been suggested that pulmonary vascular disease is a multifactorial process requiring not only a permissive genotype, but also a vulnerable cell phenotype and additional exogenous triggers (52). Pulmonary endothelial damage, for example in patients with congenital heart disease and left-to-right shunt lesions, may represent such a trigger for developing pulmonary vascular disease in susceptible individuals (53). Endothelial damage as a consequence of a high pulmonary blood flow and pressure may allow serum factors to infiltrate the vascular wall, leading to degeneration and degradation of extracellular matrix as well as release of growth factors normally quiescent in the matrix (such as transforming growth factor- β and fibroblast growth factor) (54). Endothelial damage also favours platelet activation and activation of coagulation pathways. Beyond forming a mere barrier between the media and the vascular lumen, the vascular endothelium is the source of vasoactive mediators including nitric oxide, endothelin-1 and prostacyclin (55-58). Endothelial damage has been reported to shift the balance in favour of vasoconstrictors, such as endothelin-1 and thromboxane, and to induce smooth muscle cell hypertrophy, proliferation, and failure to sustain normal apoptosis pathways, thereby resulting in the known histopathological changes seen in pulmonary vascular disease (52).

Vascular smooth muscle cell hypertrophy and proliferation are additional features of PAH and are, in fact, sometimes the only identifiable vascular lesion (59). This may be partly related to vasoconstriction. Calcium influx causes both smooth muscle cell contraction and stimulates cell proliferation (59,60). It appears, however, that smooth muscle cell hypertrophy and proliferation is mainly related to paracrine factors such as platelet-derived growth factor (61), angiopoietin (62), insulin-like growth factor (63), endothelin (64) and eicosanoids (65). In addition, smooth muscle cell proliferation has been linked to increased circulating serotonin (5-HT) levels and overexpression of the 5-HT transporter (66-69). The free calcium concentration in the smooth muscle cell cytosol regulates contraction and also proliferation. The cytosolic calcium concentration, in turn, is increased by calcium influx through voltage dependant calcium channels and their function is linked to membrane potential, largely determined by potassium channels (70). It is not surprising, therefore, that reduced potassium channel function and expression has been linked to pulmonary vascular smooth muscle cell proliferation and – by different mechanisms – reduced apoptosis (60). Pulmonary hypertension is also characterized by adventitial thickening and increased collagen and elastin production (71). Recent research has highlighted that the vascular adventitia is a highly dynamic structure, regulating vasomotor tone and extracellular matrix composition as well as expressing endothelin and other growth-factors (72). Additional abnormalities involved in the pathophysiology of PAH include immune inflammation with intrapulmonary inflammatory infiltration (73), impaired endothelial cell apoptosis and progenitor cell homing to the site of pulmonary vascular changes (74).

On a histological level, though the causes of pulmonary vascular disease may vary, the structural changes are similar, and medial hypertrophy as well as intimal thickening is usually seen (54). In severe disease, the formation of plexiform lesions is described.

Although sustained PAH is characterized by pulmonary vascular changes, the symptoms and survival prospects are determined by the ability of the right ventricle to cope with an increased pulmonary vascular resistance. Chronic right ventricular pressure overload leads to right ventricular dilatation, reducing right ventricular mass

to volume ratio and increasing wall tension according to Laplace's law . Increased wall stress and reduced systolic coronary perfusion may in turn induce right ventricular dysfunction and right ventricular hypertrophy. Over time, compensatory mechanisms of the right ventricle fail and right ventricular failure develops. In addition to the direct effect on the right ventricle, right ventricular dilation and interventricular septal shift may impact on the left ventricular shape and function, leading to biventricular dysfunction (75). Besides manifest heart failure, right ventricular hypertrophy and myocardial fibrosis (76) may predispose patients to malignant ventricular arrhythmias resulting in sudden cardiac death, an aspect of the disease that has received relatively little attention so far.

Clinical Features of PAH

Patients may present with increasing exercise intolerance, shortness of breath on exertion, tiredness, chest pain or syncope. Children are often more sick at presentation than adults (12). Failure to thrive and fatigue may be noted, but symptoms can be subtle. Leading symptom in children at the time of diagnosis is dyspnoea (7,41), followed by limitation in exercise (7). In IPAH, presentation with syncope appears to be more frequent in children (7), with almost one third of children presenting with syncope in the UK. In contrast, a Dutch study reported the rate of children who had a syncopal episode at presentation to be only 13% (41). Chest pain and peripheral oedema are rare in children but may occur in adults. The degree of disability is described by the NYHA or WHO Functional Classification. This was developed for use in adults and is used in children although it is difficult to apply in this setting. There are 4 classes. Class I is the apparently healthy, asymptomatic patient and class IV is the severely incapacitated patient in right heart failure.

On auscultation, an accentuated pulmonary component of the second heart sound is noted in nearly all patients (7). A systolic murmur may be audible, as the majority of patients with significant PHT have a degree of tricuspid regurgitation. An early-diastolic decrescendo murmur may also be noticed, generated by a regurgitant pulmonary valve. Occasionally a third heart sound may be heard and a right ventricular heave is present in the majority of children at presentation. Jugular veins

may be prominent and a visible a wave, the reflection of the atrial systole by a stiff right ventricle in diastole may be seen.

Symptoms are usually the consequence of a deteriorating right ventricular function, namely hepato-splenomegaly, peripheral or generalized oedema as central venous pressures increases. In children, however, hepatomegaly was only noted in less than a quarter of children at the time of diagnosis (7).

Clubbing, peripheral cyanosis and eventually erythrocytosis may be clinical symptoms in patients with end-stage PHT and significant hypoxaemia. Auscultation of the lungs should be unremarkable. If inspiratory crackles are heard, interstitial lung disease should be ruled out.

Other symptoms, such as Raynaud phenomenon, teleangiectasia or sclerodactyly deserve special attention, as those symptoms do occasionally indicate additional underlying problems, such as scleroderma or vasculitis.

Making a diagnosis of PAH

All children, who present with unexplained shortness of breath, lethargy or syncope deserve further investigation and pulmonary hypertension ought to be ruled out. Children are often misdiagnosed as being 'asthmatic' (77). All children with suspected pulmonary hypertension are initially assessed by clinical examination, electrocardiogram, chest X ray and echocardiography.

The electrocardiogram helps to reveal right ventricular hypertrophy or strain and shows right atrial dilatation. Voltage criteria of right ventricular hypertrophy and right axis deviation are common in patients with IPAH (78). The absence of these findings does not necessarily suggest that pulmonary artery pressures are normal, as the ECG has limited sensitivity and specificity. Due to a natural right axis deviation in infancy, the sensitivity of the ECG is even lower in this setting.

The chest X-ray allows to assess the cardiothoracic ratio and to determine if cardiomegaly is present. Furthermore it reveals, if the pulmonary arterial vasculature is abnormal. While central pulmonary arteries are expected to be enlarged, there is usually a rarefaction of peripheral pulmonary arteries, often with an abrupt calibre change.

The echocardiogram allows an estimation of pulmonary artery pressures in the majority of children (79), it provides information of the degree of right ventricular impairment, and can give information about left ventricular dysfunction, when tissue Doppler imaging is used. This problem will be studied in the work presented in this thesis.

If echocardiography fails to visualize the anatomy adequately, a contrast CT angiogram should be considered. Also, if interstitial pulmonary disease or any other aetiology is suspected a high-resolution CT scan of the chest should be performed, to confirm the diagnosis and evaluate the lung parenchyma.

For diagnosis, accurate measurement and calculation of pulmonary vascular resistance, evaluation of pulmonary vascular responsiveness, children should undergo a right heart catheterization with vasodilator testing. In children who present in a poor condition, in whom either right ventricular function had been severely affected, or who presented with frequent pulmonary hypertensive crises, institution of treatment is paramount. The aim is to stabilize a child first, to avoid any potential additional risk exposing a frail child to an invasive procedure with considerable morbidity. Once the clinical situation had improved and pulmonary hypertensive crises are controlled, a delayed catheterization is performed, acknowledging that measures are flawed and may be lower, as the child is already on treatment.

Children who are old and mature enough are asked to perform a six-minute walk test (6MWT), which can be done in our experience from the age of 4-5 years onwards. Older children (from 140 cm body height) may undergo formal cardiopulmonary exercise testing as a baseline status. Information about the six-minute walk test and cardiopulmonary exercise testing is presented and discussed in this thesis.

Management of PAH

Evidence-based treatment algorithms for adult PAH patients have been published as part of current guidelines (3) and the treatment strategies employed at the United Kingdom Service for Pulmonary Hypertension in Children (UKSPHC) have been

reported in detail recently (40). Medical treatment is constantly evolving as new medicines become available and is guided by evaluation of disease severity, the assessment of vasoreactivity and assessment of efficacy of therapy (3). Depending on the child's clinical condition and the aetiology of the PAH children presenting to the UKSPHC are given either monotherapy or combination therapy after diagnosis. Children are reassessed on a regular basis and therapy is modified when necessary. In adult practice the condition of a patient is defined as stable and satisfactory, stable but not satisfactory or unstable and deteriorating based on the clinical, non-invasive and invasive findings. These include clinical evidence of right ventricular failure, progression of symptoms, presence of syncope, WHO functional class, 6-minute walk test distance, B-type natriuretic peptide levels, echocardiographic evidence of pericardial effusion or reduced right ventricular function and haemodynamic parameters such as cardiac index and mean right atrial pressure (3,80). Based on these criteria targets are set and medical treatment is escalated if goals are not met (81). This goal-orientated strategy requires a set of established risk prognosticators, limiting its usefulness in paediatric patients where non-invasive prognosticators are less well established. In addition, invasive haemodynamic assessment is associated with an increased risk of complications (82). Although most paediatric patients require an initial cardiac catheterization to establish the diagnosis of PHT and to assess vasoreactivity, repeated right heart catheterizations are avoided and, unlike in adult patients, sequential haemodynamic parameters are generally not available to guide therapy in children. Figure 1.1 provides an overview over the treatment strategy for children with IPAH employed at the UKSPHC (7,83).

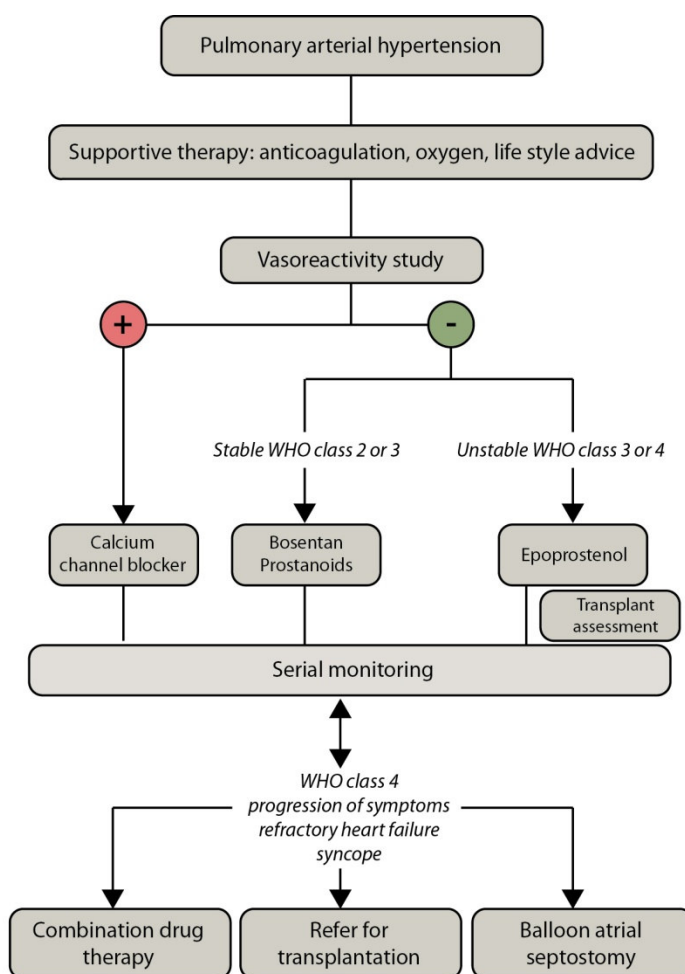


Figure 1.1. Treatment algorithm in children with pulmonary hypertension. Modified from Haworth et al. (83)

The following paragraphs provide a short overview of pulmonary hypertension therapies used in our paediatric population during the study period.

Calcium Channel Blockers

Treatment with calcium channel blockers is reserved for children who are responsive to vasodilator testing (83). The definition of an acute responder is controversial. The original definition was a fall in pulmonary vascular resistance and arterial pressure of at least 20%, without reduction in cardiac output (84). More recently, a reduction of mean pulmonary arterial pressure of more than 10 mmHg, leading to a mean pulmonary arterial pressure below 40 mmHg or a fall in pulmonary vascular resistance to near normal values has become the accepted definition (7). Using the latter definition, a retrospective review of children under follow up at the UKSPHC

suggested that only 7.4 % of IPAH and only 6.0 % of APH patients are acute responders (40). Acute responders require constant re-evaluation as children may deteriorate on a calcium channel blocker and lose vasoreactivity with time. Despite these limitations, the survival prospects of acute responders treated with nifedipine are reassuring (3,7,85-87).

Prostacyclin and derivatives

Epoprostenol (PGI₂, PGX, prostacyclin) is the synthetic sodium salt of a naturally occurring metabolite of arachidonic acid (prostaglandin). Prostacyclin is produced by the endothelium, acts as a vasodilator and has antiproliferative, platelet inhibitory and positive inotropic properties (88). Intravenous epoprostenol was the first effective treatment for severe, established PAH (58). Its efficacy has been proven in adults and in children (5,89,90). Due to its efficacy epoprostenol is prescribed to adults and children with IPAH who are in WHO functional class 3 or 4 and who do not show a positive response to acute vasodilator testing. With increasing experience, the indications in paediatric practice have widened beyond IPAH and include children with severe PAH associated with connective tissue disease, post-repair congenital heart disease, HIV associated PHT and portal PHT. According to current guidelines it is the drug of choice in patients presenting unstable or in WHO functional class 4 (3).

The half-life of Prostacyclin is only 2-3 minutes and a continuous infusion is required to maintain a constant plasma concentration. Chronic administration, therefore, generally requires a central line. Sudden 'rebound pulmonary hypertension' or even deaths are reported in abrupt delivery failure or inadvertent drug interruption of any other cause or drug withdrawal. Epoprostenol is usually well tolerated in children and adults and side effects are not excessively troublesome. Diarrhoea, flushing, jaw pain and light-headedness are described. Epoprostenol is an expensive, high maintenance therapy, which demands an ongoing commitment by the patient, parents or carers and the cooperation of the child (79).

Studies on the management of children given epoprostenol are presented in this thesis.

The subcutaneous analogue of prostacyclin (Treprostinil) is used in adults with WHO functional class 2 - 4. The main advantage is avoidance of a central access with its associated morbidity, although the drug needs to be continuously delivered via a subcutaneous infusion. The half-life of three to four hours is longer than that of the intravenous form permitting continuous subcutaneous infusion (91). It has been shown to improve exercise tolerance as demonstrated by an increase in 6-minute walk test distance (91-93). Due to pain at the injection site and the relative lack of subcutaneous tissue its use is problematic in young children.

Inhaled prostacyclin (Iloprost) has gained approval for adult IPAH patients in WHO functional class 3 and 4. Exercise tolerance improves but due to the relatively short half-life repeated inhalations are required every 2-3 hours (i.e. 6-9 inhalations/day) for a sustained beneficial effect. A disadvantage of this application form is that it is relatively time-consuming and tiring, as each inhalation takes around 15 minutes (94). Due to limited compliance and high rates of discontinuation of treatment in children (95) this therapy was very rarely used at the UKSPHC.

Inhaled Treprostinil has been recently described to be well tolerated as well as to improve exercise capacity and quality of life in adult patients with PAH (96) when added to sildenafil or bosentan therapy. Despite these encouraging early results, data in children with PAH is lacking and this drug was not used at the UKSPHC.

Oral prostacyclin (Beraprost) has been used for patients in WHO functional class 2 or 3. However, long-term data are not convincing, as development of tolerance and loss of therapeutic benefit appears to be a concern (97). This drug was not used at the UKSPHC.

Dual endothelin receptor antagonists

Bosentan is a non-selective endothelin-A and endothelin-B receptor antagonist. It is approved for the treatment of PAH adult patients in WHO functional class 3 and 4. Bosentan was shown to lead to symptomatic improvement and increase in exercise tolerance in 2002 (98). The pharmacokinetics, safety and efficacy of Bosentan treatment in children with pulmonary hypertension was reported in 2003 by Barst

and colleagues (99). Further observational reports are available, demonstrating the beneficial effect of Bosentan in children (100,101). Derangement of liver function is described (102). Liver function needs to be monitored regularly as elevation of transaminases may occur. More recently selective endothelin-A (ET_A) antagonists were introduced (Sitaxentan and Ambrisentan). Trials in adults has shown improvement of exercise tolerance (103-105). However, none of the patients included in the studies presented in this thesis was treated with a selective ET_A antagonist as currently no data are available on the efficacy and safety of ambrisentan and sitaxentan in paediatric patients.

Phosphodiesterase-5 inhibitors (PDE-5 inhibitors)

Sildenafil, a phosphodiesterase-5-inhibitor, was shown to be effective in reducing pulmonary vascular resistance as well as improving functional class and the 6-minute walk test in adult patients with PAH in the SUPER-1 trial (34). Its oral route of administration and relatively low cost makes it attractive for use in stable PAH patients. However, there are little data on the safety and efficacy of PDE-5 inhibitors in paediatric patients. A pilot study in children has suggested that it may also be beneficial in this population (106). A randomized controlled trial in children has been completed and the results should be available in the near future (83). Overall, however, the experience with sildenafil monotherapy in IPAH has been generally unsatisfactory in the UKSPHC (40). In the early days of the Service the children treated with sildenafil monotherapy who had been given this drug before transfer to the UKSPHC were extremely ill on arrival and outcome was usually poor. Given this experience, children with IPAH were not started on long-term sildenafil as monotherapy, awaiting further data from randomized controlled trials. Instead, sildenafil is used in combination with other therapies to maximally stimulate all vasodilatory pathways (7). Sildenafil also appears to have a clinical role in counteracting rebound pulmonary hypertension after withdrawal of inhaled nitric oxide (107). Additional, PDE-5 inhibitors such as Tadalafil have been developed but their use has been largely restricted to adult patients (108) until further data on safety and efficacy become available.

Combination therapies

Combinations of pulmonary hypertension specific therapies are increasingly used in patients with IPAH (109). A recent retrospective UK study suggested that a combination of intravenous epoprostenol with either bosentan or sildenafil, or both appeared to achieve the best outcome in children with IPAH (40). Although, possible drug interactions need to be considered (110,111) the increasing use of combined therapies reflects the current clinical practice of goal-orientated drug therapy with a need for more aggressive treatment (3).

Balloon atrial septostomy

Balloon atrial septostomy has been proposed as a therapy option for highly symptomatic patients refractory to advanced vasodilator therapy (112-114). The artificially created interatrial communication may function as a “pop-off” valve, allowing the right ventricle to decompress and to maintain systemic blood flow when metabolic demand is increased (115), for example during pulmonary arterial hypertensive crises, albeit at the expense of mild desaturation (116). The reported average reduction in arterial oxygen saturation in a study in a paediatric cohort was from 97.8% before septostomy to 90% after 3–6 months (113). Clinically, atrial septostomy has been shown to improve haemodynamics and quality of life as well as prolong survival (112,117-121). In the paediatric population it is especially useful in children presenting with syncope (40). Atrial septostomy is effective in abolishing syncope, and can protect against syncope by maintaining systemic cardiac output, particularly when the pulmonary arterial pressure rises acutely (113). Atrial septostomy augments systemic blood flow, potentially benefiting organs that lack the ability to autoregulate blood flow at low blood pressures. It has been suggested that a mean arterial pressure of at least 60 mmHg is required to enable cerebral autoregulation (122). In the situation of desperately poor systemic blood flow, autoregulation may not be able to successfully maintain flow to some vital territories - an increase in total flow could permit autoregulatory processes to restore perfusion to vital organs. In addition, it has been suggested that atrial septostomy increases oxygen delivery to the tissues due to an increase in cardiac output, despite the modest reduction in systemic arterial oxygen saturation septostomy (113). However, a recent analytical study revealed this does not coincide with an improved tissue

oxygenation and is merely a consequence of the way raw arterial oxygen delivery is defined (123).

The problems associated with atrial septostomy in children with PAH are addressed in studies presented in this thesis.

Transplantation

Double-lung or heart-and-lung transplantation is the therapy of last resort for patients with severe PHT, when quality of life and exercise tolerance deteriorate despite optimized maximal medical therapy. Organ availability - particularly for children - is sparse and children with PHT are competing with children listed for transplantation for other conditions such as cystic fibrosis. Waiting time is often long and deaths on the active transplant list occur. Timing is a major challenge. This issue has been addressed in the work presented in this thesis.

Survival after successful transplantation is limited and mean survival after lung-heart-and-lung transplantation nowadays is reported to be approximately 4.2 years (124).

Supportive therapy

Patients require regular assessment and follow up management at expert centres for PAH. Strenuous exercise and competitive sports should be avoided. In children without contraindications oral anticoagulation is recommended (85,125). In addition, patients with signs and symptoms of right heart failure will generally benefit from diuretics. Immunization against influenza and pneumococcal infections are recommended and endocarditis prophylaxis should be considered in patients with congenital heart defects (126,127). In addition, adequate family support is required, especially when intravenous treatment along with its high demands and commitment to the family is contemplated.

Risk stratification in PAH

Estimating the prognosis of the individual patient with PAH is paramount for optimizing medical therapy and the timing of additional therapeutic options such as

atrial septostomy and transplantation. Various studies have explored the risk factors of poor outcome in older patients with PAH. The majority of the studies were performed in adult patients with IPAH and young children were not included in these studies. The topic has been comprehensively reviewed by McLaughlin et al. (128). There is inconsistency between studies regarding the prognostic value of various risk factors but it appears that the following risk markers (sorted by increasing invasiveness) are related to poor outcome in patients with PAH.

Functional parameters

1. Advanced WHO functional class (treatment naive and on therapy) (4,7,129-133).
2. Low 6-minute walk test distance (89,134,135).
3. Low peak oxygen consumption and low peak exercise systolic and diastolic blood pressure on cardiopulmonary exercise testing (136).

Biochemical markers:

1. Elevated atrial or B-type natriuretic peptides (ANP, BNP, NT-pro BNP)
2. Elevated uric acid levels (136-138)

Electrocardiographic (ECG) measures:

1. ECG findings of increased P-wave amplitude and voltage criteria of right ventricular hypertrophy (78).

Echocardiographic parameters:

1. Presence of a pericardial effusion (134,139).
2. Right atrial area (134).
3. Elevated Doppler echocardiography right ventricular (Tei) index (140,141).

Haemodynamic measures on right heart catheterization:

1. Elevated mean right atrial pressure (4,78,130-132,134,142-146)
2. Reduced cardiac index (4,78,132,136,138,139,142,144,146,147)
3. Elevated mean pulmonary arterial pressure (4,125,130,132,136,139,144-146,148)
4. Elevated pulmonary vascular resistance (4,78,125,136,142,145,146,149)
5. Vasodilator responsiveness (131,133,142,150)

Additional risk markers include:

1. Persistence of WHO functional class 3 or 4 in patients treated with epoprostenol (130)
2. Low diffusion capacity (DLCO, < 45% of predicted) in patients with scleroderma-associated PAH (151)
3. Younger age at diagnosis in paediatric patients with IPAH (146)

Only a few studies have investigated prognostic markers specifically in children. Sandoval et al. (143) assessed 18 children below 16 years of age who had IPAH. Due to the small number of children included only increased mean right atrial pressure was found to be significantly related to survival on univariate Cox proportional-hazard analysis, while a trend towards worse outcome was seen in patients with higher functional class, signs of heart failure, male sex, vasodilator unresponsiveness, reduced mixed venous oxygen saturation, increased pulmonary vascular resistance and reduced stroke volume index. On multivariate analysis only lower stroke volume index and higher mean right atrial pressures emerged as significant predictors of worse survival, thus highlighting the importance of haemodynamic parameters for risk stratification in IPAH. Unfortunately, the authors did not assess the prognostic value of the 6-minute walk test distance in this study. Recently, a retrospective cohort study of 64 children with IPAH (<16 years of age) who presented to the UK Service for Pulmonary Hypertension in Children found that WHO functional class and low weight z-scores were the only significant predictors of death on multivariate survival analysis (7). On univariate analysis, however, right ventricular systolic pressure estimated on Doppler echocardiography, the degree of right ventricular dilatation and pulmonary vascular resistance index (naïve and with vasodilator testing) were found to be related to survival (7). These results are largely in agreement with a recent Dutch study (152).

The aims of the studies presented in this thesis

During the last two decades the outcome of patients with PHT has been transformed by a better understanding of the pathophysiology of the condition, the advent of new medical therapies and in adults, improved risk stratification. Not surprisingly,

however, research has focused on adult patient cohorts with limited data being available in children. This thesis aims to:

1) evaluate certain non-invasive tests used to diagnose and monitor children with pulmonary hypertension. As medical therapy is largely guided by patients' objective functional capacity, work focuses on the 6-minute walk test distance and parameters of cardiopulmonary exercise testing. Although the 6-minute walk test is commonly used to assess a patient's exercise capacity there are limited data on normal values of walk test distance in UK children and the 6-minute walk test distance varies considerably with the age of the child. One aim of this thesis is, therefore, to establish normal values of 6-minute walk test distance in UK children against which the performance of children with PHT can be compared in future studies. Furthermore, in older children cardiopulmonary exercise testing may be used to assess objective exercise capacity, providing additional physiologic information. However, the relationship between the 6-minute walk test distance and parameters of cardiopulmonary exercise testing in children with PAH is unclear, hampering comparison of data between the two modalities and limiting clinical application of results. Thus, a further aim was to compare these two modalities.

2) to investigate potential risk factors of mortality. These prognostic markers include echocardiographic markers of impaired biventricular myocardial function, heart rate variability and B-type natriuretic peptide

3) to study the paediatric experience with epoprostenol therapy, use of atrial septostomy and the timing of transplantation in children because data on safety and efficacy of therapeutic options are sparse in children with PAH. A case series of patients, who underwent implantation of atrial fenestrated devices is presented. Taking advantage of the opportunity afforded by having a large centre for paediatric PHT collaborating with a large, active paediatric transplantation program, considerations for selecting children for listing for lung transplantation are presented. Overall, these sub-studies provide further insight into the comprehensive assessment, risk stratification and treatment of children with PHT and should assist clinicians in providing the optimal therapy for children with this condition, which carries such a high morbidity and mortality.

2. METHODS OF ASSESSMENT

Introduction

As the majority of methods used in this thesis are routine clinical procedures, this chapter introduces the standards and methods of clinical assessment in more detail. As the same methods were used in the various studies presented in this thesis, it is hoped that this chapter helps to avoid redundancy for the reader. This chapter gives an overview of the assessment of (WHO) functional class, electrocardiography including Holter monitoring, echocardiography, six-minute walk test, cardiopulmonary exercise testing, cardiac catheterization, B-type natriuretic peptide measurement and the basic statistical methods used in this thesis.

2.1 Assessment of functional class

The assessment of functional class is based on the classification recommended by the World Health Organization (WHO) (153). It represents a modification of the well known New York Heart Association functional classification (NYHA) describing the level of functional impairment and associated symptoms in cardiovascular disease (154). An increase in NYHA/WHO class reflects a deterioration in functional capacity, suggesting that the patient suffers from more severe symptoms and has a greater restriction in physical activity. It is used in order to stratify patients who have a similar level of severity of symptoms and have a comparable degree of physical limitation into one of four groups. Though the assessment of a patient's functional class may well be influenced by the individual or physicians judgement, it represents a relatively simple approach to evaluate and categorize a patient's exercise tolerance. These classification systems were designed for use in adults.

Table 2.1. The WHO classification to describe functional status

Functional Class	Symptomatic profile
Class 1	Patients with pulmonary hypertension but without any resulting limitation of physical activity. Ordinary physical activity does not cause dyspnoea, fatigue, chest pain or near syncope.

Class 2	Patients with pulmonary hypertension resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity causes undue dyspnoea or fatigue, chest pain or near syncope.
Class 3	Patients with pulmonary hypertension resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary activity causes undue dyspnoea or fatigue, chest pain or near syncope.
Class 4	Patients with pulmonary hypertension with inability to carry out any physical activity without symptoms. These patients manifest signs of right heart failure. Dyspnoea and/ or fatigue may even be present at rest. Discomfort is increased by any physical activity.

2.2 Electrocardiogram (ECG)

Twelve lead electrocardiograms were recorded in a standardized way to assess the underlying heart rhythm and rate. The ECGs were specifically analyzed for evidence of right ventricular hypertrophy (RVH) and strain. For RVH, voltage criteria were used (R wave amplitude in V1 plus the S wave amplitude in V5 or V6). Right ventricular strain was defined as a QRS-T angle $> 90^\circ$ and a T-axis between 0 and -90° . Possible strain was defined as a QRS-T angle $> 90^\circ$ and a T axis between 0 and $+90^\circ$ as described by Park et al. (155).

2.3 Assessment of heart rate variability from 24 hour Holter ECG monitoring

Holter monitoring was performed and data were analyzed using the customized Reynolds Pathfinder Software (Hertford, UK). Parameters of heart rate variability were determined from the recordings, including SDNN (standard deviation of all normal-to-normal intervals), SDANN (standard deviation of mean values for all normal-to-normal intervals over 5 min) and RMSSD (square root of the mean square differences of successive RR intervals).

2.4 Transthoracic echocardiography

The majority of echocardiograms were performed on a Vivid 7 machine (GE, Milwaukee, USA) equipped with Matrix 3.5 and 7 MHz probes. All patients underwent a thorough assessment according to the sequential anatomical analysis approach to ascertain the underlying cardiac morphology (156). Evidence of right ventricular dilatation, hypertrophy and function was assessed. A semiquantitative graduation system for right ventricular impairment, dilatation and hypertrophy, as well as the degree of valvar regurgitation was used.

Right ventricular function was classified as being normal, mildly, moderately or severely impaired (157). Valvar regurgitation, right ventricular dilatation and hypertrophy were classified as none (0), trivial (1), mild (2), moderate (3) or severe (4).

In the presence of a complete Doppler trace envelope of tricuspid regurgitation, an estimate of the right ventricular systolic pressure is given using the modified Bernoulli equation (158).

$$\Delta P = 4 \cdot v^2$$

ΔP represents the pressure difference across the valve and v represents the maximal measured Doppler velocity. The presence of a pericardial effusion was recorded and described when present.

2.5 Six minute walk-test (6MWT)

All 6MWTs were conducted using a lap 30–50 m in length on flat, hard ground, according to the ATS guidelines (159). A portable pulse oximeter (PulsoxTM-3iA, Minolta) was used to record the transcutaneous oxygen saturation and heart rate before the start of the walk, after every minute during the test and after 1 and 3 minutes during the recovery period. The pulse oximeter was lightweight (42 g excluding batteries, wrist band and probes) and attached to the child's wrist as shown in Figure 2.1. Depending on the child's age, a finger probe or clip was used as transcutaneous sensor and attached to one finger of the same hand.



Figure 2.1. Portable pulse oximeter (PulsoxTM-3iA, Minolta), (finger-probe not shown)

The children were asked to walk up and down the measured lap at their best pace but not to run, race or dawdle. The same instructions were given to all children before undertaking the walk test. All tests were undertaken by each child separately to prevent competition. The instructor walked behind the child to make sure that the child determined the pace his/herself and also to read the oxygen saturation and heart rate as outlined above. While encouragement (e.g. 'Keep going', 'You are doing well') and announcement of time remaining were given to the children, no judgemental comments (such as 'You could go faster' or 'Slow down') were made regarding the child's performance. Weight, height, gender and ethnic background were documented. The total distance walked in 6 minutes was measured. Predicted values for 6MWT distance in boys and girls were calculated based on gender, age and height according to published data for healthy Caucasian children and adolescents (160).

2.6 Cardiopulmonary exercise tests

Cardiopulmonary exercise testing was performed on a mechanically braked bicycle ergometer (Ergoline 900) with respiratory gas exchange analysis (Medgraphics, St Paul, Minn., US). A ramp protocol comprising an initial period of loadless cycling to

permit equilibration was used. A period of active recovery (slow cycling under minimal friction load) was commenced after maximal exertion. Heart rate, blood pressure, and oxygen saturation were monitored in all children for the duration of the test. Peak oxygen uptake ($\dot{V}O_2$) and anaerobic threshold were derived from respiratory gas analysis during maximal exercise testing. Anaerobic threshold was determined by use of the modified V-slope method (161). Peak heart rate, blood pressure, and workload achieved were recorded. Predicted values for peak oxygen uptake were calculated according to published data in healthy children (162).

2.7 Cardiac catheterization studies with assessment of pulmonary vascular resistance

The majority of cardiac catheterization studies were performed at our institution. All patients were anaesthetized and ventilated for the duration of the study to achieve stable conditions.

Cardiac catheterization with pulmonary vascular responsiveness testing was performed in a standardized way. Pressures were measured directly using fluid filled catheters according to the child's size, measuring pulmonary artery pressure, and left atrial pressure or pulmonary capillary wedge pressure to derive the trans-pulmonary pressure gradient.

In the majority of cardiac catheterizations (all that were performed at Great Ormond Street Hospital), the oxygen consumption was measured – rather than assumed – with a mass spectrometer (Amis 2000, Innovision, Odense, Denmark), to achieve maximal accuracy under standard anaesthetic conditions. A considerable discrepancy between assumed and measured values, which can lead to a false assumption of pulmonary artery resistance is reported in children (163).

Pulmonary vascular responsiveness was tested at baseline and in a single second step with an increased fraction of inspired oxygen (FiO_2 0.65) and nitric oxide (NO 20 ppm).

No further measures are taken to limit catheterization time, as in adults short-term morbidity has been reported to correlate with duration of general anaesthetic time (164). Pulmonary vascular resistance was calculated according the standard equations (165).

2.8 B-type natriuretic peptide (BNP)

Venous blood samples were obtained and BNP measurements were performed using the Triage test (Biosite Diagnostics, San Diego, CA)(166) according to the manufacturer's instructions. This test uses a fluorescent-labeled antibody to BNP and has a range from 5 to 5000 pg/ml.

2.9 Statistical analysis

Throughout the thesis, non-normally distributed data are presented as median (with interquartile range or centiles), while normally distributed values are given as mean and standard deviation (SD). Parameters were tested for normal distribution by the Kolmogorov–Smirnov or the D'Agostino-Pearson test. Comparisons between groups were made using the Student's t test, analysis of variance (ANOVA) or Mann–Whitney U test for continuous variables (depending on the data distribution) or the chi-square (χ^2) test as for categorical variables. For all analyses a p-value <0.05 was considered significant. All tests were performed two-tailed. Further details on specific statistical methods used to analyze data are outlined in the according chapters.

MedCalc Version 11.1.0 (MedCalc Software, Mariakerke, Belgium), StatView 5.0 (Abacus Concepts, Berkeley, CA, U.S.A.) and R (Version 2.10.1) statistical package were used for statistical analysis (167).

2.10 Ethics approval

Ethics approval was sought for the review of patients' records and data collection for the retrospective studies. The prospective walk test study in healthy children was approved by the local ethics committee and by the Central Office for Research Ethics Committee (COREC).

3. NON-INVASIVE EVALUATION OF CHILDREN WITH PHT

Introduction

Children with pulmonary hypertension were assessed routinely as described in Chapter 2. Detailed studies were carried out on the six-minute walk test, heart rate variability, tissue Doppler imaging and B-type natriuretic peptide and the results of these studies are presented in this chapter.

3.1 SIX-MINUTE WALK TEST AND CARDIOPULMONARY EXERCISE TESTING

3.1. NORMAL SIX-MINUTE WALK TEST DISTANCE VALUES FOR HEALTHY UK CHILDREN

3.1.1 Background

The six-minute walk test (6MWT) is the distance a person can walk at a constant, uninterrupted, unhurried pace in six minutes. It is a simple method of assessing exercise capacity. This test is often used to assess patients with cardiovascular or respiratory diseases (168). In addition, it was one of the first exercise modalities approved by the U.S. Food and Drug Administration (FDA) as a clinical endpoint for prospective clinical trials in patients with pulmonary hypertension (169).

It has been shown to be an independent predictor of morbidity and mortality in adult patients with heart failure, chronic respiratory disease, cystic fibrosis (170,171) and idiopathic pulmonary hypertension (135,172). It is particularly useful for longitudinal assessment, to monitor response to treatment and to guide therapy (81).

The major advantages of the 6MWT are that it is easy to perform and can be repeated at low cost. The 6MWT is frequently used in children with pulmonary hypertension and cystic fibrosis and in those with severe cardiopulmonary diseases being assessed for transplantation (170,173,174).

Despite its frequent use, no normal values for UK children below the age of 12 years are available. This study aimed to establish normal values for UK children, who underwent the 6MWT in exactly the same fashion, as one would usually apply it to PHT patients.

3.1.2 Patients and Methods

There were 328 normal, healthy children (54% male) aged between 4 and 11 years included in this study. Most were recruited from two primary schools. In addition healthy siblings and relatives of children attending Great Ormond Street Hospital were invited to participate. Parents gave informed consent and any children with chronic disease were excluded. Children with mild asthma, not on regular bronchodilators and not excluded from physical education classes, were accepted as normal. All children willingly agreed to perform the 6MWT. Weight, height, gender and ethnic background was documented. The vast majority of children included were of Caucasian origin. The ethnic distribution was as follows:

Caucasian n= 272 (83%), Asian/ Asian-Caucasian n= 33 (10%), Afro-Caribbean/ Afro-Caribbean-Caucasian n= 23 (7%).

The test was conducted as described in detail in Chapter 2 according to ATS guidelines (159). The data were obtained by three different investigators. When walk tests were analysed according to the supervising investigator no significant difference in walk test distance could be found, suggesting that there was no relevant inter-observer variation. Thus, the data obtained by different investigators were pooled.

Statistical analysis

The relationships between age, height, weight and 6MWT distance were studied by univariate and multivariate regression analysis. To assess linearity in scatterplots, the Lowess technique (LOcally Weighted Scatterplot Smoother) was used to draw a smooth line representing the average value of distance as a function of weight and height.

3.1.3 Results

All children completed the 6MWT. The number of individuals in each age group, the gender distribution, weight, height, body mass index (BMI), heart rate at baseline, maximum heart rate, the oxygen saturation at baseline, the minimum oxygen saturation and the distance walked in 6 minutes are outlined in Table 3.1.1.

Table 3.1.1. Results of the six-minute walk test in the different age groups.

Age (years)	4	5	6	7	8	9	10	11	Total
male/female	18/18	21/19	19/21	22/18	27/18	27/22	30/18	15/15	178/150
Weight (Kg)	18±2	21±4	24±5	26±4	30±6	34±7	38±9	41±9	29±9
Height (cm)	107±4	115±6	121±6	128±5	133±8	139±7	145±8	149±7	130±15
BMI	16.0±1.3	15.7±1.7	16.5±2.5	16.0±1.8	16.8±2.6	17.8±2.8	17.8±2.8	18.4±3.4	16.9±2.6
SO ₂ baseline	97±2	97±1	97±2	97±2	97±1	98±2	98±1	98±1	97±2
SO ₂ minimum	95±2	96±2	96±2	95±3	95±2	96±2	95±2	96±2	96±2
HR baseline (bpm)	108±16	109±20	107±17	109±21	99±15	96±18	95±20	98±16	102±19
Peak HR (bpm)	139±10	140±13	141±10	142±14	133±10	133±12	130±11	132±13	136±12
6MWT (m)	383±41	420±39	463±40	488±35	483±40	496±53	506±45	512±41	470±59
Minimum	300	300	380	438	400	395	400	415	300
Maximum	472	470	600	583	600	600	601	590	601
Centiles (m)									
10	322	367	415	449	440	424	450	453	396
25	357	404	434	453	454	454	480	490	435
50	390	428	456	479	480	500	515	518	475
75	404	450	479	518	508	537	537	540	515
90	438	462	502	538	525	562	560	562	544

Values are presented as mean ± standard deviation. 6MWT= Six-minute walk test distance; BMI= body mass index; bpm= beats per minute, HR= heart rate; SO₂= transcutaneous oxygen saturation.

Height and weight increased linearly ($r=0.9$; $p<0.0001$ and $r=0.77$, $p<0.0001$, respectively) with age. Body mass indices in all age groups were on average within the normal range as outlined by the Center of Disease Control Growth charts for Children (175).

No significant drop of transcutaneous oxygen saturation was observed during the 6MWT in this cohort. The mean oxygen saturation was 97-98% at baseline, and the minimum saturation was 95-96% during the walk test. The heart rate increased from a baseline of 102±19 bpm reaching a plateau of 136±12 bpm after 1-3 minutes (Figure 3.1.1). During exercise the heart rate was higher in girls than in boys (Figure 3.1.1) ($p<0.05$).

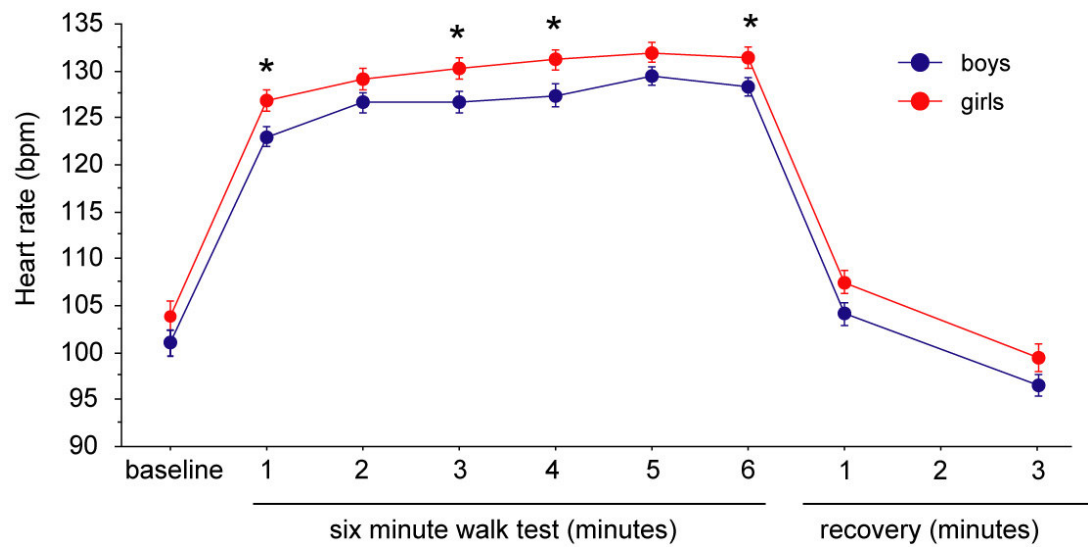


Figure 3.1.1. Heart rate at baseline, during the six-minute walk test and on recovery in boys and girls. The heart rate was significantly higher in the girls (mean±standard error; * $p<0.05$).

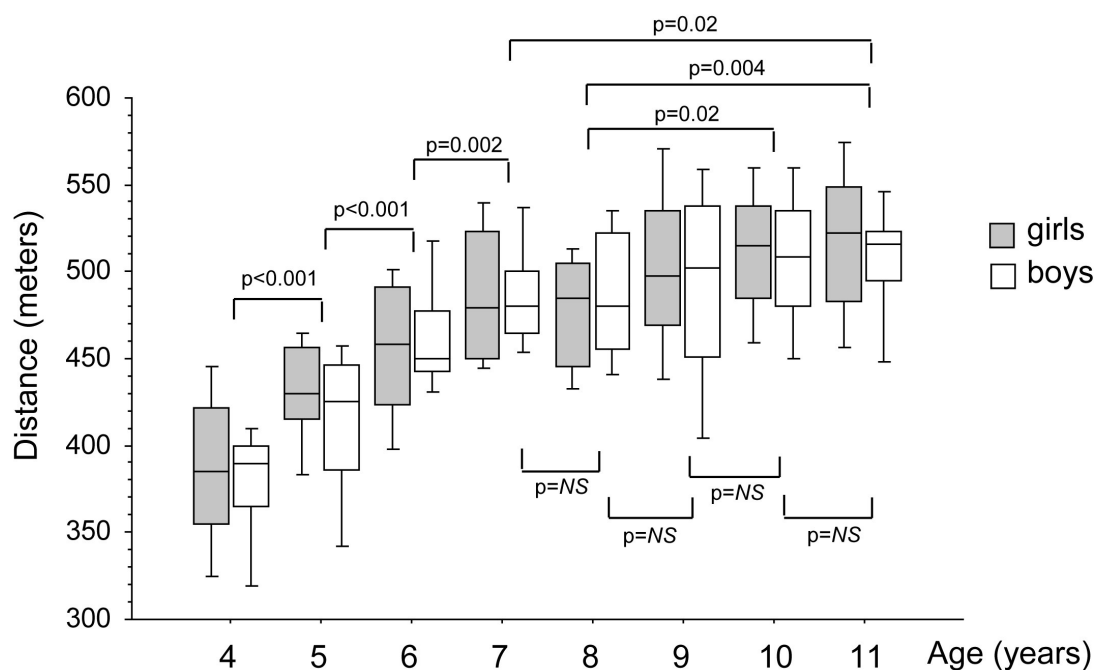


Figure 3.1.2a. The distance walked in six minutes in boys and girls in each age group. Data are shown as box-and-whiskers-plots illustrating the median, 25th and 75th centile (grey box) and 5th and 95th centile. p-values refer to the difference between various age groups, having pooled data from both sexes (unpaired t-test).

The mean distance walked increased from 383 ± 41 m at 4 years to 512 ± 41 m at 11 years, with no significant difference between boys and girls (Figure 3.1.2a, Table 3.1.1). The data from both sexes were therefore pooled (Figure 3.1.2b).

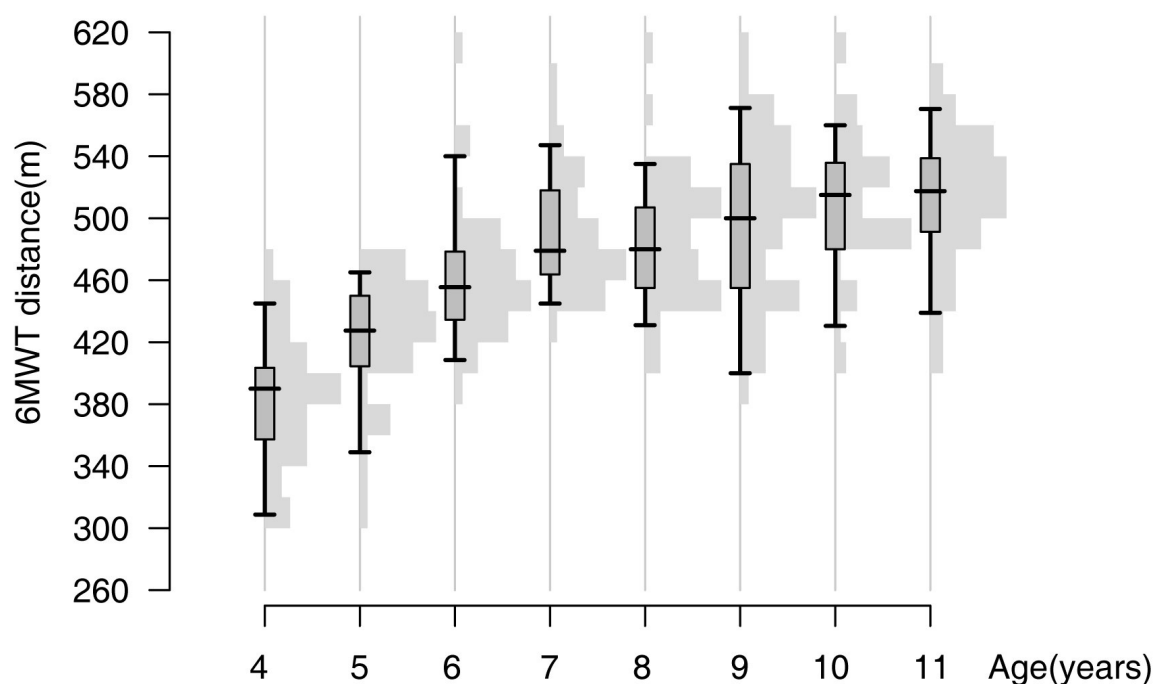


Figure 3.1.2b. Walk test difference of different age groups. Data are shown as histograms and box-and-whiskers-plots illustrating the median, 25th and 75th centile (grey box) and 5th and 95th centile.

The distance walked correlated with age ($r=0.64$, $p<0.0001$). The mean 6MWT distance increased by 37m between the age of 4 and 5 years ($p<0.001$), by 43 m between 5 and 6 years ($p<0.001$) and by 25 m between 6 and 7 years of age ($p=0.002$). Beyond 7 years of age the distance walked did not increase significantly from one year to the next, there was a significant increase however, between 7 and 11 years of age ($p=0.02$).

On univariate linear regression analysis the distance walked correlated with weight ($r=0.51$, $p<0.0001$) and height ($r=0.65$, $p<0.0001$) (Figure 3.1.3), as well as age.

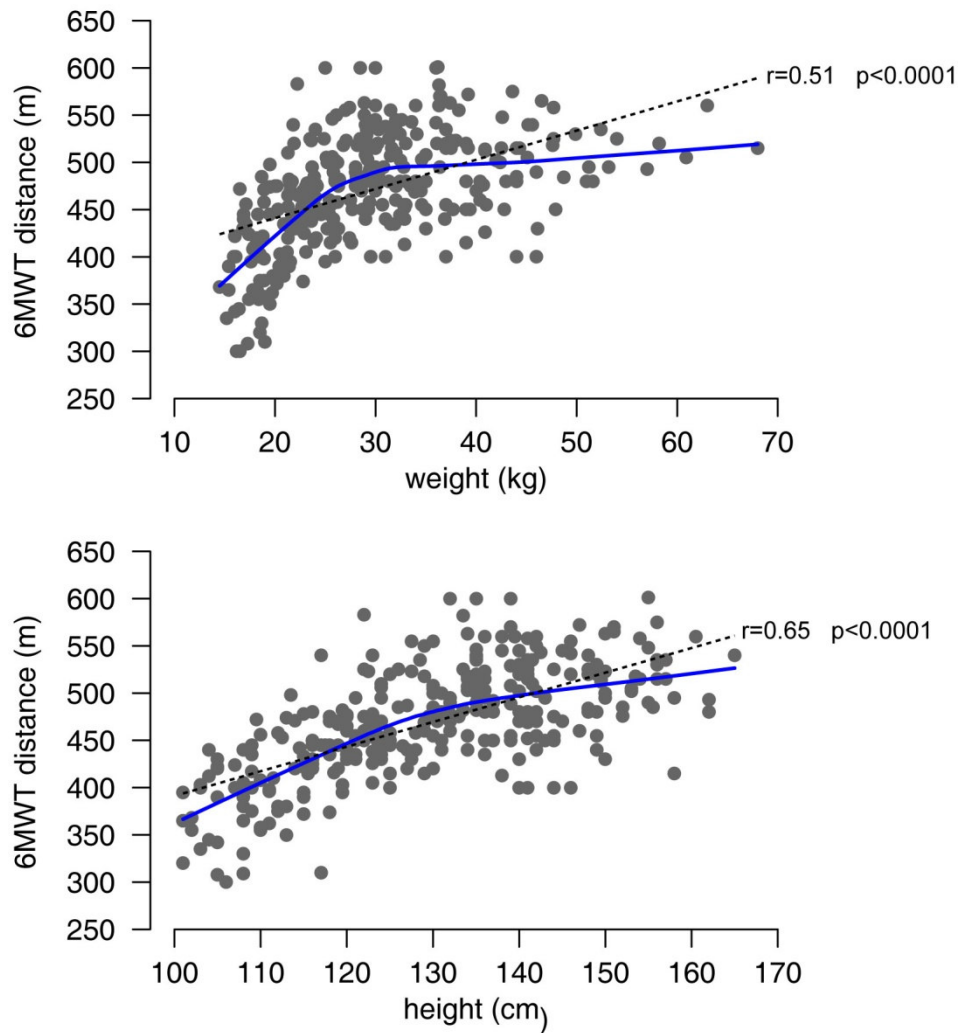


Figure 3.1.3. Relationship between distance walked in six minutes and height and weight. r and p - values were derived for a linear relationship (black, dotted line). The blue curve represents the average value of distance as a function of weight and height (Lowess technique). The fitted smooth curve shows a form of response that is clearly inconsistent with a linear relationship. It suggests that there is an approximate linear relationship up to a weight of ~30 kg and a height of ~130 cm. After these points the curve gradually merges into a more horizontal line and especially a further increase in body weight appears to be widely unrelated to an improvement in six minute walk test distance.

On multivariate analysis, 44% of the variation in distance walked by the children could be accounted for jointly by age, weight and height ($r=0.67$, $p<0.0001$). Age alone accounted for 41% of the variation ($r=0.64$, $p<0.0001$). Between different ethnic groups there was no significant difference in the analysis of walk test distance corrected for age.

3.1.4 Discussion

The six-minute walk test was established in adult populations to assess capacity to exercise, prognosis and response to therapy in PAH. It reflects a person's ability to perform day-to-day activities. However, in highly compromised patients, due to a cardiac or respiratory disease, the 6MWT may represent a maximal exercise (176). The 6MWT is also used in children with cardiopulmonary disease, but there are few data available on normal children.

The current study establishes normal values for 6WMT distance in healthy British children between 4 and 11 years of age. This study describes the increase in distance walked with age. No reference values for 6MWT distance have been available in younger children, therefore quantification of exercise intolerance in this population was largely based on data extrapolated from older individuals, which is likely prone to error. From clinical experience, it is apparent that a 6MWT can be, in principle, performed by young children, but obviously depends upon the individual child's motivation and coordination. This study illustrates, however, that with encouragement and explanation children from the age of four years perform the 6MWT well. The variation in walk distance in children of 4 and 5 years of age did not differ significantly from that observed in the older children.

This cross-sectional study suggests that there is a rapid increase in the distance walked in children aged 4 to 7 years of age with a further slower increase up to 11 years of age.

To date normal values for the 6MWT have only been reported in older cohorts. Li and colleagues reported normal values in 78 Chinese children aged between 12 and 16 years of age (177). The mean distance for the whole group was 660 ± 58 m, this distance being considerably longer than in the 11 year old children (512 ± 41 m) studied here. Unfortunately values for the individual age groups are not reported; given the age of these children the greater distance might be related to an increase in height during and after puberty. Chetta et al reported in a Caucasian cohort aged 20-50 years a mean distance of 593 m for females and 638 m for males (178). For an older age group (40-80 years) Enright reported a median walk distance of 494 m for women and 576 m for men. After correction for height there was no gender related difference, which is in keeping with the findings of the current study (179). In young

adults age was not related to 6MWT distance (179-182). In contrast to this, the results of the current study in a paediatric cohort suggest that age alone accounts for 41% of predicted walk test distance variation.

The heart rate recorded in the current study demonstrates that the 6MWT represents sub-maximal effort in healthy children. Estimated peak heart rate during maximal exercise of children between 4 and 11 years of age would be approximately 210 bpm (220bpm-age) (183). Thus the peak heart rate reached by the children included in this study was approximately 65% of the normal predicted peak heart rate for age. In the population studied here heart rate increased early during the test with a plateau after approximately 1-3 minutes. Heart rate during exercise was higher in girls than in boys. A similar observation was made by Mahon who described a lower heart rate in boys during sub-maximal exercise on a cycle ergometer (184) and by Armstrong, investigating cardiovascular responses to sub-maximal treadmill running in children between 11 and 13 years. After adjustment for body size, boys had a greater stroke volume than girls when exercising at the same level (185). The authors, therefore, speculated that girls compensated for the lower stroke volume with a significantly higher heart rate.

Knowledge of normal 6MWT distances for children should be helpful in assessing the degree of exercise limitation in children with cardiovascular disease. For example the 6MWT is commonly used in children with chronic diseases, such as pulmonary hypertension to assess response to advanced therapies (79,106). The current data shows that an increase in walk distance needs to be interpreted with caution, especially in younger children as a physiological increase could easily be misinterpreted as a response to medical therapy, while in fact it reflects the physiologic effect of growth.

Study limitations

Only one 6MWT was performed per child. It has been shown, that there might be a training effect and a longer distance might be reached on a repeated tests (181,186,187). However a high degree of test-retest reliability has recently been reported by Li et al in healthy children (177). It has recently been shown in a study of

healthy Singaporean adults that 6MWT distance cannot be predicted using reference equations derived from adult Caucasian populations (188). The current study enrolled children from different ethnic backgrounds, though the majority were of Caucasian origin. Although no significant difference could be found between the distance walked by the Caucasian children and the other ethnic groups in the current study, this limitation should be considered when extrapolating the results of this study to children of non-Caucasian origin.

In conclusion, performing a six-minute walk test is feasible and practical in young children. This study provides data on normal UK children against which the performance of sick children and the response to therapeutic intervention can be judged.

3.2 COMPARISON BETWEEN SIX-MINUTE WALK TEST DISTANCE AND PARAMETERS OF CARDIOPULMONARY EXERCISE TESTING

3.2.1 Background

The six-minute walk test (6MWT) is the distance a person can walk at a constant, uninterrupted, unhurried pace in six minutes. It represents a simple and cost-effective method of assessing exercise capacity and is used routinely to assess patients with cardiovascular or pulmonary disease (168). It has been demonstrated to be an independent predictor of morbidity and mortality in adult patients with heart failure, pulmonary disease and IPAH (171),(135,172). At many centres it is used for longitudinal assessment of patients with chronic disease, especially to monitor response to treatment and to guide therapy (81).

Cardiopulmonary exercise testing (CPET) with metabolic monitoring is regarded by many to be the gold-standard modality to assess exercise capacity (189). While the 6MWT may or may not represent a maximal exercise test, the CPET does represent a maximal exercise test and provides additional measures of exercise tolerance and disease severity such as peak oxygen consumption ($\dot{V}O_2$), oxygen consumption at anaerobic threshold ($\dot{V}O_{2AT}$) and ventilatory efficiency ($\dot{V}E/\dot{V}CO_2$). Despite its apparent advantages and the fact that $\dot{V}O_2$ predicts prognosis in adult patients with PHT (136), the use of CPET as an endpoint for prospective clinical trials in patients with pulmonary hypertension is problematic. The alleged disadvantages of CPET as compared to the 6MWT are the requirement for technical training for laboratory personnel, standardisation of CPET and calibration of the CPET equipment before testing (190).

It has been demonstrated that CPET can be performed safely in paediatric patients with PHT (191) but little is known about the relationship between 6MWT distance and variables of CPET in children with PAH.

3.2.2 Patients and Methods

Forty-one consecutive exercise studies were included in this analysis: 15 exercise tests in children with idiopathic PAH (IPAH) (mean age 13.0 ± 3.0 years; 9 female), 18 in children with PAH associated with congenital heart disease other than the

Eisenmenger syndrome (mean age 14.8 ± 2.8 years; 7 female; unrepaired heart defects $n=3$, post-repair PAH 15) and 8 in children with the Eisenmenger syndrome (mean age 11.8 ± 2.9 years; 4 female). The mean age \pm standard deviation was 13.5 ± 3.0 years. Of those with IPAH, 35% were on monotherapy and 65% were on combination therapy. Children with congenital heart disease (without the Eisenmenger Syndrome) were treated with bosentan monotherapy (40%) or combination therapy (30%). Of those with the Eisenmenger syndrome, 4 children were on either Bosentan or Sildenafil monotherapy, and one other received both drugs. All children underwent both tests without complications. Cardiopulmonary exercise testing and 6MWT were performed as described in Chapter 2.

Predicted values for 6MWT distance in boys and girls were calculated based on gender, age and height according to published data for healthy Caucasian children and adolescents (160). All patients underwent exercise testing as part of their clinical follow-up and provided informed consent before exercise testing.

Statistical Analysis

Relationships between variables of 6MWT and CPET were studied using Pearson correlation coefficient (after testing for normal distribution). To explore the relationship between variables of 6MWT-distance and CPET in scatterplots the Lowess technique (LOcally WEighted Scatterplot Smoother) using a local linear polynomial fit to provide a smooth line describing the association between 6MWT-distance and pVO_2 was used (192,193). In addition, a parametric model using fractional polynomials was fitted to the data using Generalized Additive Models for Location Scale and Shape (GAMLSS) in R as described by Rigby and Stasinopoulos (194). To describe the change in heart rate and oxygen saturation during 6MWT a linear mixed effects model with higher-than-linear ($a+x+x^{3/2}$) terms to describe the observed shapes and to take into account the repeated measurements structure of the data was fitted using the nlme package in R (195). To study the optimal cut-off value for 6MWT-distance in predicting pVO_2 , R^2 -values were calculated for the linear correlation between 6MWT-distance and pVO_2 for a subset of 6MWT-distances (lowest value to increasing cut-off) and the R^2 -values for different cut-off values of 6MWT-distance were plotted.

3.2.3 Results

Reduced exercise capacity in children with PAH

Children with pulmonary hypertension had a reduced exercise capacity as judged by 6MWT and CPET. Peak VO₂ was reduced to $31.5 \pm 12.2\%$ of the predicted value ($p < 0.0001$) (Figure 3.2.1).

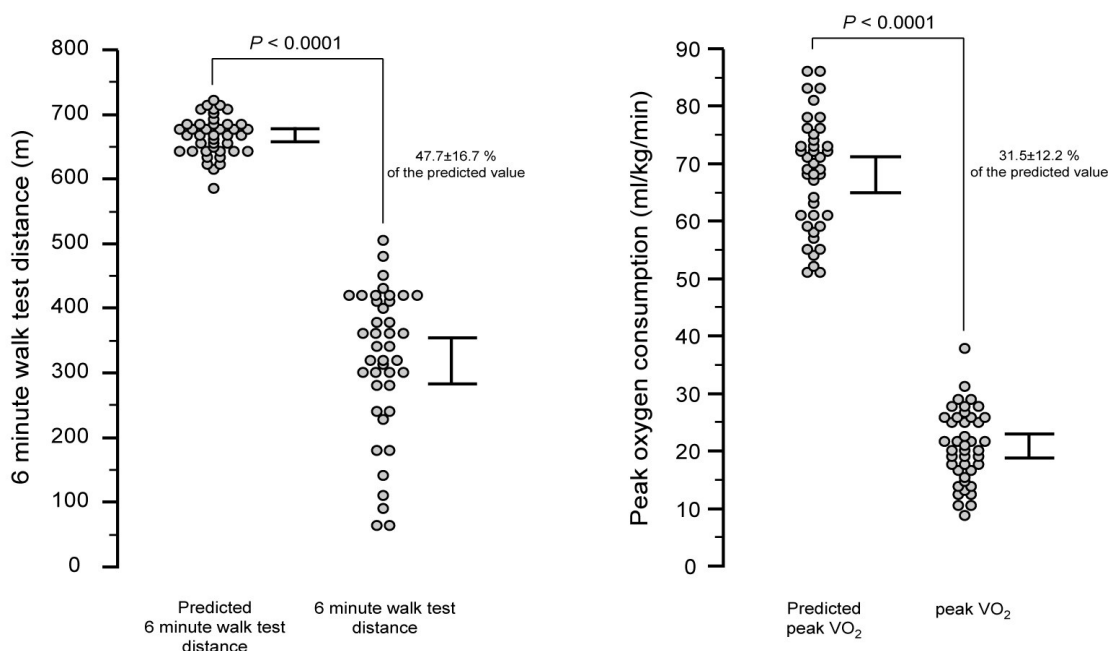


Figure 3.2.1. Predicted and measured values for 6 minute walk test distance and peak oxygen consumption (peak VO₂) in children with PAH. Markers indicate 95% confidence interval for the mean. Comparisons between groups were performed using Mann-Whitney tests. Reference values were calculated according to published data (160,162).

The 6MWT distance was also reduced to $47.7 \pm 16.7\%$ of predicted ($p < 0.0001$). In fact, none of the studied children achieved 80% of the 6MWT value predicted for healthy children. Interestingly, no significant difference in 6MWT distance could be found between children in WHO class 2 or 3 (325 ± 109 vs. 304 ± 132 m, respectively; $p = 0.67$). Nor was there a significant difference in pVO₂ between WHO class 2 or 3 (22.5 ± 6.5 vs. 20.5 ± 6.4 ml/kg/min in WHO class 2 and 3, respectively; $p = 0.39$). These observations illustrate the limitations of assessing exercise tolerance based on patients' subjective physical capacity. The 6MWT distance was lowest in the Eisenmenger population (255 ± 132 m; $39.2 \pm 21.1\%$ predicted 6MWT distance)

compared to patients with IPAH (332 ± 110 m; $50.2 \pm 16.0\%$ predicted 6MWT distance) and PAH associated with congenital heart disease other than the Eisenmenger syndrome (336 ± 102 m, $49.4 \pm 14.7\%$ predicted 6MWT distance) but the differences were not statistically significant.

Similarly, patients with the Eisenmenger syndrome (16.8 ± 5.8 ml/kg/min) had a significantly lower value for pVO_2 compared to patients with PAH associated with other forms of congenital heart disease (23.3 ± 6.7 ml/kg/min, $p = 0.03$) and the mean value was lower, although not statistically significant, than in those with IPAH (20.2 ± 5.6 ml/kg/min, $p = 0.12$). Percentage predicted pVO_2 was also lower in the Eisenmenger group ($24.3 \pm 9.7\%$) compared to patients with either PAH associated with congenital heart disease other than the Eisenmenger syndrome ($33.6 \pm 12.2\%$, $p = 0.07$) or idiopathic PAH ($32.8 \pm 12.6\%$, $p = 0.12$).

Correlations between variables of CPET and 6MWT

Both the pVO_2 and the oxygen consumption at anaerobic threshold (VO_{2AT}) correlated with the 6MWT distance ($r = 0.49$; $p = 0.001$ and $r = 0.40$, $p = 0.01$, respectively), and an inverse correlation was found between VE/VCO_2 at anaerobic threshold and the 6MWT distance ($r = -0.43$; $p = 0.005$).

However, when the relationship between the 6MWT distance and pVO_2 was investigated further using locally-weighted polynomial regression (lowess) it became apparent that a linear correlation between 6MWT distance and pVO_2 existed only at low levels of exercise capacity.

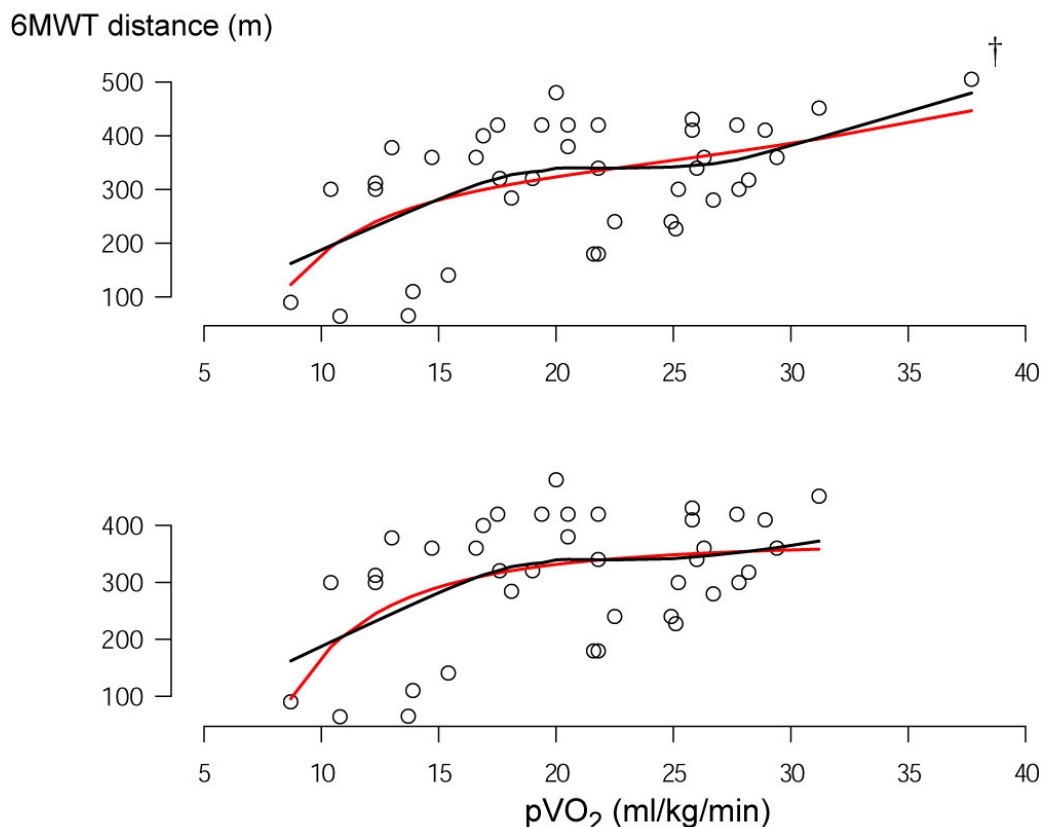


Figure 3.2.2. Locally-weighted polynomial regression (lowess – black curve) with and without exclusion of an outlier (†) showing a close to linear association between 6 minute walk test distance and peak oxygen consumption (peak VO₂) up to a 6MWT distance of approx. 300 m or 20 ml/kg/min with a plateau-phenomenon thereafter. In addition, a parametric model using fractional polynomials was fitted to the data (red curve) using Generalized Additive Models for Location Scale and Shape (GAMLSS) in R as described by Rigby and Stasinopoulos (194).

As illustrated in Figure 3.2.2 there appeared to be a close to linear association between 6MWT distance and pVO₂ up to a 6MWT distance of approx. 300 m or 20 ml/kg/min, respectively. Visually, there appeared to be a “ceiling-effect” of 6MWT distance for higher values of pVO₂.

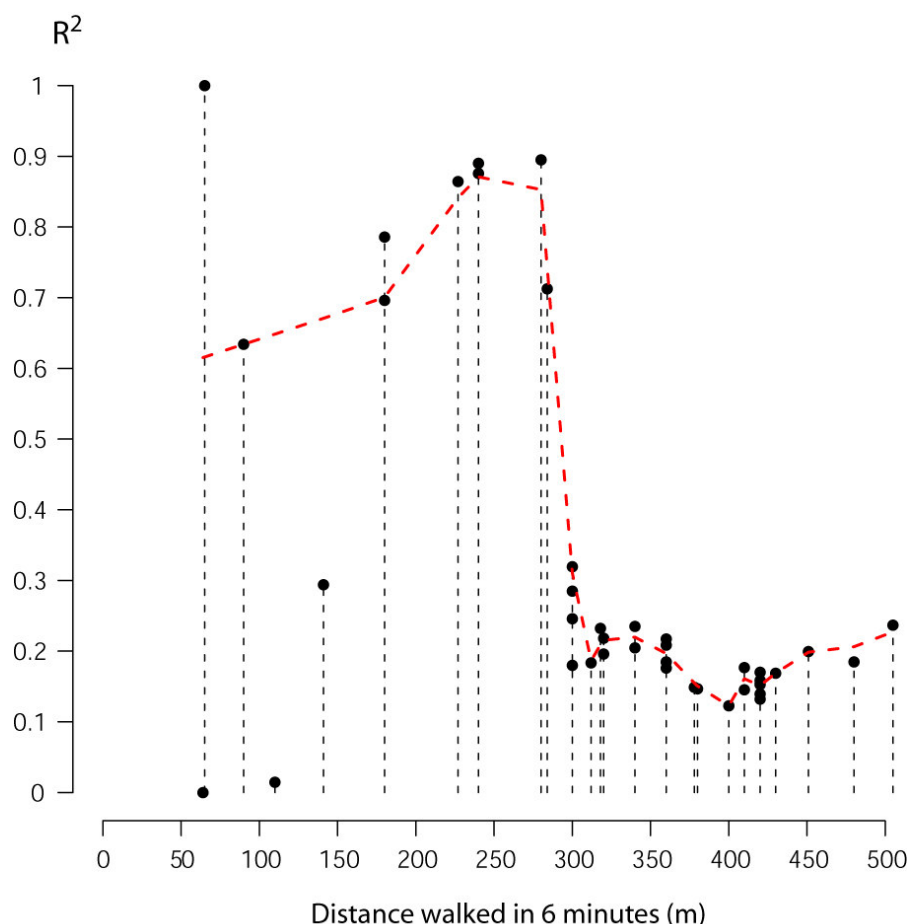


Figure 3.2.3. Plot of calculated R^2 -values for the linear correlation between 6-minute walk test (6MWT)-distance and peak oxygen consumption (pVO_2) for a subset of 6MWT-distances (lowest value to increasing cut-off) against different cut-off values of 6MWT-distance. The plot supports the notion that there is a relevant reduction in the percentage of the variation of pVO_2 explained by 6MWT-distance around a cut-off value of 300 metres and this is in agreement with the impression on visual inspection of the data. The red dotted line represents the result of a locally-weighted polynomial regression (lowess).

Based on visual inspection of the relationship between pVO_2 and 6MWT, as well as based on R^2 maximization (Figure 3.2.3) a cut-off value of 300 m was chosen to further investigate the relationship between pVO_2 and 6MWT below and above this value.

This cut-off value is in agreement with data published in adult heart failure patients, dividing patients in those with maximal and sub-maximal exercise and identifying patients with adverse outcome (176). Using this cut-off value confirmed a significant relationship between the pVO_2 and 6MWT distance up to a distance of 300m, finding

that the 6MWT distance accounted for 71% of the variation in pVO₂ when the 6MWT distance < 300 m, while there was hardly any association between 6MWT distance and pVO₂ at higher levels of 6MWT distance (Figure 3.2.4). In fact, when one apparent outlier (the child with the highest combination of 6MWT distance and pVO₂ and the highest residual value on correlation analysis – marked with † in Figure 3.2.2) was excluded no significant association between 6MWT distance and pVO₂ was found for patients with a 6MWT distance > 300 m ($p = 0.30$).

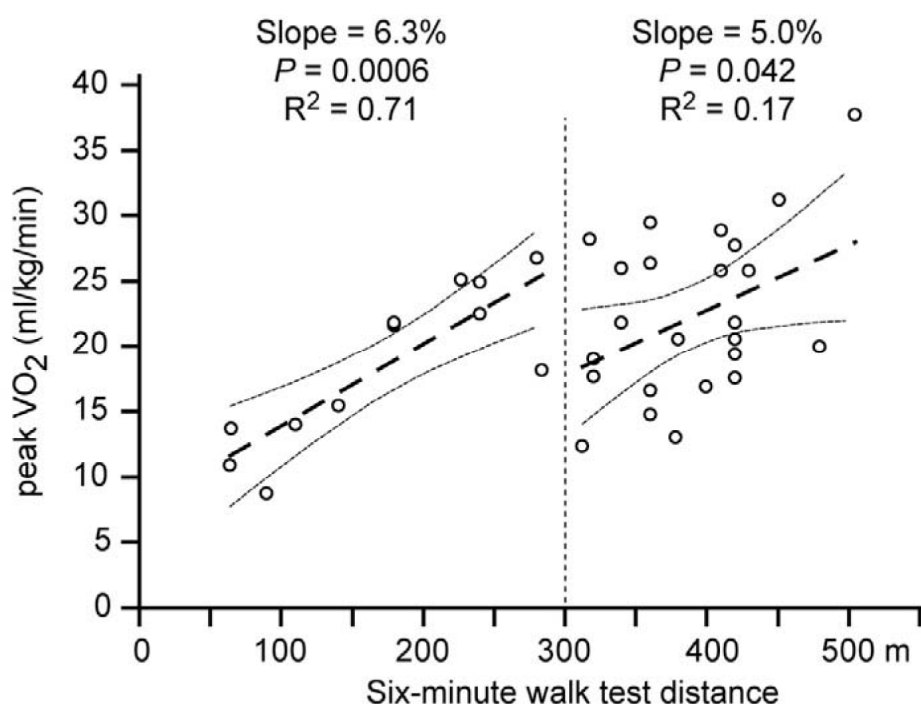


Figure 3.2.4. Linear regression comparing the association between 6 minute walk test distance and peak oxygen consumption (peak VO₂) for patients with a 6 minute walk test distance below and above 300 m separately.

Similar differences were found for VO₂AT ($p = 0.046$ [$R^2 = 0.34$] for a 6MWT distance < 300 m and $p = 0.20$ [$R^2 = 0.07$] for a 6MWT distance > 300 m) and for VE/VCO₂ at anaerobic threshold ($p = 0.02$ [$R^2 = 0.41$] for a 6MWT distance < 300 m and $p = 0.90$ [$R^2 < 0.01$] for a 6MWT distance > 300 m).

Does the 6MWT represent a maximal exercise test in patients with PAH?

To further investigate whether the 6MWT distance represents a measure of maximal exercise capacity in children with PAH we compared maximal heart rate during CPET and 6MWT.

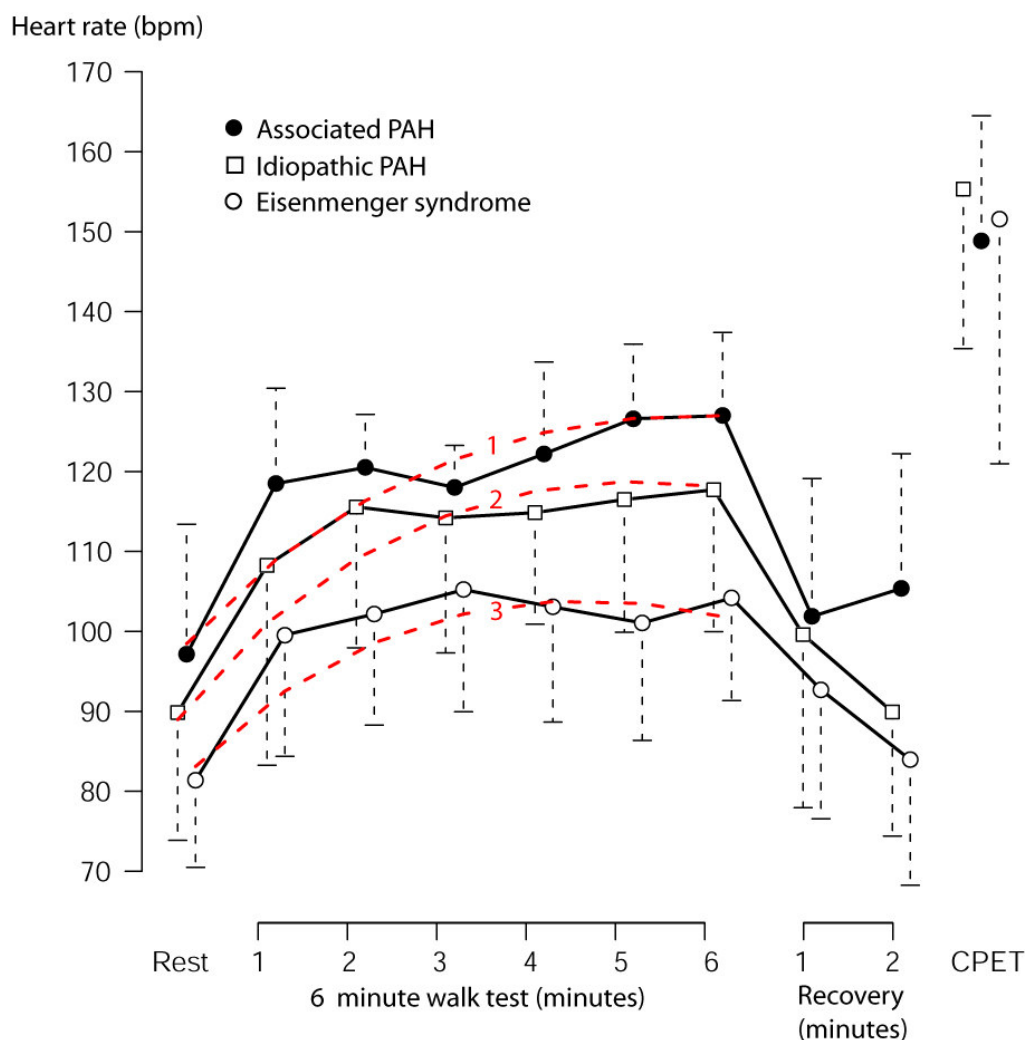


Figure 3.2.5. Heart rate (HR) profile during 6 minute walk test (6MWT) and maximal heart rate during cardiopulmonary exercise testing (CPET) in children with idiopathic pulmonary hypertension, pulmonary hypertension associated with congenital heart disease other than the Eisenmenger syndrome (“Associated PAH”) and Eisenmenger syndrome during 6 minute walk test and cardiopulmonary exercise testing. Symbols and error bars indicate mean \pm standard deviation. To describe the change in heart rate during 6MWT a linear mixed effects model with higher-than-linear ($a+x+x^3/2$) terms to describe the observed shapes and to take into account the repeated measurements structure of the data was fitted using the nlme package in R (red dotted lines) (195): *Curve 1:* $HR=95.7+15.8*t-4.3*t^{1.5}$, $p<0.002$ for all coefficients; *curve 2:* $HR=87.3+17.9*t-5.2*t^{1.5}$, $p<0.0001$ for all coefficients; *curve 3:* $HR=79.2+15.9*t-4.9*t^{1.5}$, $p<0.0001$ for all coefficients. t =time in minutes.

As illustrated in Figure 3.2.5, maximal heart rate during CPET was significantly higher in the entire patient cohort (152 ± 24 vs. 120 ± 17 /min; $p < 0.0001$), and in all three individual patient-subgroups (p-value between 0.0002 and 0.01) compared to maximal heart rate during 6MWT, supporting the notion that the 6MWT represents a sub-maximal exercise test. Furthermore, within the Eisenmenger population the minimal oxygen saturation recorded during exercise testing was lower during CPET compared to the 6MWT, although there was little difference in minimal oxygen saturation between the two tests in children with IPAH and other congenital heart disease associated pulmonary hypertension (Figure 3.2.6).

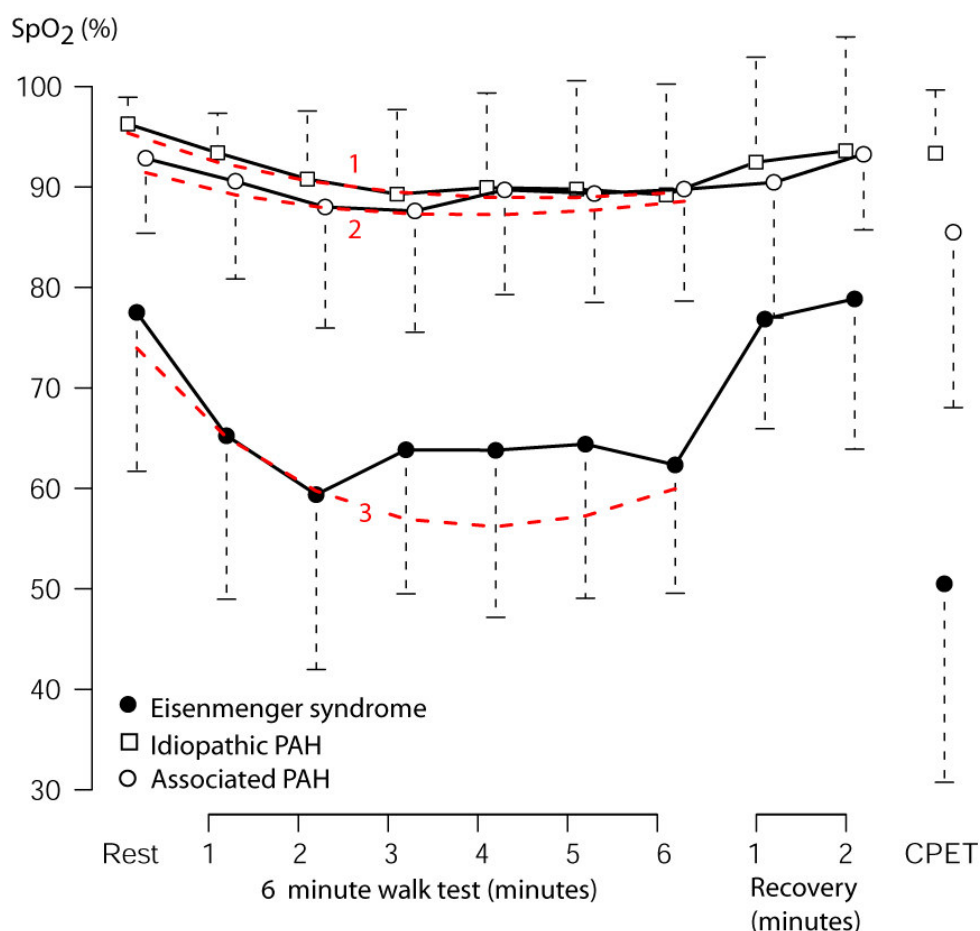


Figure 3.2.6. Transcutaneous oxygen saturation (SO₂) in children with idiopathic pulmonary hypertension, pulmonary hypertension associated with congenital heart disease (“Associated PAH”) and the Eisenmenger syndrome during the 6 minute walk test and cardiopulmonary exercise testing (CPET). Note that minimal transcutaneous oxygen saturation in Eisenmenger patients is lower during CPET compared to the 6 minute walk test. Symbols and error bars indicate mean \pm standard deviation. To describe the change in SO₂ during 6MWT a linear mixed effects model with higher-than-linear ($a+x+x^3/2$) terms to describe the observed shapes and to take into account the repeated measurements structure of the data was fitted using the nlme package in R (red dotted lines) (195): *Curve 1: $SO_2 = 95.8 - 4.5 * t + 1.4 * t^{1.5}$, $p < 0.01$ for all coefficients; curve 2: $SO_2 = 92.4 - 4.0 * t + 1.4 * t^{1.5}$, $p < 0.0001$ for all coefficients; curve 3: $SO_2 = 76.5 - 14.9 * t + 4.9 * t^{1.5}$, $p < 0.0001$ for all coefficients. t = time in minutes.*

To further investigate the relationship between maximal heart rate during CPET and 6MWT the difference between these two variables was calculated for each individual

patient. Correlating pVO_2 with the heart rate difference between CPET and 6MWT revealed a close correlation between these variables as shown in Figure 3.2.5 ($p = 0.0004$; $r = 0.53$). While some patients with a $pVO_2 < 20$ ml/kg/min had a similar or even lower maximal heart rate during CPET compared to 6MWT all children with a $pVO_2 > 20$ ml/kg/min exhibited higher maximal heart rates during CPET compared to 6MWT, consistent with the notion that the 6MWT distance represents a maximal exercise test in sicker children while it is a marker of sub-maximal exercise capacity in less compromised patients (Figure 3.2.7).

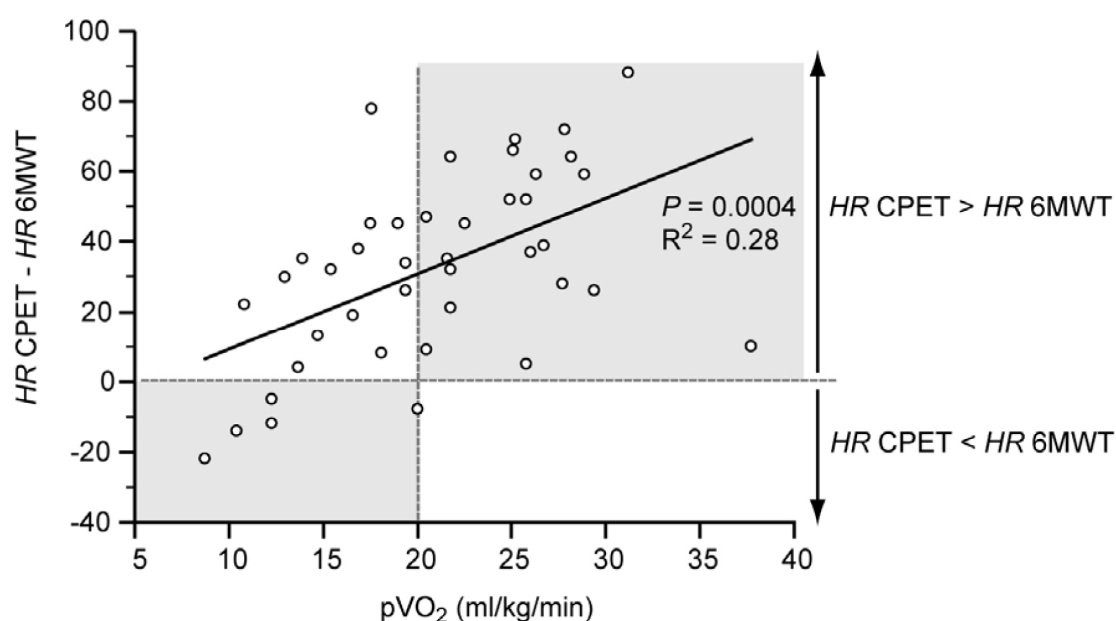


Figure 3.2.7. Scatterplot exploring the relationship between the difference in maximal heart rate during cardiopulmonary exercise testing (CPET) and 6 minute walk test (6MWT). The plot illustrates that while some patients with a peak oxygen consumption (pVO_2) below 20 ml/kg/min had similar or even lower heart rates during CPET compared to the 6 minute walk test (left lower quadrant), patients with a $pVO_2 > 20$ ml/kg/min had consistently higher heart rates during CPET compared to the 6 minute walk test and the difference in heart rate increased with increasing exercise tolerance (right upper quadrant).

3.2.4 Discussion

This study shows that children with PAH have a markedly depressed exercise capacity. In children with a 6MWT distance below ~ 300 meters or a pVO_2 below ~ 20 ml/kg/min a close correlation was found between measures of exercise capacity derived from CPET and the 6MWT distance. In contrast, there was a much weaker, if any, association between these assessments of exercise capacity in less compromised patients. Therefore, it appears that the 6MWT distance fails to reflect maximal exercise tolerance in less impaired children with PAH and that CPET may be a useful adjunct in the comprehensive assessment of patients with a 6MWT distance above 300 meters.

Peak oxygen consumption is an established and reliable measure of exercise intolerance and is widely employed in the assessment of adult patients with congenital heart disease and PAH (157,196). In the present study a significantly reduced pVO_2 and 6MWT distance was seen in children with PAH compared to reference values derived from healthy children of similar age (160). These findings are in keeping with data obtained in similar studies carried out on adult patients with PAH (106,135,197,198). However, there is little data available on the 6MWT distance and especially on CPET in children with PAH. As a consequence, considerable controversy exists regarding the merits of performing CPET in these children. While the 6MWT is easy to perform and can be repeated at low cost, CPET with metabolic monitoring requires expensive equipment, technical expertise and training of the subject. It does however, provide additional objective measures of exercise capacity such as oxygen uptake and ventilatory efficiency, which can be regarded a surrogate variables of pulmonary blood flow (189,199). In addition, VO_2 at anaerobic threshold and markers of ventilatory efficiency can be calculated based on CPET. A modest correlation between 6MWT distance and VE/VCO_2 at anaerobic threshold – a parameter of ventilatory efficiency – was found in the current study. Recently, measures of ventilatory efficiency such as VE/VCO_2 at anaerobic threshold and the VE/VCO_2 -slope have been found to be related to prognosis in various adult cardiovascular cohorts (196,200-203). Conceptually these measures are appealing due to their robustness and the fact that they are independent of patient effort. In fact,

studies in adult patients with congenital heart disease and congestive heart failure have established that VE/VCO_2 -slope is superior to pVO_2 in predicting prognosis (189,200). Whether these parameters relate to prognosis in children with PAH as they do in adults with PAH and without a persistent foramen ovale (136) remains to be established. In patients with right-to-left shunting at rest or during exercise the value of parameters of ventilatory efficiency may be limited. For example, parameters of ventilatory efficiency do not carry important prognostic information in cyanotic patients, such as patients with Eisenmenger syndrome, for reasons discussed in detail by Dimopoulos et al. (196). Briefly, it has been speculated that in cyanotic patients the limitation to exercise does not arise from poor ventricular function, vascular remodelling or autonomic dysfunction but rather from exercise induced increase in right-to-left shunting. The limitation of exercise capacity is, therefore, below that which would have been set by the usual pathophysiologic abnormalities that in turn are responsible for the impaired prognosis. Therefore, survival prospects are not as poor as would be predicted from the exercise capacity (204). However, in selected children with PAH, parameters of ventilatory efficiency may be useful in assessing disease severity, for assessing response to drug therapy and for consideration as potential endpoints in future trials on drugs used to treat PAH.

Remarkably, the correlation coefficient between 6MWT distance and pVO_2 in the current paediatric study (0.49) is almost identical with that published in a large cohort of adult patients who underwent exercise testing as part of a multicentre clinical trial evaluating sitaxsentan – an endothelin receptor antagonist ($r=0.483$) (190). Oudiz and colleagues suggested that the correlation was modest at least in part due to lack of standardization of CPET and a different level of experience at centres participating in this drug trial. The current study demonstrates that even when all tests are performed at a single institution using a standardized protocol, skilled technicians and properly calibrated CPET equipment the correlation between the 6MWT distance and pVO_2 is only modest and the 6MWT distance cannot be used as a surrogate for pVO_2 .

The heart rate recorded during the 6MWT in the current study demonstrates that the 6MWT represents sub-maximal effort in many children. Estimated peak heart rate

during maximal exercise of children with an average age of 13 years would be approximately 195 bpm (220 bpm minus age) (183). Thus the peak heart rate in the current study was approximately 60% of the normal predicted peak heart rate for age. In contrast, the maximal heart rate during CPET averaged 152 bpm, representing 78% of the predicted value. As illustrated by Figure 3.2.5 heart rate increased early during the 6 minute walk test with a plateau after 1-3 minutes consistent with the concept of a steady-state metabolism reached after this point in analogy to patients performing a sub-maximal test or a CPET below the anaerobic threshold (205). Furthermore, Figure 3.2.7 illustrates that heart rate difference between the CPET and the 6MWT was biggest in children with high values of pVO_2 , supporting the notion that the 6MWT represents a sub-maximal test in this setting with lower peak heart rate.

Clinical Implications

Knowing how far a child with PAH can walk in six minutes is helpful in assessing the degree of exercise limitation, and in evaluating prognosis and response to medical therapy. Although changes in the 6MWT distance are routinely used as an end-point in clinical trials of PAH drugs and provide valuable information when managing children with PAH, this study suggests that CPET should be considered as a complimentary test in less impaired children with PAH with a 6MWT distance of more than 300 m, because the 6MWT distance appears to represent a sub-maximal test in these children. None of the patients included in the current study suffered any adverse events during either CPET or performing the 6MWT. This suggests that exercise testing is safe even in children with PAH and this is in agreement with previous studies in adult patients with congenital heart disease and pulmonary hypertension including patients with IPAH and with the Eisenmenger syndrome (157,189). Exercise tests in children with PAH should, however, be supervised by a trained exercise physiologist and a physician with experience managing children with PAH should be present at all times. The UKSPHC operates a policy of starting performing a regular 6MWT from the age of approximately 4 years onwards. Initially these tests are performed to accustom the children to doing a 6MWT. Most children can perform a 6MWT from the age of 5-6 years reliably, although other investigators have recommended that walk tests in children with PAH should not be

performed below the age of 7 years for safety reasons (206). In the present study cardiopulmonary exercise tests were performed on a bicycle ergometer and this requires a minimal height of 140 cm.

There appears to be no doubt that in healthy individuals the 6MWT represents a sub-maximal exercise test, mainly reflecting the patient's ability to perform day to day activities. Roul and colleagues, investigating adult patients with heart failure, suggested that in highly compromised patients the 6MWT may represent a maximal exercise test (176). As in the present paediatric study, the 6MWT distance in adults in heart failure correlated with pVO_2 only when the 6MWT distance was less than 300 m. In addition, the 6MWT distance was predictive of survival in heart failure patients walking less than 300 m in 6 minutes. The present study establishes for the first time that pVO_2 relates closely to the 6MWT distance in children with PAH who have a poor exercise capacity (i.e. a 6MWT distance below approx. 300 m or a pVO_2 below approx. 20 ml/kg/min). For higher values of 6MWT distance this association is weak or absent. Therefore when assessing the effect of medical therapies on exercise capacity in children with higher exercise capacity a CPET should be the preferred exercise testing modality.

Study limitations

Predicted values for the 6MWT distance were derived from a study published recently by Geiger (160) and colleagues who used a measuring wheel to record the walking distance. This represents a slight modification from conventional practice. It is difficult to know whether and to what extent this would have influenced their results. Future studies will focus on studying exercise capacity in PAH of different aetiologies and attempting to correlate performance with outcome. This was a cross-sectional analysis and further longitudinal studies are required to assess whether serial CPET or 6MWT represent effective efficacy endpoint for pulmonary hypertension trials. Further prospective studies are clearly required to assess whether CPET alone or in combination with the 6MWT distance is superior to the 6MWT distance in guiding therapy and assessing prognosis in children in PAH.

In conclusion, performing 6-minute walk tests and CPET in children with PAH is feasible and safe. The 6MWT reflects maximal exercise capacity in PAH children with a 6MWT distance of less than 300m or a pVO₂ of less than 20 ml/kg/min. CPET should be considered as a complimentary test in children whose exercise tolerance is well above these thresholds.

3.3 HEART RATE VARIABILITY

3.3.1 Background

Little is known about heart rate variability (HRV) in patients with pulmonary hypertension. Autonomic dysfunction is however thought to be an important feature of severe PAH and has been described in adult patients with idiopathic PAH (IPAH) and with the Eisenmenger syndrome. Two studies have reported an increase in sympathetic tone (207,208) and an early loss of circadian rhythm has been demonstrated (208). Folino reported lower values of HRV in 9 adults with IPAH compared to healthy controls (207). An abnormal HRV has been shown to be a powerful and independent predictor of adverse prognosis in paediatric patients with congenital heart disease without PAH (209-211), adults with ischaemic heart disease (212-216) and in the general population (217). HRV data can be derived from conventional Holter-ECG recordings, which are frequently performed as part of routine care in patients with PAH.

The prognostic value of impaired cardiac autonomic nervous activity in children with PAH is unknown. The objective of the current study was to assess the prognostic value of HRV for risk stratification in children with PAH.

3.3.2 Patients and Methods

Forty-seven children (27 male; mean age 11.4 ± 5.5 years) with PAH were included in this retrospective study [IPAH $n=21$, associated PAH (APAH) $n=26$]. Cases of APAH included 21 with congenital heart disease, 4 with chronic or interstitial lung disease and 1 child with vasculitis. The children underwent Holter-ECG for a variety of reasons. These included a history of syncope in 11 children, presyncopal attacks in 6, uncertain chest pain in 5, palpitations in 6, an increase in shortness of breath and reduction in exercise tolerance in 8, and an abnormal 12-lead ECG (bradycardia, ventricular ectopics) in 7 children. In 4 other patients Holter ECG recording formed a routine part of the assessment for transplantation. Holter monitoring was performed for a mean duration of 23.76 ± 2.0 hours (Reynolds Pathfinder Software, Hertford, UK). Parameters of heart rate variability were determined from the recordings, including SDNN (standard deviation of all normal-to-normal intervals), SDANN

(standard deviation of mean values for all normal-to-normal intervals over 5 minutes) and RMSSD (square root of the mean square differences of successive RR intervals). The clinical features assessed included WHO Functional Class, a six-minute walk (6MWT) (total distance walked and minimal arterial oxygen saturation during exercise), semi-quantitative echocardiographic study of right ventricular function (normal=1b; mildly=2; moderately=3; severely=4 impaired) [(157,218)], and at cardiac catheterization, the mean pulmonary artery pressure (PAP), pulmonary vascular resistance index (PVRI) at baseline and following vasodilator testing. Baseline demographic data are shown in Table 3.3.1.

Table 3.3.1. Selected baseline characteristics

Characteristic	All patients (n=47)	Dead or Tx (n=17)	Alive (n=30)	P Value
Age (years)	11.4 ± 5.5	10.2 ± 6.1	12.1 ± 5.0	0.35
Gender male / female	27/20	12/5	15/15	0.29
% IPAH patients	44.7 %	58.8 %	36.7 %	0.24
WHO II / III / IV (n)	17 / 29 / 1	1 / 15 / 1	16 / 14 / 0	0.003
6MWT distance (m)	292.2±123.4	182.3±67.6	351.7±104.6	<0.0001
History of syncope	26.1 %	27.5 %	23.5 %	1.00
RV function 1 / 2 / 3 / 4†	9 / 19 / 14 / 4	0 / 3 / 10 / 3	9 / 16 / 4 / 1	0.0003
RV dilatation 1 / 2 / 3 / 4†	1 / 9 / 19 / 16	0 / 1 / 9 / 7	1 / 8 / 10 / 9	0.23
RV hypertrophy 1 / 2 / 3 / 4†	13 / 28 / 5 / 1	1 / 13 / 3 / 0	12 / 15 / 2 / 1	0.054
Mean PA pressure (mmHg)	58.4±23.2	61.6±15.5	56.3±27.3	0.33
PVR baseline (WU)	22.1±13.1	26.8±14.3	19.2±11.6	0.38
PVR NO/FiO ₂ 0.6 (WU)	17.9±2.0	23.0±13.7	14.8±9.8	0.16
Right atrial pressure (mmHg)	8.3±3.8	6.8±1.8	9.0±4.4	0.31

p-values (Mann-Whitney U-test/ Chi-square test) for comparison between event-free survivors and patients who died or underwent transplantation. FiO₂ = inspiratory oxygen concentration; HRV = heart rate variability; NO = nitric oxide; PVR = pulmonary vascular resistance; RMSSD = square root of the mean square differences of successive RR intervals; SDANN = standard deviation of mean values for all normal-to-normal intervals over 5 minutes; SDNN = standard deviation of all normal-to-normal intervals; Tx = transplantation. WHO = World Health Organization functional class; WU = Wood units (=80 dyn·s·cm⁻⁵). † indices of right

ventricular hypertrophy, size and function were quantified semi-quantitatively as: normal=1; mildly=2; moderately=3; severely=4 impaired. Plus-minus values are mean \pm standard deviation

After a period of follow up the patients were divided into two groups, those still alive and those who died or had had a lung or heart-lung transplant. Data from all the children were analysed and then data from those with IPAH and APAH were analysed separately.

Statistics

To compare different predictive values, receiver operating curve analysis (ROC) was performed and areas under the curve (AUC) for sensitivity and specificity were constructed. Kaplan-Meier cumulative survival plots were constructed to illustrate the results. The relationship between co- variables and survival was initially studied by univariate Cox proportional hazard analysis. Significant parameters were subsequently included into bivariate Cox proportional hazard models in a stepwise forward selection procedure. The hazard ratio with 95% confidence interval (CI) and p-values are presented. Hazard ratios for continuous variables apply per unit of the analysed variable.

3.3.3 Results

The mean follow up period after Holter-ECG recording was 19 ± 11.5 months (1.8-49.1 months). During this time ten children died (4 with IPAH), and seven were successfully transplanted (6 with IPAH). Two of those who died had been listed for transplantation, both with IPAH. Death or transplantation was used as the combined endpoint (10 IPAH, 7 APAH). All 47 children were on advanced therapies for the treatment of pulmonary arterial hypertension. None of the patients received antiarrhythmic drugs.

For the whole group, the children who died or underwent transplantation had significantly lower values for SDNN, SDANN and RMSSD than the survivors (Table 3.3.2, Figure 3.3.1).

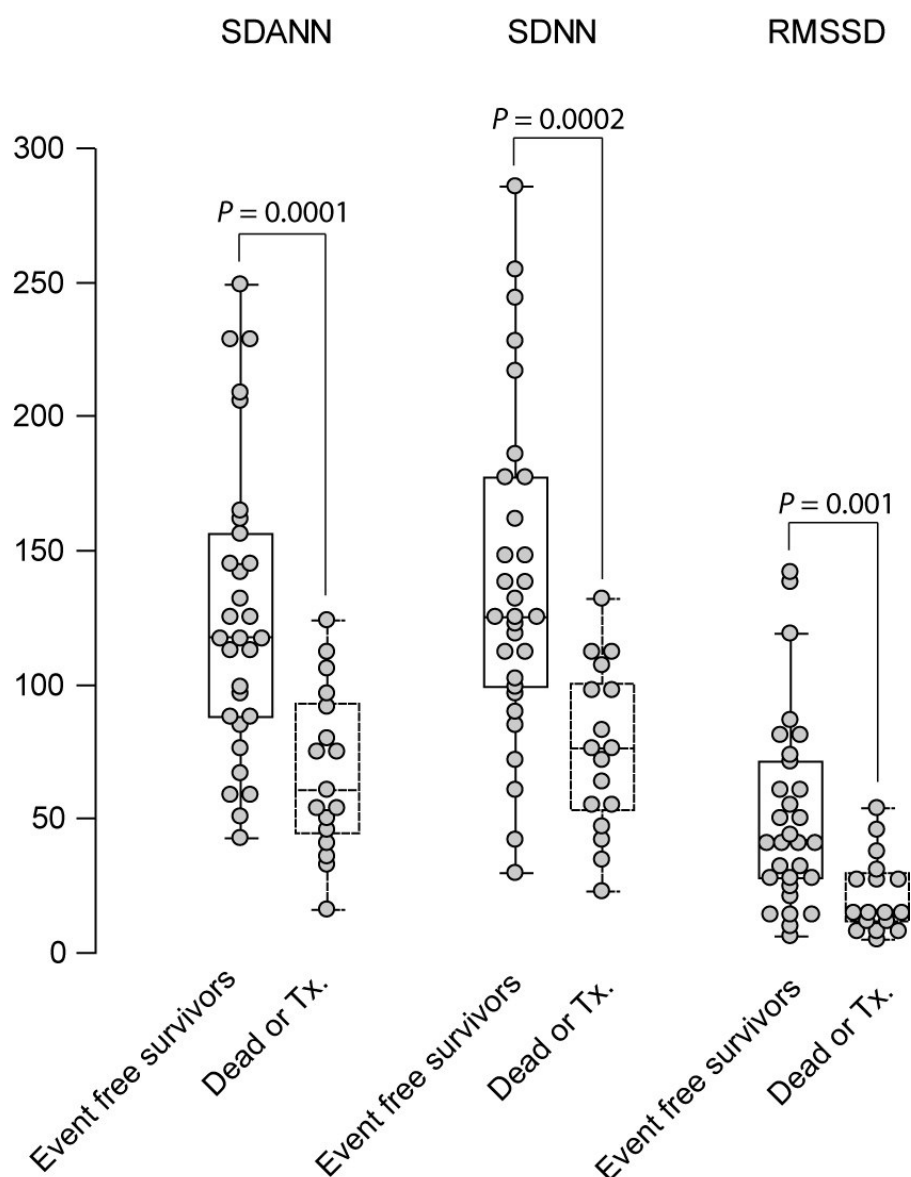


Figure 3.3.1 Box and whiskers plot diagram with parameters of HRV (SDANN, SDNN and RMSSD) of patients who died or were transplanted versus the event-free survivors.

When subdivided into patients with IPAH or APAH, lower values were seen in patients who died or underwent transplantation in both groups (Table 3.3.2).

Parameters of HRV were not significantly different between boys and girls and no correlation with patient age was found (Table 1). All parameters of HRV were significantly lower in children in WHO Functional Class III than Class II (SDANN: 87.5 ± 43.3 vs. 139.4 ± 62.1 ; $p=0.002$; SDNN: 93.1 ± 45.3 vs 157.4 ± 63.8 ; $p=0.0005$; RMSSD: 32.3 ± 28.6 vs 56.9 ± 34.3 ; $p=0.004$). The HRV also decreased as 6MWT distance decreased: $r=0.551$; $p=0.0009$, $r=0.610$; $p=0.0003$, $r=0.529$; $p=0.0015$ for

SDANN, SDNN and SDNN respectively. Correlation coefficients between 6MWT and HRV were better in IPAH than in the whole group of 47 children: $r=0.716$, $p=0.0008$; $r=0.814$, $p=0.0008$ and $r=0.864$, $p=0.0004$ in SDANN, SDNN and SDNN respectively (Figure 3.2.2).

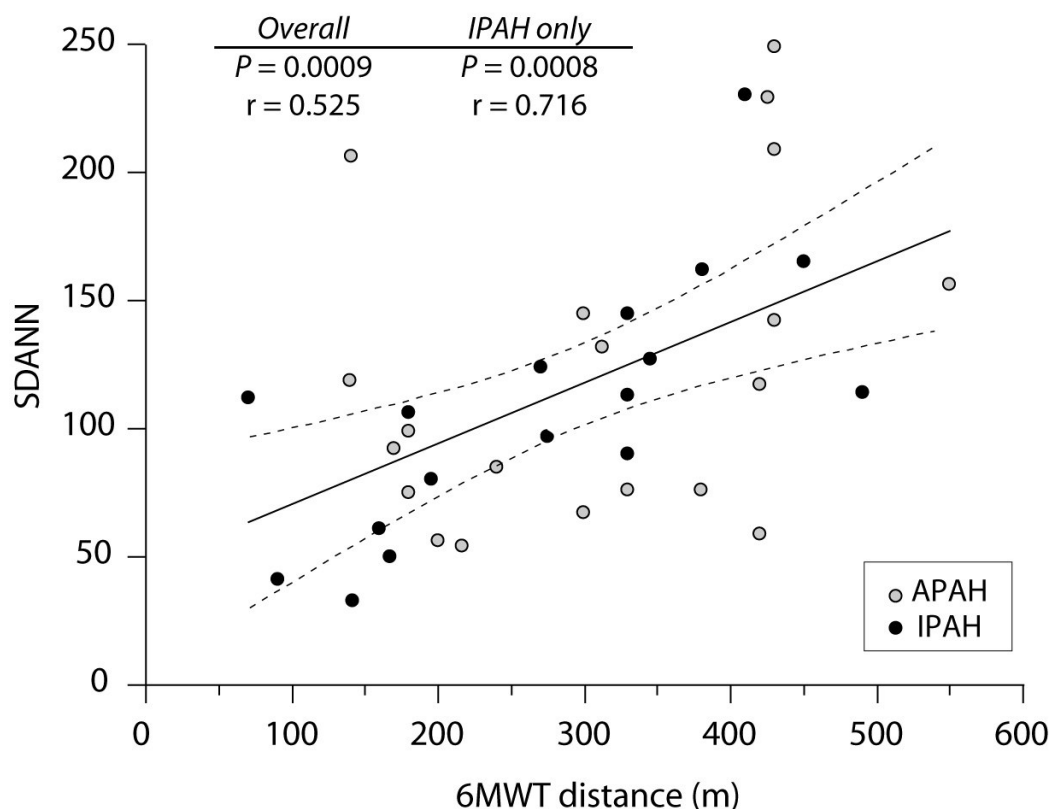


Figure 3.3.2 Correlation of SDANN and 6MWT for patients with APAH and IPAH.

Parameters of HRV did not correlate with either the semi-quantitative assessment of right ventricular function or the haemodynamic findings. Nor did any HRV parameter correlate with the occurrence of either supraventricular (median 2.0, IQR 0-31.5) or ventricular ectopic beats (median 5.0, IQR 0-71.5).

On univariate Cox-proportional-hazards analysis, for the whole group all parameters of HRV predicted death or need for transplantation ($p<0.01$ for all) (Table 3.3.3). This was also true of the IPAH subgroup, ($p<0.005$) but in the APAH subgroup only SDANN was related to the endpoint ($p=0.037$). Considering mortality alone, SDANN and SDNN were predictive for the whole group ($p<0.01$). In addition, WHO functional class, six-minute walk test distance, echocardiographic assessment of right

ventricular hypertrophy and function and PVRI at baseline and on vasodilator testing also predicted the combined end point (Table 3.3.3). The frequency of ventricular and supraventricular ectopic beats was not related to outcome in the whole group, IPAH or APAH, nor the arterial oxygen saturation at rest or history of syncope (Table 3.3.3).

Receiver-operating characteristics (ROC) curve analysis demonstrated that all three parameters of HRV were significantly related to the combined endpoint (SDANN [ms] 0.822 [95% CI 0.682-0.918], SDNN [ms] 0.784 [95% CI 0.639-0.890], RMSSD [ms] 0.666 [95% CI 0.514-0.797]).

As SDANN, SDNN and RMSSD correlated with each other (SDANN vs SDNN $r=0.983$, $p<0.0001$; SDANN vs RMSSD $r=0.656$, $P<0.0001$; SDNN vs RMSSD $r=0.714$, $P<0.0001$) and provide similar information we used SDANN (having the greatest AUC) for all subsequent analyses. A cut-off value (representing highest sensitivity and specificity) of 112 ms was identified as being the best predictor of patients who met the endpoint criteria, both for the whole patient group and for the IPAH subgroup (entire group: Sensitivity 94.1%, specificity 63.3%; IPAH: Sensitivity 90.0%, specificity 72.7%). For the whole group, patients with a SDANN-value <112 ms had a 5.8 fold increased risk of death/transplantation (event free survival at 24 months: 41.7% vs. 90.5% in those >112 ms); $p=0.008$) and a 7.2 fold increased risk of death (survival at 24 months: 57.9% vs. 95.0%; $p=0.03$) (Figure 3.3.3).

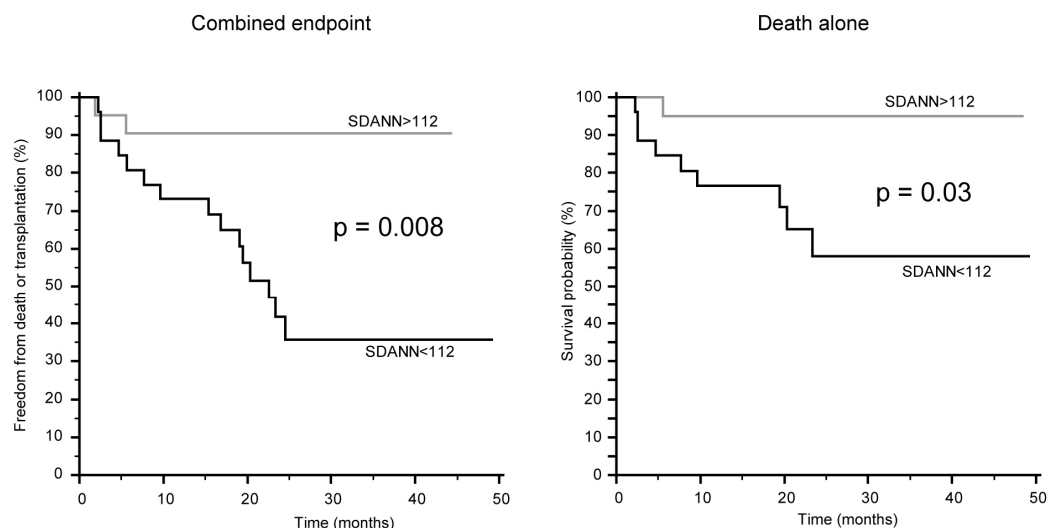


Figure 3.3.3 Kaplan Meier curves for combined endpoint and survival alone.

Extending the analysis, bivariate Cox-proportional-hazards analysis (Table 3.3.4) showed that SDANN was a superior predictor of the end-point than either WHO Functional Class or the PVRI, both at baseline and on vasodilator testing. It was comparable to the echocardiographic assessment of right ventricular function and hypertrophy. Only the 6MWT distance was superior to SDANN for prediction of death or transplantation in the whole group. Findings in the IPAH subgroup were similar but in addition to the 6MWT, the PVRI on vasodilator testing was also superior to SDANN as a prognostic marker (Table 3.3.4). In the APAH population, WHO functional class was a slightly better predictor of the end-point than SDANN but the 6MWT was not. The limited prognostic value of the 6MWT in those with APAH may be due to the lower number of deaths and transplantations in this subgroup. SDANN predicted outcome independently of right ventricular hypertrophy and PVRI at baseline and with vasodilator testing (Table 3.3.4).

Table 3.3.2 Comparison between parameters of heart rate variability between patients who died or underwent transplantation

	SDANN			SDNN			RMSSD		
	Dead or Tx	Alive	p Value	Dead or Tx	Alive	p Value	Dead or Tx	Alive	p Value
All patients (ms)	70.7±30.9	119.2±57.2	0.0001	79.5±30.5	129.9±63.6	0.0002	22.3±11.7	47.7±34.5	0.0001
IPAH (ms)	68.9±34.1	132.7±45.9	0.001	73.4±33.3	149.2±58.6	0.0008	13.9±6.0	49.3±32.5	0.002
APH (ms)	66.6±27.6	124.1±60.9	0.020	79.4±28.9	132.6±64.2	0.024	33.3±14.8	52.6±38.0	0.285

(Tx) and event-free survivors. *P*-values (Mann-Whitney U-test/ Chi-square test) for comparison between event-free survivors and patients who died or underwent transplantation. APH = pulmonary hypertension associated with congenital heart disease; IPAH = idiopathic pulmonary hypertension; RMSSD = square root of the mean square differences of successive RR intervals; SDANN = standard deviation of mean values for all normal-to-normal intervals over 5 minutes; SDNN = standard deviation of all normal-to-normal intervals.

Table 3.3.3 Univariate predictors of death or need for transplantation in all children.

Variable	Hazard Ratio (95% confidence interval)	p value	χ^2 Value
Parameters of HRV			
SDANN (ms)	0.974 (0.958 – 0.990)	0.002	10.01
SDNN (ms)	0.980 (0.968 – 0.992)	0.002	10.05
RMSSD (ms)	0.956 (0.925 – 0.988)	0.008	7.03
Parameters of functional capacity			
Six-minute walk test distance	0.986 (0.979 – 0.994)	0.0006	11.67
WHO class 3 (vs. WHO 2)	10.790 (1.438 – 80.947)	0.02	5.24
Echocardiographic parameters			
RV dilatation †	1.474 (0.786 – 2.763)	0.23	1.45
RV hypertrophy †	3.088 (1.332 – 7.164)	0.009	6.83
RV function †	3.929 (2.025 – 7.621)	0.0001	16.22
Haemodynamic parameters			
Mean PA pressure (mmHg)	1.012 (0.991 – 1.033)	0.28	1.18
PVR baseline (WU)	1.055 (1.008 – 1.105)	0.021	5.30
PVR NO/FiO ₂ 0.6 (WU)	1.061 (1.011 – 1.113)	0.019	5.72
Right atrial pressure (mmHg)	0.711 (0.416 – 1.096)	0.12	2.36
Other parameters			
Arterial oxygen saturation (%)	0.993 (0.928 – 1.063)	0.84	0.41
Clinical history of syncope	0.937 (0.307 – 2.861)	0.94	0.13
Supraventricular ectopic beats	0.465 (0.205 – 1.056)	0.07	3.31
Ventricular ectopic beats	0.736 (0.386 – 1.404)	0.36	0.86

FiO₂ = inspiratory oxygen concentration; HRV = heart rate variability; NO = nitric oxide; PVR = pulmonary vascular resistance; RMSSD = square root of the mean square differences of successive RR intervals; SDANN = standard deviation of mean values for all normal-to-normal intervals over 5 minutes; SDNN = standard deviation of all normal-to-normal intervals; WHO = World Health Organization functional class. WU = Wood units (=80 dyn·s·cm⁻⁵). † indices of right ventricular hypertrophy, size and function were quantified semi-quantitatively as normal=1; mildly=2; moderately=3; severely=4 impaired.

Table 3.3.4 Bivariate predictors of death or need for transplantation in children.

Variable	All patients		IPAH		APAH	
	Hazard Ratio (95% confidence interval)	P value	Hazard Ratio (95% confidence interval)	P value	Hazard Ratio (95% confidence interval)	P value
SDANN (ms)	-	-	-	-	*	-
6 MWT distance	0.986 (0.979 – 0.994)	0.0006	0.981 (0.966 – 0.995)	0.01	*	-
SDANN (ms)	0.973 (0.957 – 0.990)	0.0018	0.975 (0.955 – 0.995)	0.0013	-	-
WHO class 3 (vs. 2)	-	-	-	-	20.21 (1.75 – 234.4)	0.017
SDANN (ms)	0.975 (0.959 – 0.990)	0.0014	0.975 (0.955 – 0.995)	0.0026	0.933 (0.888 – 0.980)	0.006
RV hypertrophy†	2.582 (1.204 – 5.539)	0.015	-	-	14.38 (1.92 – 109.88)	0.01
SDANN (ms)	0.977 (0.962 – 0.993)	0.0045	0.976 (0.958 – 0.993)	0.0077	-	-
RV function†	3.709 (1.871 – 7.349)	0.0002	6.8532 (1.285 – 36.562)	0.025	4.19 (1.62 – 10.82)	0.003
SDANN (ms)	0.969 (0.951 – 0.987)	0.0007	0.978 (0.957 – 0.999)	0.0036	-	-
PVRI baseline (WU)	-	-	-	-	0.951 (0.914 – 0.989)	0.01
SDANN (ms)	0.969 (0.951 – 0.986)	0.0006	-	-	-	-
PVRI NO/FiO ₂ 0.6 (WU)	-	-	1.093 (1.0137 – 1.179)	0.0021	0.951 (0.914 – 0.989)	0.01

6MWT = six-minute walk test; 95% CI = 95% confidence interval; APAH = pulmonary hypertension associated with congenital heart disease; FiO₂ = inspiratory oxygen concentration; IPAH = idiopathic pulmonary hypertension; NO = nitric oxide; PVRI = pulmonary vascular resistance index; SDANN = standard deviation of mean values for all normal-to-normal intervals over 5 minutes; WHO = World Health Organization functional class; WU = Wood units (=80 dyn•s•cm-5). † indices of right ventricular hypertrophy, size and function were quantified semi-quantitatively as normal=1; mildly=2; moderately=3; severely=4 impaired. * For the APAH cases the 6MWT distance was not significantly related to outcome on univariate Cox proportional hazard analysis, and was therefore not included into the bivariate model.

3.3.4 Discussion

The purpose of this study was to assess the prognostic value of HRV in children with PAH. We found that measures of HRV (SDANN, SDNN and RMSSD) were predictive of death or transplantation in a group of children with IPAH and APAH with a mean age of 11.4 years. An SDANN of less than 112 ms was associated with an unfavourable outcome. WHO Functional Class, the 6MWT distance, the echocardiographic assessment of RVH and function and the PVR at baseline and on vasodilator testing were all independent predictors of the combined end-point. Bivariate analysis however, showed that HRV was either a superior or comparable predictor of outcome with the exception of the 6MWT, which was a superior predictor.

This is, to our knowledge, the first time that a low HRV has been associated with an adverse outcome in children with PAH but it is a well recognised feature of left ventricular failure (214-216) and has been noted in a small number of adults with IPAH (207). It indicates autonomic dysfunction and appears to be a feature of ventricular dysfunction. The right ventricle is severely compromised in patients with advanced PAH. It is well recognised, that the integrity of the right ventricular function rather than the degree of pulmonary vascular injury is the main determinant of symptoms and survival (219). Right ventricular mass, volume, and function were recently shown to be independent predictors of mortality and treatment response in adults with IPAH (220). The left ventricle is also compromised as the interventricular septum hypertrophies and shifts to compress the left ventricle.

The cause of autonomic dysfunction is uncertain. Left ventricular failure is generally associated with an increase in sympathetic activity and downregulation of the beta-adrenergic receptors (221). This receptor is also downregulated in pulmonary hypertensive monocrotaline treated rats (222), by 57% in the right ventricle and 22% in the left ventricle. When the sympathetic innervation of the heart was demonstrated in adult patients with IPAH using ^{123}I metaiodobenzylguanidine there was a low heart:mediastinal activity ratio in the left ventricle (223). The ratio correlated with total pulmonary vascular resistance, right ventricular ejection fraction, and survival. Chronically hypoxic rats had a low HRV associated with an increase in sympathetic activity (224). Neuroendocrine activation in human PAH is evidenced by an increase

in ANP, BNP, ET-1 and norepinephrine, changes which relate to NYHA Functional Class and the development of congestive cardiac failure (218). Summarizing the published evidence, ventricular dysfunction appears to be associated with heightened neuroendocrine activity associated with downregulation of the beta- adrenergic receptor. The frequency of supraventricular and ventricular ectopic beats did not relate to HRV. In adults with IPAH vagal activity appeared greater in those with recurrent syncope(207) although this was not seen in our paediatric population.

In the present study HRV correlated with the 6MWT and was lower in those in WHO Functional Class III than II. These assessments are generally thought to reflect well-being and disease progression in PAH to the extent that they have been used as end-points to determine whether patients have benefited from drug administration. The 6MWT correlates with several parameters of cardiopulmonary exercise testing and as in the present study in children, is a predictor of outcome in adults (135,169). Using ROC analysis, the extent to which the 6MWT was a predictor in adults was similar to that seen in the present paediatric series (225). Determination of HRV could be an adjunct to the 6-minute walk test in older children and be a realistic, more reliable prognostic indicator in children too young to perform a walk test reliably. The WHO /NYHA functional classifications applied to children have the disadvantage of relying on the parents' opinion of their child's physical limitations and the interpretation of their opinion by the physician. Even in adult studies these functional classifications are generally used as a secondary endpoint or a part of a combined endpoint in clinical trials (169,226).

Analysing the data from the more homogeneous subgroup of children with IPAH we found that the PVR on vasodilator testing as well as the 6-minute walk test was a better prognostic indicator than HRV. In adults with IPAH the baseline PVR was shown to be a predictor of survival on univariate analysis in several studies before the advent of pulmonary hypertension specific therapies (4,142) but not in epoprostenol treated patients (130,131). All our patients had received pulmonary hypertension specific therapies, 20 of them on epoprostenol. Unlike previous adult studies neither the mean PAP nor the mean right atrial pressure (130) was predictive of outcome, the right atrial pressure being relatively low in all children and the PAP consistently high.

In children with APAH the HRV and all clinical and haemodynamic parameters were predictive of outcome, as in IPAH, but the results of bivariate analysis were different. WHO Functional Class and the echocardiographic assessment of the right ventricle were superior to HRV, while the 6MWT and PVR were not. The inferiority of an assessment of exercise capacity and PVR might be explained by the diversity of the different types of congenital heart and lung disease included in this group. The limitations of using WHO Functional Class particularly in children have been noted above.

The echocardiographic assessment of right ventricular hypertrophy and function predicted outcome in both IPAH and APAH, as in adult studies (169,227). It was not however superior to HRV in predicting outcome, with the single exception of right ventricular hypertrophy in APAH. The predictive value of echocardiography might have been better had we used a more sophisticated interrogative and analytical approach, such as determining tricuspid annular plane systolic excursion rather than using a semi-quantitative assessment. But echocardiographic variables generally reflect the long-term consequences of PAH rather than recent change. At the Venice 2003 WHO Symposium on Pulmonary Hypertension it was concluded that echocardiographic variables were helpful as secondary rather than as primary endpoints in clinical trials, helping validate the clinical benefit or otherwise of a therapeutic intervention (169). HRV may be a more exact measure of current status.

The principle limitations of the present study are that we did not study HRV in consecutive children presenting to the service in order to establish the incidence of abnormalities at presentation and did not carry out longitudinal studies on individual children. A prospective longitudinal study would have established whether a change in HRV correlated with, or could predict, change in clinical status rather than just death or transplantation. Prediction of arrhythmias, syncope, need for atrial septostomy would be particularly helpful. We did not find a correlation between HRV and syncope but adults with IPAH and recurrent syncope appeared to have greater vagal activity (207). Also, the analytical assessment of HRV could be improved. We used time domain parameters but spectral analysis might enhance the prognostic power of HRV.

In conclusion, the present study shows that a low HRV is predictive of death or transplantation in children with PAH. HRV can be studied easily and repeatedly even in small children. Its use as a non-invasive tool to monitor clinical progress deserves further study, as does its own potential as an independent risk factor for cardiac arrhythmia and sudden death.

Whether beta-blockade would be helpful in these patients is unknown. Theoretically beta-blockade could reduce the increase in L-type calcium current and cytosolic calcium transients in response to beta-adrenergic surges and so reduce calcium mediated arrhythmias and risk of sudden death.

3.4 ECHOCARDIOGRAPHY AND TISSUE DOPPLER IMAGING

3.4.1 Background

Despite the use of new pulmonary hypertension-specific medicines, PHT remains a progressive disease without a cure and significant mortality. It is eventually right ventricular performance that determines exercise tolerance and prognosis (219). Due to its complex morphology and myocardial architecture and dependence on loading conditions, however, objective quantification of right ventricular function remains challenging (228,229). Furthermore, right ventricular dilatation, hypertrophy and dysfunction affect the left ventricle and its filling dynamics. These ventricular-ventricular interactions in severe PAH are poorly understood.

This study tested the hypothesis that in children in whom the right ventricle is chronically exposed to high pulmonary pressures, left ventricular function may be impaired due to underfilling, although this has not been seen on conventional (2D / M-mode) echocardiography. This study also aimed to assess ventricular interactions using both conventional echocardiographic techniques as well as colour-coded tissue Doppler imaging (TDI), a method known to sensitively detect myocardial dysfunction (230).

3.4.2 Patients and Methods

Forty-one children (18 male) with a mean age of 7.9 ± 5.6 years with PAH and a structurally normal heart were assessed using echocardiographic techniques including tissue Doppler imaging. In addition 44 age-matched healthy controls (23 male) were investigated (mean age 7.7 ± 4.1 years, p vs. patients = 0.82). Patient demographics are presented in Table 3.4.1.

Table 3.4.1 Patients' demographics

	Controls (n=44)	PHT (n=41)	
	Mean \pm SD	Mean \pm SD	p - Value
Age (years)	7.7 \pm 4.1	7.9 \pm 5.6	ns
Height (cm)	126.1 \pm 23.7	117.6 \pm 34.2	ns
Weight (kg)	31.1 \pm 16.5	26 \pm 17.5	ns
Heart rate (bpm)	97 \pm 28	105 \pm 23	ns

bpm= beats per minute, cm=centimetre, kg=kilogram, ns= not significant, PHT= pulmonary hypertension, SD=standard deviation

Twenty-seven children included were diagnosed with IPAH, whereas 14 children had a structurally normal heart and associated PAH (interstitial and chronic lung disease n=6, connective tissue or autoimmune disease n=5, late closure of a persistent arterial duct n=2, drug [busulphan] associated n=1).

In 31 children the presence of significant pulmonary hypertension was confirmed on cardiac catheterisation. In the remaining 10 children echocardiographic assessment and estimation of right ventricular systolic pressures clearly indicated the presence of pulmonary hypertension. Only patients with a maximum tricuspid regurgitation velocity of 3 m/s or greater were included into this study. All patients were treated with advanced specific PAH therapies.

Conventional echocardiography

All patients underwent a standard echocardiographic assessment as described in Chapter 2. In addition, on two-dimensional (2D) and M-mode echocardiography right and left atrial areas (apical 4-chamber view), diameters of the tricuspid, mitral (apical 4-chamber view), pulmonary (tilted parasternal long axis view) and aortic valve (parasternal long axis view) and branch pulmonary arteries (high parasternal short axis view) were measured; all measurements were expressed in z-scores for body surface area (231).

To quantitatively determine the degree of interventricular septal shift the left ventricular eccentricity index (LVEI) was quantified from a parasternal short axis view, using the ratio of the diameter of the left ventricular cavity perpendicular to the interventricular septum and its diameter on a perpendicular view as described previously (232,233). From M-mode parasternal long axis views, right and left ventricular end-diastolic and systolic dimensions and interventricular septal as well as posterior left ventricular free wall measures were obtained. Ejection fraction was assessed using the Teichholz formula as described in previous reports (234). An M-mode measurement from an apical 4-chamber view of the tricuspid valve annulus was performed to assess the tricuspid annular plane systolic excursion (TAPSE) (235,236).

Using Doppler techniques, in presence of a complete Doppler envelope, the tricuspid valve maximal velocity, was documented to estimate pulmonary artery pressures/ right ventricular pressures according to the modified Bernoulli equation. Pulmonary

acceleration time was determined from the pulmonary artery pulsed Doppler trace signal.

Tissue Doppler imaging

TDI spectral analysis velocity measures were performed at tricuspid valve level of the right ventricular free wall, of the left ventricular free wall at the level of basal mitral annulus and the interventricular septal crest. Three cardiac cycles were recorded and measures at tricuspid valve level were taken at breathhold in all cooperative children. Imaging was optimized to acquire a tissue Doppler frame rate of >160 f/s.

Tissue Doppler imaging data was compared with conventional echocardiographic parameters reflecting pulmonary artery pressures (pulmonary arterial acceleration time, tricuspid regurgitation [TR] velocity, TR severity [0= none, 1=trivial, 2=mild, 3=moderate, 4=severe TR]) and right ventricular functional parameters such as TAPSE, semiquantitative right ventricular function (1=normal, 2=mildly, 3=moderately, 4=severely impaired), and left ventricular eccentricity index as quantitative expression of the degree of interventricular deviation, caused by the interaction of ventricular pressure-loading conditions.

3.4.3 Results

No significant difference in patients' gender, age, weight, height or heart rate was found between normal healthy controls and children with PAH (Table 3.4.1).

Conventional echocardiographic measures of ventricular dimensions and function were significantly different in PAH patients and normal children. As expected, planimetric measurements of bi-atrial areas (right: 12.4 ± 6.2 vs. 9.6 ± 2.5 cm²; $p=0.02$, left: 7.6 ± 3.7 vs. 9.2 ± 2.8 cm²; $p<0.04$), pulmonary artery (PA) dimensions (right PA 1.3 ± 0.5 vs. 1.1 ± 0.29 cm; $p=0.002$; left PA 1.4 ± 0.5 vs. 1.1 ± 0.27 cm; $p<0.01$), tricuspid (2.6 ± 0.75 vs. 2.2 ± 0.19 cm; $p<0.01$) or mitral valve diameter (1.8 ± 0.59 vs. 2.1 ± 0.45 cm; $p=0.03$) and diastolic right intraventricular diameter (2.7 ± 1.3 vs. 1.8 ± 0.8 cm; $p<0.01$) were abnormal in PAH patients. (Figure 3.4.1)

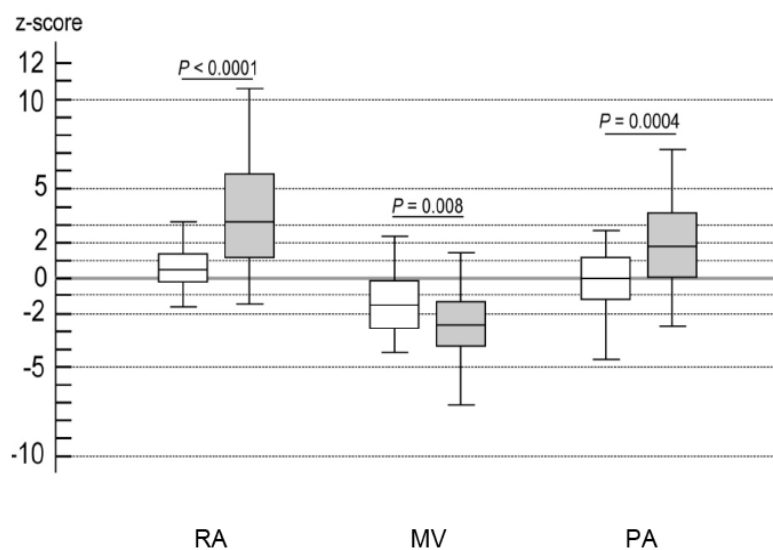


Figure 3.4.1 Comparison of z-scores for right atrial area (RA), mitral valve (MV) and mean pulmonary artery diameter (PA) in controls (white bars) and children with pulmonary hypertension (grey bars).

The pulmonary acceleration time (65 ± 23 vs. 120 ± 32 ms; $p < 0.0001$) was shorter in children with PHT, and the tricuspid annular plane systolic excursion (1.4 ± 0.34 vs. 1.9 ± 0.23 cm; $p < 0.0001$) was significantly decreased. In the left ventricle, the ventricular eccentricity index was also found to differ significantly from normal healthy controls as shown in Table 3.4.2.

Table 3.4.2 Baseline echocardiographic data in control subjects and patients with pulmonary hypertension (PHT).

	Controls n=44 Mean \pm SD	PHT n=41 Mean \pm SD	p - Value
RA z-score	0.8 \pm 1.7	3.8 \pm 3.7	<0.0001
LA z-score	0.7 \pm 2.3	-0.6 \pm 1.6	0.02
TV z-score	0.1 \pm 1.6	2.6 \pm 4	0.002
PV z-score	-0.24 \pm 2.0	1.99 \pm 2.3	<0.0001
RVIDd (cm)	1.83 \pm 0.72	2.73 \pm 1.29	
RVIDs (cm)	1.5 \pm 0.52	3.6 \pm 6.5	
TR velocity (m.s⁻¹)	2 \pm 0.4	4.5 \pm 1	< 0.0001
PA acceleration (msec)	119.7 \pm 31.8	65.3 \pm 22.7	< 0.0001
MV z-score	-1 \pm 1.7	-2.7 \pm 2.3	0.01
LVEId	1 \pm 0	1.6 \pm 0.5	< 0.0001
LVEIs	1.1 \pm 0.1	2.1 \pm 0.8	< 0.0001
TAPSE (cm)	1.9 \pm 0.2	1.4 \pm 0.3	< 0.0001
EF (%)	58.7 \pm 12.6	66.3 \pm 16.6	ns

EF = ejection fraction, IVS = interventricular septum, LA = left atrium, LVEId and -s = left ventricular eccentricity index in diastole and systole, MV = mitral valve, PA = pulmonary artery, PV = pulmonary valve, RA = right atrium, RVIDd and -s = diastolic and systolic right ventricular inflow diameter, TAPSE = tricuspid annular plain systolic excursion, TR = tricuspid regurgitation, TV = tricuspid valve, SD = standard deviation. ns = non significant.

Patients had a significantly larger LVEI compared to normal controls. On conventional Teicholz measurements, the mean left ventricular ejection fraction was higher in the children with pulmonary hypertension compared with normal children (66.3 \pm 16.6 vs. 58.7 \pm 12.6 %). However, this trend did not reach statistical significance (p=0.065).

Tissue Doppler measures of biventricular function

Children with PAH had lower systolic (S) (tricuspid 0.11 \pm 0.03 vs. 0.13 \pm 0.02; p<0.0001; septal 0.06 \pm 0.02 vs. 0.08 \pm 0.01; p <0.0001) and early diastolic (E) velocities at the tricuspid and septal level (tricuspid 0.12 \pm 0.04 vs. 0.19 \pm 0.15; p<0.0001; septal 0.072 \pm 0.03 vs. 0.14 \pm 0.02; p<0.0001). Despite preserved left ventricular function on conventional M-mode echocardiography - calculated using the Teicholz formula - we found that left ventricular systolic performance was markedly impaired on TDI in children with PAH. Tissue Doppler LV velocities (S,

E, *A*) were impaired in children with PAH (*S*: 0.06 ± 0.018 vs. 0.09 ± 0.02 ; $p < 0.0001$; *E*: 0.11 ± 0.04 vs. 0.18 ± 0.05 ; $p < 0.0001$; *A*: 0.06 ± 0.02 vs. 0.07 ± 0.017 ; $p = 0.0015$), as shown in Table 3.4.3 and Figure 3.4.2 and illustrated on examples in Figure 3.4.3.

Table 3.4.3 Tissue Doppler Imaging parameters. In control subjects and patients with pulmonary hypertension (PHT): Comparisons were performed between normal controls and patients with PHT.

	Controls (n=44) Mean \pm SD	PHT (n=41) Mean \pm SD	P– Value
<i>Lateral tricuspid valve level</i>			
S TV (m/s)	0.13 ± 0.02	0.11 ± 0.03	< 0.0001
E TV (m/s)	0.19 ± 0.15	0.12 ± 0.04	< 0.0001
A TV (m/s)	0.11 ± 0.02	0.11 ± 0.03	Ns
<i>Interventricular septal level</i>			
S IVS (m/s)	0.08 ± 0.01	0.06 ± 0.02	< 0.0001
E IVS (m/s)	0.14 ± 0.02	0.07 ± 0.03	< 0.0001
A IVS (m/s)	0.07 ± 0.01	0.06 ± 0.01	Ns
<i>Lateral mitral valve level</i>			
S MV (m/s)	0.09 ± 0.02	0.06 ± 0.02	< 0.0001
E MV (m/s)	0.18 ± 0.05	0.11 ± 0.04	< 0.0001
A MV (m/s)	0.07 ± 0.02	0.06 ± 0.02	0.0015

A = late diastolic velocity, *E* = early diastolic velocity, *IVS* = interventricular septum, *MV* = mitral valve, *ns* = non significant, *PA* = pulmonary artery, *S* = systolic velocity, *SD* = standard deviation, *TV* = tricuspid valve

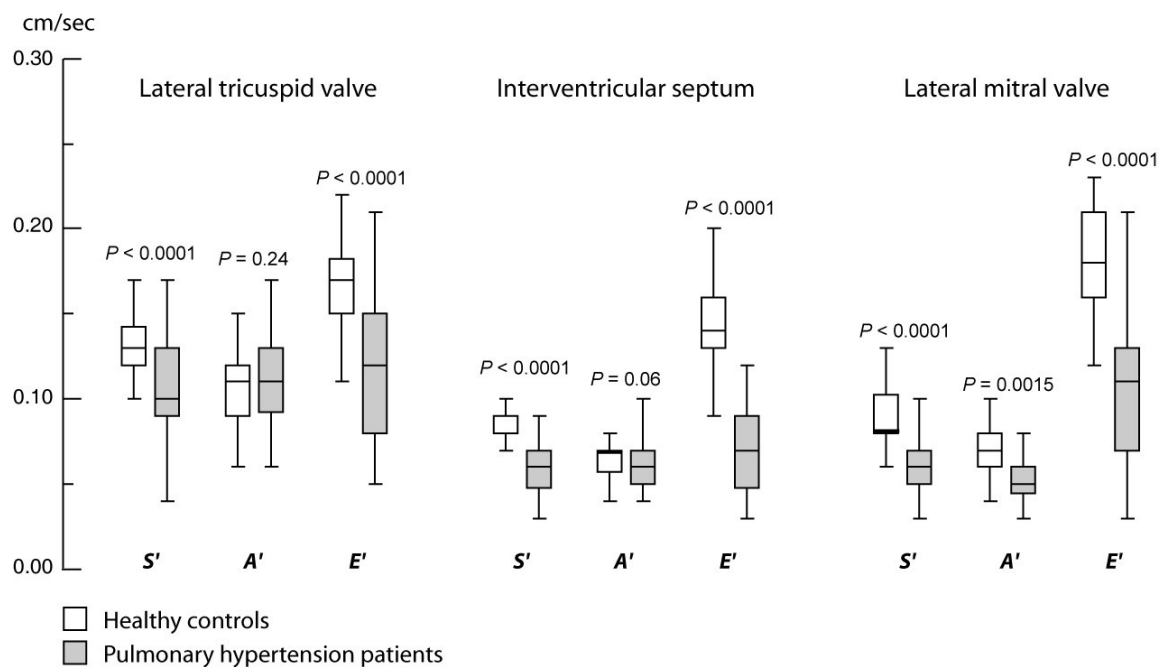


Figure 3.4.2 Comparison of spectral tissue Doppler velocities of controls and children with pulmonary hypertension at the lateral tricuspid, septal and lateral mitral valve level. S' indicates systolic velocity, E' early and A' late diastolic velocity.

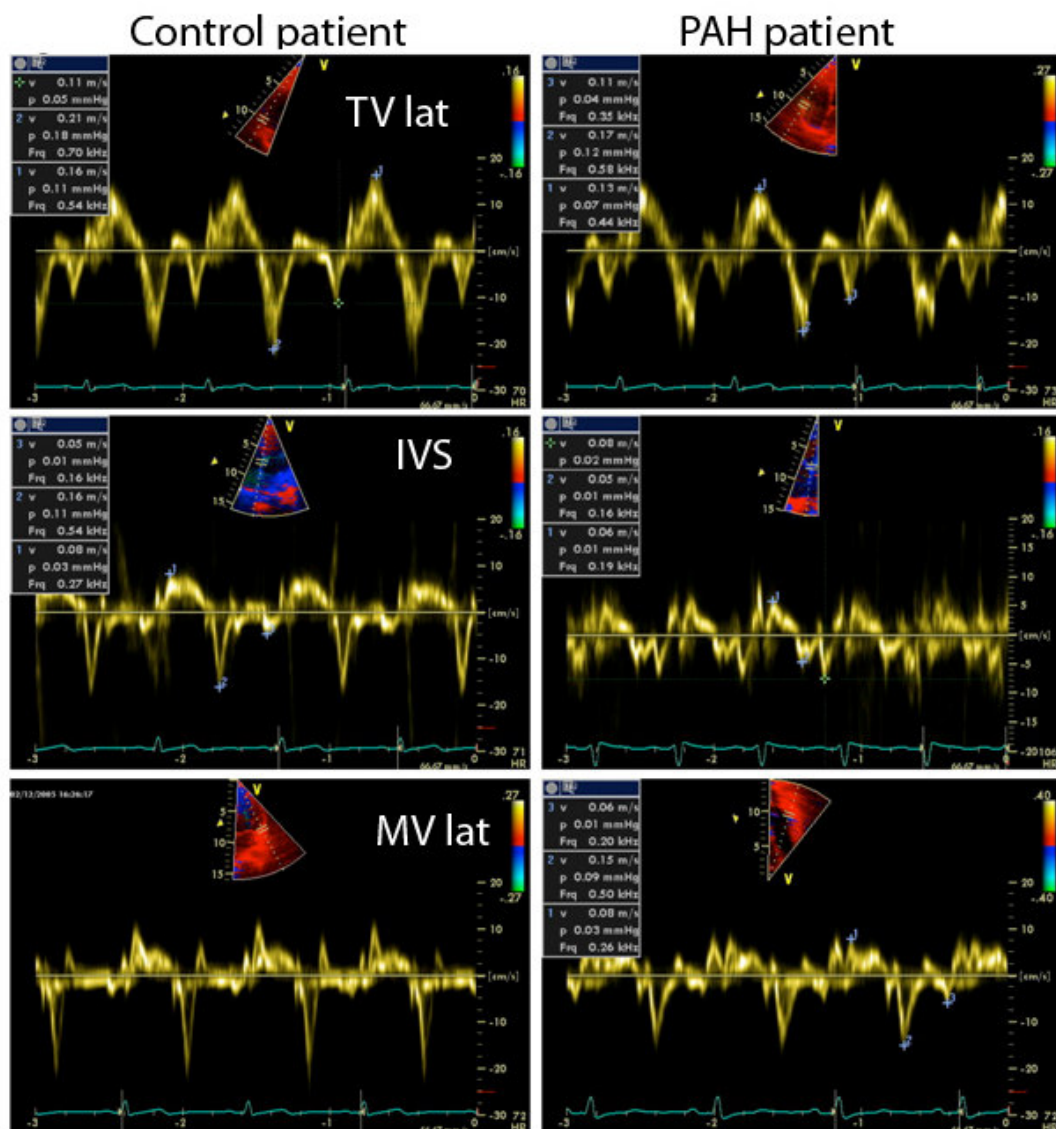


Figure 3.4.3 Representative spectral tissue Doppler images at lateral tricuspid (TV lat), septal (IVS) and lateral mitral valvar (MV lat) level in a control patient and a child with PAH.

Although left ventricular function is preserved on conventional M-mode echocardiography using the Teichholz formula, children with pulmonary hypertension showed marked impairment of left ventricular systolic performance on TDI. Systolic and early diastolic (*S*, *E*) Tissue Doppler velocities were reduced in children with PHT at all measured levels compared to the normal controls. The *a* wave was reduced at tricuspid and septal level, whereas the *a* velocity at mitral valve level was not significantly different to normal controls.

Correlations between conventional echocardiographic and tissue Doppler Imaging

Higher values for systolic tissue Doppler (*S*) velocities at tricuspid ($r=0.42$, $p=0.016$) and septal ($r=0.52$, $p=0.002$) level were associated with a longer pulmonary acceleration time (a marker of less severe PAH). Systolic tissue Doppler (*S*) velocity at tricuspid ($r=0.56$, $p=0.0002$) and septal level ($r=0.36$, $P=0.02$) were also associated with higher TAPSE values. At mitral valve level no relationship was found between the systolic function (*S* wave velocity) and Pac. In contrast, a shorter Pac was related to a lower early diastolic (*E*) ($r=0.44$, $p=0.01$) and a higher atrial (*A*) ($r=-0.38$, $p=0.03$) tissue Doppler velocity, suggesting a higher degree of diastolic dysfunction in patients with more severe PAH.

Tissue Doppler velocities of systolic and diastolic function measured at interventricular septal were significantly associated with semi-quantitative right ventricular function [(*S*): ($r=-0.34$, $p=0.03$); (*E*): ($r=-0.63$, $p<0.0001$); (*A*): ($r=-0.39$, $p=0.01$)], whereas no such association could be established at the tricuspid valve or mitral level (Table 3.4.4).

Table 3.4.4 Correlations between systolic velocities (S) at lateral tricuspid (TV lat.), interventricular (IVS) and lateral mitral (MV lat.) level and echocardiographic parameters of degree of pulmonary hypertension, right ventricular dysfunction on conventional echocardiography and measures of left ventricular eccentricity.

	S TV lat. (cm/sec)	S IVS (cm/sec)	S MV lat. (cm/sec)
PAC time (ms)	r=0.42; P=0.016	r=0.52; P=0.002	r=-0.10; P=ns
TAPSE (cm)	r=0.56; P=0.0002	r=0.36; P=0.02	r=0.25; P=ns
TR velocity (m/s)	r=0.24; P= ns	r=-0.07; P= ns	r=-0.09; P= ns
TR severity (1-4) *	r=-0.02; P= ns	r=-0.22; P= ns	r=-0.23; P= ns
RVF (1 – 4) *	r=-0.17; P= ns	r=-0.34; P=0.03	r=-0.23; P= ns
dp/dt (mmHg/s)	r=-0.18; P= ns	r=-0.23; P= ns	r=-0.19; P= ns
LVEI syst.	r=-0.15; P= ns	r=-0.22; P= ns	r=-0.28; P= ns
LVEI diast.	r=-0.01; P= ns	r=-0.21; P= ns	r=0.003; P= ns

PAC = pulmonary acceleration time, TAPSE = tricuspid annular plain systolic excursion, TR = tricuspid regurgitation, RVF = right ventricular function, dp/dt = right ventricular pressure increase, LVEI syst. and – diast. = left ventricular eccentricity index in systole and diastole. *r* = Pearson correlation coefficient; * = Spearman rank correlation.

3.4.4 Discussion

Although left ventricular function appeared to be preserved on conventional 2D- and M-mode echocardiography, TDI analysis of longitudinal left ventricular wall motion suggested an impaired left ventricular function in children with PAH. This finding may reflect the detrimental impact of right ventricular dysfunction, dilatation and hypertrophy, on left ventricular function (i.e. ventricular-ventricular interaction) and cardiac output in patients with pulmonary hypertension. Septal flattening and leftward deviation (characteristic “D-shaped” left ventricle) do occur as a consequence of increased right ventricular pressures and an underfilled left ventricle. In this context, it is noteworthy, that despite similar degree of pulmonary hypertension, patients with Eisenmenger syndrome generally present with a better preserved right ventricular function and less severe septal deviation than patients with pulmonary hypertension and a structurally normal heart. These observations might help explain the better outcome of the Eisenmenger patients compared to patients with idiopathic pulmonary hypertension (237) and emphasize the importance of understanding the close interaction between biventricular geometry and function.

This study compares data of children with PAH and normal healthy controls, focusing on measures of longitudinal contractility using tricuspid annular displacement (TAPSE) on M-mode echocardiography and TDI measures at annular and septal basal myocardial segments. These parameters of longitudinal function are particularly useful in assessing right ventricular systolic function.

The left ventricle ejects a significant proportion of its stroke volume as a result of torsional shape changes. In contrast, right ventricular stroke volume grossly depends on longitudinal shortening (238). Anatomical studies demonstrated that the deeper right ventricular muscle fibres are predominantly arranged in a longitudinal fashion from the tricuspid valve annulus to the apex (239). Therefore, longitudinal tricuspid annular motion and velocity closely reflect the right ventricular free wall function and may precede circumferential right ventricular dysfunction. Furthermore, parameters of longitudinal right ventricular function can be assessed quantitatively. As expected, in children with PHT geometrical biplanar measures of the right heart were increased compared to normal healthy controls. In addition, tissue Doppler measures for assessment of both systolic and diastolic ventricular function were impaired in the patient group, which is in keeping with the findings of a recent study on adults with PHT (240). Paediatric data are sparse and only one recent study, investigating 15 infants with PHT secondary to congenital diaphragmatic hernia, made observations similar to those in our present study (241).

The reduction in *E*-wave velocity across the mitral valve on spectral Doppler analysis in patients with PHT is suggestive of filling impairment. Left ventricular filling is compromised by the septal deviation of the hypertrophied and pressure overloaded right ventricle. Thus, the left ventricular myocardium is not working at the optimal level of the Frank-Starling curve (242-244). The TAPSE and pulmonary artery flow acceleration time (PAC) did not correlate with left ventricular S wave velocity but the PAC-time correlated with transmitral E and A wave velocities at mitral valve level. These findings suggest that pulmonary hypertension had a greater impact on the diastolic rather than the systolic cycle of the left ventricle. In patients with severe PHT and who had a shorter PAC, diastolic E and A Doppler filling

patterns were markedly lower, suggestive of a significantly reduced left ventricular preload.

Echocardiographic assessment of right ventricular function and volumes based on conventional volumetric variables such as ejection fraction remains a challenge due to the complicated geometry of the right ventricle (75,245). Cardiac magnetic resonance imaging may be a more accurate method to assess right ventricular function, however due to the relatively long acquisition time it requires general anaesthesia or heavy sedation in young children, which are a considerable risk for patients with pulmonary hypertension. Echocardiographic techniques allow direct evaluation of cardiac function by assessing myocardial deformation. Longitudinal function can be assessed by measuring annular plane systolic excursion and tissue velocities. One possible limitation of using annular plane systolic excursion and tissue velocity is that these parameters could be affected by tethering effects. This could lead to an overestimation of myocardial function. This could represent a limitation of the current study. However, while this is a potential concern in patients with ischaemic cardiomyopathy and regional wall motion abnormalities, tethering effects are less likely in patients with PAH, where right ventricular function is uniformly affected. In addition, a recent study has demonstrated a good correlation between tricuspid annular peak systolic velocity and RV ejection fraction (246).

Tissue Doppler echocardiography has emerged as a quantitative modality to assess ventricular function. Unlike conventional visual assessment of right ventricular function, requiring extensive training, experience and subjective interpretation, tissue Doppler echocardiography uses easily identifiable cardiac structures and velocities can be quantified objectively. Tissue Doppler imaging techniques may therefore allow to demonstrate sub-visual changes of the right and left ventricular function. However, prognostic implication of TDI is still unclear and more studies are required in children and adults. Due to the prospective design of the study and the short follow-up period the number of children, who died ($n=2$) or underwent transplantation ($n=3$) was low. Therefore, this study is not suited to provide information on the prognostic value of the studies echocardiographic variables.

There is ongoing controversy on the potential benefits of speckle-tracking based assessment of myocardial deformation compared to tissue Doppler echocardiography. While the latter is angle dependent, speckle-tracking is theoretically angle independent. However, as axial resolution is superior to lateral resolution, speckle-tracking perpendicular to the ultrasound beam may be suboptimal. Furthermore, in the current study we focused on longitudinal function, which can be equally well assessed by both modalities.

In conclusion, despite not being evident on conventional 2D echocardiography, LV systolic performance is impaired in children with pulmonary hypertension. Quantitative TDI assessment of ventricular function and biventricular interactions in this setting might provide further insights into the mechanisms leading to end stage PHT, and may encourage clinicians to optimize anti-failure treatment earlier in the course of the disease process. Further studies with a longer follow up period are required to assess the potential value of tissue Doppler echocardiography in assessing prognosis and guiding therapy in children with pulmonary hypertension.

3.5 B-TYPE NATRIURETIC PEPTIDE

3.5.1 Background

Pulmonary hypertension leads to right heart failure and the prognosis was extremely poor until new pulmonary hypertension-specific medicines were introduced over the last two decades. These drugs have improved quality of life and survival. Monitoring progress, assessing the need for escalation of therapy and determining the time to transplantation, however, remains a challenge, especially in children where invasive assessment is associated with considerable risks (82). In adult patients B-type natriuretic peptide (BNP) has been shown to be a useful diagnostic tool in patients with cardiac or pulmonary disease (247,248). BNP has been shown to predict both left and right ventricular dysfunction (247,249-251). Previous studies have also shown that BNP correlates closely with New York Heart Association functional class (247,252,253) and haemodynamic parameters (254,255). In adult patients with IPAH, previous publications have demonstrated that BNP correlates with the mean pulmonary artery pressure (PAP), total pulmonary vascular resistance index (PVRI) (252,256), right atrial pressure and right ventricular end-diastolic pressure (257). Nagaya et al. have also shown BNP values at baseline to be predictive of survival in adults with IPAH (137). There are no comparable data in children with PHT but elevated levels have been described in children with congenital heart disease (258-262), Kawasaki disease (263), cardiomyopathy (264) and heart failure (265,266).

BNP is synthesized as pro-BNP - a prehormone - predominantly by ventricular cardiomyocytes. It is cleaved into the biologically active BNP, which represents the C-terminal fragment and the biologically inactive N-terminal fragment (NT-proBNP). Both NT-proBNP and active BNP have been used as biomarkers for diagnosis and risk-stratification in patients with cardiovascular conditions, and their diagnostic significance is comparable (267,268). BNP-production is augmented by an increase in pressure and/or volume overload (250), resulting in increased myocardial wall stretch. Synthesis of BNP can also be increased by tachycardia, glucocorticoids, thyroid hormones and vasoactive peptides (250,269,270).

This study tested the hypothesis that plasma BNP levels are elevated in children with PHT, relate to WHO functional class and predict death or the need for transplantation.

3.5.2 Patients and Methods

Fifty patients (32 male) from the UK Pulmonary Hypertension Service for Children seen between 01/2004 and 09/2006 were studied. Baseline characteristics are shown in Table 3.5.1. Patients had a mean age of 8.4 ± 5.1 years (range 0.3-18.4). Twenty-seven children (14 male) had IPAH. Twenty-three children had pulmonary hypertension associated with other diseases (APH) including congenital cardiac disease (n=17) four patients with lung disease, and one patient each with vasculitis and HIV. All children were treated with advanced specific therapies for pulmonary hypertension (epoprostenol, bosentan and sildenafil) or a combination of these drugs. Twenty-seven patients were on monotherapy (epoprostenol n=4, bosentan n=15, sildenafil n=8). Eighteen children were on dual therapy (epoprostenol and bosentan n=12, bosentan and sildenafil n=6) and 5 children were on triple therapy (epoprostenol/inhaled prostanoid, bosentan and sildenafil).

The children were followed-up for a mean of 14.0 ± 7.5 months (range: 0.2-33.1) after measuring the BNP level. All children underwent a physical examination, assessment of WHO Functional Class and transthoracic echocardiography at the time of BNP sampling. A concomitant six-minute walk test was available in 14 children who were well and old enough to do the test reliably. The walk test was performed in a standardized matter as described in the General Methods section.

Fourteen patients underwent a cardiac catheterization study with assessment of the pulmonary vascular resistance at the time of BNP sampling. The mean PAP was 62.4 ± 29.7 mmHg and the mean PVRI was 19.5 ± 13.2 units m². On vasodilator testing with inhaled nitric oxide during cardiac catheterization the mean PVRI was 16.2 ± 12.2 units m² (Table 3.5.1).

Table 3.5.1 Selected characteristics. Patients are classified into those who died or underwent transplantation and those who survived.

Characteristic	All patients (n=50)	Combined endpoint (n=14)	Event free survival (n=36)
Age (years)	8.4 ± 5.1	7.6 ± 4.0	8.7 ± 5.53
Gender (male / female)	32/ 18	11 / 3	21/ 15
Weight (kg)	30.5 ± 23.1	33.6 ± 33.9	29.2 ± 17.5
Age at diagnosis (years)	5.2 ± 4.5	4.9 ± 4.0	5.3 ± 4.7
IPAH / Associated PAH	27 / 23	7 / 7	20 / 16
WHO II / III / IV	20 / 26 / 4	0 / 11 / 3	20 / 15 / 1 **
BNP (pg/ml)	143.5 ± 236.2	229.0 ± 235.4	110.3 ± 236.2
Six minute walk distance (m)	286.9 ± 91.1	233.8 ± 70.0	310.3 ± 90.6 *
Oxygen saturation at rest (%)	91.4 ± 9.0	88.4 ± 11.2	92.5 ± 7.8
<u>Echocardiography</u>			
RV dilatation I / II / III / IV	1 / 11 / 21 / 17	0 / 1 / 5 / 8	1 / 10 / 16 / 9
RV dysfunction I / II / III / IV	5 / 28 / 11 / 6	0 / 5 / 5 / 4	5 / 23 / 6 / 2 *
RV hypertrophy I / II / III / IV	1 / 20 / 26 / 3	0 / 4 / 9 / 1	1 / 16 / 17 / 2
TR severity I / II / III / IV	17 / 15 / 16 / 2	3 / 4 / 7 / 0	14 / 11 / 9 / 2
TR velocity (m/sec)	4.5 ± 0.9	4.5 ± 0.6	4.5 ± 1.0
<u>Invasive haemodynamic data</u>			
	(n=13)	(n=1)	
Mean PAP baseline (mmHg)	62.4 ± 29.7	46.0	63.7 ± 30.6
Right atrial pressure (mmHg)	9.1 ± 7.0	5	9.5 ± 7.3
PVRI baseline	19.5 ± 13.2	12.5	20.0 ± 13.6
PVRI nitric oxide	16.2 ± 12.2	6	17.1 ± 12.3

Mean ± standard deviation are given. t-test, Mann-Whitney U-test or Chi-square test were made for comparison between patients who died/underwent transplantation or were listed for transplantation and the remaining population. *BNP = B-type natriuretic peptide. IPAH = idiopathic pulmonary arterial hypertension. PAP = pulmonary arterial pressure. PVRI = pulmonary vascular resistance index. RV = right ventricular. TR = tricuspid regurgitation. WHO = World Health Organization Functional Class (II-IV). Combined endpoint= death, listing for transplantation/transplantation*

** p<0.001 * p<0.05

Right ventricular function, dilatation and hypertrophy were assessed at the time of BNP sampling by echocardiography and described semi-quantitatively as being normal, mildly, moderately or severely impaired (113,218). Tricuspid regurgitation was described as trivial, mild, moderate and severe (I-IV). Right ventricular systolic

pressure was estimated from the tricuspid regurgitation signal using the modified Bernoulli equation.

BNP measurements were performed on EDTA blood using the Triage test (Biosite Diagnostics, San Diego, CA) (166) according to the manufacturer's instructions. This test uses a fluorescent-labelled antibody to BNP and has a range from 5 to 5000 pg/ml (254). BNP values were correlated with gender, WHO Functional Class, echocardiographic and haemodynamic findings, the six-minute walk test and outcome. For normal adults, 99.5% have a value less than 100 pg/ml. In children, Koch and colleagues (271) reported a mean BNP value (Triage test) in normal children older than 2 weeks of age (n=152) of 7.0 ± 5.9 (SD) pg/ml in boys and 10.1 ± 8.6 (SD) pg/ml in girls. No BNP value was greater than 32.7 pg/ml. Law and colleagues (261) reported a mean value of 22 ± 5 (SEM) pg/ml, range 5 to 63 pg/ml in 16 children age 0.3 to 18.5 years, mean 10.1 years. Soldin (272) evaluated 808 children, using the same Triage test, and found a maximum 97.5th centile value of 173 pg/ml in children over 1 year of age.

Statistical analysis

As there was evidence of significant departure from the normal distribution log transformed BNP values were used for all correlation analyses. For statistical analysis, WHO class was considered as a continuous variable and the Spearman rank correlation was used to estimate correlation between WHO class and log transformed BNP values. To determine the optimal cut-off value of BNP the areas under the curve (AUC) for sensitivity and specificity were calculated using receiver-operating characteristics (ROC) analysis to assess the prognostic accuracy of BNP values. Kaplan-Meier survival plots were constructed to illustrate the association between BNP and survival using death or transplantation as the end-point.

3.5.3 Results

The mean BNP of all the children with PH was significantly increased, 143.5 ± 236.2 pg/ml (range <5-1250) ($p < 0.01$) compared with a normal mean value described by Koch (271). There was no significant difference between those patients with IPAH and those with APH (mean 119.9 ± 168.9 pg/ml, range <5-644.0, versus 171.1 ± 298.4 pg/ml, range <5-1250, respectively; $p = 0.60$). However, considering the children individually and taking 32.7 pg/ml (271) as the upper limit of normal, 56% of all

children with PH had an abnormally high value and the proportion of those with an elevated level was greater in IPAH than APH, 70% v. 39%. There was no difference between the results for boys and girls ($p=0.45$).

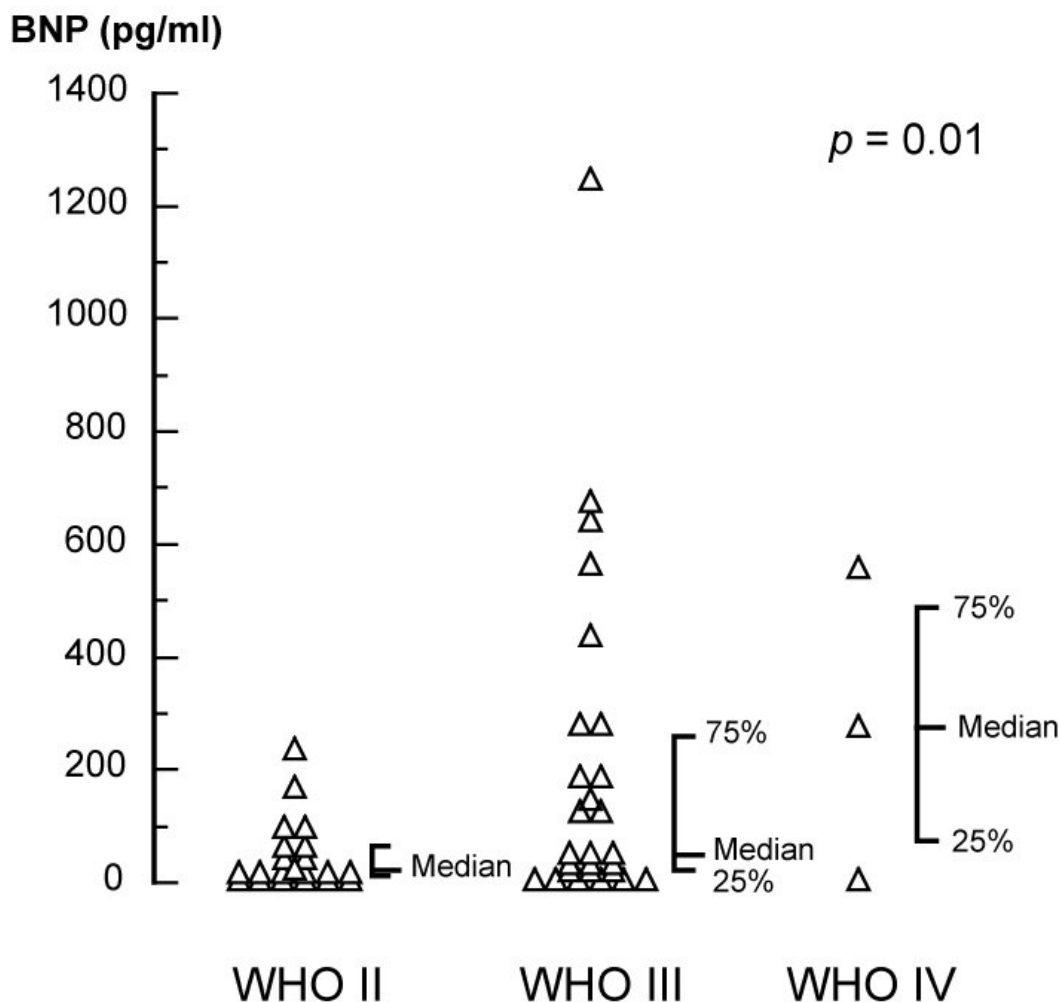


Figure 3.5.1 Distribution of B-type natriuretic peptide levels related to World Health Organization Functional Class. Bars and error markers represent median, lower and upper quartile. Kendall's Tau rank correlation was used for comparison.

There was a positive association between BNP value and WHO Functional Class as illustrated in Figure 3.5.1. Patients in Functional Class 3 and 4 had significantly higher BNP values compared with those in a lower class (50.8 ± 61.3 ; 196.9 ± 291.2 ; 280.0 ± 276.5 in WHO class 2, 3, and 4, respectively; $p=0.01$). The patients on intravenous epoprostenol alone or in combination ($n=21$) had a significantly higher BNP than those on oral therapies (256.1 ± 324.7 vs. 61.9 ± 76.8 pg/ml, $p=0.01$).

BNP levels correlated with the echocardiographic findings, including right ventricular dilatation ($p<0.01$), right ventricular hypertrophy ($p<0.01$) and right ventricular function ($p<0.01$). When the IPAH and APH groups were studied independently both groups showed a similar significant relationship. A small pericardial effusion was seen in two cases. There was no correlation between BNP value and patient age ($p=0.53$), oxygen saturation at rest ($p=0.53$) or six-minute walk test distance ($p=0.26$) for the whole group, nor when the IPAH and APH were analyzed separately.

In children with IPAH there was a significant positive correlation between the mean PAP in an inspired oxygen concentration of 30%, as well as PAP and PVRI when on inhaled nitric oxide ($n=9$; $r=0.72$, $p=0.03$; $r=0.68$, $p=0.044$ and $r=0.78$, $p=0.02$, respectively) and BNP. In contrast, there was no correlation between BNP and haemodynamic findings in patients with APH ($n=5$).

During the mean follow up period of 14.0 ± 7.5 months (range 0.2-33.1 months) seven patients died, six with APH (interstitial lung disease $n=1$; congenital heart disease $n=5$) and one patient with IPAH. Five children underwent a lung- or heart-lung transplantation (4 IPAH, 1 lung disease). Two patients (both IPAH) were listed for transplantation and are still waiting for a donor organ offer (Table 3.5.1).

The mean BNP in the survivors was not significantly different from that in children who were later transplanted or died ($P=0.15$) (Table 3.5.1, Figure 3.5.2), nor was there a significant difference in age, gender, weight, systemic arterial oxygen saturation, echocardiographic and haemodynamic parameters. The six-minute walk test, ($P=0.01$) and the WHO Class ($P=0.0004$) were however, significantly better in children who survived (Table 3.5.1). The survivors walked 70 meters (median) further than those who subsequently underwent transplantation or died.

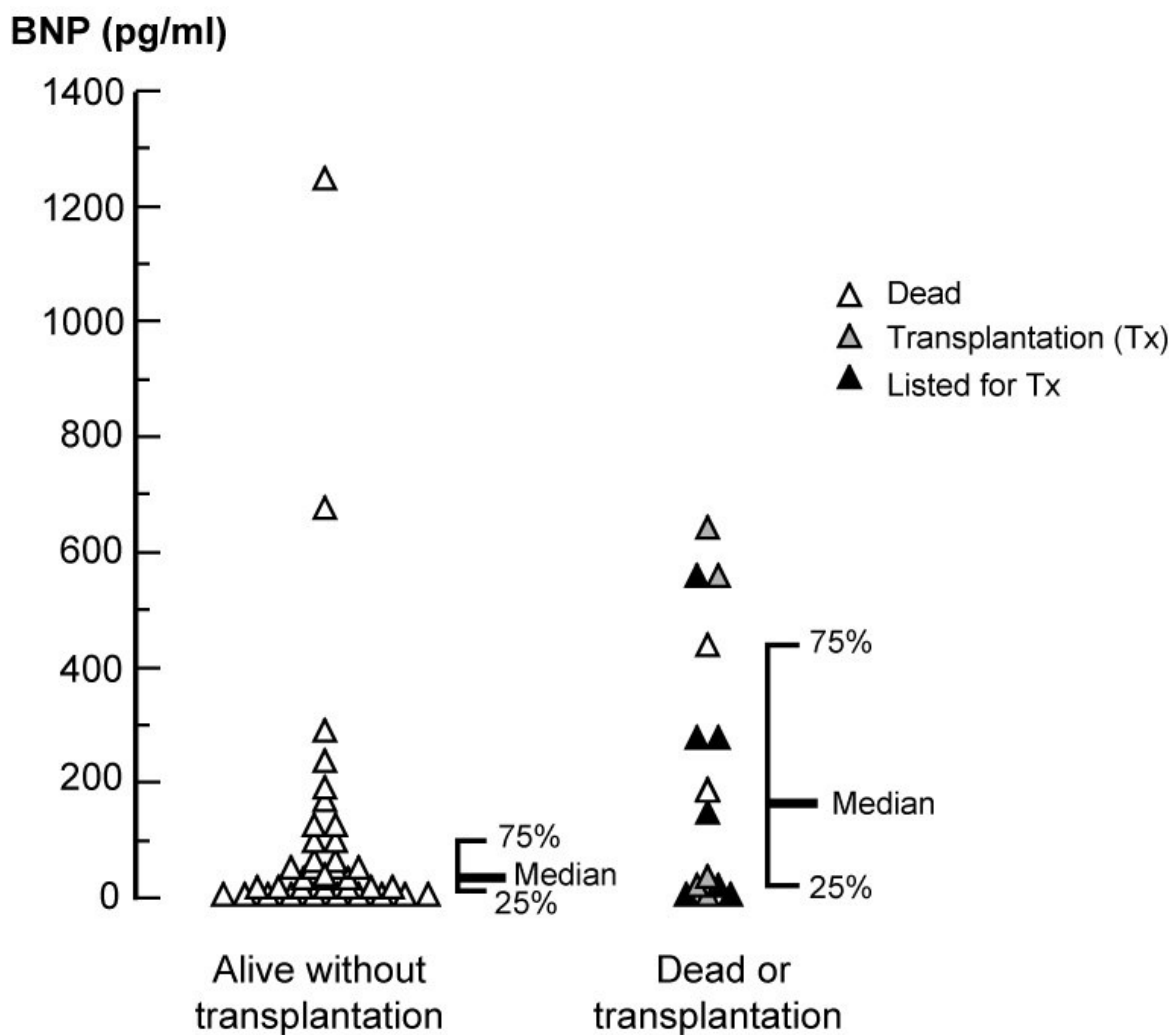


Figure 3.5.2 Distribution of B-type natriuretic peptide levels by outcome. Patients were classified into survivors and those who with a combined endpoint (death, transplantation or admission to the active transplant list). Bars and error markers represent median, lower and upper quartile.

A BNP value above 130 pg/ml was identified as the cut-off value best predicting a combined end-point of death or transplantation/listing for transplantation but there was a limited sensitivity of 57.1% and a specificity of 83.3% in the whole cohort. Analyzing patients with IPAH and APH separately showed a higher sensitivity of 71.4% and a specificity of 90.0% ($p < 0.028$) for the children with IPAH; Log-rank analysis of survival time showed a trend, which was not significant ($p = 0.07$).

In contrast no association between BNP value and outcome was found in patients with APH (sensitivity was 42.9%, specificity 81.2%, $p = 0.85$), the Log-rank test for survival time was not significant ($p = 0.37$).

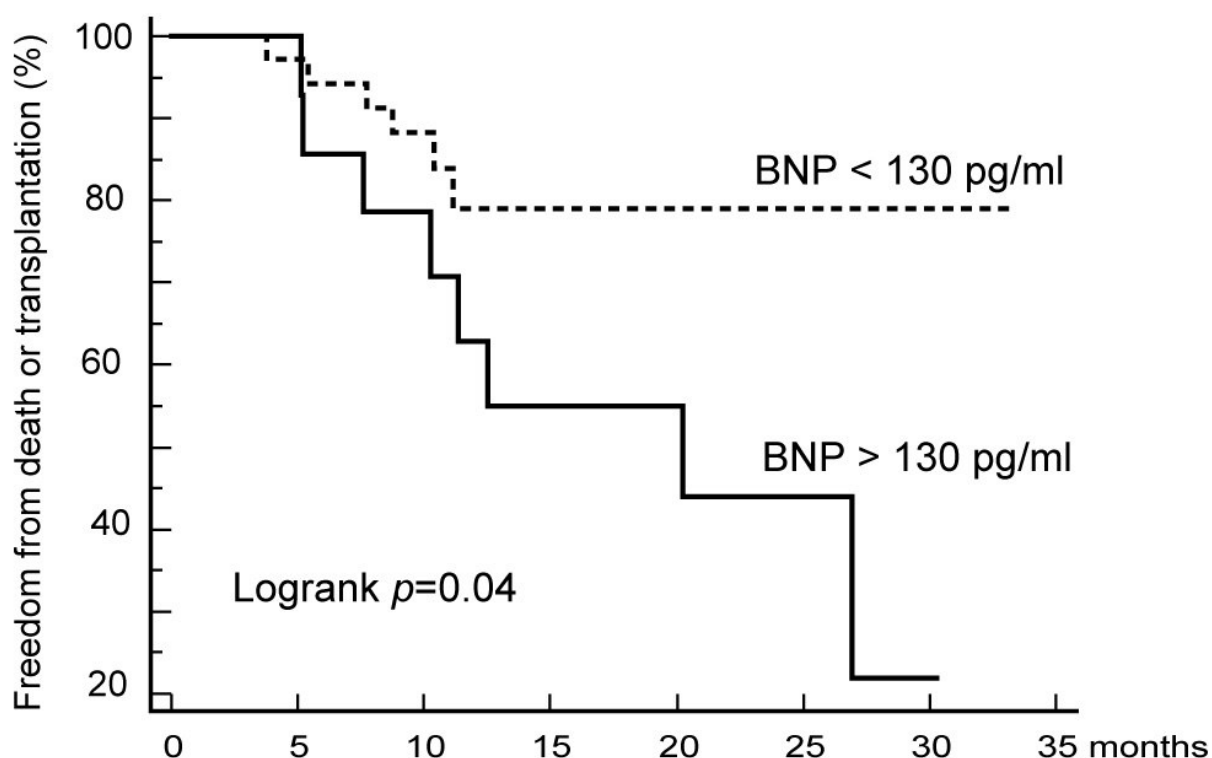


Figure 3.5.3 Kaplan–Meier plots for patients classified according to endpoint of admission to the active transplantation list, transplantation or death and a B- type natriuretic peptide value below or above 130 pg/ml.

Kaplan-Meier survival curves were constructed to illustrate the better outcome of patients with BNP values less than 130 pg/ml at baseline (Figure 3.5.3). Figure 3.5.2 illustrates the substantial overlap in BNP values between patients who survived and those who were listed/underwent transplantation or died. The mean BNP level was not significantly different in children who died or were transplanted ($p=0.15$) (Table 3.5.1). Four patients who died (IPAH $n=1$; interstitial lung disease $n=1$; congenital heart disease $n=2$) and three who had a transplant (IPAH $n=3$, lung disease $n=1$), had lower BNP levels, than the 97.5th centile for normal as given by Soldin (272). The time between BNP measurement and death or transplantation was 3.8- 20.3 months. There was no correlation between BNP value and the time difference between BNP sampling and death or transplantation ($p= 0.46$).

3.5.4. Discussion

This study shows that the BNP levels correlate with functional status in children with PHT. The BNP level was higher in those patients requiring intravenous epoprostenol treatment rather than oral therapies. These findings are similar to those in adults, where higher BNP levels have been reported in patients with greater physical disability (273). In the current study BNP levels did not correlate with 6-minute walk test distance, probably because children were tested over a wide age range and underlying diagnosis was heterogeneous. There was no correlation with age and BNP levels were not different between boys and girls. The main limitations of this study are that BNP levels were not measured at the time of cardiac catheterization and longitudinal data is not available, thus making it impossible to relate BNP values directly to response to therapy and disease progression.

Plasma BNP correlated with echocardiographic measures of right ventricular performance, including right ventricular dilatation, hypertrophy and ventricular function. In a study of 55 adults with pulmonary hypertension, including 36 with IPAH, BNP correlated positively with diastolic right ventricle/left ventricle ratio area, inversely with acceleration of right ventricular ejection time and with the presence of a pericardial effusion (274). In children with congenital heart disease, BNP or NT-BNP values correlated with echocardiographic features of heart failure and right ventricular volume overload (266). The majority of published studies have related BNP to the hemodynamic rather than the echocardiographic findings. In the present study children with IPAH showed a positive correlation between the BNP level and the mean PAP and PVRI on inhaled nitric oxide at cardiac catheterization. This implies that a correlation is best achieved in children when the pulmonary vascular resistance is at its lowest and most stable. Also, correlations between BNP and hemodynamic status might have been better had there been no delay between cardiac catheterization and blood sampling. In adult patients with IPAH, one study reported a correlation between the BNP level and mean PAP, mean right atrial pressure, right ventricular end diastolic pressure and total pulmonary vascular resistance index (137,257) while another study found an association between BNP and PAP and pulmonary vascular resistance (252). In the current study no correlation was found between BNP and the haemodynamic parameters in children with APH. This is perhaps surprising given that an elevated BNP has previously been reported

in PHT associated with both congenital heart disease and in children with acute respiratory distress associated with congestive heart failure (265,275). The patients included in the current study might have been expected to have had higher BNP levels because they had a pressure overloaded right ventricle rather than the volume overloaded right ventricle of a ventricular septal defect. Lin et al and Nagaya et al (257,276) found that patients with RV pressure overload due to IPAH and thromboembolism have higher BNP levels than those with right ventricle volume overload due to an atrial septal defect .

Evaluating the response to therapy and likely time to deterioration and the need for transplantation is extremely difficult, particularly in children. Using receiver-operating characteristics (ROC) analysis to assess the prognostic accuracy of BNP value revealed that children with elevated BNP values >130 pg/ml had a worse prognosis. The BNP level was shown to be an independent predictor of mortality in adult patients with IPAH. In this adult population a baseline BNP value of 150 pg/ml has been described as the best cut-off value distinguishing survivors and non-survivors (137). In adults with chronic heart failure higher concentrations of BNP were associated with increased mortality, independent of age, functional class and ejection fraction (277). In the current study however, the sensitivity of BNP (57%) was too low to be clinically useful. There were six patients with a BNP value lower than our derived cut-off value (<130pg/ml), who died or required transplantation. For the subgroup of children with IPAH sensitivity and specificity of BNP predicting death or need for transplantation was better. In the subgroup of children with APH, BNP was found to be less useful with a lower sensitivity and specificity. In addition, the BNP level may have been influenced by therapy. All children in the present cohort were receiving either epoprostenol, bosentan, sildenafil or a combination of these drugs. It has been suggested that long term treatment with epoprostenol (257) can lead to a reduction in the BNP level, suggesting that BNP might be a potential non-invasive tool to monitor the efficacy of advanced therapies in longitudinal studies (169).

BNP is regarded as a useful biomarker of right ventricular dysfunction, but its role in the pathobiology of pulmonary hypertension is uncertain. BNP is synthesized and secreted by the ventricular myocardium, little is stored and rapid gene expression

with de novo synthesis regulates secretion. The main stimulus for secretion is increased myocardial wall stress (257,269,270,278,279). Like ANP, BNP is a pulmonary vasodilator causing elevation of intracellular cGMP and is essential in preventing myocardial hypertrophy and fibrosis (280,281). Humans exposed to hypoxia had an attenuated increase in mean PAP and PVR when infused with BNP (282). These and other studies indicate the potential benefit of enhancing BNP activity, either by using natriuretic peptide analogues / recombinant BNP, or reducing the breakdown of the endogenous product via inhibition of neutral endopeptidase or both. In addition, BNP promotes natriuresis and suppresses plasma aldosterone, attributes which are relevant to the management of patients with a failing right ventricle (283).

In conclusion, considering 32.7 pg/ml as the upper level of normal value of BNP in healthy children as reported by Koch, the current study illustrates that BNP levels are elevated in 56% of children with PHT. 74% of children with IPAH had an elevated BNP as compared with 34% of those with APH. A high BNP level in children with pulmonary hypertension should raise concerns about the optimization of medical therapy. A low BNP however, does not necessarily indicate that a patient is well and stable. The findings in the present study indicate that BNP determination can be a useful adjunct to the assessment of clinical status but should not be used in isolation as independent screening tool to predict outcome.

4. TREATMENT OF CHILDREN WITH PULMONARY HYPERTENSION

4.1 EPOPROSTENOL THERAPY

4.1.1 Background

Epoprostenol therapy was the first available effective treatment for patients with severe established PHT. In a 12-week prospective, randomised trial, efficacy was proven in adults with IPAH. The trial demonstrated an improvement of exercise tolerance, quality of life, haemodynamics and survival (89).

Data of the first paediatric trial were published in 1999, suggesting a treatment benefit also for children (5). Traditionally, epoprostenol is given to adults with IPAH and with PHT associated with connective tissue disease and to children with IPAH, all of whom are in WHO functional class 3 – 4 and do not show a positive response to acute vasodilator testing at cardiac catheterisation. Over the last decade and with increasing experience, the use and indications in paediatric practice have widened. Epoprostenol has been successfully used in children with PHT, other than IPAH, such as connective tissue disease, congenital heart disease other than the Eisenmenger Syndrome and other types of APAH.

This study reviews the UKSPHC experience with epoprostenol therapy in 39 children with PHT. The majority of children were referred to the UKSPHC after its establishment in 2001-2. The study focuses on treatment indications, efficacy, response to therapy and the associated morbidity due to the permanent central venous access (Hickman line) needed for the administration of epoprostenol. .

4.1.2 Patients and Methods

All patients were regularly assessed before and after initiation of epoprostenol treatment. A careful recent clinical history was obtained, exploring day-to-day life, physical activities and participation in activities with peers, such as playgroup, kindergarten or school. Special consideration was paid to the social and family environment, to judge whether epoprostenol treatment along with its high demands and commitment to the family/ child's carers was in the child's best interest and could be facilitated.

In addition to a physical examination, weight and height were recorded, and an electrocardiogram and transthoracic echocardiography were performed on every visit to clinic or ward. The functional status was assessed by WHO Functional Class. A 6MWT was performed in children old enough to do the test reliably. The test was performed in a standardised manner by an experienced investigator monitoring the distance walked and the transcutaneous systemic arterial oxygen saturation at baseline and on exertion as outlined in the general methods section (159). Cardiac catheterisation was performed under general anaesthesia (see Chapter 2) before instituting treatment in all children. Electrocardiograms were analysed specifically for evidence of right ventricular hypertrophy (RVH) and strain. On transthoracic echocardiography, evidence of right ventricular dilatation, hypertrophy and function was assessed and documented as outlined in Chapter 2.

Statistics

Kaplan–Meier survival plots were constructed from the start of epoprostenol treatment. Censoring events were transplantation and cessation of epoprostenol. Z-scores of weight were calculated by $z = (\text{measured value} - \text{reference mean}) / \text{reference SD}$. Reference values were taken from CDC (Center of Disease Control) growth charts (175).

4.1.3 Results

Between 1997 and 2005, 39 children were treated with epoprostenol. Patient demographics are shown in Table 5.1.1. Of these children, 25 had IPAH, whereas associated pulmonary hypertension (APH) occurred in 14 children, including postoperative congenital heart disease, cardiomyopathy, connective tissue disease, chronic lung disease or HIV (Table 4.1.1).

The male:female ratio was 1:1.8 for IPAH and 1.3:1 for APH. The median age at the onset of therapy was 5.4 years (range 4 months–7 years).

Table 4.1.1. Patients' characteristics.

Patients	n=39
Male:Female	17:22
Age (median)	5.4 years (4 months - 17 years)
Weight	21.2 ± 15.3 kg
Aetiology of PH	
IPAH	n=25
PH associated with	
• CHD	n=6
• Cardiomyopathy	n=3
• Connective tissue disease	n=2
• Chronic/interstitial lung disease	n=2
• HIV	n=1
WHO functional class	
1 / 2 / 3 / 4	0 / 0 / 27 / 12
Medication before commencement of Epoprostenol	
• Bosentan	8
• Sildenafil	6
• Calcium channel blocker	6
• Bosentan and sildenafil	2
• Bosentan and calcium channel blocker	2
Pulmonary haemodynamics at onset of treatment	
• Mean pulmonary arterial pressure	59 ± 17 mmHg (25-88)
• Mean systemic arterial pressure	62 ± 14 mmHg (42-95)
• Right atrial pressure	8.1 ± 5.2mmHg (3-28)
• Mean left atrial pressure/ PCWP	9.4 ± 4.6 (3-23)
• PVRI - baseline	23.3 ± 11.6 units.m ² (3.5- 49.8)
• PVRI -100% FiO ₂	20.4 ± 10.8 units.m ² (8.7- 44.0)
• PVRI - 65%FiO ₂ /20 ppm NO	19.5 ± 11.1 units.m ² (2.5-39.0)
• Pulmonary arterial saturation	65.9 ± 12.5 % (23-87)
• Systemic saturation	96 ± 4.7 % (83-100)

Data are shown as mean ± standard deviation. In addition the range is presented in brackets.
CHD = congenital heart disease; HIV = human immunodeficiency virus infection; IPAH = idiopathic pulmonary arterial hypertension; NO= nitric oxide; ppm= parts per million; PCWP= Pulmonary capillary wedge pressure; PH = pulmonary hypertension; PVRI= Pulmonary vascular resistance index; SD = standard deviation; WHO = World Health Organization Functional Class; m²= meter squared

Children were treated with epoprostenol when they presented severely symptomatic in WHO Class III or IV, or had failed to improve or to sustain an initial improvement

on oral therapy for PAH. In total, 24 children had previously been treated with bosentan, sildenafil or a calcium channel blocker.

Baseline status

All patients were severely symptomatic; 27 patients were in WHO functional class 3 and 12 were in class 4 (Fig 5.1.1).

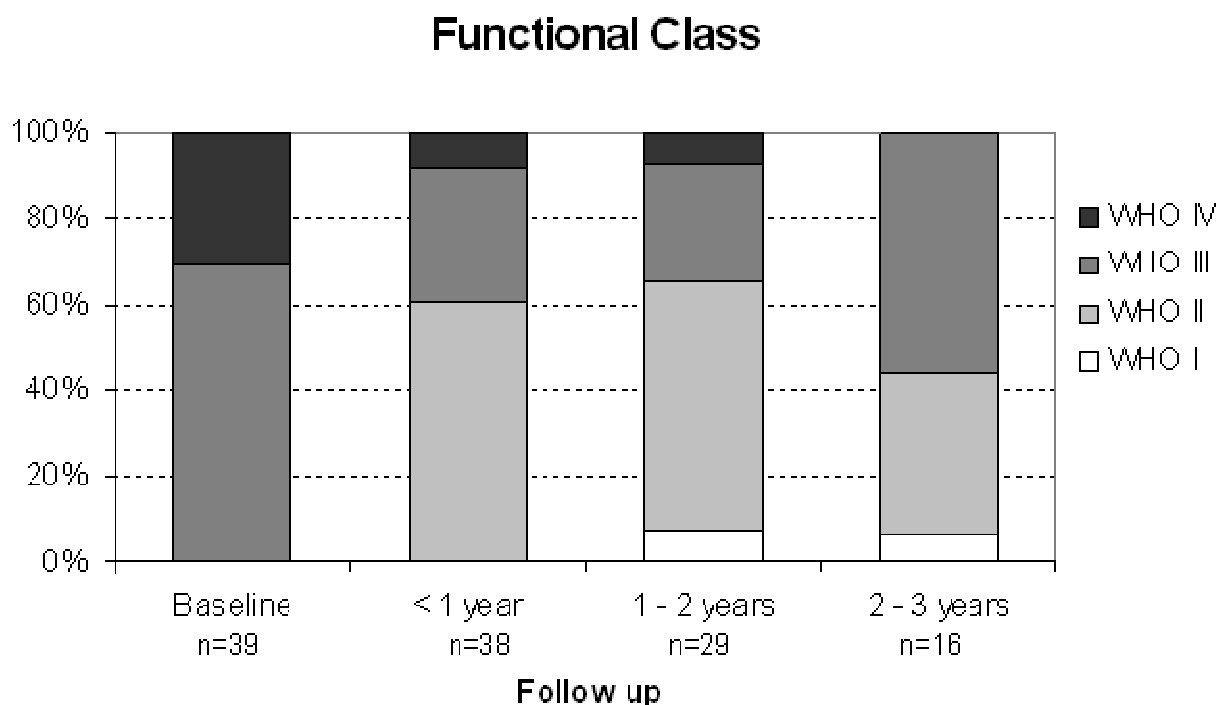


Figure 4.1.1.Improvement in functional class (WHO) on epoprostenol therapy:Percentage of children in each WHO functional class at the start of the treatment with epoprostenol and after 1, 2 and 3 years. During the first year, no child died and one underwent transplantation, three children died during the second year, and three died and two had a transplant during the third year.

At the time of establishment of epoprostenol, the median weight of all children was 16.0 kg (*mean [SD] 21.0 [15.3] kg*). The children were underweight and had a mean z-score of -1.55 [1.74] range -2.14 to -0.96). In all children, the electrocardiogram showed evidence of right ventricular hypertrophy (RVH). The amplitude of the R wave in V1 plus the S wave in S₅ or S₆ was 43.5 (20.7) mV. Evidence of right ventricular strain was present in 14 children and ‘possible strain’ in another 13 cases.

Transthoracic echocardiography showed RVH in all children, associated with right ventricular dilatation in 33 children. Right ventricular systolic function was impaired

in 32 children. None of the children a significant pericardial effusion. Doppler pressure estimations of right ventricular pressure could be obtained in 34 children. Mean velocity was 4.6 m/s, suggesting a mean right ventricular systolic pressure of 84 mmHg (plus right atrial pressure) according to the modified Bernoulli equation. Pulmonary regurgitation was present in 23 patients, but was not severe in any child.

Table 4.1.2 Echocardiographic parameters at baseline and during epoprostenol treatment.

	Baseline	Epoprostenol therapy
Right ventricular function		
Normal	7	11
Mildly impaired	17	13
Moderately impaired	13	12
Severely impaired	2	3
Tricuspid regurgitation		
None	2	0
Trivial	3	9
Mild	30	18
Moderate	3	11
Severe	1	1
V_{max} tricuspid valve	4.6±0.9 m/s (n=34)	4.2±0.8 m/s (n=36)
Pulmonary regurgitation		
None	16	18
Trivial	7	6
Mild	13	14
Moderate	3	1
Severe	0	0
End diastolic PR velocity	2.9±0.6 m/s (n=16)	2.8±0.5 m/s (n=12)

PR = pulmonary valve regurgitation; V_{max} = maximal velocity.

At cardiac catheterisation, the mean pulmonary arterial pressure (PAP) was 59 (SD 17) mmHg. In 26 children the PAP equalled or exceeded the systemic arterial pressure (see Table 4.1.1).

The mean pulmonary vascular resistance index (PVRI) was 23.3 (11.6) units/m² in the 34 children in whom it was assessed. The lowest PVRI was seen in a child with cardiomyopathy. The child had an elevated PAP, was thought to be a 'high risk'

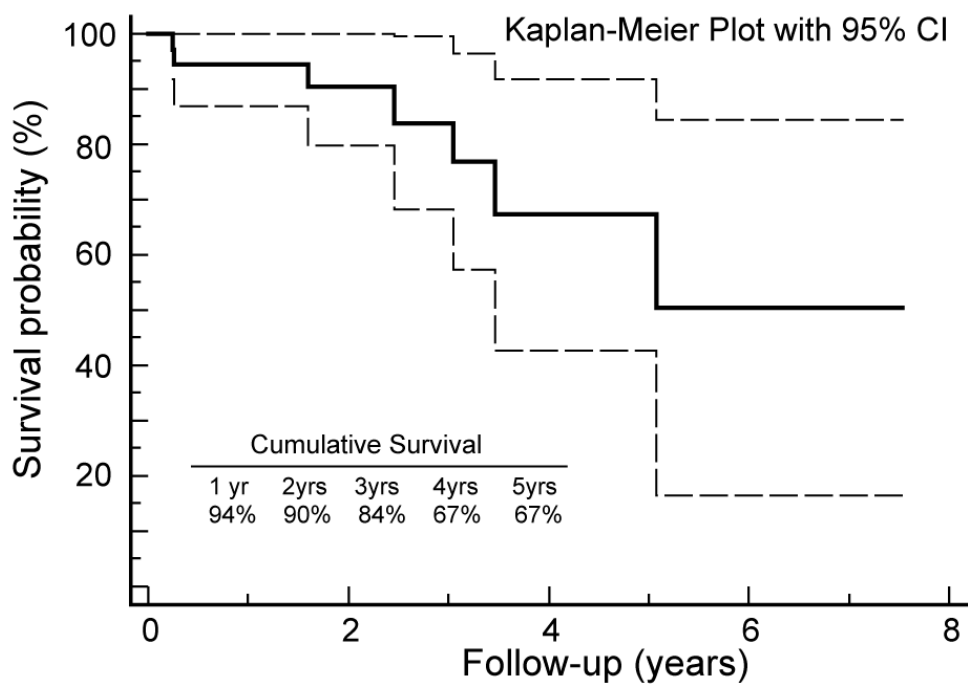
candidate for heart transplantation alone and, in an attempt to avoid heart–lung transplantation, was treated with epoprostenol for a month and then successfully underwent heart transplant. There was little change in PVRI in children who were tested either with inhaled nitric oxide (n=31) or with an increased inspired oxygen concentration (n = 18) (Table 4.1.1).

In all children a Hickman line was placed under general anaesthesia and connected to a portable infusion pump (CADD legacy pump, Pharmacia Deltec, St Paul, Minnesota, USA). In each patient the drug was uptitrated according to the clinical response. The initiation dose in children we use is 2 ng/kg/min, a dose that is usually well tolerated.

The rate was subsequently increased, with the rate of increase depending on the severity of the disease, clinical response to therapy and the development of side effects. In this series, the mean dose of epoprostenol was 29.6 (15.2) ng/kg/min (range 6–63 ng/kg/min). The lowest dose was given to a child with cardiomyopathy who underwent heart transplant 1 month later. The mean dose given to children with IPAH was 32.5 (12) ng/kg/min (10–63 ng/kg/min). All children were kept in hospital under observation until clinical improvement was evident. Subsequently the parents received instructions how to further elevate the dose, so that further increments (and adjustments for weight gain) could be carried out at home.

The mean follow-up time was 27 (21) months (range 1– 90 months). The Kaplan–Meier analysis showed that the cumulative survival of all patients who received epoprostenol at 1, 2 and 3 years was 94%, 90% and 84%, respectively. The cumulative survival is illustrated in Figure 4.1.2a. Survival of patients with IPAH at is shown in Figure 4.1.2b, their survival at 1, 2 and 3 years was 96%, 91% and 83%, respectively.

a)



b)

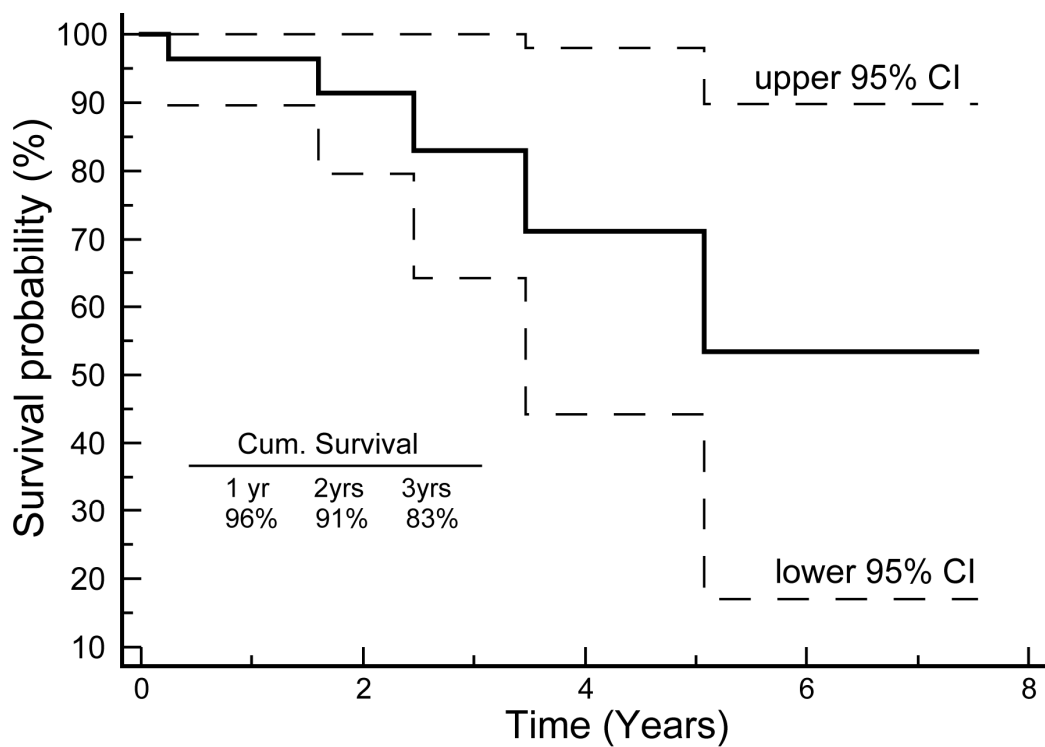


Figure 4.1.2a and 4.1.2b Kaplan–Meier cumulative survival plots with 95% CIs of (a) all children treated with epoprostenol and (b) those diagnosed as having IPAH. Children were censored at the time of transplant and when epoprostenol treatment ceased.

Censoring events were transplantation and cessation of epoprostenol. In total, 32 of the 39 patients survived, four having been on epoprostenol treatment for more than 5 years. Seven patients died after receiving treatment for a median of 29 months (range 3–61 months). Four patients with IPAH died from disease progression despite maximum therapy. There was one death in a child with severe systemic sclerosis and two from corrected congenital heart disease.

In six children, who had been on nifedipine, when epoprostenol treatment was commenced, nifedipine was discontinued, because of possible compromise of right ventricular function (284). Similarly, sildenafil was withdrawn in four unstable, sick children to avoid hypotension at initiation of epoprostenol treatment. Once established on epoprostenol, however, additional therapies were added in 14 children: bosentan in 8, sildenafil in 5 and both in 1 child. In total, 17 children with syncope or pre-syncope had an atrial septostomy (285).

Epoprostenol was discontinued in 10 children, eight of whom underwent transplantation. Six of the transplanted children had IPAH and two had a cardiomyopathy. Of those transplanted children, four children with IPAH had a double lung transplantation, and two received a heart and lung transplantation. One child with a cardiomyopathy had a heart and lung transplant and one a heart transplant. In these eight children, epoprostenol therapy was started at a median age of 4.8 years (range 1.2–16.8) and the mean duration of treatment before transplantation was 26.2 (16.4) months (range 1–51). For patients with IPAH, the mean duration of treatment was 32 (13.9) months, and for those with cardiomyopathy it was 1 and 17.2 months. Two patients could be successfully transitioned onto either bosentan or nifedipine. One child of these children had IPAH, presented with a cardiac arrest and was treated with epoprostenol before endothelin receptor antagonists were available. She was successfully transitioned onto a dual endothelin receptor antagonist. The other child was electively treated with epoprostenol after a late surgical closure of atrial and ventricular septal defects. She had suffered from severe postoperative pulmonary hypertensive crises. Her cardiac catheterisation study 7 months after surgery showed a normal PAP and PVR and she was transitioned onto nifedipine.

The mean WHO functional class improved significantly during the first year of therapy, from 3.6 to 2.6 ($p=0.001$), and remained stable up to and during the third year of treatment at 2.6 (Figure 4.1.1). During the first year, an improvement in functional class was observed in 30 children; another 4 were stable, 3 deteriorated and 2 died. In addition, the children gained weight. For weight, the mean z score improved significantly from -1.55 (1.74) (range -2.14 to -0.96) to -1.16 (1.8) ($p=0.03$) during follow-up.

A 6MWT was undertaken in patients old enough to perform the test reliably. Twelve children performed the test before they started epoprostenol therapy, 16 children performed their first test on treatment. For these 28 children the mean (SD) initial distance was 250 (93) m. After a follow-up of 11.4 (7.1) months, the distance walked increased to 327 (105) m ($p=0.003$), as illustrated in Figure 4.1.3.

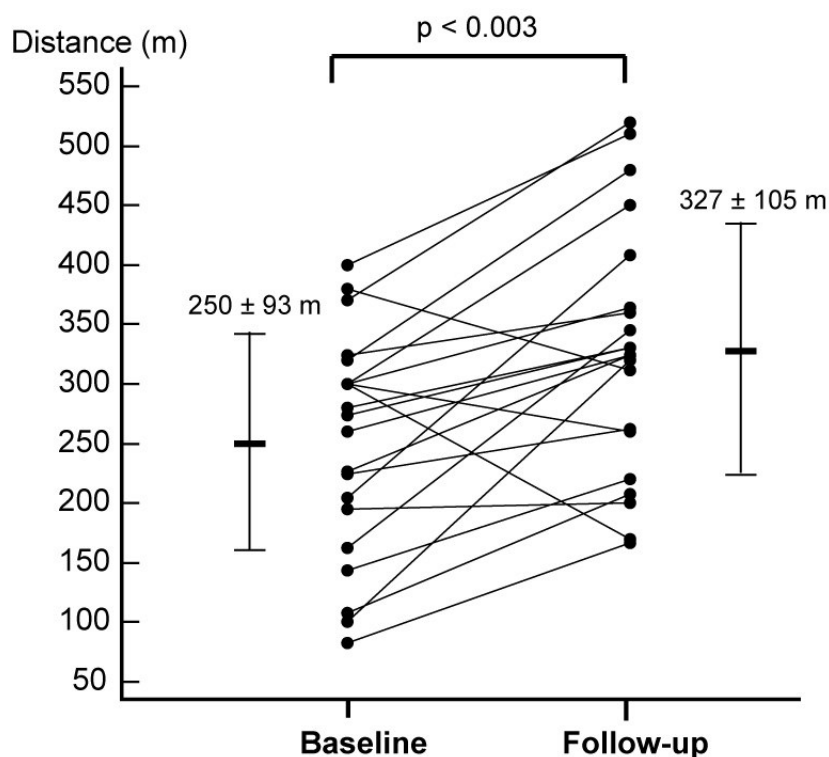


Figure 4.1.3 Six-minute walk test results at baseline and on treatment after 11.4 months of follow-up.

Electrocardiography was unchanged and did not show any evidence of a significant reduction in either RVH (39.7 (21.2) vs 43.5 (20.7) mV, $p = 0.07$) or right ventricular strain.

Echocardiographic right ventricular function improved during the first year of treatment in 12, was unchanged in 19 and deteriorated in 8 patients (Table 4.1.2).

At baseline, the tricuspid regurgitant jet velocity was 4.6 (0.9) m/s and 4.2 (0.8) m/s on treatment, suggesting a non-significant reduction in the systolic right ventricular pressure ($p=0.08$). There was little change in frequency or severity of pulmonary or tricuspid regurgitation on epoprostenol treatment: all in all, 37 children had tricuspid regurgitation and 23 patients had pulmonary regurgitation before treatment while 39 children had tricuspid regurgitation (TR) and 21 pulmonary regurgitation on treatment. The mean end-diastolic velocities as estimated by the pulmonary regurgitation jet, and before and after treatment they were 2.9 (0.6) m/s and 2.8 (0.5) m/s, respectively.

Administration of epoprostenol was unproblematic in 24 patients. There were only 0.33 Hickman line changes per patient year on treatment. In total, the line required replacement in 15 patients. The reasons included: infection on 20 occasions, a leaking line in 5 and catheter dislocation on 4 occasions. Courses of antibiotic treatment were required on 43 occasions, primarily for local site infections. Only on six occasions blood cultures were positive in four patients (*Pseudomonas* $n = 1$, Methicillin-resistant *Staphylococcus aureus* (MRSA) $n = 1$, other than MRSA *Staphylococcus aureus*, $n = 3$, *Staphylococcus aureus* and *Candida* in 1).

4.1.4 Discussion

This study shows that epoprostenol therapy represents an effective treatment for children with severe PHT. The natural history in children with PHT is worse than in adults. Historical data from a registry on IPAH of the National Institutes of Health suggested survival rates in adults of 68%, 48% and 34% at 1, 3 and 5 years respectively, while median survival was 2.8 years (286). In children without treatment median survival is less than 1 year from the time of diagnosis (5,7). The current study illustrates that on treatment with epoprostenol the survival in children is at least comparable to that reported in adults. In the current study the survival in children with IPAH was 96%, 91% and 83% at 1, 2 and 3 years, respectively. These data compare favourably with those of other published series. Survival rates in one study on adults were 87.8%, 76.3% and 62.8% at 1, 2 and 3 years (131), and in a

more recent study the survival rates were 91%, 84% and 75% at 1, 2 and 3 years, respectively (287). A study of 35 children on epoprostenol treatment stated survival figures of 94%, 81% and 61% at 1, 5 and 10 years (288).

The cohort reported here includes 36% of children with PHT which was not classified as 'idiopathic', and these children responded equally well to epoprostenol. A proportion of them had associated PHT secondary to congenital heart disease. Closure of a cardiac defect in the presence of an elevated pulmonary vascular resistance is an recognized risk and well known to accelerate the development of severe pulmonary vascular obstructive disease (289,290). Thus treatment with epoprostenol appears to be a logical step in these patients. Adult studies have shown its usefulness in patients with PHT associated with connective tissue disease and HIV (291-293). It is encouraging that the children in this series also benefited from epoprostenol treatment. This study also shows that the functional class and 6-minute-walking-test improve on epoprostenol therapy. The children gained weight, even improving their z-scores. In addition, echocardiographic right ventricular function remained stable on therapy.

Despite the absence of a positive response to acute vasodilator testing in the catheterization laboratory, long-term treatment with epoprostenol was successful in this cohort of children with severe PHT. This is in agreement with the experience of other investigators (130,294). Overall, the clinical condition of the patients usually improved soon after the initiation of treatment. In adults with IPAH, the benefit of epoprostenol treatment is also most apparent soon after starting therapy, with only little additional improvement thereafter (131). It is hypothesized that early improvement might be due to the positive inotropic effect of epoprostenol (88). In patients with IPAH who receive epoprostenol, an early increase in cardiac output is well documented (89,131,133). Clinical improvement in the current study was accompanied by favourable changes in right ventricular function as assessed by echocardiography. The function either improved or did not deteriorate in 31 of the 39 children. In addition, electrocardiographic measures of RVH and ECG signs of strain did not deteriorate, which against the background of pulmonary vascular disease being a chronic progressive disease with a poor prognosis, could be considered a therapeutic success.

Fourteen children remained on bosentan or sildenafil at the start of epoprostenol therapy, and another 14 children were given a second disease specific therapy when their clinical condition deteriorated. As pulmonary vascular obstructive disease can develop rapidly in children, maximum early intervention is desirable. The aim is to improve survival, enhance the quality of life and eventually be able to offer transplantation when medical treatment fails. Epoprostenol treatment plays an important role particularly in young children, helping them maintain their physical status and enabling them to grow and survive to reach an age when lung or heart and lung transplantation becomes feasible. Treatment can also be considered as a pharmacological ‘bridge to transplantation’ until a suitable organ becomes available.

Epoprostenol treatment represents an invasive ‘high maintenance’ therapy and requires careful consideration and assessment of the risk–benefit ratio. The international experience with epoprostenol has shown that it is the most effective treatment in severely symptomatic patients with IPAH. It has been successfully used as a rescue therapy in patients failing to respond to oral therapies such as bosentan and sildenafil.

The policy of the UKSPHC when this study was carried out was to select children for epoprostenol treatment because they were clinically unstable, were in WHO functional class 3 or 4, or had either failed to respond to oral or were considered to be too sick and unlikely to respond quickly to oral therapy. These conditions comply with the management guidelines for IPAH prepared by the European Society of Cardiology (3) and the American Heart Association (37,295). Unfortunately, less invasive therapies such as the epoprostenol analogue treprostinil or inhaled iloprost are of limited value in children for reasons discussed in Chapter 1. In the present study, there was no mortality associated with the invasive mode of administration of epoprostenol itself. As anticipated, morbidity was primarily due to infections, which were generally limited to site infections.

In conclusion, the results of this study indicate that epoprostenol therapy slows the progression of pulmonary vascular disease and can be effective in both children with IPAH and in those with APH. Epoprostenol therapy improves survival compared to historical controls, improves WHO functional class and exercise tolerance and

improves the ability to thrive in children with severe PHT. It is thought that epoprostenol can either slow down or stabilise the pulmonary vascular disease process, at least for a time. The particularly rapid course of the disease in childhood suggests that children should be treated promptly and effectively as soon as they present to the physician. Overall, this study supports the notion that epoprostenol represents an effective, feasible therapy with an acceptable morbidity even in young children with severe PHT.

4.2 ATRIAL SEPTOSTOMY – THE UKSPHC EXPERIENCE BETWEEN 1997 and 2005

4.2.1 Background

Despite advances in medical therapy PHT remains an incurable progressive disease, eventually leading to right heart failure, functional incapacity, and death. The symptoms are non-specific and therefore presentation is late. In adult patients atrial septostomy has been shown to be beneficial for selected patients (4,9,296,297). The procedure permits right to left shunting, decompresses the right heart and can increase left ventricular preload during an acute rise in pulmonary vascular resistance such as a pulmonary hypertensive crisis. Observed beneficial effects in adult populations include prolonged survival with deferral of transplantation and improved quality of life, symptoms, and haemodynamic function (112,117,119,120,298). Nevertheless, atrial septostomy has been associated with a considerable risk of intraprocedural and periprocedural mortality of up to 30% in some series (112,119,298). As experience using this procedure in children with pulmonary hypertension is limited a number of issues require clarification in paediatric patients. These include the optimal timing of the procedure, associated morbidity and mortality, and long term effectiveness. This study was performed to review the UKSPHC experience with atrial septostomy in the treatment of children with severe IPAH.

4.2.2 Patients and Methods

From the institutional database 20 children with severe PAH who underwent an interventional cardiac catheterisation to create an interatrial communication were identified. Nineteen patients had IPAH, nine boys and 10 girls. One girl had pulmonary hypertension associated with HIV (Table 5.2.1). The mean age at atrial septostomy was 8.4 years with a range between 0.25 and 17 years.

All patients were highly symptomatic, 9 in WHO functional class 3 and 11 in class 4 as shown in Table 5.2.1. The majority (65%) had recurrent syncope, the remaining patients (35%) were in right ventricular failure despite treatment. All patients had systemic arterial oxygen saturations of at least 90%. Among the 7 children who could perform a six-minute walk test, the mean distance walked was 326 m (range 160– 432 m). At the time of the septostomy the treatment regimens of the children was as follows: 17 children were receiving specific pulmonary hypertensive

treatment as a single or dual therapy and 3 sick children were catheterised with septostomy when they first presented to the Service after being treated for 24 hours. Transthoracic echocardiography showed a tricuspid regurgitant jet velocity of at least 4 m/s in 17 children and 3.1–3.4 m/s in the remaining patients. Seven patients had moderately or severely impaired right ventricular function (Table 4.2.2). Cardiac catheterisation (Table 4.2.2) was performed under general anaesthesia. The mean pulmonary artery pressure approached or exceeded the mean systemic arterial pressure in 15 patients. The mean pulmonary vascular resistance index was 23 units/m². Nineteen patients were not found to be responders on acute vasodilator testing with a combination of 65% oxygen and nitric oxide. One patient, however, who had several cardiac arrests before and on admission to hospital had a positive vasodilator response with the pulmonary artery pressure falling to one third systemic arterial pressure. Overall, the mean right atrial pressure of the patients was 9.3 mm Hg (range 3–19 mm Hg).

The mean time from diagnosis to atrial septostomy was 2.5 years (range one month to 16 years). Eight of 20 (40%) patients had the septostomy performed within six months of the diagnostic cardiac catheterisation. Three children were so severely symptomatic when they presented that it was thought safer to begin treatment with intravenous epoprostenol for a week or more and then perform an atrial septostomy in the same session as the diagnostic cardiac catheterisation study, to avoid repeat general anaesthesia. Three other children admitted for atrial septostomy, were felt to be unstable, particularly due to their right ventricular function on echocardiography, and epoprostenol was started the day before the procedure and maintained subsequently.

Table 4.2.1 Patient characteristics.

Patient	Gender	Age at procedure (years)	Diagnosis	WHO Class	Indication	Therapy before procedure	Subsequent Therapy
1	M	0.25	IPAH	III	Syncope	PGI2 Sild	PGI2 Sild
2	M	1.5	IPAH	III	RVF	PGI2	PGI2
3	F	2.5	IPAH	III	Syncope	Bos	Bos PGI2
4	F	3	IPAH	III-IV	Syncope, RVF	Ilopr Nifed	Ilopr Nifed
5	M	3.5	IPAH	IV	Syncope	nil	PGI2
6	F	4	IPAH	III-IV	Syncope	Bos Sild	PGI2 Bos Sild
7	M	5	IPAH	IV	RVF	PGI2 Bos	PGI2 Bos
8	M	5.5	IPAH	IV	RVF	Nifed PGI2	Nifed PGI2
9	M	5.5	IPAH	IV	Syncope	nil	PGI2
10	F	6	IPAH	III	Syncope	Nifed	PGI2
11	F	6.5	IPAH	IV	RVF	PGI2 Sild	PGI2
12	F	9	IPAH	III	Syncope	Nifed	PGI2
13	M	9.5	IPAH	III	RVF	nil	PGI2
14	F	12.5	IPAH	III	Syncope	Bos	Bos
15	M	13.5	IPAH	IV	Syncope	Nifed	Nifed
16	F	14	IPAH	III-IV	Syncope	Bos, Sild	Bos, Sild
17	F	15.5	IPAH	IV	RVF	PGI2	PGI2
18	F	16.5	IPAH	III-IV	Syncope	Bos	Bos
19	M	17	IPAH	III	RVF	Nifed	PGI2
20	F	16.5	APH (HIV)	III	Syncope	Bos	PGI2, Bos

IPAH = idiopathic pulmonary arterial hypertension; Associated PH = secondary pulmonary hypertension.

Bos = bosentan, Nifed = nifedipine, PGI2 = epoprostenol, Sild = sildenafil, RVF = right ventricular failure.

4.2.3 Results

Technique of atrial septostomy

The interventional procedure was performed under fluoroscopic and transoesophageal echocardiographic guidance with general anaesthesia in all children. The atrial septostomy was carried out using different techniques as shown in Table 5.2.3. Eight children underwent graded balloon septostomy, two blade septostomy, and a combination of a blade plus graded balloon septostomy was employed in the remaining three patients. More recently, a graded balloon approach was followed by the introduction of a custom made fenestrated atrial septal defect (ASD) Amplatzer device, which was implanted in seven patients and is described in detail in the next section.

Procedural complications

An adequate interatrial communication could be successfully created in all but one patient (case 20). The mean size of the created communication – as measured by echocardiography immediately post procedure - was 7 mm. Table 4.2.3. shows the size of the communication for all patients. The procedure was uncomplicated in 16 cases. Relevant complications occurred in four patients but all eventually made a good recovery. One case was complicated by arrhythmias and cardiac arrest just before the atrial septostomy (case 3). The patient remained intubated, and mechanically ventilated until a successful graded balloon septostomy was carried out 13 days later. In another patient (case 6) who had presented with syncope the implantation of a 5 mm fenestrated ASD Amplatzer device was complicated by a cardiac arrest, and the patient required mechanical ventilation for 13 days. In case 17 insertion of a fenestrated device was complicated by hypoxaemia and acidosis leading to an emergency re-intubation and mechanical ventilation for five days. As mentioned above the procedure failed in one patient (case 20). In this patient there was considerable systemic arterial desaturation from 98% at baseline to 58% during graded balloon septostomy. The patient required prompt implantation of a 14 mm Amplatzer ASD occluder device. This patient had a high right atrial pressure of 19 mm Hg. The patient was mechanically ventilated for 24 hours and eventually discharged on epoprostenol and remains well.

Outcome

The mean systemic arterial oxygen saturation decreased from 98% before septostomy to 90% after 3–6 months ($P=0.01$). In addition, WHO functional class improved with 6 children moving from WHO class 4 to 3, and four from WHO class 3 to 2 ($P<0.001$). In contrast, no significant change was found in the 6-minute walk test distance. Echocardiography showed an improvement in right ventricular function in seven patients and no deterioration in the rest. The velocity of the tricuspid valve regurgitant jet remained unchanged. A bidirectional flow was generally seen at rest across the atrial communication. All patients could be discharged home after the procedure. The majority of patients were treated with continuous epoprostenol infusions as shown in Table 4.2.1. Recurrent syncope abolished in all except one child (case 1) who died two months after the procedure. The seven other patients in right heart failure improved as judged clinically. In one case (patient 19), chest pain

did not improve after the first septostomy and a second, successful procedure was performed a few months later, resolving symptoms. In case 17 a second procedure with a fenestrated device insertion was done 16 months after the first improving right ventricular function.

After a mean follow up of 2.1 years (one month to 6.7 years) clinical improvement has been maintained in 18 of the 19 patients with a successful atrial septostomy; the remaining patient is also well and at college. Two children since underwent bilateral lung transplantation (cases 5 and 9). In addition to the baby who died at 5 months of age, one patient (case 10) died suddenly five years later with a severe episode of haemoptysis.

Table 4.2.2 Echocardiographic and hemodynamic parameters before atrial septostomy

Patient	Echo. RVF score (1-4)	Mean PAP/SAP (mmHg)	RAP (mmHg)	PVR (units.m ²)
1	2	58/57	7	19
2	3	53/56	5	14
3	2	65/61	10	26
4	2	71/67	-	23
5	2	56/47	6	38
6	3	25/54	3	12
7	4	70/65	18	13
8	2	71/58	7	14
9	2	66/59	9	27
10	3	80/75	-	22
11	4	63/45	8	39
12	2	56/74	3	5.6
13	2	80/71	10	27
14	2	59/74	12	12
15	-	78/69	9	20
16	2	53/73	7	18
17	3	49/50	18	21
18	2	49/75	13	13
19	2	71/61	3	14
20	3	80/63	19	22

Echo. RVF = echocardiographic assessment of right ventricular function [11], PAP = pulmonary arterial pressure; PVR = pulmonary arterial resistance; RAP = right atrial pressure; SAP = systemic arterial pressure;

Table 4.2.3 Procedure type and immediate outcome

Patient	Type of Procedure	Size [mm]	Ventilator days
1	BAS	5	0
2	Fen. ASD device	5	0
3	BAS	5	12
4	Blade/BAS	10	0
5	BAS	5	0
6	Fen. ASD Device	5	13
7	Fen. ASD Device	5	0
8	Blade/BAS	7.5	0
9	BAS	5	0
10	Blade	6	0
11	BAS	10	0
12	BAS	7	0
13	Fen ASD device	8	0
14	Fen. ASD device	8	0
15	Blade	4.5	0
16	Fen ASD device	7	0
17	Fen. ASD device	6	10
18	BAS	7	0
19	Blade/BAS	8	0
20	BAS	14	7

Fen. = fenestrated, *ASD* = atrial septal defect; *BAS* = balloon atrial septostomy; *Blade* = blade atrial septostomy

4.2.4 Discussion

This study shows that atrial septostomy can be performed safely and is effective in children with IPAH. None of the patients died and the procedure was successful in 19 of 20 patients. Syncope was abolished and right heart failure improved in the majority of patients. In addition, WHO functional class and right ventricular function improved. Longer term outcome is also reassuring, with 17 of the 19 children who had an atrial septostomy being alive after a mean follow up of 2.1 years. Two patients have had a successful bilateral lung transplantation highlighting the potential role of atrial septostomy as bridge to transplantation in selected patients. Overall, the mean systemic arterial oxygen saturation dropped by approximately 8% points. In one patient severe right to left shunt immediately after creation of the interatrial communication lead to severe cyanosis and required emergency closure of atrial septal defect. The right atrial pressure was high in this case at 19mmHg. This case highlights the problem of high right atrial pressures. In fact, current guidelines recommend to avoid atrial septostomy in patients with a right atrial pressure above 20 mmHg. This case, however, illustrates that severe complications can also occur at right atrial pressures that are lower, but approaching this threshold. Although much

has been speculated about a possible improvement of tissue oxygen delivery as a consequence of successful atrial septostomy, it appears that the main effect is the ability to shunt right-to-left during periods of high pulmonary resistance and elevation of pulmonary artery pressure or when a higher systemic cardiac output is required. It has been said that the inter-atrial communication allows the patient to maintain an adequate systemic cardiac output at the expense of a (modest) reduction in oxygen saturation (123). This notion is supported by the results of the current study showing that syncope was abolished in the majority of children.

In conclusion, this study demonstrates that atrial septostomy improved symptoms and quality of life in a group of severely symptomatic children with IPAH. The procedure was effective with no fatalities. Complications can, however, arise in these fragile patients and atrial septostomy should only be performed in experienced centres. As the atrial communication can close spontaneously regular follow up is essential.

4.3 ATRIAL SEPTOSTOMY – EFFICACY AND LONG-TERM PATENCY OF FENESTRATED AMPLATZER DEVICES

4.3.1 Background

The use of fenestrated atrial devices is appealing in patients with PHT who undergo atrial septostomy in an attempt to guarantee long-term patency of the atrial communication. Fenestrated Amplatzer devices (Amplatzer, AGA Medical, Golden Valley, MN) have been successfully used for ASD closure, whilst maintaining a small atrial communication of predefined size in various patient groups, for example the elderly (299,300). Fenestrated devices have also been used in patients after the Fontan operation to create an interatrial communication percutaneously in order to abolish problems caused by an elevated central venous pressure, such as protein-losing enteropathy or persistent pleural effusions (301,302). Fenestrated atrial devices are especially appealing in patients with PHT (285). Despite the obvious need for this type of device, custom-made fenestrated occluders are no longer commercially available. Use of modified Amplatzer devices could be a feasible alternative. Experience with fenestrated devices in the paediatric population, however, is limited. This study presents the indications, the procedural and long-term complications and data on the long-term patency and efficacy of custom-made and modified Amplatzer devices in a group of children with PAH.

4.3.2 Patients and Methods

Ten children (5 male) between 1.5 and 15.5 years of age (median 4.5 years) had a fenestrated Amplatzer device inserted between 2003 and 2007. Table 5.3.1 provides details to patients' demographics. Six patients had custom-made fenestrated devices inserted (modified by the manufacturer MM, Special order Amplatzer, AGA Medical, Golden Valley, MN, Figure 4.3.1), while modified standard atrial septal occluders were used in four patients of because custom-made fenestrated devices were no longer available. Written consent was obtained from all parents before the interventional procedure after extensive explanation of the risks and benefits of the insertion of a fenestrated device. The Amplatzer device consists of Nitinol metal with smooth rounded disks and polyester fabric inside the meshed discs. A fenestration was created through the occluding portion of the disc using 4.0 sutures and removal of fabric (Figure 4.3.2). The device was subsequently checked for

deformation and it was ensured that it could be easily stretched and regained its double disc shape without any deformation. The device was loaded onto the introducing sheath, and delivered by the technique used for atrial septal occluders. In children with an intact atrial septum, transatrial septal puncture was performed using a standard needle, dilator, and sheath combination (St Jude Medical, Fullerton, CA) under transoesophageal echocardiographic monitoring. With release of the device the hat sprang back into the disc shape. No further dilatation of the self-fabricated fenestration was necessary in those four patients who received a modified device. Flow across the fenestration was demonstrated by echocardiography at the time of the interventional procedure.

All children were regularly followed in outpatient clinic and assessed with transthoracic echocardiography to monitor the patency of the device. Right ventricular function was assessed semiquantitatively by echocardiography and quantified as normal, mildly, moderately or severely impaired, as described previously (218,285). Patients' functional class was assessed using the WHO classification (1-4). Special attention was paid to those symptoms, which had lead to the insertion of the device.

Table 4.3.1 Patients' data.

	Age [years]	Sex	Diagnosis	Indication for Device	WHO pre	WHO post	RVF pre	RVF post	Symptom resolved
1	1.3	M	T 21, PHT	ASD II	3	2	1	1	Y
2	1.5	M	PHT	RV-failure	3	3	3	2	Y
3	3.1	M	T 21, PHT	ASD II	2	2	1	1	Y
4	3.1	F	ECMO	Offload LA	4	-	4	-	Y
5	4	F	PHT	Syncope	3	3	3	2	Y
6	5	M	PHT	RV-failure	3	3	4	3	Y
7	9.5	F	PHT	RV-failure	3	2	3	2	Y
8	12.5	M	PHT	Syncope	3	3	2	2	Y
9	14	F	PHT	Syncope	3	2	2	2	Y
10	15.5	F	PHT	RV-failure	4	3	3	3	Y

ASD II = secundum type atrial septal defect, ECMO= extracorporeal membrane oxygenation, F=female, LA= left atrium, M=male, N= no, PHT= pulmonary hypertension, RV= right ventricular, RVF= Right ventricular function (semiquantitatively described as) 1= normal, 2= mildly, 3= moderately, 4= widely impaired. T 21= Trisomie 21, WHO= Functional class according to World Health Organization (1-4), Y=yes,

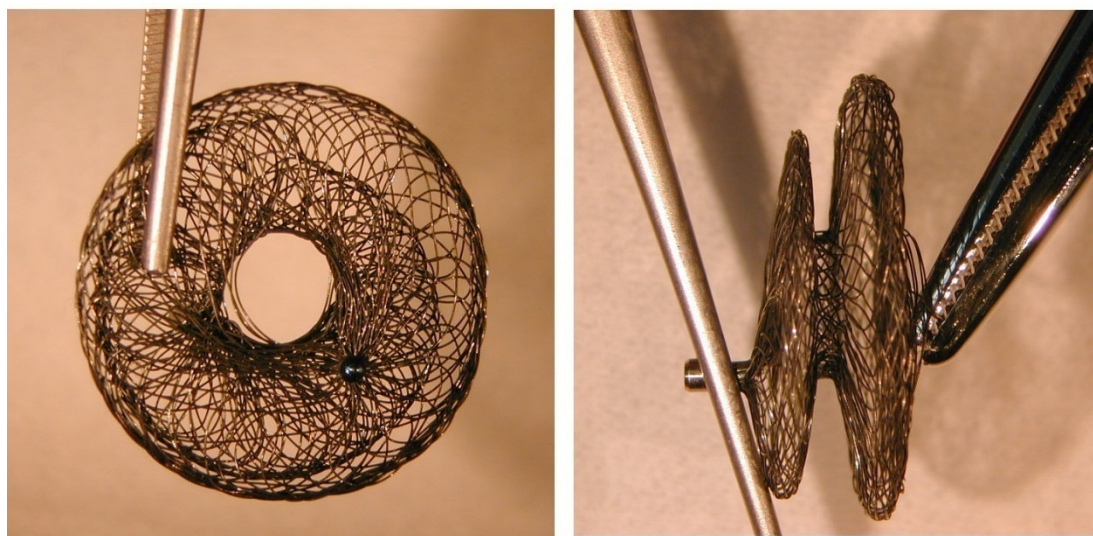
**Figure 4.3.1** Custom-made fenestrated Amplatzer device.

Table 4.3.2 Haemodynamic data and device follow-up.

	Device	Size (mm)	Fen size (mm)	PAP/AoP	PVRI	RAP	LAP	Anticoag./ Antiplatelet	Follow-up (months)	Device Patency
1	modified	12	5	26/60	-	8	-	Aspirin	19.5	N
2	custom	10	5	53/56	14	5	9	Aspirin	26.7	Y
3	modified	14	5	34/62	3.6	-	-	Aspirin	23.8	N
4	modified	11	5	-	-	-	-	Heparin	0.1	Y
5	custom	11	5	25/54	19	3	5	Aspirin	28.5	N
6	custom	10	5	70/65	13	18	-	Warfarin	28.2	Y
7	custom	14	8	80/71	27	10	10	Warfarin	31.0	Y
8	custom	14	8	61/72	17	12	-	Warfarin	24.0	Y
9	modified	9	7	53/73	18	7	14	Warfarin	17.8	N
10	custom	14	6	49/50	21	18	-	Warfarin	23.6	Y

Anticoag.= Anticoagulation therapy, AoP= Mean Aortic pressure, Fen. = fenestration, mm= millimetre, N= no, PAP= Mean pulmonary artery pressure, PVRI = pulmonary vascular resistance index, RAP= right atrial pressure, Y=yes,

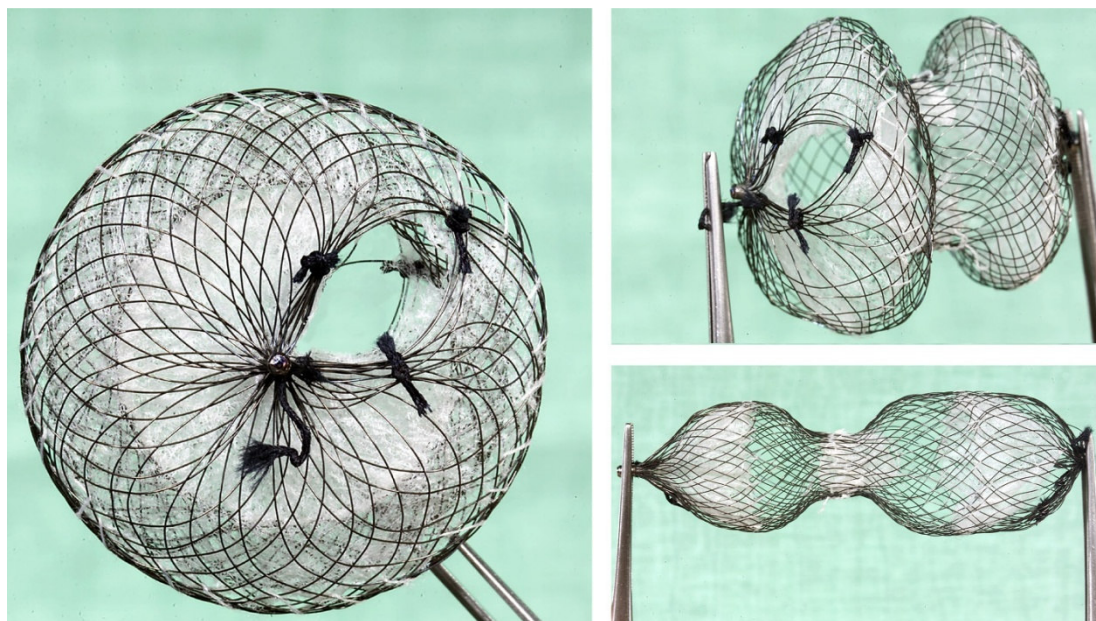


Figure 4.3.2 Modified standard Amplatzer atrial septal occluder with self-made fenestration.

4.3.3 Results

In nine patients, devices were implanted for PHT. Seven of those patients underwent atrial septostomy with fenestrated device insertion (four children for right heart failure, three for recurrent syncope). The remaining two patients were children with Down's syndrome and a haemodynamically relevant atrial septal defect. In addition, one patient with dilated cardiomyopathy on extracorporeal membrane oxygenation had a fenestrated atrial device inserted to offload the left atrium. Patients with pulmonary hypertension were all in WHO functional class 3 or 4, whereas the two patients with pulmonary hypertension and haemodynamically relevant atrial septal defect were in functional class 2 and 3, respectively. The patient on ECMO was classified as functional class 4.

Catheterization and device delivery was successful in all patients without any procedural complications. All modified Amplatzer devices were easily stretched and were loaded without any problems onto the delivery system. Devices were well seated and an adequate flow across the created fenestration could be demonstrated immediately after device delivery in all patients with transthoracic or transoesophageal echocardiography (Figure 4.3.3).

In those patients who had a fenestration created for right heart failure, right ventricular function improved (Table 4.3.1). One child underwent heart-lung-transplantation 23.6 months after device insertion. At the time of transplantation the device was found to be still patent. Episodes of syncope abolished in two of three patients who received a fenestrated device for syncope. One child presented with a recurrent syncope two years after device implantation. Echocardiography at this time confirmed that the fenestration was no longer patent. The two patients with Down syndrome, who received the device to reduce shunting through a significant atrial septal defect but to maintain a small communication as a blow-off valve, are well and thriving. The patient on EMCO, in whom an atrial communication was created to offload the left atrium, was transplanted three days after the intervention. As a consequence, this child was not included in the long-term follow-up.

In 8 patients the device remained in situ after a mean follow-up of 24.5 months (range: 17.5 -31.0 months). Device patency was confirmed in 5 patients (all custom-made) after 26 months follow up (range: 23.6 – 31 months), 4 children being treated with warfarin, one child with aspirin. Device patency could not be demonstrated in 4 other children after a median follow-up of 10 months (range: 2.8 – 14.5 months). One custom-made and three modified devices occluded. Of these children three were treated with aspirin, one was treated with warfarin.

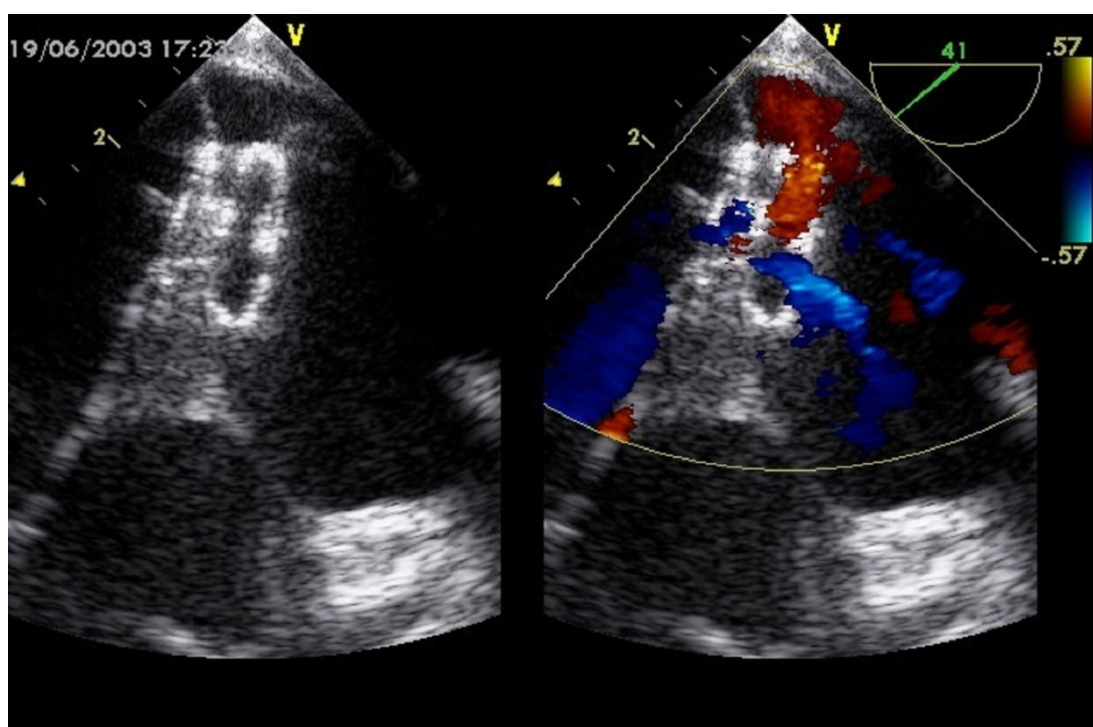


Figure 4.3.3 Transesophageal echocardiogram demonstrating left to right shunt across fenestration of the device.

4.3.4 Discussion

In this case series the implantation of fenestrated atrial devices was found to be feasible and clinically effective but long-term patency could not be guaranteed. In addition, the results indicate that anticoagulation with warfarin may be more effective than aspirin in preventing closure of the fenestration.

In patients with PHT improved survival has been reported in patients with a native atrial communication (303). The creation of an atrial septostomy is an established treatment in selected patients with intact atrial septum (112,118,119,296). The majority of patients in the present case series had PHT and were treated with advanced therapies, and all showed symptomatic benefit from the creation of an inter-atrial communication. Right heart failure and syncope were reduced. Previous studies have shown that atrial septostomy improves haemodynamics and prolongs survival in patients with severe PAH (112,117-121). In the paediatric population it may be especially useful in children presenting with syncope (40). Atrial septostomy allows augmenting systemic blood flow, potentially benefiting organs that lack the ability to autoregulate blood flow at low blood pressures. It has been suggested that a

mean arterial pressure of at least 60 mmHg is required to enable cerebral autoregulation (122). Interestingly, atrial septostomy has been demonstrated to be especially effective in abolishing syncope. Atrial septostomy could protect against syncope by maintaining systemic cardiac output, particularly when the pulmonary arterial pressure rises acutely (113). In the situation of desperately poor systemic blood flow, autoregulation may not be able to successfully maintain flow to some vital territories - an increase in total flow could permit autoregulatory processes to restore perfusion to vital organs. In addition, it has been suggested that atrial septostomy increases oxygen delivery to the tissues due to an increase in cardiac output, despite the modest reduction in systemic arterial oxygen saturation septostomy (113). However, a recent analytical study revealed this does not coincide with an improved tissue oxygenation and is merely a consequence of the way raw arterial oxygen delivery is defined (123). According to current guidelines atrial septostomy should, thus, be considered in PAH who continue to be symptomatic despite optimal medical PAH treatment (3).

Alternative indications for a fenestrated ASD device include reducing a left to right shunt in patients prone to develop pulmonary vascular disease. In fact, two of the patients had Down syndrome with significant left to right shunts through native atrial septal defects. Both patients had echocardiographic evidence of right ventricular volume overload and pulmonary hypertension (with mean pulmonary arterial pressures of 26 and 34 mmHg, respectively). It was felt, therefore, that complete closure of the atrial septal defect could be detrimental and potentially result in significant morbidity. It is recognized that children with Down syndrome have a reduced number of alveoli and pulmonary capillaries leading to rarefaction of the pulmonary vasculature (304). As a consequence, volume overload of the pulmonary circulation is detrimental and can result in pulmonary vascular disease (305,306). Though development and progression of pulmonary vascular disease is difficult to foresee in this patient group (307), use of a fenestrated device was recommended. Maintenance of a small communication is useful in offloading the right ventricle as pulmonary vascular disease progresses. Closure of a significant atrial septal defect with standard devices would make an atrial septostomy later in life technically impossible but insertion of a fenestrated device addressed this problem. This approach may also be taken with children without Down Syndrome but other risk

factors for progressive pulmonary vascular disease, such as chronic lung disease and pulmonary dysplasia (307,308).

It is perhaps no surprise that manufacturer modified devices seem superior to the operator modifications of standard atrial septal occluders. Fenestrations in 3 of the 4 operator modified devices were occluded on long-term follow-up in the current series. However, the mechanisms for closure of fenestrations are not entirely clear. The most likely reason for closure of fenestrations within atrial septal devices is tissue in-growth and thrombosis. In modifying custom-made devices, the created communication more often had an oval or uneven shape rather than the desired circular opening, probably due to the memory of the nitinol wires. Therefore, it can be speculated that a more turbulent flow through an uneven opening might have made fenestrations more susceptible to spontaneous closure. Interestingly, explanted prototype manufacturer modified devices have shown that the dimension of the fenestration may also reduce as a result of loss of metal shape memory (309). This observation provides the rationale for further catheter intervention to re-establish or improve patency of fenestrations. The use of endovascular stents is an obvious alternative but this technique adds complexity to the procedure and may lead to complications such as injury to the atrial wall.

The optimal anticoagulation policy for atrial septal occluders to prevent thrombotic adhesions to the surface of the device is unclear. At Great Ormond Street Hospital, younger children with PHT receive Aspirin, while older children are anticoagulated with warfarin as part of their standard treatment for pulmonary hypertension. Most of the children included in this series were already on such treatment at the time of the procedure. In this series 3 of the 4 children in whom the communication closed were on aspirin there were too few patients to draw a definitive conclusion.

In conclusion, implantation of fenestrated atrial devices is technically feasible and safe in children with PHT. However, continued patency of the fenestration is not assured. This study points out the need for manufactured shunt devices, providing a safe and effective possibility of treating patients who would benefit from such a device.

4.4 TRANSPLANTATION IN CHILDREN WITH PULMONARY HYPERTENSION

4.4.1 Background

Advanced medical therapies have transformed quality of life and outcome in patients with IPAH (89,131,288). Despite these advances in medical therapy, however the long-term outlook is poor (4,131). When medical therapies are exhausted lung (LTx) or heart-lung (HLT_x) transplantation remains the only option for end-stage disease. The outcome of pediatric lung/ heart-and-lung transplantation has improved recently as a result of increased experience and better regimens of immunosuppressant therapy. However, transplantation does not represent a cure, as it involves the risk of early and late rejection, bronchiolitis obliterans syndrome, drug related problems and lymphoproliferative disease. As a consequence, contemporary registry data suggested a median survival following pediatric lung/ heart-lung transplantation of 4.3 years (124). Deciding when to place a child on the active transplant list, therefore, remains a challenge. Optimal timing represents a balance between obtaining a donor organ in time, and being offered a graft while the child remains relatively well on medical treatment, bearing in mind the inherent risks of transplantation. At present there are no clear criteria for the optimal timing at which a child with IPAH should be listed for transplantation.

The aim of this retrospective case series was to review the clinical experience with children with IPAH referred for transplantation assessment between 1/2002-6/2007, when the UKSPHC was established. The characteristics and outcome of children listed for transplantation were compared with those of patients who were not listed in order to identify distinguishing features between the two groups.

4.4.2 Patients and Methods

All IPAH patients are regularly reviewed by the UKSPHC team. Those patients treated with advanced medical therapies, including continuous intravenous epoprostenol, are referred to the Transplantation Team for assessment. The decision as to whether or not a child should be listed for transplantation is based on the clinical judgement of both physicians and surgeons.

Data on children with IPAH who were, and who were not listed for transplantation were analysed retrospectively. The two groups were compared focusing on differences in age at diagnosis, weight, height, functional class (WHO 1-4), resting oxygen saturation (SpO_2), minimal SpO_2 during 6MWT, 6MWT distance, drug treatment, serum-BNP (Triage-test, Biosite), the forced expiratory volume in one second (FEV_1), functional vital capacity (FVC), semiquantitative echocardiographic assessment of right ventricular function (1=normal, 2=mildly, 3=moderately, 4=severely impaired), mean baseline pulmonary artery pressure (mPAP), mPAP/ mean aortic pressure (mAP) ratio, pulmonary vascular resistance index (PVRI) and the PVRI on vasodilator testing (100% FiO_2 and 20 ppm NO) and cardiac index.

After transplantation children were treated according to a standardized immunosuppressive regime. As a routine, induction immunosuppressive therapy with basiliximab is used and following transplantation a combination of tacrolimus, mycophenolate mofetil and steroid treatment is given. All patients undergo regular routine follow-up surveillance transbronchial biopsy according to a set protocol (310). Depending on the histopathological findings action is taken to treat early signs of rejection and prevent the potential onset of obliterative bronchiolitis.

Statistical analysis

For statistical analysis, WHO class and echocardiographic measures were considered as a categorical variable and the Spearman rank correlation was used. For all analyses a two-sided p-value of <0.05 was considered statistically significant. Statistical analyses were performed using the StatView 5.0 (Abacus Concepts, Berkeley, CA, U.S.A.) and MedCalc 8.2.1 (MedCalc Software, Mariakerke, Belgium) packages.

Table 4.4.1 Patient Demographics.

	All patients	Listed for Tx	Not listed	p-value
n (male)	14	7	7	
Gender (male/ female)	8 / 6	5 / 2	3 / 4	n.s.
Age at Dx (years)	3.7 (0.4-9.5)	4.5 (0.9-6.1)	3.0 (0.4-9.5)	n.s.
Age at follow-up (years)	8.6 (4.6-14.5)	7.6 (4.6-14.5)	9.0 (5.6-13.1)	n.s.
Weight (kg)	23.3 (13.5-41.1)	19.0 (13.5-35.4)	24.1 (17.0-41.1)	n.s.
Height (cm)	120 (92-155)	110 (92-155)	124 (110-151)	n.s.
FU after Tx	-	2.2 (0.21-2.39)	-	-
Age at FU (Tx patients)	-	7.4 (4.7-10.7)	-	-
Epo+Bosentan (+Sildenafil)	11 (3)	6 (1)	5 (2)	
Warfarin/ Aspirin	8 / 6	3 / 4	5 / 2	
Atrial communication (ASD/ BAS/ intact IAS)	8 / 4 / 1	4 / 3 / 0	2/ 4/ 1	

ASD = Atrial septal defect, BAS = Balloon atrial septostomy, cm = centimeter, Dx = diagnosis, Epo = Epoprostenol, FU = follow up, IAS = interatrial septum, kg = kilogram, n = number of patients, n.s. = not significant, Tx = transplantation.

Table 4.4.2 Patient Characteristics.

	All patients	Listed for Tx	No listed	p-value
WHO at assessment	2.9 (2-4)	3.5 (3-4)	2.5 (2-2.5)	0.0006
SO ₂ at rest	95 (83-100)	98 (86-98)	98 (83-100)	n.s.
6MWT minimal SO ₂	84 (52-93)	76.5 (52-82)	89 (84-93)	0.0001
6 MWT distance	269 (40-515)	154 (40-310)	330 (300-515)	0.0023
FEV1 %	81 (49-110)	94 (60-110)	73 (49-100)	n.s.
FVC%	81 (45-132)	87.5 (66-132)	78 (45-85)	n.s.
BNP (pg/ml)	74.0 (7.6-1210.0)	410 (7.6-1210.0)	37.6 (10.5-241)	n.s.
Echocardiographic RVF	2.8 (2-4)	3.0 (2-4)	2.0 (2-4)	n.s.
Mean PAP	64 (25-80)	66 (56-71)	64 (25-80)	n.s.
PAP/ AoP ratio	1.2 (0.5-1.9)	1.2 (1.1-1.5)	1.0 (0.5-1.9)	n.s.
RAP	7.7 (3-12)	9 (4-12)	6.5 (3-10)	n.s.
PVRI baseline (WU*m ²)	26.8 (12-44)	34.4 (13.5-39)	26.6 (12-49)	n.s.
PVRI vasodilator testing (WU*m ²)	24.5 (10.5-39)	34 (15.5-39)	14.6 (10.5-33)	0.03
Cardiac index (l/min/m ²)	1.9 (1.3-3.1)	1.9 (1.3-3.1)	2.0 (1.5-2.6)	n.s.

AoP = Aortic pressure, BNP = B-type natriuretic peptide, CI= Cardiac index, FEV1 % = Forced expiratory volume in one second, FVC% = Functional vital capacity, l= liter, m² = meter squared, min = minute, ml = milliliter, n.s. = not significant, PAP = Pulmonary artery pressure, pg = picogram, PVRI = Pulmonary vascular resistance index, RAP = Right atrial pressure, RVF = Right ventricular function, SO₂= saturation, Tx = transplantation, WHO = World Health Organization, WU = Wood units, 6MWT = Six-minute walk test.

4.4.3 Results

Since 2002 fourteen children (8 male) with IPAH were referred for transplantation assessment. The patient demographics are presented in Table 4.4.1.

All patients were treated with pulmonary hypertension-specific medicines such as epoprostenol and bosentan (dual endothelin-receptor-antagonist). Five patients also received sildenafil (phosphodiesterase-5-inhibitor). Seven children were listed for transplantation. The median time from diagnosis to listing was 3.6 years (1.4-9.3). Five children were subsequently transplanted (LTx n=3; HLTx n=2). Their median waiting time was 81 days (16-301) and the median age at transplantation was 5.4 (4.5-8.3) years. Two patients died on the active transplant list after being listed for 81 and 202 days, respectively. Seven children were considered to not be sick enough for listing, on a clinical basis. These patients remained under close clinical review and were reassessed for transplantation on a regular basis. All patients remained alive and stable on combination therapy.

Children who were listed for transplantation were in a worse functional class (WHO 3.5 vs 2.5; $p=0.0006$). These patients had a lower minimal oxygen saturation on exercise (76.5 vs 89%; $p=0.0001$) and the distance walked in six-minutes was lower compared to those not listed (154 vs 330m; $p=0.0023$). Echocardiographic RV-function was worse in those listed (4 vs 3; $p=0.03$), and pulmonary vascular resistance index (PVRI) was higher on vasodilator testing (34 vs 14.6 u.m ; $p=0.03$). The mean baseline PVRI of the listed patients was also higher than in those not listed although the difference was not statistically significant (Table 5.4.2). Age at diagnosis and at assessment/listing, weight, height, mPAP, mPAP/mAP ratio, cardiac index, resting SO_2 , FEV1%, FVC% and serum-BNP did not differ between the two groups as shown in Table 4.4.2.

The median postoperative mechanical ventilation time was 6 days (2-59), stay in the intensive care unit was 6 days (4-10) and total hospitalization time 24 days (16-81) in those children who underwent transplantation. The patient with the longest hospitalization time required a temporary tracheostomy due to slow weaning from ventilation and prolonged oxygen requirement. The patient, however, made a good recovery and is now doing well. In one child who underwent bilateral lung transplantation the atrial septostomy created before transplantation for recurrent

syncope became haemodynamically significant in the setting of a normal pulmonaryarterial pressure. The ASD was not closed at the time of transplantation, as the size of the ASD was underestimated on echocardiography due to equalisation of right and left atrial pressures and minimal net shunt in this setting. The child subsequently underwent surgical closure of the atrial septal defect 19 months following the transplantation after an attempt at interventional closure had failed.

All 5 transplanted patients were alive after a median follow-up of 2.8 (0.7-2.9) years, with a good functional outcome (WHO function class 1). None of the children has clinical evidence of obliterative bronchiolitis or post-transplant lymphoproliferative disease. Weight has improved in some children, but the change in z-score did not reach statistical significance ($p=0.06$; Figure 4.4.1) (175).

Table 4.4.3 Patients transplanted.

Time Dx to listing (years)	3.6 (1.4-9.3)
Age at admission to Tx list	5.9 (3.9-13.8)
Days on list	81 (16-301)
Age at Tx (years)	5.4 (4.5-8.3)
FU after Tx	2.2 (0.21-2.39)
Time ventilated	6 (2-59)
Days O ₂ post extubation	5 (0-9)
ICU stay	6 (4-12)
Hospitalized	24 (14-81)
CPB time	160 (130-243)
X clamp time	190 (0-243)
Age at FU (Tx patients)	7.4 (4.7-10.7)
FEV1 % post Tx	100 (82-103)
FVC% post Tx	109 (99.9-114)

CPB= Cardio-pulmonary bypass, Dx= Diagnosis, ICU=Intensive Care Unit, FEV1 % = Forced expiratory volume in one second, FVC% = Functional vital capacity, FU=Follow up, Tx=Transplantation, O₂=Oxygen, X clamp= Cross clamp

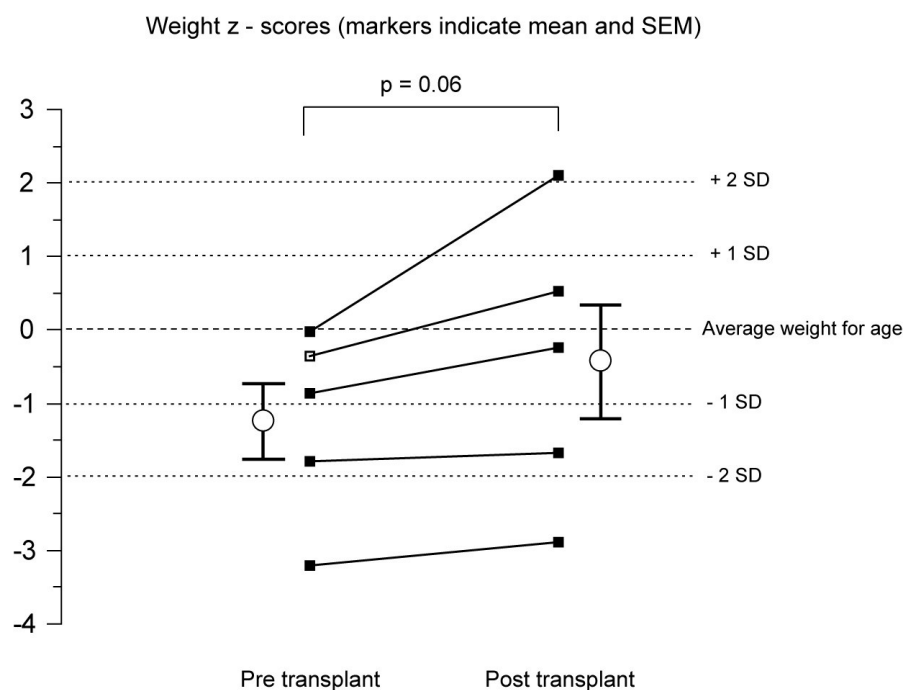


Figure 4.4.1 Thriving of children after transplantation

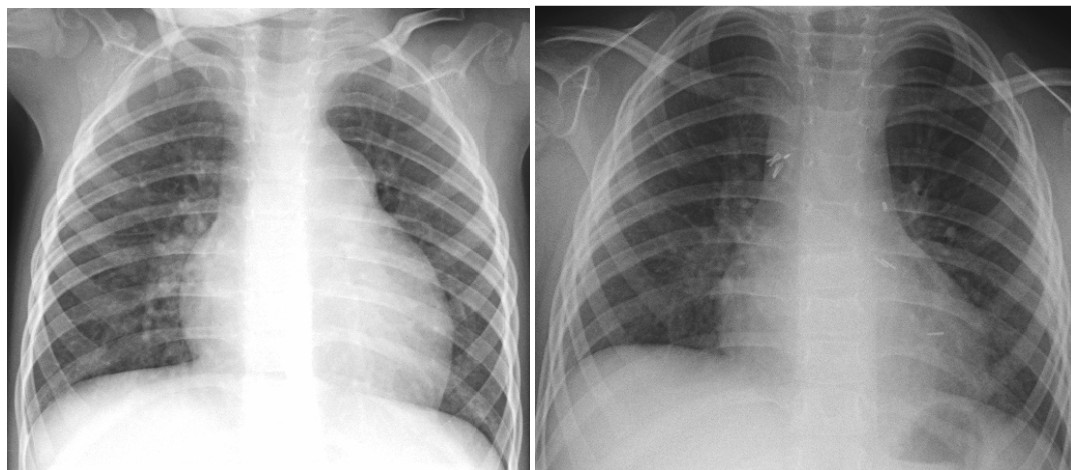


Figure 4.4.2 Example of improvement in radiographic appearance comparing pre and post transplantation chest X-rays in a child with IPAH. Left: Chest X- rays of a 5 year old boy, before (a) and 4 weeks after (b) bilateral lung transplantation showing in a) cardiomegaly with prominent central pulmonary arteries and b) a normal cardiac silhouette and pulmonary-vascular markings.

4.4.4 Discussion

The decision as to whether or not a child should be listed for transplantation was based on clinical judgement. All the IPAH patients included in the current study were on maximum medical therapy. This retrospective analysis showed that the children listed for transplantation had a worse WHO functional class, walked less far in 6 minutes, had a worse right ventricular function as judged by transthoracic echocardiography and a less favourable haemodynamic response to vasodilator testing at cardiac catheterisation. Five of the seven children listed underwent lung or heart-lung transplantation and are alive and well. In addition, all the children considered too well to be listed are still alive. Only the children who were listed but not transplanted died. All the transplanted patients have an exercise capacity comparable to their peers as judged by their parents are back at school or nursery.

There are no established criteria for the optimal time at which children with IPAH should be listed for transplantation, unlike other diseases (311-313). Children with IPAH would normally be considered for transplantation if death within 6 months to 2 years seems likely (314). In principle, the decision to list a child with IPAH for transplantation is similar to that used for adult patients, namely a poor short term prognosis/or a poor quality of life despite optimal medical therapy, but unlike objective criteria to guide clinicians are sparse in children. Survival probability models for adults have been developed, based on data obtained from the National Institutes of Health registry on IPAH and waiting times for single lung transplantation according to the United Network for Organ Sharing (315). The 6MWT and cardiopulmonary exercise testing have prognostic implications and cut-off values for 6MWT distance and peak oxygen consumption have been described (135,136). Adults whose exercise tolerance is less than these derived cut-off values are expected to have a survival benefit from transplantation. In children, however, the feasibility of doing a six-minute walk test and cardiopulmonary exercise testing is influenced by the child's age and level of understanding (316). In addition, the association between 6MWT distance and outcome is less clear in children. Although a good correlation of WHO functional class with outcome has been described in children listed for transplantation by other paediatric transplantation centres (317), categorization into a functional class is confounded by the parents' and clinicians' perception, particularly in younger children and may be misleading.

It has been suggested that haemodynamic evaluation – providing objective measurements – may be more helpful in assessing the need for transplantation for lung transplantation in children with IPAH. The product of the mean right atrial pressure and pulmonary vascular resistance may allow for the estimation of probability of death at 1 and 2 years after catheterization (318). This probability factor was derived by combining two previously known equations, one predicting survival in adults with PHT and one based on a retrospective study of a heterogeneous group of children with PHT (IPAH and congenital heart disease). There was a significant association between the predictive scores and death before transplantation (317). The major limitation of this approach is the necessity of carrying out cardiac catheterisation on patients with severe pulmonary hypertension (82,164) and the fact that this score was developed before the advent of oral advanced therapies or the widespread use of epoprostenol. Non-invasive approaches are clearly preferable but their prognostic value is limited at present. In the current study a semi-quantitative assessment of right ventricular function demonstrated a greater degree of functional impairment in those listed for transplantation. More refined techniques such as tricuspid annular excursion, tissue-Doppler imaging or speckle tracking echocardiography may prove to be more helpful in future (319,320), as may evaluation of right ventricular function by cardiac magnetic resonance imaging (321,322).

Especially in paediatric patients there is a shortage of donor organs and a long waiting time can be expected. Regular reassessment every 3-6 months is mandatory to ensure that cardiac function remains reasonable and a double-lung transplantation, rather than heart-and-lung-transplantation, is still feasible; as well as to re-evaluate clinical and nutritional status and to evaluate end-organ function. The current approach at Great Ormond Street Hospital London is to offer double-lung transplantation, not heart-and-lung-transplantation in the expectation that right ventricular function will recover.

The obvious limitations of this study are its retrospective nature and the relatively small number of patients included. It is, of course, impossible to know how those patients who were transplanted would have fared without transplantation.

In conclusion, this study emphasises the difficulties of assessing children with IPAH for lung transplantation and indicates the need to define objective listing criteria for these patients as part of multi-institutional efforts. Nevertheless, it is reassuring that clinical judgement appears to have been correct in deciding who should and should not have been listed for transplantation. Outcome after transplantation in contemporary children with IPAH has been encouraging. Though transplantation is not a cure, it represents a worthwhile choice, giving the child a good quality of life.

5. DISCUSSION

The clinical picture and outcome of severe PHT has been transformed by the introduction of new disease-specific therapies, but it is more difficult to manage children than adults with this condition. Evidence-based treatment algorithms have been developed for use in adults and more recently have been modified for use in children. Choice of therapy depends on an assessment of clinical status and, as outlined in Chapter 1 of this thesis, it is difficult to quantify clinical status and response to treatment in children. The traditional endpoints used in adult studies, such as change in exercise capacity or the six-minute walk test distance are often difficult to apply in a paediatric population. A further difficulty is defining ‘treatment success’ in patients with a chronic progressive disease. Gaining stability or avoiding further deterioration may well be regarded as treatment success in this setting, since pulmonary vascular disease is a progressive disease and a decline of exercise tolerance and progressive worsening of symptoms is to be expected. Nowadays, the major challenge for clinicians is to know how to risk stratify patients, how and when to adjust medication and when additional therapies, such as atrial septostomy or listing for transplantation, are required. In view of an anticipated longer waiting time for a suitable organ in children, particularly for younger children, outweighing the inherent risks of transplantation and the chance of getting an organ in time is challenging.

Taking advantage of the rare opportunity afforded by having a single UK centre for paediatric PHT this thesis attempts to clarify the potential clinical value of non-invasive prognosticators and to evaluate the two most aggressive forms of pulmonary hypertension therapy, namely epoprostenol and atrial septostomy in children with PAH. The results of these studies have indicated how best to proceed in evaluating and treating children with pulmonary hypertension.

Evaluation starts with describing the child’s clinical state. How ill and how incapacitated is the child. The WHO or NYHA Functional Classification designed for use in adults is also used in children, as it has been in the work presented in this thesis, but it has severe limitations when used in children. It does not take into

account age, development, size, or maturation. In future it will be important to design an age-specific functional classification for children.

A progressive reduction in exercise capacity is a feature of sustained PAH. Exercise capacity has been well studied in adults with PAH but not in children. Most randomized controlled adult trials in the setting of PHT have used the 6-minute walk test distance as a primary endpoint and it is an important parameter for guiding therapy. Unfortunately, the value of the 6-minute walk test distance is less well established in paediatric patients. It cannot obviously be used in young children but even in older children there are concerns that the test may be age dependent and affected by growth, maturation and level of understanding. The present study demonstrated that performing a 6MWT is feasible and can be done reliably in children as young as 4 years of age. The studies in this thesis aimed to establish normal values for the 6-minute walk test distance in UK school children between 4 and 11 years of age. They provide data against which the exercise tolerance of sick children can be compared. A limitation of the study is that only one walk test was performed by each child and so possible training effects could not be studied. Also, due to limitations in time and resources the number of children needed to create meaningful gender and age specific percentile curves for 6-minute walk test distance in UK children could not be recruited. In addition, the children were of different ethnicities, although mainly Caucasian, and ethnic specific data are needed.

As cardiopulmonary exercise testing with gas exchange monitoring is regarded by many as the gold standard for assessing exercise capacity the relationship between parameters of cardiopulmonary exercise testing and the 6-minute walk test distance was also investigated. We found that both tests are feasible and safe to perform, supervised by an experienced medical team. In adults the 6MWT is considered as a test, which assesses exercise capacity at sub-maximal level. The results of this thesis, however, demonstrate that in children the 6MWT distance actually reflects maximal rather than sub-maximal exercise in those who cannot walk more than 300 m in 6 minutes, or who cannot reach a peak oxygen consumption above 20 ml/kg/min. CPET should be considered as a complimentary test in children, particularly when their 6MWT is above the 300m threshold. Interestingly, in adults the current

European guidelines include a 6-minute walk test distance below 300 metres as one of the criteria for escalation of PHT therapy (3).

There were several limitations in the present studies. While most children at the UKSPHC regularly undergo a 6MWT only a limited number were referred for CPET, based on the treating physician's discretion. The patients included in this study may, therefore, be a biased sample. Furthermore, the predicted normal values of 6MWT used in the present study were derived from a publication in 564 children, published by Geiger in 2007 (160). The authors of this study employed a measuring wheel to assess the walk test distance and this represents a slight modification of the conventional technique of performing a 6MWT, as used in our studies. While it appears unlikely that this modification alters the results of the test significantly, this possibility cannot be excluded. We used the normal values reported by Geiger because the 6MWT studies on UK children were still ongoing at that time. Due to the limitations of the equipment (bicycle) we were unable to offer cardiopulmonary exercise testing to children with a body height of <140 cm. There is an ongoing effort to develop smaller machines, for example, toddlers' go-cars with computer simulators that are suitable for younger children who can cooperate with a breathing mask.

Symptoms and outcome in PHT are mainly determined by the ability of the right ventricle to cope with a chronically elevated afterload and this, in turn, affects left ventricular function. Therefore the study of biventricular function in children with PHT is important. Using tissue Doppler echocardiography, this thesis demonstrates that despite not being evident on conventional 2D and M-mode echocardiography, left ventricular systolic and diastolic performance is impaired in children with PHT. This finding probably reflects the detrimental impact of right ventricular dysfunction, dilatation and hypertrophy, on the left ventricle (i.e. ventricular-ventricular interaction). The work highlights interesting parallels with patients who have congenital heart disease. In tetralogy of Fallot, for example, left ventricular dysfunction – due to ventricular-ventricular interaction – was thought to be a simple bystander of right ventricular dilatation and systolic impairment (323). More recently it has emerged that left ventricular systolic dysfunction is independently related to an increased risk of sudden cardiac death in patients with tetralogy (324). As patients

with tetralogy of Fallot and PHT share important pathophysiologic features which increase the propensity for malignant arrhythmia, such as right ventricular enlargement, myocardial fibrosis and autonomic dysfunction (76,323,325-327), one may speculate that left ventricular dysfunction may be prognostically adverse in children with PHT. This question should be addressed in future studies. Speckle tracking echocardiography may provide additional insights into the pathophysiology of biventricular dysfunction. Tissue Doppler echocardiography provided important insights into cardiac physiology and pathophysiology in the present study, but experience shows that it has failed to establish itself in clinical practice over the last 15 years. In contrast, it appears that speckle tracking echocardiography has the potential to become part of clinical routine, mainly due to its ability to provide simple measures of global systolic function (328). This approach could be helpful. A recent study showed that global systolic strain – which can be obtained semi-automatically using contemporary echocardiography systems – is superior to ejection fraction in predicting prognosis in adult heart failure patients (329). Further studies should therefore, assess the prognostic value of these novel speckle tracking echocardiography derived measures of biventricular function in children with PHT.

Unfortunately, in the present echocardiographic study the follow-up time was short and therefore the number of endpoint events was insufficient to make it possible to investigate the prognostic value of tissue Doppler echocardiography. The choice of other possible prognostic markers was guided by the two most common modes of death in this population, namely progressive heart failure and sudden – presumably cardiac related – death (330).

Electrocardiographic parameters of autonomic dysfunction have emerged as powerful prognostic markers of sudden death in several cardiac conditions (212-216). As sudden death is not uncommon in PHT and the pathophysiologic substrate for malignant arrhythmias is likely present in the right ventricle of PHT patients (76) this study investigated the prognostic value of parameters of heart rate variability (HRV) in children with PHT. Parameters of HRV are objective markers and can be measured easily and repeatedly even in small children. Determination of HRV indicated that a low HRV is predictive of death or transplantation. An abnormal HRV has been shown to be a powerful and independent predictor of adverse

prognosis in paediatric patients with congenital heart disease (209-211), in adults with ischaemic heart disease (212-216) and in the general population (217). It reflects autonomic dysfunction and is related to the risk of malignant arrhythmias (217). The current study indicates that parameters of HRV carry prognostic information independently of 6-minute walk test distance. It is tempting to speculate that parameters of HRV may be more suited to predict arrhythmic events, while the 6-minute walk test distance relate to the risk of heart failure related complications. Therefore, the use of these non-invasive tools in combination in the setting of paediatric PHT is conceptually appealing and deserves further study. The main limitations of the present study are that HRV was not studied in consecutive children with PHT, but rather children who were referred for a Holter-ECG based on symptoms or resting-ECG evidence of arrhythmia. Therefore this is a biased sample. Future studies should include longitudinal studies of HRV in all children with PHT. It would be interesting to measure additional parameters of autonomic dysfunction such as heart rate turbulence and baro-reflex sensitivity, potentially providing supplemental prognostic information (331).

B-type natriuretic peptide is a recognized marker of myocardial dysfunction and is related to mortality in various cardiovascular cohorts (332,333). This thesis therefore investigated the prognostic value of BNP in children with PHT. It emerged that BNP levels were elevated in 60% of children with PHT. This proportion was higher in children with IPAHA than in children with associated PHT (73% versus 46 %, respectively). Although BNP was related to mortality and need for transplantation, it became apparent that BNP may not be sensitive enough to guide PHT therapy. While a high level of BNP should raise concerns about the optimization of medical therapy, a low BNP does not necessarily indicate that a patient is well and stable. The results of this study indicate that routine BNP measurement can be a useful adjunct to the assessment of clinical status but should not be used in isolation as independent screening tool to predict outcome. Repeat measures of BNP or NT-pro BNP in a longitudinal patient follow-up however, have been used in adults to assess response to therapy (334) and their prognostic value in paediatric PHT patients requires further investigation.

In summary, it is hoped that knowledge of the value and limitations of non-invasive prognostic markers such as exercise capacity, echocardiographic measures of ventricular function, HRV and BNP will eventually assist clinicians in selecting adequate treatment options for children with PHT and help to risk stratify patients. The need for further study is self-evident. It is unlikely that any single measure will prove to be the ideal prognosticating factor. Having optimised all current techniques, combining relevant material selected from specific investigations to produce a combined prognostic factor may be the way forward.

A further aim of this thesis was to evaluate certain treatment options in children with severe PAH, namely continuous intravenous epoprostenol treatment and atrial septostomy. This study shows that epoprostenol treatment is feasible in children, appears to slow disease progression and to improve survival. In addition, epoprostenol improves WHO functional class, exercise tolerance and ability to thrive in children with PAH, thereby improving quality of life despite the morbidity and potential complications associated with a continuous epoprostenol infusion. Thus epoprostenol represents an effective, feasible therapy with an acceptable morbidity in young children with severe pulmonary hypertension. The majority of children were also receiving sildenafil and an endothelin receptor antagonist. Therefore all the three signalling pathways known to be abnormal in pulmonary vascular disease were being modified. The use of aggressive combination therapy in children needs further study particularly with regard to whether or not it should be started when a child first presents with IPAH.

Creation of an atrial communication has been advocated to abolish syncope and unload the compromised right ventricle in patients with PHT. The retrospective review of a series of children who underwent atrial septostomy showed that this intervention is feasible in children with PAH and can improve symptoms. The procedure is only safe however, if the right atrial pressure is not high, as illustrated in one of our syncopal patients. The right atrial pressure was 19 mm Hg and the systemic arterial oxygen saturation fell from 98% at baseline to 58%. An Amplatzer ASD occluder device was inserted immediately, with no ill effects. The current guidelines recommend that atrial septostomy should not be done in patients presenting with a right atrial pressure above 20 mmHg (3). There is also an inherent

risk of creating too large a communication, inducing severe cyanosis. In the current study oxygen saturation fell on average from 97.8% before septostomy to 90% after 3–6 months. Fenestrated devices may reduce the risk of oversizing the atrial septostomy and the retrospective study described in this thesis showed that implantation of fenestrated devices is feasible and safe in children. However, custom-made as well as self-made fenestrated devices can close spontaneously with time. This study highlights the need for new, improved fenestrated devices. Given the far superior survival prospects of Eisenmenger patients compared to patients with IPAH (335) it is tempting to contemplate ways to create an “Eisenmenger-like” physiology in patients with IPAH. In addition to creating shunts non-invasively at atrial level, recently it has been suggested that IPAH patients (including children) with suprasystemic pulmonary arterial pressures may benefit from the creation of an anastomosis between the left pulmonary artery and the descending aorta (i.e. Potts anastomosis) (336,337). However, current knowledge on the long-term effects and the adequate dimensions of such shunts are limited and further research using analytical models, experimental animals and clinical data is required before this technique before considering its use in children with PHT. Operating on a child with a high pulmonary arterial pressure is extremely dangerous.

Deciding when to list a child for lung transplantation is difficult. Keeping the child well on medical treatment for as long as possible is desirable because the mean survival remains relatively poor, although it has improved over the last decade due to better immunosuppression strategies and post-transplant patient care,. Also, there are few donor organs available. This present study showed that the decision as to whether or not a child should be listed for transplantation is subjective and chiefly based on clinical judgement. In retrospect, a worse WHO functional class, lower 6-minute walk test distance, more impaired right ventricular function as judged by transthoracic echocardiography and a less favourable haemodynamic response to vasodilator testing at cardiac catheterisation emerged as the main criteria related to listing for transplantation. It is reassuring that, despite being a subjective decision based on a combination of parameters all the children considered too well to be listed are still alive while only children who were listed but not transplanted died. This suggests that the currently employed process of evaluating and risk stratifying

patients is effective but highlights the pressing need for more objective markers and defined criteria to allow comparison of outcomes between transplantation centres.

6. CONCLUSION

The studies presented in this thesis highlight that the optimal non-invasive risk marker for children with pulmonary vascular disease has yet to be found and the ideal treatment option for this devastating and progressive disease does not exist. There is no cure at the present time. Awareness of the limitations and weaknesses of prognostication markers should be helpful in improving patient care and in the design of future studies. Using combinations of different non-invasive markers is likely to be more helpful but further studies are required to create and validate risk models for children with PAH.

An ideal risk prognosticator should be non-invasive, highly specific, sensitive and cost-effective. One day there may be an ideal risk prognosticator but until then combining current, recognised prognosticating factors may help improve patient management, together with clinical judgement and regular re-evaluation of the patient. Ventricular dysfunction and heart failure are associated with considerable morbidity in the setting of PHT and are a principal cause of mortality. Unfortunately, traditional markers of ventricular dysfunction such as ejection fraction have clear limitations in the setting of right ventricular disease and alternative markers of right ventricular dysfunction should be sought. Assessment of longitudinal ventricular function, such as TAPSE, tissue Doppler imaging as used in Chapter 3 of the thesis or speckle tracking based parameters used alone or in combination with BNP measurements (a serum marker of ventricular dysfunction) may be a way forward. In view of the echocardiographic limitations in assessing right ventricular function, MRI may be a promising non-invasive alternative to quantify ventricular function and dimensions. The studies in this thesis have demonstrated left ventricular systolic dysfunction in children with PHT and MRI assessment should focus on left ventricular impairment and biventricular interaction. Technical advances in MRI are desirable to avoid the need for heavy sedation or general anaesthesia.

An accurate assessment of clinical status and quality of life is essential in guiding the management of a child. A functional classification designed specifically for children is needed, rather than trying to apply the WHO or NYHA classification designed for adults. Consideration of quality of life is important in a disease which cannot be

cured. Quality of life questionnaires (e.g. SF-6) or scores have been used in children with PHT (338). As expected, the scores for physical performance using the SF-6 questionnaire were low but the psychosocial scores were significantly higher. Comparing the answers and judgement of the older children who were asked to complete the questionnaire (e.g. SF-6) themselves and those filled in by their parents revealed interesting differences of perception. The children's judgement of their quality of life often seemed more optimistic than the parents' impression of the child's quality of life. Despite various assessment modalities, individual definition and perception of quality of life does differ tremendously. Perception can be influenced by religious, ethical and personal attitudes, which are beyond the scope of a scientific approach. Also, an individual's expectations change in the setting of chronic disease. The clinician should aim to provide children and parents with the best possible objective information, evidence and available scientific data. An empathic relationship with the families, ongoing support and comfort, link and introduction to support systems (such as the Pulmonary Hypertension Association UK and community networks) and, last but not least, an open mind to the individual patient's and family's needs and wishes should be paramount and should lead the counselling process.

Given the better survival prospects in the current era the focus of outcome measures should move from classical mortality related end-points, such as death and transplantation to morbidity using composite endpoints such as hospitalization, need for escalation of therapy or other parameters of clinical worsening. Quality of life and exercise capacity are additional important markers of morbidity. Exercise capacity has been extensively used as an endpoint in trials in adult patients with PHT, mainly in the form of 6-minute walk test distance. Using raw 6-minute walk test distance may be relatively unproblematic in adults, but as shown in the present thesis, the 6-minute walk test distance is age and gender dependent in normal children. In order to compare the 6-minute walk test distance of patients with normal values, it is necessary to have percentiles or at least the mean and standard deviations of normal values in order to calculate z-scores.

In view of the still devastating outlook and poor prognosis new medicines are urgently needed. Traditional randomized placebo controlled trials are unethical

however, because there are drugs on the market which give symptomatic improvement and increase survival. Alternative concepts and study designs are needed. Economic constraints are obvious, although potentially less dominant in paediatric practice compared to adult services. However, given the cost implications of disease targeting therapies, cost utility analyses are required and preliminary data are emerging in adult patients (339).

In conclusion, it is hoped that this thesis will contribute to a better understanding of pulmonary hypertension in children, advance studies on risk stratification and inform clinicians about treatment options in children with pulmonary hypertension.

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List of publications arising from the work in this thesis

Chapter 3

Lammers AE, Hislop AA, Flynn Y, Haworth SG.

Six minute walk test in healthy children.

Arch Dis Child. 2008; 93(6): 464-8.

Lammers AE, Diller GP, Odendaal D, Taylor S, Derrick G, Haworth SG.

Comparison of Six-minute Walk Test and Cardiopulmonary Exercise Test Performance in Children with Pulmonary Hypertension

Arch Dis Child 2010 - *in press*.

Lammers AE, Munnery E, Hislop AA, Haworth SG.

Heart Rate Variability Predicts Outcome in Children with Pulmonary Hypertension

Int J Cardiol. 2010; 142: 159-65.

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Prognostic value of B-type natriuretic peptide in children with pulmonary arterial hypertension.

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Chapter 4

Lammers AE, Hislop AA, Flynn Y, Haworth SG.

Epoprostenol treatment in children with severe pulmonary hypertension

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Lung transplantation in children with idiopathic pulmonary arterial hypertension.

Pediatr Pulmonol. 2010; 45: 263-9.