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Pulmonary hypertension in children

– Aspects of vasodilator therapy and novel imaging

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DEPT OF EXPERIMENTAL MEDICAL SCIENCE | FACULTY OF MEDICINE | LUND UNIVERSITY



Pulmonary hypertension in children

– Aspects of vasodilator therapy and novel imaging

Ida Jeremiasen



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DOCTORAL DISSERTATION

For the degree of Doctor of Philosophy (PhD) at the Faculty of Medicine,
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Abstract

Background: Pulmonary hypertension (PH), i.e. high blood pressure in the lung, can be a lethal condition. In children, PH is often associated with congenital heart defects (CHD) or developmental lung disease. Pulmonary vasodilator therapy is used in children, but guidelines are often based on results from clinical trials in adults and on expert opinion.

Aims: To investigate whether pulmonary vasodilator therapy is beneficial in children with PH secondary to complex CHD in different stages of palliation for single ventricle physiology. To map the use of pulmonary vasodilator therapy in young children in Sweden, and particularly for children born preterm and diagnosed with bronchopulmonary dysplasia (BPD). To provide a detailed morphological 3D visualisation of pulmonary veno-occlusive disease in children and investigate potential differences in mutation carriers versus non-carriers.

Methods: Medical records were used to study the effects of pulmonary vasodilator therapy retrospectively in children with PH and CHD with single ventricle physiology. Data on paediatric prescription of pulmonary vasodilators in Sweden during a 10-year period were obtained from national registries and additional details were studied for children born preterm. Synchrotron-based phase-contrast imaging, combined with histology, immunohistochemistry and *in situ* hybridisation, was used to visualise pulmonary vascular changes three-dimensionally in pulmonary veno-occlusive disease.

Results: Saturations improved and pulmonary artery pressures decreased in children in different stages of single ventricle physiology treated with pulmonary vasodilators. The paediatric use of vasodilators in Sweden was explored, and sildenafil as monotherapy was found to be the most commonly used. BPD in children born preterm and CHD were the most common associated conditions. In children born preterm suffering from BPD, vasodilator treatment was often initiated without prior catheterisation. Synchrotron-based 3D imaging provided unique details and demonstrated different pulmonary vascular remodelling in pulmonary veno-occlusive disease in an eukaryotic translation initiation factor 2 α kinase 4 (*EIF2AK4*) mutation carrier compared to a non-carrier.

Conclusions: Pulmonary vasodilator therapy appears to be safe to use in children and positive effects were seen, but the extent of treatment is substantial, even in very preterm children, despite a lack of evidence of its efficacy. Synchrotron-based phase-contrast 3D imaging is a valuable tool for understanding vascular remodelling in different types of PH and, by using this technique, we confirmed differences in sporadic versus heritable pulmonary veno-occlusive disease.

Key words: Pulmonary hypertension, children, vasodilator therapy, synchrotron, bronchopulmonary dysplasia, pulmonary veno-occlusive disease, congenital heart defect

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Pulmonary hypertension in children

– Aspects of vasodilator therapy and novel imaging

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Cover photo by Ida Jeremiasen. Severely diseased pulmonary arteriole from a patient with pulmonary veno-occlusive disease.

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MADE IN SWEDEN 

To Martin, Hugo and Jakob

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Papers included in this thesis

Jeremiasen I., Tran-Lundmark K., Idris N., Tran P-K., Moledina S. Pulmonary Vasodilator Therapy in Children with Single Ventricle Physiology: Effects on Saturation and Pulmonary Arterial Pressure. *Pediatric Cardiology*. 2020;41(8): 1651-1659. **(Paper I)**

Jeremiasen I., Naumburg E., Westöö C., Weismann C.G., Tran-Lundmark K. Vasodilator therapy for pulmonary hypertension in children: a national study of patient characteristics and current treatment strategies. *Pulmonary Circulation*. 2021;11(4):1-8. **(Paper II)**

Jeremiasen I., Tran-Lundmark K., Dolk M., Naumburg E. Outpatient prescription of pulmonary vasodilator therapy to preterm children with bronchopulmonary dysplasia. *Acta Paediatrica*. 2023;112:409-416. **(Paper III)**

Jeremiasen I., Peruzzi N., Lampei E., Meyer S., Akyürek L.M., Gebre-Medhin E., Mutgan C., Neubert L., Jonigk D., Galambos C., Tran-Lundmark K. Synchrotron-based phase-contrast micro-CT combined with histology to decipher differences between hereditary and sporadic pediatric pulmonary veno-occlusive disease. In manuscript **(Paper IV)**

Papers not included in this thesis

Norvik C., Westöö C.K., Peruzzi N., Lovric G., van der Have O., Mokso R., **Jeremiasen I.**, Brunnström H., Galambos C., Bech M., Tran-Lundmark K. Synchrotron-based phase-contrast micro-CT as a tool for understanding pulmonary vascular pathobiology and the 3-D microanatomy of alveolar capillary dysplasia. *Am J Physiol Lung Cell Mol Physiol*. 2020;318: L65–L75.

Westöö C., Norvik C., Peruzzi N., van der Have O., Lovric G., **Jeremiasen I.**, Tran P-K., Mokso R., De Jesus Perez V., Brunnström H., Bech M., Galambos C., Tran-Lundmark K. Distinct types of plexiform lesions identified by synchrotron-based phase-contrast micro-CT. *Am J Physiol Lung Cell Mol Physiol*. 2021;320: L000–L000.

Abbreviations

ACKR1	Atypical chemokine receptor 1
<i>ACVRL1</i>	Activin A receptor like type 1
ASD	Atrial septal defect
aSMA	Alpha smooth muscle actin
<i>BMPRI1B</i>	Bone morphogenetic protein receptor type 1B
<i>BMPR2</i>	Bone morphogenetic receptor type 2
BNP	Brain natriuretic peptide
BPD	Bronchopulmonary dysplasia
cAMP	Cyclic adenosine monophosphate
<i>CAVI</i>	Caveolin 1
CCB	Calcium-channel blockers
cGMP	Cyclic guanosine monophosphate
CHD	Congenital heart defect
CMR	Cardiac magnetic resonance imaging
CPET	Cardiopulmonary exercise testing
CT	Computerised tomography
CTEPH	Chronic thromboembolic pulmonary hypertension
CXR	Chest X-ray
DNA	Deoxyribonucleic acid
ECG	Electrocardiogram
<i>EIF2AK4</i>	Eukaryotic translation initiation factor 2 α kinase 4
<i>ENG</i>	Endoglin
ERA	Endothelin receptor antagonist
ET	Endothelin
ETA	Endothelin type A
FC	Functional class
FUEL	Fontan Udenafil Exercise Longitudinal
GA	Gestational age

GOSH	Great Ormond Street Hospital
HIV	Human immunodeficiency virus
HLHS	Hypoplastic left heart syndrome
HPAH	Heritable pulmonary arterial hypertension
HR	High resolution
HRCT	High-resolution computerised tomography
IP/PGI2	Prostacyclin/glandin I2
IPAH	Idiopathic pulmonary arterial hypertension
<i>KCNK3</i>	Potassium two pore domain channel subfamily K member 3
LFT	Liver function test
LVEF	Left ventricular ejection fraction
MRI	Magnetic resonance imaging
mLAP	Mean left atrial pressure
mRNA	Messenger ribonucleic acid
mPAP	Mean pulmonary arterial pressure
NO	Nitric oxide
<i>NOTCH3</i>	Neurogenic locus notch homologue protein 3
PAH	Pulmonary arterial hypertension
PAP	Pulmonary arterial pressure
PAWP	Pulmonary artery wedge pressure
PCH	Pulmonary capillary haemangiomas
PDA	Patent ductus arteriosus
PDE-5	Phosphodiesterase-5
PDE-5i	Phosphodiesterase-5 inhibitor
PH	Pulmonary hypertension
PHVD	Pulmonary hypertensive vascular disease
PVOD	Pulmonary veno-occlusive disease
PVR	Pulmonary vascular resistance
PVRi	Pulmonary vascular resistance index

Qp	Pulmonary flow
RNA	Ribonucleic acid
SGA	Small for gestational age
sGC	Soluble guanylate cyclase
<i>SMAD9</i>	Suppressor of mothers against decapentaplegic 9
SNQ	Swedish Neonatal Quality Registry
STARTS-1	Sildenafil in Treatment-Naive Children, Aged 1-17 Years, With Pulmonary Arterial Hypertension
SWEDCON	SWEDish registry of CONgenital heart disease
<i>TBX4</i>	T-box transcription factor 4
TCPC	Total cavo-pulmonary connection
TGF- β	Transforming growth factor-beta
VQ	Ventilation/perfusion
WHO	World Health Organization
WU	Wood units

Introduction and background

As a paediatric cardiologist, I diagnose and treat children suffering from pulmonary vascular disease. The underlying conditions differ from those seen in adults, but current paediatric treatment guidelines are still largely based on studies performed in adult patient populations. This thesis project focused on pulmonary hypertension (PH) in children, and studied the extent of use and effects of pulmonary vasodilator treatments. A novel technique to visualise the pulmonary vasculature three-dimensionally was also used, in combination with other methods, to explore the vascular remodelling in heritable versus sporadic pulmonary veno-occlusive disease (PVOD).

Pulmonary hypertension

PH in children, i.e. elevated blood pressure in the lungs, is a rare condition. Estimates suggest a prevalence of approximately 20 cases per million children¹. The definition of PH in children was updated recently and is now defined as a mean pulmonary artery pressure (mPAP) of > 20 mmHg in children aged > 3 months²⁻⁴. For a definite diagnosis, the pressure must be measured by right heart catheterisation. However, in children, who are small and often fragile, indirect measurements from cardiac ultrasound are often used to diagnose PH. Clinical features of paediatric PH are nonspecific, but often patients describe having chest pain and shortness of breath, particularly during exercise, and episodes of syncope.

PH is a complex and often chronic condition with high morbidity and mortality. The aetiologies in children differ compared to those in adults and the onset can occur at any age. Globally, left-sided heart failure and lung diseases causing hypoxia are the most common causes of PH⁵. Children more often have pulmonary arterial hypertension (PAH) of idiopathic or familial origin or PH associated with congenital heart disease or developmental pulmonary disease⁶.

A paediatric classification of PH was first introduced in 2011⁷. In 2013, the paediatric aetiologies were incorporated into the adult World Health Organization (WHO) PH classification⁸, which was later updated in 2019². PH is categorised into five main groups and further subgroups (**Table 1**). Group 1 PH, PAH, includes precapillary pulmonary vascular conditions with an elevated pulmonary vascular

resistance (PVR) of >3 Wood units (WU) and a pulmonary artery wedge pressure (PAWP) of <15 mmHg. See section: “*Diagnostics in pulmonary hypertension/cardiac catheterisation*” for explanations of PVR and PAWP. Group 2 PH is caused by left heart disease leading to a postcapillary vascular condition. Group 3 PH occurs secondarily to lung disease, Group 4 PH occurs as a result of pulmonary arterial obstructions, and Group 5 PH is associated with other multifactorial mechanisms, including complex congenital heart defects (CHD). In the latter three groups, there is often a combination of pre- and postcapillary vascular pathology.

Table 1

The WHO classification of pulmonary hypertension. LVEF, left ventricular ejection fraction; PAH, pulmonary arterial hypertension; PH, pulmonary hypertension; PVOD/PCH, pulmonary veno-occlusive disease/pulmonary capillary haemangiomatosis; WHO, World Health Organization.

Main group	Subgroup	
1		PAH
	1.1	Idiopathic PAH
	1.2	Heritable PAH
	1.3	Drug- and/or toxin-induced PAH
	1.4	PAH associated with: 1.4.1 Connective tissue disorder, 1.4.2 HIV infection, 1.4.3 Portal hypertension, 1.4.4 Congenital heart disease, 1.4.5 Schistosomiasis
	1.5	PAH long-term responders to calcium channel blockers
	1.6	PAH with overt features of venous/capillary (PVOD/PCH) involvement
	1.7	Persistent PH of the newborn
2		PH due to left heart disease
	2.1	PH due to heart failure with preserved LVEF
	2.2	PH due to heart failure with reduced LVEF
	2.3	Valvular heart disease
	2.4	Congenital/acquired cardiovascular conditions leading to post-capillary PH
3		PH due to lung diseases and/or hypoxia
	3.1	Obstructive lung disease
	3.2	Restrictive lung disease
	3.3	Other lung disease with restrictive/obstructive pattern
	3.4	Hypoxia without lung disease
	3.5	Developmental lung disorders
4		PH due to pulmonary artery obstructions
	4.1	Chronic thromboembolic PH
	4.2	Other pulmonary artery obstructions
5		PH with unclear and/or multifactorial mechanisms
	5.1	Haematological disorders
	5.2	Systemic and metabolic disorders
	5.3	Others
	5.4	Complex congenital heart disease

This thesis explored paediatric PH in general, but with a particular focus on PVOD (subgroup 1.6), PH secondary to bronchopulmonary dysplasia (BPD, subgroup 3.5) and complex CHD (subgroup 5.4).

Pulmonary anatomy

Normal pulmonary vascular anatomy

The pulmonary artery originates from the right ventricle and delivers desaturated blood to the lung. Human lungs consist of three main lobes in the right lung and two in the left. Each lung lobe is divided further into segments that have many small secondary lung lobules, which are approximately 1-2.5 cm in size (**Figure 1**). Every secondary lobule has its own distal pulmonary artery for delivery of desaturated blood and bronchiole, dividing into between three-to-five terminal bronchioles, for air supply. A secondary lung lobule is built by up to 30 acini supplied by smaller arterioles and respiratory bronchioles, with 1500-4000 alveoli in each acinus. The alveoli are surrounded by small capillaries responsible for exchange of oxygen and carbon dioxide in the lung. The secondary lung lobules are divided by interlobular septa, which contain the pulmonary veins and lymphatic vessels. The pulmonary veins carry the oxygenated blood back to the heart. It is worth noting that the pulmonary vascular tree is considerably shorter compared to that of the systemic circulation, but it still receives the same amount of blood. Therefore, low vascular resistance and high compliance are essential for the correct functioning of the lungs.

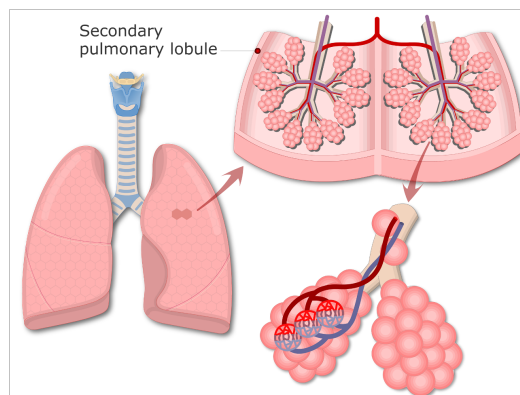


Figure 1

Pulmonary structure and vasculature. The left lung is divided into two, and the right lung into three main lobes. Each lobe consists of segments and numerous secondary pulmonary lobules supplied by a main pulmonary artery and bronchiole. Each secondary pulmonary lobule consists of smaller acini which contain the capillary network and alveoli for gas exchange. *Illustration taken from www.getbodysmart.com*

The bronchial circulation in the lung consists of small arteries arising from the aorta and from intercostal arteries. Blood flow through the bronchial arteries represents 1-3% of the total cardiac output⁸. Bronchial arteries mainly supply the bronchi with oxygenated blood, but they also oxygenate the walls of larger vessels through the vasa vasorum, a network of small vessels in the adventitia of pulmonary arteries and veins⁹. The main part of the bronchial circulation drains to the left atrium of the heart through connections with pulmonary veins, but approximately one-third drains through systemic veins to the right atrium.

A normal pulmonary artery has three layers, as is the case with all arteries in the body¹⁰⁻¹² (**Figure 2**). The innermost intima consists of a single layer of endothelial cells; the middle layer, the tunica media, contains smooth muscle cells, and the outermost tunica externa or adventitia consists of collagen fibres, the vasa vasorum and a variety of cell types including fibroblasts and macrophages among others. Larger arteries are muscularised and have an internal and an external elastic membrane lining the medial layer. These arteries are always found together with an airway of the same size. Smaller arteries lack an internal elastic membrane, are termed arterioles (<100 µm), and are found in alveolar ducts. Arterioles only have a thin or no muscular layer, and capillaries are normally totally non-muscularised.

The normal pulmonary venous structure is similar to that of the arteries, but without well-defined elastic membranes, and normal veins have a thinner muscular media layer. Smaller venules in acini are difficult to distinguish morphologically from arterioles. Therefore, it is important to be able to track them to where they connect to larger veins, which are always located in the interlobular septa.

Foetal lung development

Lung development starts early in embryogenesis with the formation of two lung buds, and is completed at approximately 4 weeks of gestation¹³. A series of branching follows and, by 17 weeks, a rudimentary structure of the *conducting bronchioles* has formed. During the canalicular stage, which occurs between 16-26 weeks of gestation, the early *respiratory bronchioles* are formed. Alveolar ducts are formed during the so-called saccular stage, which starts at approximately 24 weeks and continues until term. Alveolarisation is a late process, starting around 36 weeks' gestation and continues postnatally until the age of 10 or more years. At the same time as the airways develop, pulmonary arteries are formed from the right ventricle, which then branch and run along the bronchioles. It is essential to bear these stages of development in mind when considering pulmonary morbidity as a result of premature birth.

Vascular remodelling in pulmonary hypertension

The vascular remodelling in CHD-associated PH and idiopathic PAH was first described histologically and classified by Heath and Edwards in 1958¹⁴. Later, modifications to the classification were made, among others by Wagenvoort and Wagenvoort^{12, 15}.

The vascular remodelling process is complex, but arterial remodelling can be summarised in terms of muscular medial hypertrophy in combination with concentric intimal thickening in pulmonary arteries adjacent to the airways (**Figure 2**). The lumen is narrowed. Cells in the neointima are often gradually replaced by fibrosis. In some cases, the arterial wall develops fibrinoid necrosis, inflammatory cells may be seen, and the internal elastic membrane is often destroyed. The smaller arterioles (in alveolar ducts) become muscularised and will resemble the larger arteries. The resulting increased pressure leads to right heart remodelling, and eventually dysfunction.

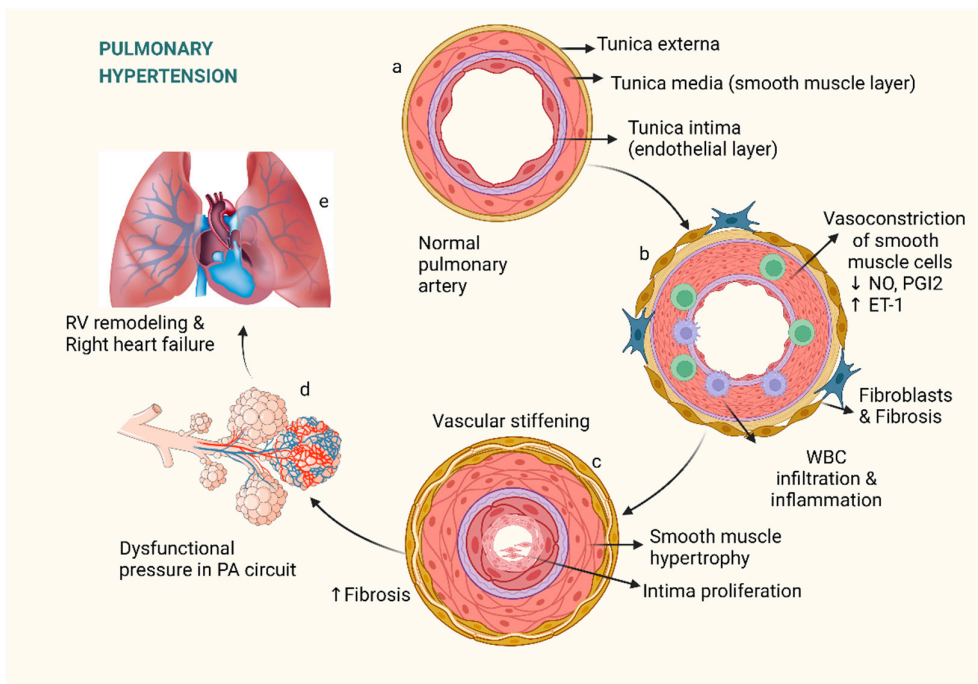


Figure 2

Pulmonary arteries and right heart remodelling in pulmonary hypertension (PH). A. Normal pulmonary artery B and C. Vascular remodelling in PH leading to increased pressure (D) and right heart failure (E). NO, nitric oxide; PGI₂, prostaglandin I₂; ET-1, endothelin-1; RV, right ventricle; PA, pulmonary artery; WBC, white blood cells. *Illustration by Beckman T et al. Pharmaceuticals, 2022, licensed by <http://creativecommons.org/licenses/by/4.0/>*¹⁶.

With progression of disease, plexiform lesions may develop. Plexiform lesions are vascular structures connected to severely diseased pulmonary arteries with several lumens in which the blood can pass through. Some of the lesions are occluded pulmonary arteries with re-canalisation, and these lesions can resemble an organised thrombus, but may be distinguished from the latter by the destroyed internal elastic membrane. With a thrombus, this is not usually seen. Previous studies have also suggested that at least some plexiform lesions are connected to the bronchial circulation¹⁷. Recently, we studied plexiform lesions in idiopathic PAH using synchrotron-based phase-contrast micro-computerised tomography (CT) imaging, and a novel classification of four types of different plexiform lesions was described¹⁸. Type 1 lesions are found in supernumerary arteries, monopodial branches of pulmonary arteries without accompanying bronchioles, and connect to the vasa vasorum; type 2 are tortuous transformations of intrapulmonary bronchopulmonary anastomoses; type 3 appear at abrupt ends of pulmonary arteries, and type 4 represent total or partly occluded arteries with re-canalisation (**Figure 3**). Three-dimensional imaging confirmed that type 1 and 2 plexiform lesions have connections to the bronchial circulation and may function as a pressure off-loading mechanism, as also suggested previously by others¹⁷.

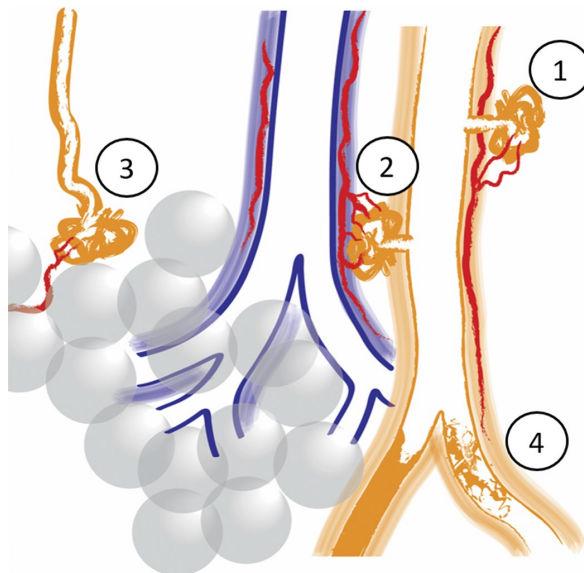


Figure 3

Description of plexiform lesions. Type 1 are found in monopodial branches from pulmonary arteries connecting to the vasa vasorum; type 2 seem to be the result of tortuous transformation of intrapulmonary bronchopulmonary anastomoses; type 3 are found at abrupt ends of distal pulmonary arteries; and type 4 are occluded pulmonary arteries with re-canalisation. Airway in blue, pulmonary artery in orange, bronchial artery in red. *Illustration with permission from Westöö et al., Am J Physiol Lung Cell Mol Physiol. 2021*¹⁸.

The vascular lesions in PH are pre-capillary in PAH compared to post-capillary in PVOD and left heart disease. Plexiform lesions are not seen in patients with PVOD¹⁹, but commonly in PAH. Right heart remodelling and failure are the result of the increased pulmonary artery pressure, and will be a consequence in both situations.

Angiogenesis, i.e. development of new blood vessels, is a normal process in developing tissue, but is also stimulated in pathological conditions, such as PH²⁰. The most frequently described mechanism for angiogenesis is *sprouting*, which is stimulated by hypoxia-induced angiogenetic growth factors. In sprouting, a vascular sprout is formed from a pre-existing vessel and forms a new vessel. *Intussusceptive angiogenesis* is another mechanism for angiogenesis in which a pre-existing vessel divides into two separate lumens. This process occurs more quickly than sprouting. The rare lung developmental disorder, alveolar capillary dysplasia, has been shown to have a high prevalence of intussusceptive angiogenesis and it has also been reported to occur in patients with PVOD²¹.

The true process and underlying mechanisms of vascular remodelling in PH are still not known fully, and most likely differ significantly between the different types of PH.

Diagnosics in pulmonary hypertension

There is a risk of delayed diagnosis of PH because the condition is rare and, in addition, clinical symptoms are often non-specific and variable among individuals. The diagnosis of PH should be considered in a young child with signs of “failure to thrive”, decreased WHO functional class, and symptoms such as dyspnoea at rest or during exercise, syncope and chest pain. These are the most frequently described signs and symptoms of PH⁶. Functional class (I-IV) is a classification of the severity of symptoms during activity, where functional class I is characterised by mild symptoms, and class IV refers to severe impact on activity, with symptoms at rest.

Diagnostic tools for PH include both non-invasive and invasive examinations and imaging²². One example of a diagnostic algorithm for children is shown in **Figure 4**. Although PH is defined as a mPAP of >20 mmHg measured by means of heart catheterisation, the individual investigation should be adapted to each unique child by the responsible paediatric cardiologist. In some cases, catheterisation is considered to be too high a risk to carry out on a small fragile child, and medications are initiated without a prior invasive pressure measurement. This approach probably occurs more often in children compared to adults. Lung biopsy, a high-risk procedure, does not form part of the suggested algorithm in children and is contraindicated in most cases.

The most frequently used investigations are discussed in more detail below.

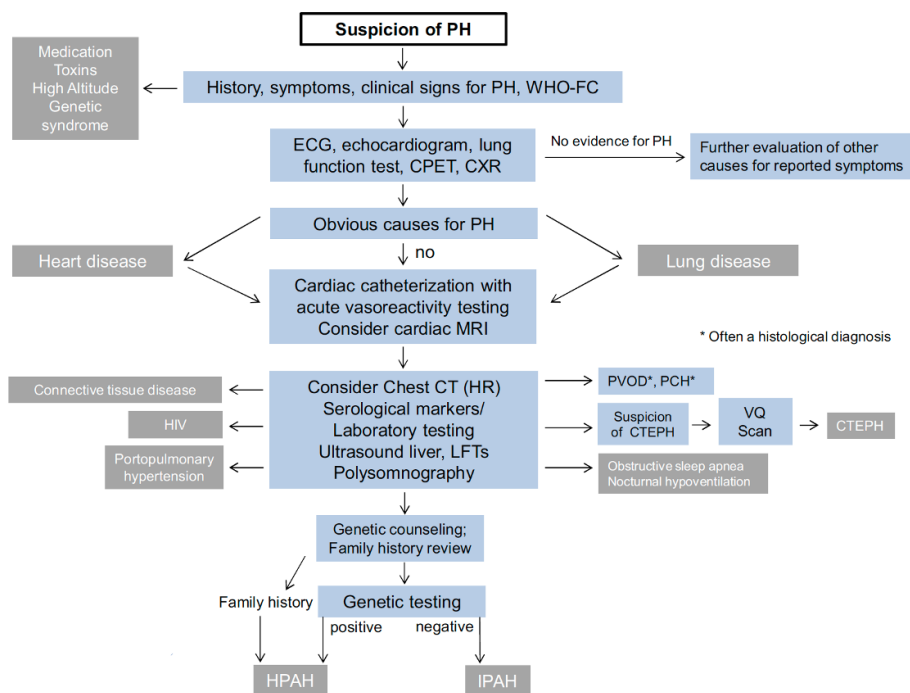


Figure 4

Diagnostic algorithm for children with a suspicion of PH. CPET, cardiopulmonary exercise testing; CT, computerised tomography; CTEPH, chronic thromboembolic pulmonary hypertension; CXR, chest X-ray; ECG, electrocardiogram; FC, functional class; HIV, human immunodeficiency virus; HPAH, heritable pulmonary arterial hypertension; HR, high resolution; IPAH, idiopathic pulmonary arterial hypertension; LFT, liver function test; MRI, magnetic resonance imaging; PCH, pulmonary capillary haemangiomas; PVOD, pulmonary veno-occlusive disease; VQ, ventilation/perfusion; WHO, World Health Organization. Reprinted with permission from Hansmann et al., *J Heart Lung Transpl.* 2019².

Electrocardiogram

Electrocardiogram (ECG) in long-standing PH reveals signs of right heart hypertrophy that are not specific to the condition. Arrhythmias may occur, but are not common. The precordial leads show tall R-waves in V1-V3, corresponding deep S-waves in V4-V6, and occasionally delayed intraventricular conduction with wide QRS complexes. The limb leads show right atrial enlargement with tall P-waves predominantly in the inferior leads and a right deviation of the electrical axis (**Figure 5**).

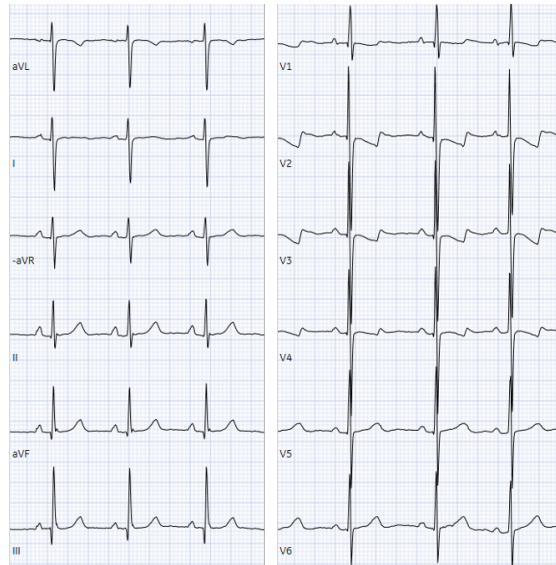


Figure 5

Electrocardiogram from a 6-year-old child with pulmonary hypertension due to pulmonary veno-occlusive disease showing signs of right heart hypertrophy with prominent P waves, right deviation of the electrical axis, prominent R waves in V1-V3 and corresponding deep S waves in V4-V6. *Illustration by the author.*

Echocardiography

Echocardiography is a non-invasive tool that uses ultrasound to evaluate the right ventricular adaptations to PH. As described in the expert consensus statement by Koestenberger *et al.*, an echocardiographic study should include an estimation of the PAP from the velocity of the tricuspid insufficiency, measurement of the pulmonary regurgitation jet, flattening of the interventricular septum, eccentricity index of the left ventricle (this becomes D-shaped in patients with PH), measurement of the increased ratio between the right and left ventricular diameter, and assessment of whether there is rapid acceleration velocity in the pulmonary artery doppler flow²³. The longitudinal systolic evaluation of the right ventricle is measured through the movement of the atrioventricular plane. The right atrium will be enlarged, the ventricle hypertrophied and, in late stages of the disease, the function will also be affected and the ventricle dilated. Echocardiography is also important to rule out left heart disease as a cause of PH.

Chest X-ray

A total of 90% of adult patients with PH have an abnormal chest X-ray with typical signs of right heart enlargement and prominent central pulmonary arteries, but more sparse distal arteries. In PVOD or PH secondary to left heart disease, the chest X-ray may show signs of pulmonary oedema and/or septal thickening.

Computerised tomography

CT of the chest is easily accessible, except for the need for sedation in smaller children, and with modern CT the amount of radiation transmitted is almost negligible. Chest CT is used mainly in PH to exclude anatomical obstructions, PVOD, parenchymal disease (for example BPD) or thromboembolic disease, and is used widely. Conventional CT angiography with intravenous contrast medium is a superior method of imaging arteries and veins. High-resolution CT (HRCT) is used without intravenous contrast medium and is a technique to enhance image resolution. HRCT is perfect to assess the pulmonary parenchyma and presence of interstitial disease. The two techniques complement each other.

Cardiac catheterisation

Cardiac catheterisation is the gold standard for diagnosing PH, and its use is indicated in most PH patients for haemodynamic measurements, to determine disease severity and confirm the diagnosis. A catheter is usually placed via the femoral vein and advanced to the right heart to measure the atrial and right ventricular pressure, and then advanced further to the pulmonary artery to measure the mPAP. As mentioned previously, a mPAP of >20 mmHg is considered diagnostic for PH²⁻⁴. By advancing the catheter as far as possible in a pulmonary artery until it is wedged in a small distal branch, the pulmonary artery wedge pressure (PAWP), an estimation of the left atrial pressure, can be measured, as shown in **Figure 6**. The PAWP is normally $<10-12$ mmHg. The PAWP is normal (or at least <15 mmHg) in pre-capillary forms of PH, such as idiopathic and hereditary PAH, and in PVOD, but is increased (>15 mmHg) in PH secondary to left heart disease.

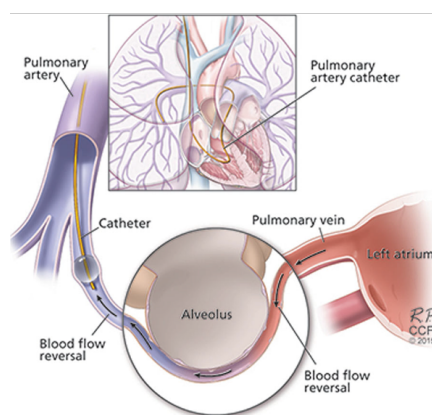


Figure 6

Right heart catheterisation to measure the pulmonary artery wedge pressure. The catheter is placed as far as possible into the pulmonary artery into a wedged position. *Illustration from Khirfan et al., Resp Research. 2019, <https://doi.org/10.1186/s12931-018-0969-7>, <http://creativecommons.org/licenses/by/4.0/>*

In order to calculate the pulmonary and systemic blood flow, the *Fick method* is used, whereby the difference in oxygen concentration and assumed oxygen consumption are factors in the equation. The *thermodilution method* is an alternative for cardiac output calculation, whereby the change in temperature over time is recorded, after injecting cold saline. It is important to distinguish pressure from resistance. Pulmonary vascular resistance (PVR) is calculated from the modified Ohm's law, i.e. $R(\text{resistance}) = V(\text{voltage})/I(\text{current})$:

$$\text{Resistance (PVR)} = \frac{\Delta\text{Pressure (mPAP} - \text{mLAP)}}{\text{Flow (Qp)}}$$

In children, PVR is usually expressed in relation to body surface area as indexed WUs, i.e. mmHg/L/min/m². A normal indexed PVR should be <2 WU. An indexed PVR of >3 WU is pathological and typical in pre-capillary PH (for example, PAH). The vasodilators oxygen and nitric oxide (NO) are often used to test pulmonary vasoreactivity during catheterisation. This is a valuable tool to assess responsiveness to vasodilator therapy in PH patients, and is also used for risk stratification prior to cardiac surgery.

General anaesthesia is often required in children undergoing cardiac catheterisation, which increases the risk of this type of investigation. In selected cases, such as very fragile and small children (<2-5 kg), it is reasonable to postpone or even omit cardiac catheterisation and, instead, rely on other diagnostic tools².

Genetic testing

Genetic variants are found in patients with hereditary PAH and knowledge is constantly expanding for WHO group 1 PH²⁴. For other groups of PH, the role of genetic testing is still uncertain. Eight genetic variants have been found to be associated with childhood idiopathic and familial PAH, and were described in 2016 in an expert consensus statement by Pattathu *et al.*: activin A receptor like type 1 (*ACVRL1 [ALK1]*), bone morphogenetic protein receptor type 1B (*BMPR1B*), bone morphogenetic receptor type 2 (*BMPR2*), caveolin 1 (*CAV1*), endoglin (*ENG*), potassium two pore domain channel subfamily K member 3 (*KCNK3*), neurogenic locus notch homologue protein 3 (*NOTCH3*) and suppressor of mothers against decapentaplegic 9 (*SMAD9*)²⁴. The first five variants are associated strongly with PH, while the latter three still require further investigation. Pathological variants (mutations) in the gene for *BMPR2* are the most common. They are associated with familial PAH in approximately 75% of patients, and found in 25% of cases of PAH which had been classified as idiopathic. Childhood onset of PAH is common in *BMPR2* mutation carriers. Another variant, the eukaryotic translation initiation factor 2 α kinase 4 (*EIF2AK4*) bi-allelic mutation, has been found exclusively in

patients with PVOD and was present in approximately 29% of participants in a PVOD cohort²⁵. Recently, the genetic variants in T-box transcription factor 4 (*TBX4*), and the less common *BMP9* and *BMP10*, have been added and are also associated with childhood onset of PAH²⁶⁻²⁹. The *TBX4* variant is associated with small patella syndrome. The overall genetic correlation in PH patients between genotype and phenotype has not been elucidated fully. In hereditary PAH with *BMPR2* mutations, the penetrance was 27% but, with a three-fold higher prevalence of PAH in females compared to males, the estimates were adjusted to 41% and 14%, respectively³⁰. Genetic counselling is important to guide families affected by PAH in treatment and follow-up.

Medical therapy for pulmonary hypertension

Before initiating treatment in a child with PH, a risk stratification process should be performed and the patient categorised as having a low or high risk of the condition, based on a combination of clinical symptoms and diagnostic investigations. An example of a risk stratification tool is shown in **Table 2**.

Table 2

Classification of risk into low or high risk depending on clinical and diagnostic investigations. BNP, brain natriuretic peptide; CMR, cardiac magnetic resonance imaging; PVRi, pulmonary vascular resistance index; WHO, World Health Organization; WU, Wood units.

Determinants of risk	Low risk	High risk
Clinical evidence of right failure	No	Yes
Progression of symptoms	No	Yes
Syncope	No	Yes
Growth	Normal	Failure to thrive
WHO functional class	I, II	III, IV
Serum proBNP	Normal	Elevated
Echocardiography or CMR	Minimal right heart enlargement and normal systolic function	Severe right heart enlargement and systolic dysfunction
Cardiac catheterisation	Positive vasoreactivity test among other parameters	Severely increased PVRi > 15 WU/m ² among other parameters

Pulmonary vasodilator therapy

Pulmonary vasodilators act on smooth muscle cells in pulmonary arterioles, mainly through three different pathways: the NO, endothelin and prostacyclin pathways (**Figure 7**)^{16, 31}. They can be taken orally, through inhalation, intravenously or subcutaneously, depending on disease severity and type of vasodilator. The short half-life of some drugs necessitates the use of continuous infusion. Pulmonary

vasodilators are well tolerated in paediatric patients with few adverse effects. It is important to note that pulmonary vasodilator therapy is mainly a symptomatic treatment that protects the right ventricle, relieves symptoms, and prolongs survival to some extent. It has, however, not been proven to modify the vascular remodelling process in humans.

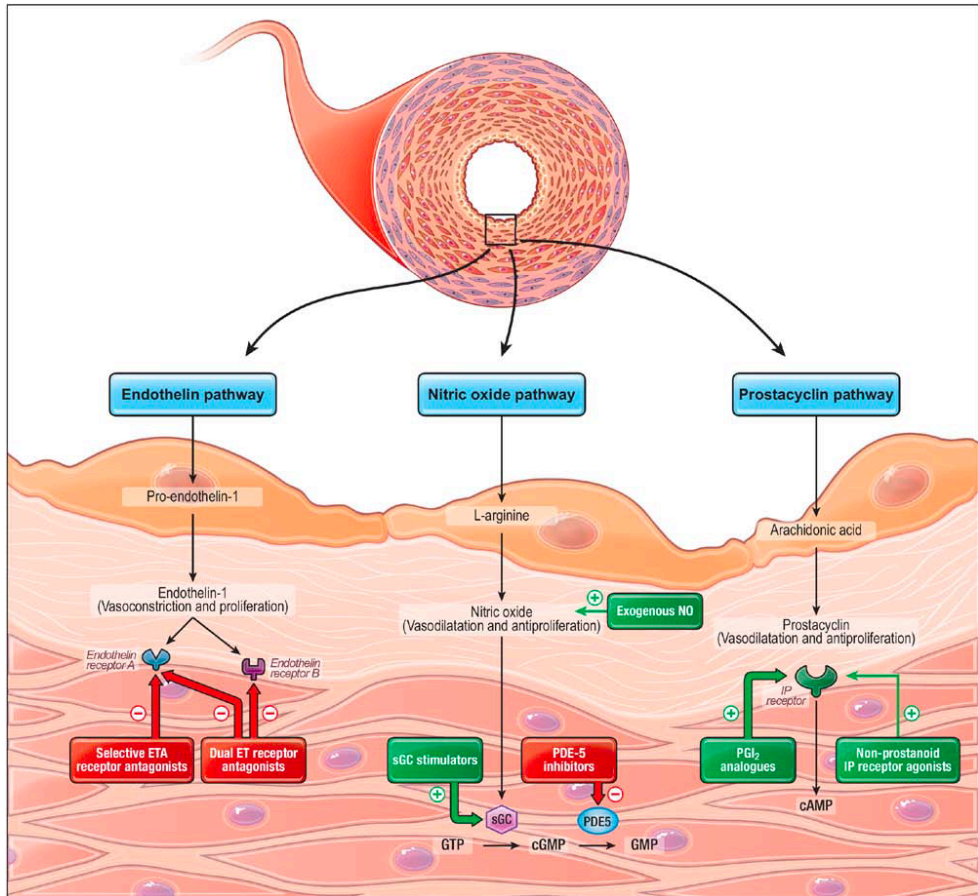


Figure 7
The three main pathways (endothelin-1, nitric oxide and prostacyclin) for pulmonary vasodilator therapy in pulmonary hypertension. cAMP, cyclic adenosine monophosphate; ET, endothelin; ETA, endothelin type A; IP/PGI₂, prostacyclin/glandin I₂; cGMP, cyclic guanine monophosphate; PDE-5, phosphodiesterase type 5; sGC, soluble guanylate cyclase. *Reprinted with permission from Humbert et al., Circulation. 2014*³¹.

Endothelin pathway

Endothelin-1 (ET-1) is produced by endothelial cells and acts through the endothelin receptors A and B as a potent vasoconstrictor with proliferative effects on smooth

muscle cells. Bosentan, and the more novel macitentan, act as dual **endothelin receptor antagonists (ERA)** on endothelin receptors A and B, resulting in reduced ET-1 signalling and hence vasodilatation. Ambrisentan is a selective type A ERA with a lower risk of liver injury as an adverse effect, compared to bosentan.

Nitric oxide pathway

NO, produced by L-arginine, activates soluble guanylate cyclase (sGC), leading to increased cyclic guanosine monophosphate (cGMP). cGMP acts as a potent vasodilator on smooth muscle cells and is metabolised by phosphodiesterase-5 (PDE-5). The most commonly used oral pulmonary vasodilator in children is sildenafil, a **PDE-5 inhibitor (PDE-5i)**. Inhibition of PDE-5 leads to increased levels of cGMP and hence vasodilatation and possibly also reduced proliferation of smooth muscle cells. More novel PDE-5i include tadalafil, vardenafil and avanafil, of which only tadalafil has been approved for paediatric use in PH in Sweden. Another more novel pulmonary vasodilator is riociguat, an **sGC stimulator**. Riociguat stimulates sGC receptors directly and also sensitises the enzyme sGC for NO, leading to increased levels of cGMP and vasodilatation.

Prostacyclin pathway

The third pathway is the prostacyclin pathway with its mediator cyclic adenosine monophosphate (cAMP). Prostacyclin is produced by endothelial cells from arachidonic acid via prostacyclin synthase and binds to prostaglandin receptors. cAMP is increased by prostacyclin binding to prostaglandin receptors, leading to vasodilatation. Patients with PH have decreased levels of prostacyclin synthase, prostacyclin metabolites and prostacyclin receptors in the lungs. Epoprostenol is the most widely used **prostacyclin analogue**, but is no longer available on the Swedish market. It must be given as a continuous intravenous infusion (half-life of 3-5 min) with careful monitoring because abrupt interruption of the medication may result in life-threatening rebound PH. Examples of other prostacyclin agonists include iloprost for inhalation, treprostinil for subcutaneous or intravenous infusion, and the more novel selexipag for oral use. The latter is a **non-prostanoid receptor agonist**, structurally distinct from prostacyclin, but with the same mechanism of action.

Pulmonary vasodilators are used in paediatric PH, but guidelines are often still based on experience derived from adult clinical trials and on expert experience and opinion. Few randomised, controlled paediatric studies are available, because PH is such a rare and heterogenous disease. Paediatric studies are important because of a different disease course and distribution of aetiologies in children compared to those in adults, and because potential toxicities and optimal dosing have not been studied widely³². Initiation of oral single or combination therapy with PDE-5i and/or ERA is recommended and approved in guidelines in paediatric patients diagnosed with low- or intermediate-risk PAH – the only clear guideline at present². For high-risk

patients in PH group 1, PAH triple therapy including a prostacyclin analogue is recommended. In many cases, pulmonary vasodilators are still used off-label in children. **Table 3** summarises the currently available drugs on the Swedish market for treating pulmonary hypertension.

Table 3

Pulmonary vasodilator drugs currently available on the Swedish market. PDE-5i, phosphodiesterase-5 inhibitor; sGC, guanylate cyclase stimulator; ERA, endothelin receptor antagonist. *Information from www.fass.se 2023.*

Generic	Commercial example®	Administration	Mechanism of action	Approved for paediatric use
Sildenafil	Revatio, Sildenafil	Oral	PDE-5i	Yes
Tadalafil	Adcirca	Oral	PDE-5i	Yes
Riociguat	Adempas	Oral	sGC	No
Bosentan	Tracleer	Oral	ERA	Yes
Ambrisentan	Ambrisentan	Oral	ERA	No
Macitentan	Opsumit	Oral	ERA	No
Treprostinil	Remodulin	Intravenous, subcutaneous or inhalation	Prostacyclin analogue	No
Iloprost	Ilomedin	Inhalation	Prostacyclin analogue	No
Selexipag	Uptravi	Oral	Non-prostanoid receptor agonist	No

Other medical therapies

Oxygen

Prior to, or in parallel with, the initiation of pulmonary vasodilator therapy, other treatments are sometimes administered. Oxygen is a potent vasodilator and a class IIb recommendation in paediatric patients with an oxygen saturation of <92%. It is complicated to administer oxygen outside hospital and, therefore, it is used mainly in the management of the acute or end-stage phases of the disease.

Calcium-channel blockers

Calcium-channel blockers (CCB) are used mainly for their antihypertensive effect on the systemic circulation, but they also affect the pulmonary vasculature. CCBs act on Ca²⁺ channels in smooth muscle cells by modulating influx and thereby reducing vasoconstriction. A good long-term response with high-dose CCB treatment has been shown to occur in approximately 5% of adult patients with PAH³³. Acute vasoreactivity testing with NO and oxygen during cardiac catheterisation must be performed before initiation of CCB treatment to ascertain that the patient is a good candidate for such treatment³⁴. The response criterion for

this testing is a reduction in mean PAP of >10 mmHg to an absolute value of <40 mmHg. Responders to acute vasodilator testing showed a mortality of 6%, compared to 45% in non-responders, at 5-year follow-up³⁵. A reason to be cautious and restrict CCB treatment to responders is the risk of cardiac ventricular dysfunction with these drugs. This risk is not seen with other pulmonary vasodilator therapies.

Diuretics

Diuretics are used to reduce volume overload arising from heart defects with a left-to-right shunt or to treat pulmonary oedema. As a result of high PVR, patients with PH may be preload-dependent and, to avoid any potential impact on cardiac function, caution is required when administering diuretic treatment.

Anticoagulation

Anticoagulation may be used as additional therapy in selected patients with PH, but the benefit in children is unclear. Individual assessment should be made, depending on whether the patient is considered to be hypercoagulable, or is prone to bleeding complications².

Conditions associated with pulmonary hypertension

This thesis focuses on PH associated with complex CHD and the developmental lung disorder BPD, both common causes of PH in children. The thesis also explores the more uncommon condition of PVOD. Below is a description of these selected conditions.

Congenital heart defects: single ventricle physiology

Approximately 1 in 100 children are born with a CHD. Most of these conditions are mild and do not require any intervention, or resolve with time as the child grows. Some CHDs will be cured by surgical correction. A small number of CHDs are complex and these patients will require life-long surveillance and, in many cases, repeated surgeries for palliation.

Single ventricle physiology and palliation

Children with different types of heart defects in which only one of the cardiac ventricles can carry the cardiac output, are palliated to so-called “single ventricle physiology”. The only existing ventricle in a single ventricle heart could be of right, left or ambiguous (undetermined) morphology (**Figure 8**).

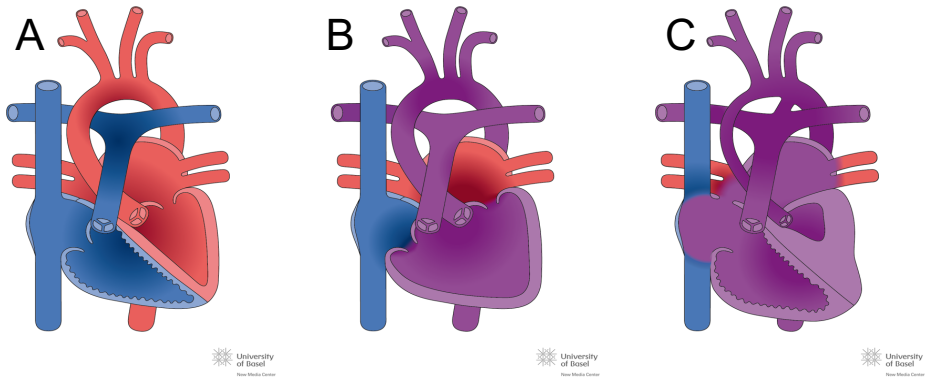


Figure 8

Examples of single ventricle hearts. A. Normal anatomy for comparison. B. Single ventricle heart with undetermined morphology of the ventricle. C. Single ventricle heart with mitral atresia and hypoplastic left heart syndrome (HLHS), in which the ventricle that needs to carry the cardiac output is of morphological right origin. Illustrations taken from <http://www.chd-diagrams.com>

As a result of the heterogenicity, every child is unique and the most accurate treatment choice is often a challenge to implement.

Figure 9 describes the staged surgical approach to attain a state of single ventricle physiology in a patient with a single ventricle heart. At birth, most of these children will require cardiac surgery to ensure adequate blood flow to both the pulmonary and systemic circulations. In some of these cases, a so-called Norwood procedure will be required, which involves making a shunt to the pulmonary circulation, and repairing the systemic outflow tract (**Figure 9A**). The shunt is connected from the right ventricle to the pulmonary artery (Sano shunt), as illustrated, or from the right subclavian artery to the pulmonary artery (Blalock-Taussig shunt) to allow desaturated blood to reach the pulmonary circulation. When the child reaches an age of approximately 4-6 months, the next surgical stage is a Glenn procedure (**Figure 9B**). At this time, the superior vena cava vein is connected to the pulmonary circulation, as a result of which venous blood from the cranial part of the body can reach the pulmonary circulation directly. Total cavopulmonary connection (TCPC) is the third and, for single ventricle physiology creation, the last surgical stage. The inferior vena cava vein is connected to the pulmonary circulation via an extracardiac tunnel (**Figure 9C**) and the heart is thereby completely bypassed by desaturated blood, which can now reach the pulmonary circulation directly. The surgery is usually performed at an age of 1.5-3 years in order to allow the pulmonary circulation to adapt and be able to allow the passive flow. At this age, this is the first time that the child will have full saturation.

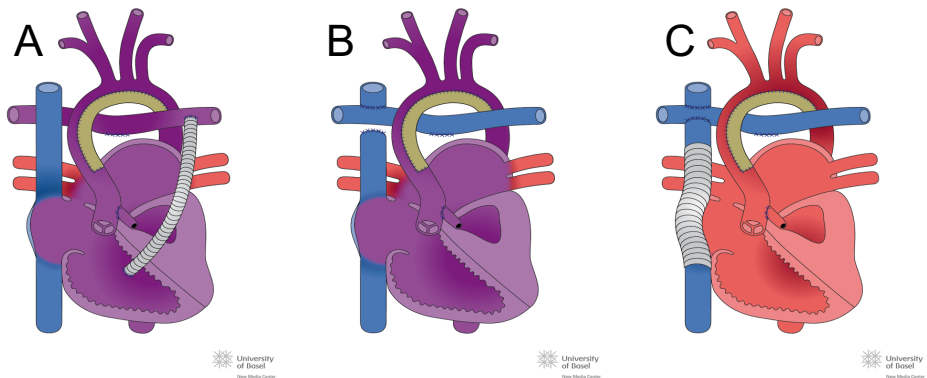


Figure 9

Univentricular/single ventricle staged surgery. A. Norwood procedure with Sano shunt. B. Glenn procedure. C. Total cavopulmonary connection. *Illustrations taken from <http://www.chd-diagrams.com>*

Morbidity and mortality have improved since the start of the modern TCPC approach in the early 1990s. Early postoperative mortality after TCPC is now reported to be as low as 0.5%, but long-term morbidity from, for example, PH, arrhythmias, sudden cardiac death, ventricular failure and protein-losing enteropathy, are problems that require medical care in approximately a few percent of cases, to up to 20% in reported studies³⁶. Patients with single ventricle hearts also report lower health-related quality of life compared to healthy individuals^{37, 38}. TCPC palliation enables survival for patients, but requires life-long surveillance.

Pulmonary vascular disease in single ventricle physiology

The pulmonary circulation in single ventricle physiology is dependent on a passive flow and, therefore, a low pulmonary vascular resistance is important. The risk of pulmonary vascular disease increases over time with loss of the normal pulsatile flow, and even a mildly elevated mean PAP can be deleterious in patients with single ventricle physiology. Loss of pulsatility has been associated with endothelial dysfunction³⁹. The vasoconstrictor ET-1 has also been shown to be overexpressed in failing single ventricle patients⁴⁰. Other factors for the risk of elevated pulmonary pressure are abnormal development of the pulmonary vasculature as a result of reduced flow in foetal life, or maldistributed flow causing patchy pulmonary hypoplasia after shunt surgery, and mechanical obstruction from abnormal vascular connections or from surgical scarring⁴¹. A pressure of more than 15 mmHg (compared to the definition of PH of a pressure of >20 mmHg) and/or combined with impaired ventricular function is considered to increase morbidity^{42, 43}. Therefore, treatment with pulmonary vasodilator therapy is an attractive option as part of the management of these children. So far, PDE-5i are used most commonly, but ERA may be alternatives in view of the current knowledge of the underlying

vascular pathology. Complex CHD is included in WHO group 5 PH. The true prevalence of significant pulmonary vascular disease in complex CHD is not known, but eventually most patients will suffer from some degree of pulmonary vascular pathology.

In summary, complex CHD is a heterogenous and rare group of heart conditions in children, and the development of pulmonary vascular disease often complicates the management of these patients. The true prevalence and underlying mechanisms of PH in complex CHD are still not understood fully, and the benefits or potential risks of pulmonary vasodilator therapy require further investigation.

Bronchopulmonary dysplasia

BPD is the most common pulmonary condition in preterm children and is a developmental lung disease associated with premature birth⁴⁴. BPD was first described in the 1960s, and was originally defined as an oxygen demand at 36 weeks of corrected gestational age (GA) or at 28 days of postnatal age⁴⁵. Modifications refined the diagnosis into mild, moderate or severe categories, depending on the ventilatory support required at 36 weeks of GA⁴⁶. Recently, an even more refined definition was suggested, whereby the mode of respiratory support is defined, regardless of oxygen use⁴⁷. This definition provides a better assessment of the severity of the disease, and its long-term prognosis. Chest X-rays of patients with BPD show typical signs of partly hyperinflated lungs in combination with widespread dense opacities (**Figure 10**).



Figure 10

Chest X-ray from a preterm child at 37 weeks of gestational age diagnosed with severe BPD with typical radiological findings of hyperinflated lungs and widespread dense opacities. *Picture adapted from www.radiologykey.com*

The incidence of premature birth in Sweden, defined as delivery before 37 weeks of gestation, is approximately 4%⁴⁸. The incidence of BPD has increased as the survival of extremely preterm children has improved, and the estimated incidence of BPD is almost 80% for children born at 22-24 weeks of gestation, and 20% for those children born after 28 weeks of gestation⁴⁹. Numbers depend on inclusion criteria and vary between studies.

Pathophysiology and risk factors for BPD

BPD is characterised by disturbed lung development, leading to immature alveolar and vascular structures (**Figure 11**). Surfactant is a substance that lowers surface tension in alveoli, and helps to keep them open at exhalation. Before the introduction of surfactant about 30 years ago, the pathological changes in patients with BPD were even more severe. The term “new BPD” refers to the time after the introduction of surfactant administration.

The underlying cause of BPD, and the subsequent increased risk of PH, is multifactorial. Prematurity increases the risk of ceased alveolar development, especially in children born at a GA of less than 28 weeks. Intrauterine growth restriction is also a risk factor, and children born small-for-gestational age (SGA) have been found to have a doubled risk of BPD⁵⁰. Furthermore, decreased angiogenesis in BPD patients may contribute to a hypoplastic pulmonary vascular tree. Infections, both antenatal, but specifically postnatal sepsis, have also been associated strongly with an increased risk of BPD⁵¹. Postnatal management also influences the risk of BPD. Mechanical ventilation with high pressure and large tidal volumes increases the risk of damage to the immature lung, and oxygen therapy stimulates the formation of cytotoxic metabolites, which the antioxidant pathways of the preterm child cannot handle. High ventilatory support avoiding mechanical ventilation is a promising management strategy that aims to reduce the pulmonary ventilation/perfusion mismatch commonly seen in patients with BPD⁵².

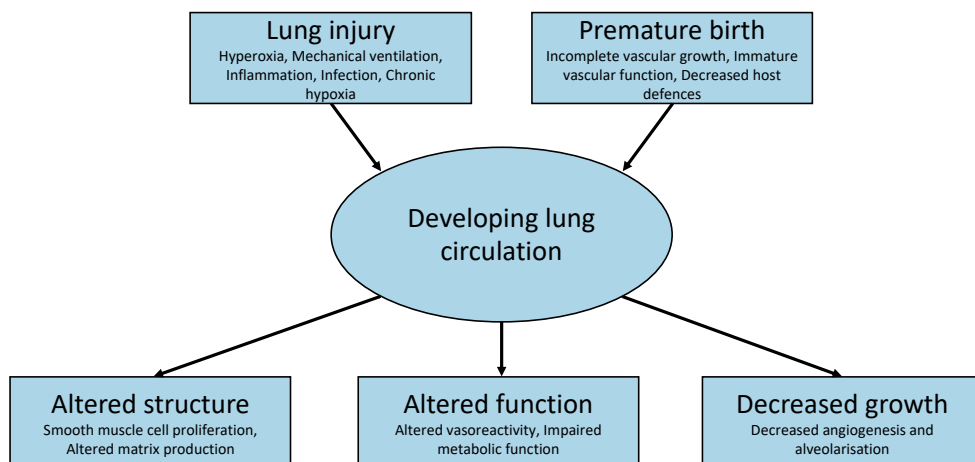


Figure 11

Summary of common abnormalities affecting the pulmonary circulation in patients with bronchopulmonary dysplasia, predisposing to the development of pulmonary hypertension. *Illustration produced by the author.*

Pulmonary hypertension in BPD

PH in patients with BPD, particularly the severe form of the disease, is associated with considerable morbidity and mortality and is included in the WHO group 3 PH. Mortality up to 40% the first 2 years of life has been reported, with the highest risk of death occurring during the first 6 months of life⁵³.

The PH in patients with BPD is believed to be caused by the arrest of pulmonary vascular growth, in combination with an increased PVR, as a result of the hypoxaemia that occurs in BPD patients. Studies have reported an incidence of PH of 18-42% in children with BPD, and as much as 60-80% in extremely preterm children (born at 22-24 weeks' gestation)⁵⁴⁻⁵⁶. Signs of PH on echocardiography have been detected as early as 7 days of age in BPD patients, and screening for PH in such children has been recommended⁵⁶. These findings indicate that PH may have already started to develop at an early stage after.

A patent ductus arteriosus is common in preterm children. The increased shunt flow to the lungs has not been considered to increase the risk of PH in patients with BPD⁵⁷. However, this has been debated and the recommendation to wait for shunt closure of a patent ductus arteriosus should be discussed further and studied in detail.

Management and outcome in BPD

Treatment strategies for BPD aim to minimise the lung damage and support the patient's well being. The administration of surfactant, plus less ventilatory and oxygen support, combined with the use of antenatal steroids to promote lung maturation, are probably the most important measures identified so far in managing the acute phase of the disease. The potential positive effects of pulmonary vasodilator therapy for PH in patients with BPD have not been studied extensively enough. Some symptomatic effects could be expected but, as described in **Figure 11**, most of the underlying mechanisms of vascular injury are not improved by administering pulmonary vasodilators. BPD is still a condition that is associated with considerable morbidity, both in the short and long term. Many children with BPD develop asthma, recurrent infections and decreased lung function with reduced exercise capacity. Even though mortality rates have improved over time, the long-term morbidity and prevalence of BPD seem to have remained stable⁵⁸.

In summary, BPD is a common condition in preterm children, and is one of the most common causes of childhood PH, but the underlying mechanisms are multifactorial and understudied. The benefits from the administration of pulmonary vasodilator therapy, and the extent of its use, are largely unknown at present.

Pulmonary veno-occlusive disease

Compared to the disease conditions discussed previously, PVOD is even more uncommon, and only represents approximately 2% of all causes of PH in children⁶. The pulmonary veins and venules are affected predominately, but also the arteries and capillaries (**Figure 12**)⁵⁹. PVOD is always associated with the development of PH and is classified in WHO subgroup 1.6: PAH with overt features of venous/capillary (PVOD/pulmonary capillary haemangiomas [PCH]) involvement.

The estimated incidence and prevalence of PH as a result of PVOD in adults and children is less than one per million but, because of misclassification of PAH, the true incidence of the condition is probably still unknown⁵⁹. Unlike PAH, which has a higher incidence in females, there is an equal gender distribution in patients with PVOD.

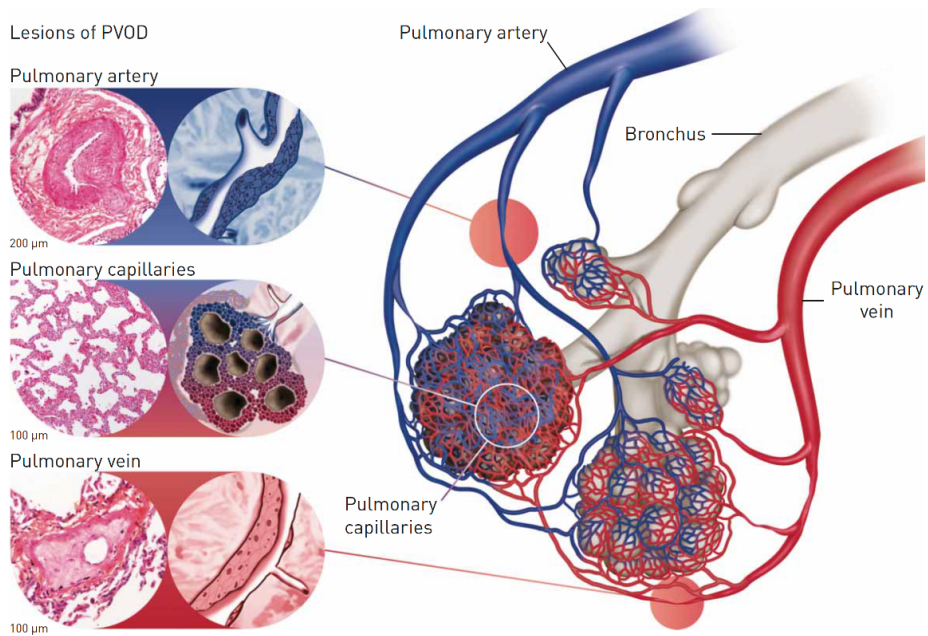


Figure 12

Vascular changes in patients with pulmonary veno-occlusive disease (PVOD). *Reproduced with permission from Montani et al. Eur Respir J. 2016⁵⁹.*

Histological and pathological findings in PVOD

Histological findings in PVOD have been described in numerous smaller studies⁶⁰. Veins and venules typically show fibrous thickening of the intima and, to some extent, medial hypertrophy with occlusions of the lumen (**Figure 13A**). This, in turn, leads to capillary remodelling and muscularisation, risk of pulmonary oedema and eventually PH. Arteries are affected too with some intimal fibrosis and medial hypertrophy, but less severely than veins and without occlusions (**Figure 13B**). There seem to be differences in the severity and pathology of the disease. Histological examination of *EIF2AK4* mutation carriers (see below) has shown the presence of significantly more intimal fibrosis and less severe medial hypertrophy of pulmonary arteries and more muscular hyperplasia of interlobular septal veins compared to that of non-mutation carriers⁶¹.

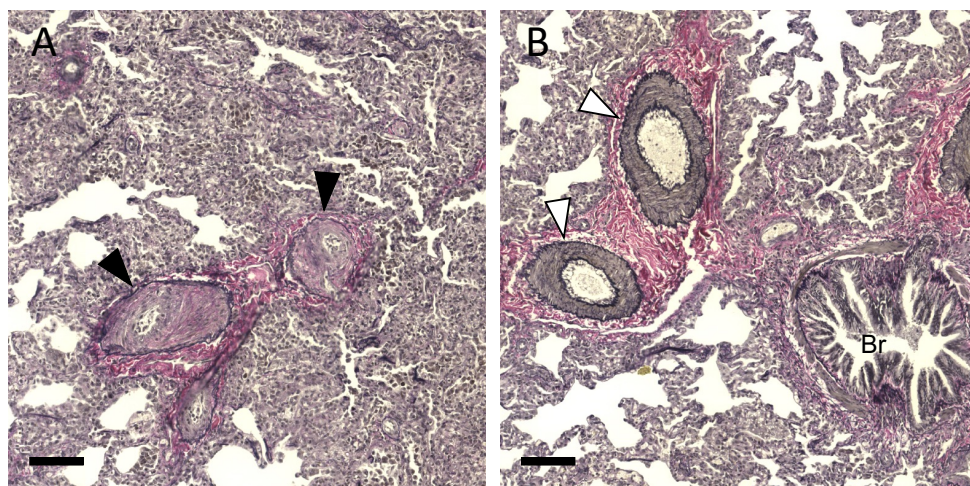


Figure 13

Histology (elastic van Gieson stain) in pulmonary veno-occlusive disease. A. Almost total occlusion of veins and alveolar septal thickening. B. Medial hypertrophy in arteries adjacent to the airway. Black arrowheads mark veins; white arrowheads mark arteries; Br, bronchiole; scale bar 100 μm . *Illustration produced by the author.*

Pulmonary capillary hemangiomatosis (PCH) is an angioproliferative condition that has pathological, clinical and genetic similarities to PVOD¹⁹. The capillary bed expands, and PCH is defined as \geq two rows of capillaries within the alveolar walls⁶². The two conditions overlap, and it has been proposed that PCH occurs secondarily to the occlusions of larger vessels seen in PVOD^{19, 62}. PVOD and PCH are listed as a single entity in the WHO classification, as described in Table 1⁶³.

Aetiology and genetics in PVOD

Different aetiologies for PVOD have been described. Bi-allelic mutations in *EIF2AK4* were first described in 2014²⁹. It has been shown to be restricted to PVOD patients and is found in approximately 29% of cases²⁵. Mutation carriers present at an earlier age with PVOD, but otherwise display a similar clinical disease course to that of non-mutation carriers. A few case reports have also suggested that the *BMPR2* mutation can be present in patients with PVOD^{64, 65}. Other described associations to, or causes of PVOD, are occupational exposure to the organic solvent trichloroethylene⁶⁶, stem cell transplantation⁶⁷ and thrombosis²¹. These causes probably pose only a minor risk in children, who are too young to have been exposed to them. Many cases of PVOD are still referred as to idiopathic in origin.

Clinical features and diagnostics in PVOD

Typical clinical signs and symptoms of PVOD in children are cyanosis on exercise or rest, and fatigue. A clinical prediction score to diagnose PVOD/PCH has been proposed in adults⁶⁸. Nine characteristics of the disease were identified, of which desaturation on exercise, findings of ground-glass opacities and thickening of interlobular septa on HRCT, reduced diffusion capacity of carbon monoxide measured after a 6-minute walk test, and pulmonary oedema as a result of the use of vasodilators, were typical for the diagnosis of PVOD. Right heart catheterisation is often used to diagnose PH in PVOD patients and to exclude left heart disease, but for a definite diagnosis of PVOD/PCH, a biopsy of pulmonary tissue is required for pathological examination.

Management of PVOD

Lung transplantation is the only cure for PVOD, and is associated with considerable mortality and morbidity. Mean survival time for patients with PVOD who do not undergo transplantation is 1-2 years⁶⁹. Time on the waiting list for an organ can be extensive, especially in children, and it can be challenging to treat the rapid disease progression while the patient is waiting for a donor organ. Medical treatment is limited, and the use of oxygen and pulmonary vasodilator therapy needs to be balanced in order to avoid the development of pulmonary oedema, while, at the same time, trying to reduce the PH. Most patients also receive diuretics to reduce the volume load. In many cases, patients respond to treatment with steroids to some extent.

In summary, PVOD is a rare condition in adults and children, and always causes PH. Various vascular changes are described, and there seem to be differences in the disease manifestations between mutation carriers and non-carriers.

Aims

The overall aim for this thesis was to gain improved knowledge on current pulmonary vasodilator therapeutic strategies, as well as on the morphology of vascular changes and causes of pulmonary hypertension in children.

Specific aims were:

To investigate whether pulmonary vasodilator therapy is beneficial in children with complex congenital heart defects (CHD) and pulmonary hypertension (*Paper I*).

To map patient characteristics, diagnoses and nationwide prescription patterns of pulmonary vasodilator therapy in young children in Sweden (*Paper II*).

To map nationwide prescription patterns of pulmonary vasodilator therapy in children born preterm and diagnosed with bronchopulmonary dysplasia (BPD) in Sweden (*Paper III*).

To provide a refined morphological visualisation of pulmonary veno-occlusive disease (PVOD) in children and to investigate differences between an *EIF2AK4* mutation carrier and a non-carrier (*Paper IV*).

Patients and methods

Paper I

Paper I was a retrospective study based on medical records. The study was the result of a collaboration with the United Kingdom Service for Pulmonary Hypertension in Children at Great Ormond Street Hospital (GOSH) in London. All children with single ventricle physiology CHD aged <18 years, who had been admitted to the service and treated with pulmonary vasodilator therapy over a period of 14 years (2004-2017), were reviewed. Information on diagnoses, clinical features, diagnostics, use of pulmonary vasodilators and concomitant therapy, and outcome were obtained. Medical records in the United Kingdom differ from those in the Swedish system. Some parts of the medical records in the United Kingdom that we viewed were digitalised, but there were also paper records still in existence and a system of “letters” containing medical information passed between the referring physician and the specialist centre, which comprised valuable condensed medical information. All available medical and surgical notes were reviewed retrospectively, and data were entered into Microsoft Excel spreadsheets and analysed using Student’s t-test statistics for comparisons and mean and range values.

Since the condition of single ventricle physiology in combination with PH is rare, the collaboration with a centre as large as GOSH was very advantageous in order to be able to collect data on paediatric cases. We also considered including data from similar Swedish patients treated in Lund. As already mentioned, the medical record systems between the two countries differ, and the available information was variable. The potential additional cases from Lund were few, because Lund compared to GOSH is a small centre, and diagnostics and patient follow-up differed between the two locations. In view of this heterogenicity, we decided not to add cases from Lund to the GOSH cohort.

Papers II and III

Both Papers II and III were epidemiological studies based on data from national Swedish registries. All children aged <7 years, who had had at least one outpatient prescription of a pulmonary vasodilator drug in Sweden during a period of 11 years

(2007-2017), were selected for inclusion in Paper II. The age limit of 7 years was chosen because the Swedish Prescribed Drug Register (see below) only began in 2005, and was not comprehensive until a few years later. All children in both studies were categorised according to the WHO classification of PH, and analyses regarding pulmonary vasodilator drugs and other patient characteristics were performed. For Paper III (which reported on a subgroup of patients from the Paper II study), all children born preterm and diagnosed with BPD were selected for further analysis. The program SPSS statistics Version 26 software (IBM) was used for analysis in Papers II and III.

Swedish registers

Sweden is unique in assigning a 12-digit identification number to each person in the country at birth. The number is used for all official authorities, and for population-based registries. With the identification number, linkage between registries is possible. Sweden has a well-structured system of national official registers. All are held by the Swedish National Board of Health and Welfare, Socialstyrelsen. Selected open statistics are available for all citizens to search via a web page. For more detailed statistics, it is possible to obtain data with ethical permission. The data collection is regulated by law, and is reported to the registers by health care givers and pharmacies. Registers used for the papers in this thesis are described below.

The Swedish Medical Birth Register was established in 1973 and covers more than 99% of all pregnancies that lead to childbirth in Sweden. The Register contains information on pregnancies, births and newborn children. In Papers II and III, information on the date of and GA at birth, gender, birth weight, malformations and neonatal diseases was collected.

The Swedish Prescribed Drug Register was established in 2005 and covers all prescribed medications that are dispensed at pharmacies in the country. Information on the dates of prescriptions, dosing, and date that the prescription was dispensed was obtained for patients in Papers II and III.

The Swedish Patient Register covers more than 99% of information on inpatient care and more than 80% of outpatient specialist care. The Register was first established in 1964 and underwent later developments. Information on diagnoses, surgeries, hospital and length of stay in hospital are examples of information that the Register holds.

The Swedish Death Register was established in 1961 and contains information on date and time of death, cause of death, place of death and diagnoses. For Papers II and III, the date and cause of death were obtained from this Register.

Paper IV

Paper IV was a study of the clinical characteristics and vascular changes in two children with PVOD. Both children were investigated and treated in 2017 at the Department of Paediatric Cardiology in Lund. Medical records were reviewed retrospectively for clinical characteristics, treatment, laboratory results, diagnostic investigations and disease course. An advantage of this study was that the investigators Ida Jeremiasen and Karin Tran-Lundmark were both involved in the care of the children. This minimised the risk of errors and misunderstandings from occurring. Tissue from lung transplantation was obtained for further analyses using a combination of histology, immunohistochemistry, *in situ* hybridisation and synchrotron-based phase-contrast micro-CT. Five blocks from each patient were used. Clinical results, histology and imaging results for the two children were compared. However, the low patient number did not permit us to perform further statistical analysis.

Histology, immunohistochemistry and *in situ* hybridisation

Tissue preparation

In order to be able to use and analyse explanted tissue from lung transplantation, a structured approach to preparation is required. Formalin that hardens and preserves the tissue is used for fixation directly after the lung dissection. Alcohol is used to remove all excess water from the tissue, after which xylene is used as a “clearing agent” to remove the alcohol and to establish optimal transparency of the tissue. Finally, paraffin is used to embed the tissue samples in blocks with a tissue size of 1-2 cm diameter and a few mm in depth. The blocks should be stored dry and in the dark at room temperature, and can be used for further analyses many years after the initial tissue preparation.

Histology

Histology is the study of microscopic structures in tissue. Thin sections from the paraffin-embedded tissue were sliced using a microtome (approximately 4 µm thick slices) and mounted on glass slides. Standard protocols for staining with haematoxylin and eosin and elastic van Gieson were used in Paper IV. Some examples of stainings are shown in **Figure 14**. Haematoxylin and eosin is the gold standard for histological analysis. Haematoxylin stains nuclei dark purple and eosin stains the extracellular matrix and cytoplasm pink (**Figure 14A**). Elastic van Gieson stain is used to highlight elastic fibres (**Figure 14B**).

Historically, a classic microscope was used for analysis. Nowadays, instead, the prepared slides are often digitalised using a slide scanner (Aperio ScanScope digital

slide scanner, Leica Microsystems, Wetzlar, Germany) and the software QuPath was used for the analysis in Paper IV.

Immunohistochemistry

Immunohistochemistry is a process in which proteins (or antigens) are identified in tissue by using antibodies that bind specifically to, and highlight, the desired antigen. Antibodies can be produced by injecting animals with part of the protein under investigation. The immune response in the animals will produce antibodies specific to the desired protein. There are many different antibodies available on the market. For Paper IV, immunohistochemistry for podoplanin was used to label lymphatic vessels, atypical chemokine receptor 1 (ACKR1) was used to detect venous endothelium, and alpha smooth muscle actin (aSMA) was used to label smooth muscle cells (**Figures 14C and D**).

In situ hybridisation

Hybridisation is the combination of different agents to create a hybrid. *In situ* hybridisation is a technique that uses labelled deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) to identify specific DNA or messenger RNA (mRNA) in tissue. The mRNA is made from the DNA code in the cell nucleus and carries information to the cytoplasm of the cell about how to produce proteins. One advantage of *in situ* hybridisation compared to immunohistochemistry is that the actual place of production of a protein can be localised. For Paper IV, *in situ* hybridisation was used for tenascin-C, a glycoprotein expressed in extracellular matrix during development or injury, that has been identified as a marker for angiogenesis. The RNAscope Technology (Advanced Cell Diagnostics, Newark, CA) apparatus and technique was used according to the manufacturer's instructions.

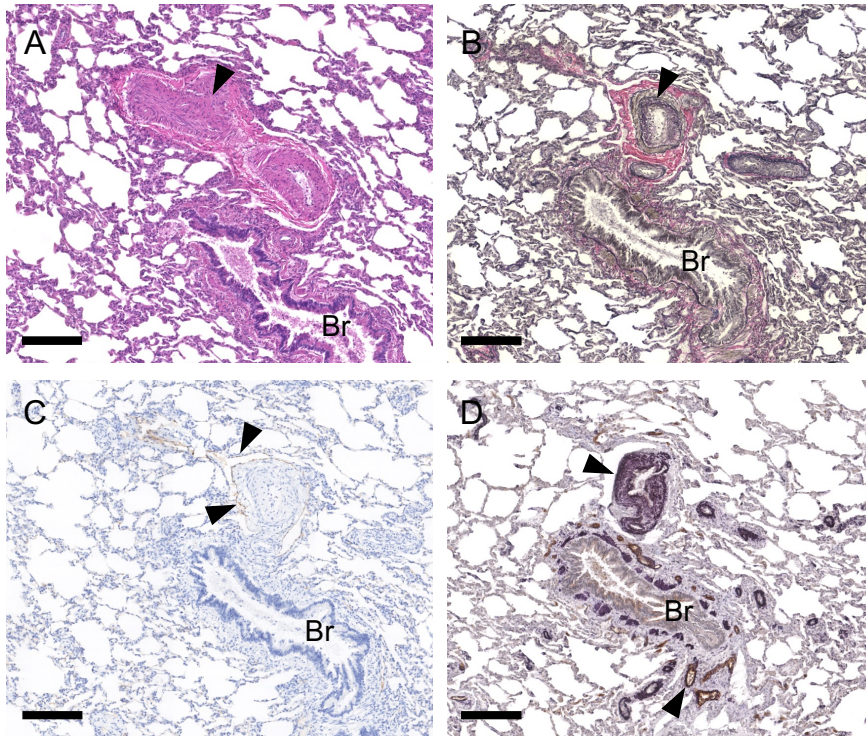


Figure 14

Examples of different stainings highlighting parts of a bronchiole, hypertrophied arterioles, expanded lymphatics and thickened alveolar septae in a patient with pulmonary veno-occlusive disease depending on the staining. A. Haematoxylin and eosin for nuclei in dark purple and extracellular matrix and cytoplasm in pink in a thickened arteriole. B. Elastic van Gieson for elastin in black and collagen in pink. C. podoplanin for expanded lymphatics in brown. D. atypical chemokine receptor 1 (ACKR1) for venous endothelium in brown and alpha smooth muscle actin (aSMA) for thickened arteriolar media in purple. Br, bronchiole; Black arrow, highlight the specific marker in each stain. Scale bar A-D, 200 μm , E, 100 μm . *Illustration produced by the author.*

Synchrotron-based phase-contrast micro-CT

In Paper IV, synchrotron-based phase-contrast micro-CT was used to visualise the pulmonary vasculature. The advantage of this technique compared to common histology is the possibility to reconstruct images to create 3D visualisations and, in Paper IV, this was of great use to enable a deeper understanding of the distribution of remodelling in arteries and veins.

A synchrotron is an electron accelerator that is used to produce photons/X-rays. Soft tissue, such as pulmonary tissue, exhibits low and homogeneous X-ray attenuation and conventional attenuation-based CT cannot produce sufficient contrast to visualise details within the vascular walls. Synchrotron radiation consists of highly coherent X-rays which enable the detection of phase-shift information. This

enhances soft tissue contrast and can generate images down to the micrometer scale. The X02DA TOMCAT beamline at the Swiss Light Source at the Paul Scherrer Institute (Villigen, Switzerland) was used to perform the visualisations. This was a project in collaboration with radiation physicists.

The set-up for synchrotron imaging is shown in **Figure 15**. In order to perform a micro-CT scan, formalin-fixed paraffin-embedded tissue samples were mounted on a rotating sample holder. Five blocks were used from each patient in Paper IV. To choose the area of interest for scanning, a standard histological staining from each paraffin block was used to guide the operator in selecting three-to-four areas of interest. The chosen areas were then marked by wax towers placed on top of the paraffin blocks. The object was scanned with X-rays and data were collected with a detector at all angles. Each scanned area of interest measured 4.2 mm x 4.2 mm x 3.5 mm.

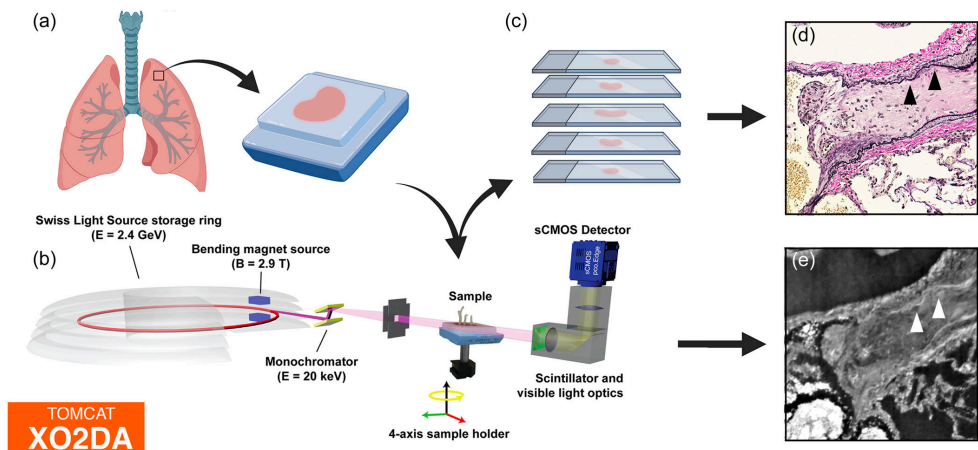


Figure 15

Synchrotron imaging and histology set-up and design, using pulmonary tissue. A, C, D. Pulmonary tissue is embedded in paraffin blocks, sliced and mounted on glass slides for histology. B, E. Synchrotron set-up using the X02DA TOMCAT to create 3D volumes. *Illustration by Van der Have O et al., Pulmonary Circulation, 2023⁷⁰, licensed by <http://creativecommons.org/licenses/by/4.0/>*

Image data of the scanned tissue were saved as stacks of TIFF files composed of 2160 image sections at a 16-bit pixel depth. The TIFF files enable visualisation at any angle for analysis and creation of 3D images. The software programs FIJI (ImageJ by National Institutes of Health, Bethesda, Maryland, USA) and Amira (Thermo Fisher Scientific, Waltham, MA, USA) were used for reconstructions⁷¹. Our research group recently published an article describing the technique⁷².

Results and discussion

Paper I

Pulmonary vasodilator therapy in children with single ventricle physiology: effects on saturation and pulmonary arterial pressure

The aim of this study was to investigate whether pulmonary vasodilator therapy is of advantage in children with complex CHD and PH.

Thirty-six children with single ventricle physiology and PH were analysed. The underlying primary anatomical CHD varied among the patients and they had different comorbidities that may have contributed to a dysfunctional single ventricle physiology. Some of the comorbidities and risk factors were late or too loose pulmonary banding, interventions for stenoses of pulmonary artery branches, restrictive atrial septum and arrhythmias. None of the patients included in the study had significant ventricular dysfunction or significant atrioventricular valve insufficiency. Some patients never advanced through all stages of the Norwood and Glenn procedures, or underwent a TCPC, because of the complexity of the disease which made it impossible to continue with surgery. The need for a low pulmonary pressure and vascular resistance is crucial to be able to proceed to a TCPC⁴¹⁻⁴³.

The effects of pulmonary vasodilator therapy were evaluated by oxygen saturation and the mean PAP was measured at cardiac catheterisation before and after the start of treatment. If confounding factors were found between the two time points, as for example, catheter interventions, the values were not used. There was a positive effect on oxygen saturation with primary vasodilator therapy, but no additional effect was seen with add-on therapy (**Figures 16A and B**). A decrease in mean PAP was noted between the start of treatment and follow-up for patients at all surgical stages (**Figure 16C**).

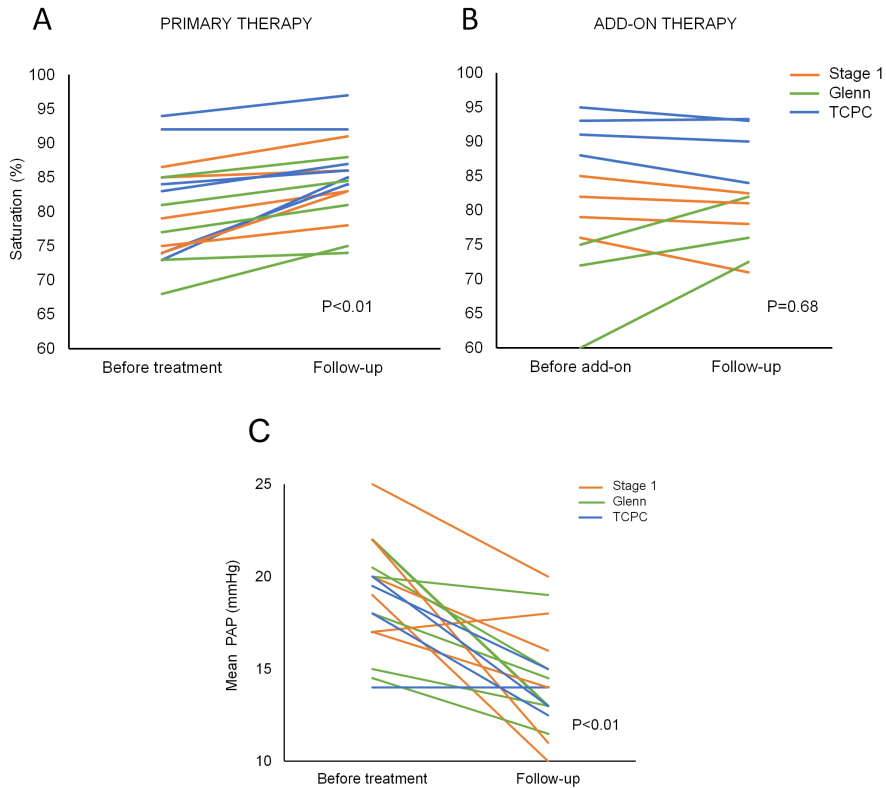


Figure 16

Results from Paper I. A. There was an increase in oxygen saturation before treatment with primary pulmonary vasodilator therapy and at follow-up, which was not seen with add-on therapy (B). C. Mean pulmonary artery pressure before treatment and at follow-up showed a significant decrease at all surgical stages. *Illustration reproduced from Jeremiasen et al., Pediatr Cardiol. 2020⁷³, licensed by <http://creativecommons.org/licenses/by/4.0/>*

Oxygen saturation and mean PAP were chosen as parameters in our study as they are indirect measurements of the ability of blood to flow through the pulmonary vasculature. A mean PAP of >15 mmHg and/or impaired ventricular function has been shown to increase the risk of failure after TCPC^{36, 42, 43, 74}. The indication for treatment with pulmonary vasodilator therapy in this study was an elevated PAP or, in the TCPC group, protein-losing enteropathy, plastic bronchitis, cyanosis and reduced exercise capacity. Long-term positive effects observed of an increased oxygen saturation from 80% to 85%, and a decreased mean PAP from 19 mmHg to 14 mmHg are not certain, and require further evaluation. The patients reported improved well-being during the study period. Other studies reported similar findings of improved clinical outcome and haemodynamic measurements with pulmonary

vasodilator therapy, but it is generally difficult to compare results between studies because of the heterogeneity of the patients⁷⁵⁻⁸⁴. The Fontan Udenafil Exercise Longitudinal (FUEL) trial by Goldberg *et al.*, involving 400 participants with single ventricle physiology who received the pulmonary vasodilator udenafil for 26 weeks, was a larger study which showed improved measures of exercise performance in patients who received the active drug⁸⁴. All the other referred studies were smaller case reports.

In our cohort, sildenafil was the most widely used vasodilator, followed by bosentan. Combination therapy was common. Other vasodilators were rarely used. Both sildenafil and bosentan have been approved for paediatric use, but so far only for the treatment of patients with PAH. Off-label use is common and is based on the subjective judgement of the responsible physician. This could explain why some patients were treated with these drugs, even in the absence of a high mean PAP at cardiac catheterisation. Other concomitant treatment was common in all patients. The polypharmacy that we encountered in the study reflects the fact that the patients in our cohort were severely ill with many comorbidities.

Sixteen children had already undergone a TCPC at the start of the study. Of the remaining 20 children, another seven progressed in surgical stage, and five of them all the way to undergoing a TCPC. On its own, pulmonary vasodilator therapy is not enough to enable a patient with PH to progress in surgical stage, but may have beneficial effects.

Total mean follow-up time of treatment with pulmonary vasodilator therapy was 4.7 years. No serious side effects were observed in our study, which was an encouraging sign considering the quite long follow-up period. Reported side effects, including priapism in one patient taking sildenafil, and elevated liver enzymes or renal failure in two patients treated with bosentan, were reversible.

Paper II

Vasodilator therapy for pulmonary hypertension in children: a national study of patient characteristics and current treatment strategies

The aim of this study was to map patient characteristics, diagnoses and nationwide prescription patterns of pulmonary vasodilator therapy in children with PH in Sweden.

The study included 233 children. The majority were stratified to WHO group 3 or 5, and had PH associated with BPD or CHD (**Figure 17**). CHD as the primary cause for pulmonary vasodilator therapy accounted for 42% of the total group, or 67% if both primary and secondary causes were included. A Dutch study reported that 52%

of cases of PH were related to CHD, of which the majority of patients were in WHO group 1⁸⁵. The recently updated classification of PH moved complex CHD into WHO group 5, which makes it more difficult to compare the studies⁴.

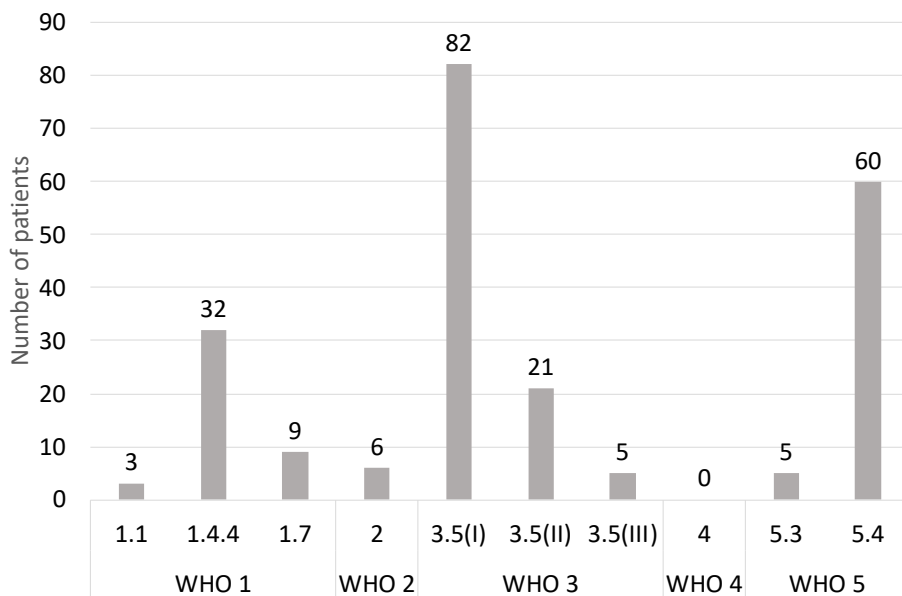


Figure 17

World Health Organization (WHO) classification of children aged < 7 years with prescribed pulmonary vasodilator therapy. 1.1, Idiopathic pulmonary arterial hypertension (PAH); 1.4.4, PAH associated with congenital heart disease (CHD) shunt; 1.7, persistent pulmonary hypertension of the newborn; 2, left heart disease; 3.5(I), bronchopulmonary dysplasia; 3.5(II), diaphragmatic hernia; 3.5(III), developmental lung disorder; 4, pulmonary artery obstructions; 5.3, other multifactorial mechanism; 5.4, complex CHD. *Illustration by Jeremiasen et al., Pulm Circ. 2021⁸⁶, licensed by <http://creativecommons.org/licenses/by/4.0/>*

This study was based on prescriptions instead of the diagnosis of PH or treatment at a PH centre, and may provide a more realistic perspective on the actual use of pulmonary vasodilator therapy in children. Sildenafil was the most commonly used pulmonary vasodilator, prescribed to 96% of patients as single or combination therapy (**Figure 18**). The second-most commonly used vasodilator drug was bosentan (12%), followed by more sporadic use of iloprost, macitentan, treprostinil and riociguat. No child was prescribed ambrisentan, avanafil, tadalafil or vardenafil during the study period. Monotherapy with sildenafil or bosentan has been reported to be safe to use⁸⁷⁻⁸⁹. Combinations of vasodilators were used in 13% of patients in our study. Recent guidelines favour upfront combination therapy in paediatric patients with heritable or idiopathic PAH with a high-risk profile². In children with

a lower-risk profile, or in patients with PH associated with other conditions in which the evidence for treatments effects is not as strong, a staged approach with initial monotherapy is preferred.

There was a distinct decline in prescriptions of sildenafil in 2014 (**Figure 18**). The reason for this could be that the Sildenafil in Treatment-Naive Children, Aged 1-17 Years, With Pulmonary Arterial Hypertension (STARTS-1) study showed an increased mortality rate with high-dose sildenafil in children with PAH⁹⁰. Based on the result, the US Food and Drug Administration issued recommendations against the use of sildenafil, unless there was a strong indication. The recommendations were later revised when the STARTS-2 study showed a favourable survival rate for all children treated with sildenafil⁹¹. Most of the pulmonary vasodilators used in Paper II were prescribed to children with PH other than PAH, but the STARTS-1 and 2 study results probably affected their overall use. High doses of sildenafil and other pulmonary vasodilators should be avoided, as no obvious improvement has been shown and because of the risk of side effects.

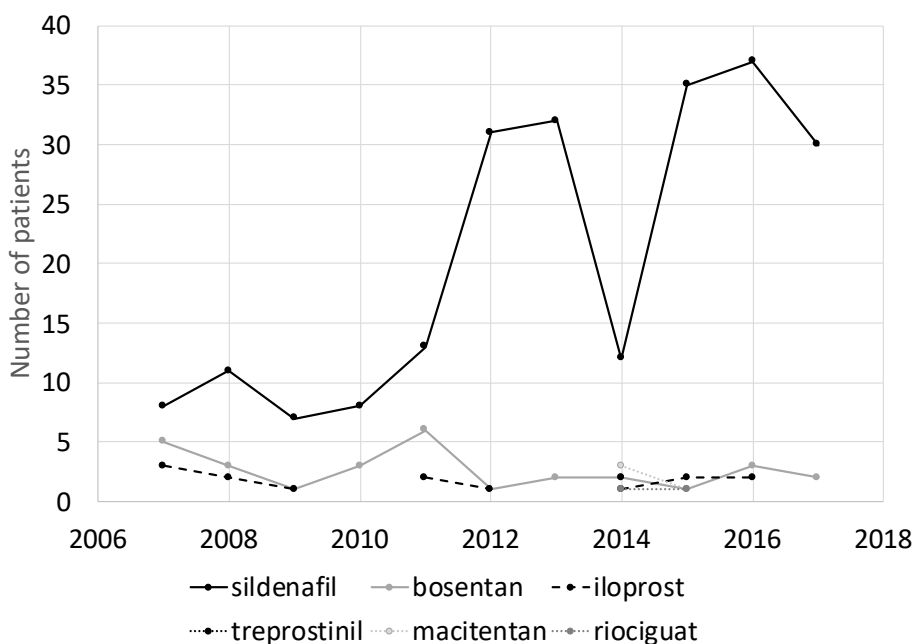


Figure 18

Number of patients with pulmonary vasodilator initiation per vasodilator drug and per year. *Illustration by Jeremiasen et al., Pulm Circ. 2021⁹⁶, licensed by <http://creativecommons.org/licenses/by/4.0/>*

The median age at the first prescription of vasodilator treatment was below 1 year in 61% of our patients. The registries used for the study did not contain information on potential side effects or reasons for discontinuation, but the majority (64%) of patients were treated for less than 1 year. For a definite diagnosis of PH, cardiac catheterisation is required. Only 39% of patients in our study had undergone cardiac catheterisation and often the primary indication for the procedure seemed to be to close an intracardiac shunt. Young children are fragile, and often present with comorbidities that are not seen in adults. Therefore, diagnosis of PH based on other diagnostic methods is reasonable in young children, and extrapolation from adult treatment guidelines should be made with caution.

In our study, mortality was highest in patients in WHO group 5, i.e. complex CHD, with 20% dying, compared to 13% in the total cohort. Similar mortality has also been described in other studies, especially for PAH^{92, 93}. This group of children present with comorbidities, such as prematurity, syndromes, as Down syndrome, and other conditions that increase the risk of fatal outcome.

Paper III

Outpatient prescription of pulmonary vasodilator therapy to preterm children with bronchopulmonary dysplasia

The aim of this study was to map nationwide prescription patterns of pulmonary vasodilator therapy in children born preterm and diagnosed with BPD in Sweden.

Seventy-four children were included. Eight patients classified as having BPD in Paper II were excluded because of refined inclusion criteria. The majority (73%) were born extremely preterm at GA 22-27 weeks, 27% were born at 28-36 weeks and 19% were small for GA. The risk of PH has been reported to be as high as 60-80% in extremely preterm children, compared to the overall prevalence of 18-42% in all patients with BPD^{54, 56}. In most children in our study, pulmonary vasodilator therapy was prescribed early in life, at a median age of 7 months, or 3 months when corrected for prematurity (**Figure 19**), but had already been terminated at a median corrected age of 4.6 months. Since the study reported on the use of outpatient prescriptions, the actual start of treatment was probably even earlier. Hence, extremely young and small children were treated.

Consensus is lacking as to whether chronological or corrected age should be used in the definition of PH³. We argue that corrected age should be used since accurate knowledge of pharmacodynamics and pharmacokinetics in very preterm children is unknown. Sildenafil has been considered to be safe to use in children aged <2 years, but available studies did not include preterm children⁹⁴.

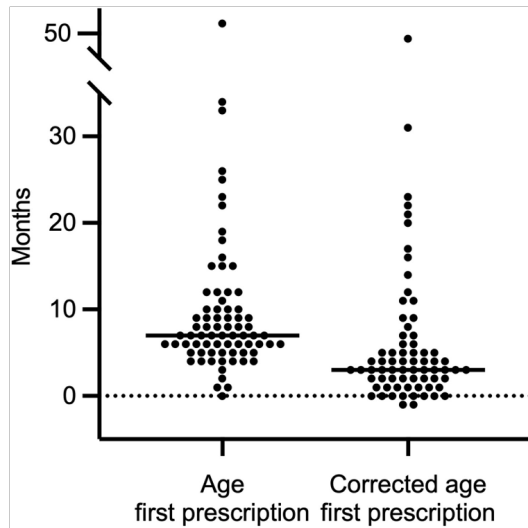


Figure 19

First pulmonary vasodilator prescription by age and by corrected age in months. *Illustration by Jeremiasen et al., Acta Paediatrica, 2023, licensed by <http://creativecommons.org/licenses/by/4.0/>*

As in Paper II, sildenafil was the most frequently prescribed vasodilator in Paper III (93%) and, compared to Paper II, mono therapy was even more common (86.4%). Other therapy was also prescribed frequently, especially inhalations, which nearly every patient had been prescribed. As described previously, BPD is a condition with variable vascular and pulmonary pathology and the underlying mechanisms for the development of PH are not understood fully^{55, 56}. The incomplete vascular growth in preterm children is probably difficult to improve with the use of vasodilators. On the other hand, increased vascular resistance, as a result of the vascular injury from hyperoxia and mechanical ventilation, may be helped by the use of pulmonary vasodilators⁴⁴.

Cardiac shunting increases the volume load on the pulmonary vasculature and the risk of PH. A patent ductus arteriosus (PDA) was present in 39% of patients in our study, and 69% of these were closed by device or surgery. A substantial number of the closures were performed after pulmonary vasodilator treatment had been initiated. An atrial septal defect (ASD) was diagnosed in 34% of patients, of which only a few were closed during the study period. It is logical to consider that pulmonary vasodilator therapy in combination with an open shunt may worsen the PH if the shunting is left-to-right. Studies have demonstrated an increased risk of intraventricular haemorrhage, necrotising enterocolitis and BPD in preterm children with PDA as well as ASD⁹⁵. The benefit of early closure on the development of PH has been shown for ASD⁹⁶, but so far has not been proven convincingly for PDA^{57, 97, 98}. We suggest that the effects of shunts on the pulmonary vasculature of preterm

children should be studied in more detail. A shunt that causes overcirculation may potentially be harmful, but a right-to left shunt may serve as a pressure relief valve when required.

Other studies reported a mortality rate of 40% or more in BPD-associated PH⁵³. The latter study by Khemani *et al.* found a mortality rate of 9%, but only studied children who had survived the first critical time in life and had been discharged from hospital. Five of the seven children who died in this study were considered to have a pulmonary cause of death.

Paper IV

Synchrotron-based phase-contrast micro-CT combined with histology to decipher differences between hereditary and sporadic pediatric pulmonary veno-occlusive disease

The aim of this study was to provide a refined morphological visualisation of PVOD in children, and to investigate differences between *EIF2AK4* variant mutation carriers and non-carriers.

Two children were studied. At admittance, patient 1 was a 7-year-old boy and patient 2 was a 6-year-old girl. Both patients were diagnosed with severe PVOD and, at the time of lung transplantation at the age of 8 years, they had similar clinical characteristics with PH combined with severe hypoxia, but they presented with a different clinical course, histological findings and genetic background.

Diagnostic investigations and treatment

An initial contrast-enhanced chest-CT in patient 1 showed scattered not well-defined ground-glass opacities, interlobular septal thickening (septal lines) and diffusely enlarged mediastinal lymph nodes (**Figure 20A**). Treatment was begun with oxygen, diuretics and pulmonary vasodilators. Cardiac catheterisation performed after the initiation of treatment showed high pressures with a mPAP of 64 mmHg and an indexed PVR of 14 WU/m², with little beneficial effect following the administration of NO and oxygen. Triple therapy with pulmonary vasodilators resulted in clinical worsening and pulmonary oedema (**Figure 20C**). Genetic testing was negative.

A contrast-enhanced chest-CT and a HRCT in patient 2 showed numerous centrilobular ground-glass opacities, interlobular septal thickening, and enlarged mediastinal lymph nodes (**Figures 20B and D**). Cardiac catheterisation performed before treatment initiation showed extremely high pressures with a mPAP of 90 mmHg and an indexed PVR of 17.6 WU/m² with some reversibility following

administration of NO and oxygen. Treatment was begun with a combination of oxygen, diuretics, low dose sildenafil, CCB, propranolol and pulsed steroids. The patient did not develop pulmonary oedema, but the vasodilation was less aggressive compared to in patient 1. Genetic testing showed that the patient was homozygous for an *EIF2AK4* (c.4603_4606delAGTG, p.(Ser1535Cysfs*2)) mutation.

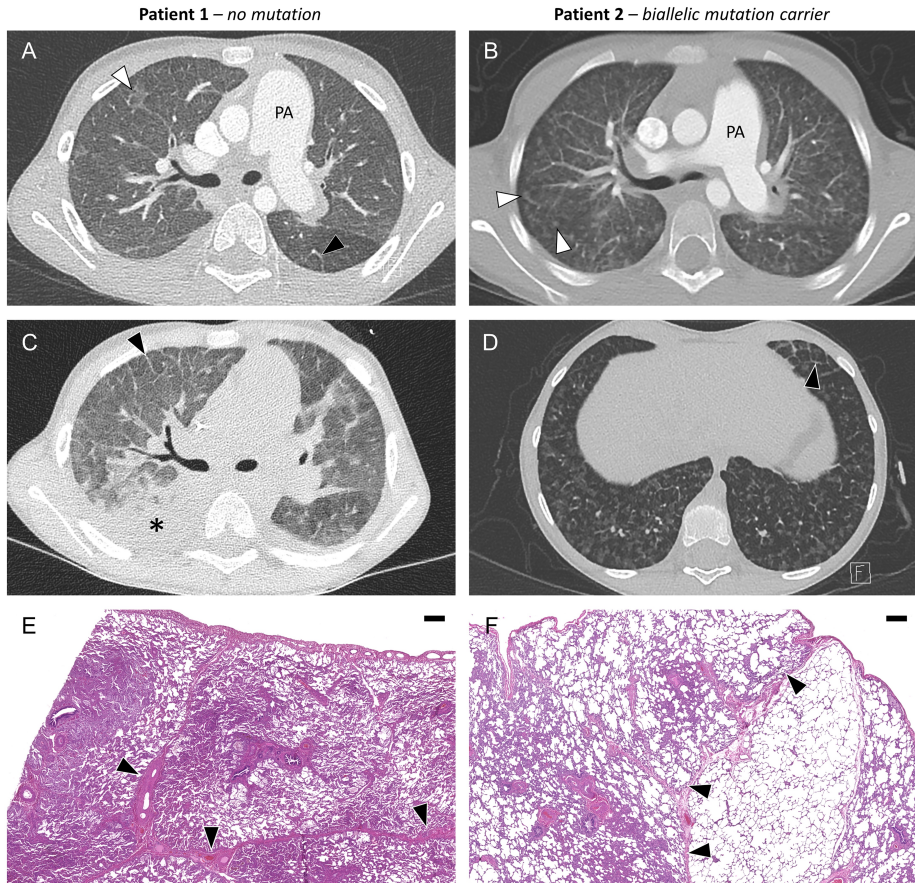


Figure 20

Chest computerised tomography (CT) and histological overviews for patient 1 (A, C, E) and patient 2 (B, D, F). A. Chest CT with intravenous contrast at admission showing septal lines and scattered poorly defined ground-glass opacities. B. Chest CT with contrast at admission showed sparse septal lines and numerous nodular centrilobular ground-glass opacities. C. High-resolution computerised tomography (HRCT) after pulmonary vasodilator therapy showed oedema, more pronounced septal lines and a pleural effusion. D. HRCT at admission at a level close to the diaphragm where the septal lines were more prominent compared to other parts of the lung. E. Haematoxylin and eosin staining with generally thickened alveolar walls and thickened septa and pleura. F. Haematoxylin and eosin staining with focally thickened alveolar walls around broncho-vascular bundles. Less pronounced septal thickening. Scale bar = 400 μ m. Black arrowheads, septal lines. White arrowheads, ground-glass opacities. *pleural effusion. PA, pulmonary artery. *Illustration from Jeremiasen et al., Paper IV; in manuscript, unpublished.*

Histopathology

Patient 1, in whom no mutation was found, had markedly diseased occluded pulmonary veins and medial hypertrophy in the arteries, but not much arterial neointima formation was seen (**Figures 21A, C and E**). His case was clearly different from patient 2, the mutation carrier, in whom the pulmonary veins in interlobular septae only showed some mild wall thickening and intimal fibrosis, but no occlusions (**Figures 21B, D and F**). However, patient 2 had pronounced arterial intimal fibrosis and medial hypertrophy. Dual lumens were also observed within the same arteriolar adventitia. A more general alveolar septal thickening was seen in patient 1 compared to patient 2, in whom the changes were more patchy.

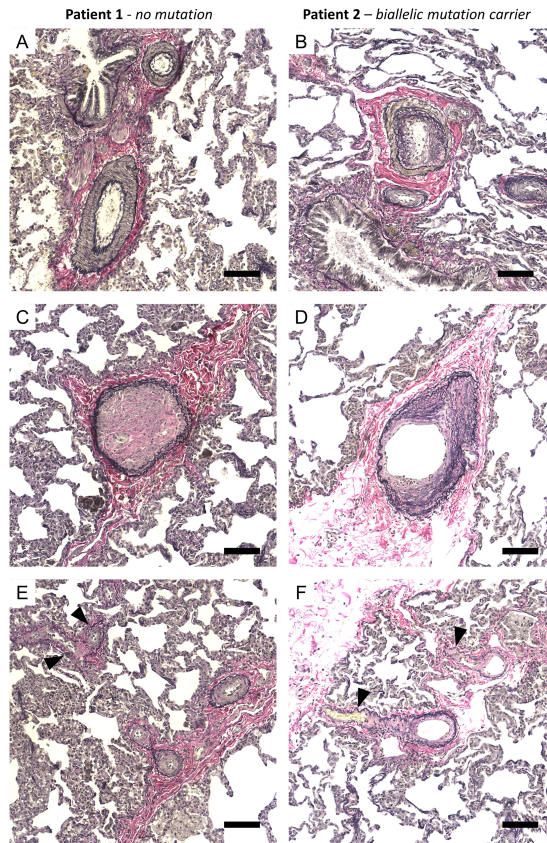


Figure 21

Elastic van Gieson staining shows vascular changes in pulmonary veins and arteries in patient 1 (A, C, E) and patient 2 (B, D, F). A. Pulmonary artery with medial hypertrophy and intact internal elastic lamina. B. Pulmonary artery with medial hypertrophy, partly fragmented internal elastic lamina and intimal fibrosis. C. Occluded pulmonary vein. D. Patent pulmonary vein with intimal fibrosis. E. Pre-septal venules with marked intimal fibrosis. F. Patent pre-septal venules with minimal intimal fibrosis. Scale bar = 100 μ m. Black arrowheads, pre-septal venules. *Illustration from Jeremiasen et al., Paper IV; in manuscript, unpublished.*

Both patients in our study had signs of PCH that was widespread, but particularly prominent close to the pleura in patient 1 compared to a more patchy distribution, often close to diseased arteries, in patient 2. The capillaries in the alveolar septae were muscularised in both patients but, again, patient 2 had a more patchy distribution of PCH focused around diseased arteries. PCH has been found in the majority of PVOD patients¹⁹ and its distribution has been described previously as patchy in mutation carriers and more diffuse in non-carriers⁶¹, as we found in our cases. We speculate that the PCH in patient 1 may have been caused by capillary congestion secondary to the obstructions in interlobular septal veins, explaining the more general distribution, compared to the patchy pattern of PCH around remodelled arteries seen in patient 2.

Podoplanin staining indicated the presence of dilated lymphatics in the interlobular septa in both patients, but they were more prominent in patient 1. Dilated lymphatics were also observed around diseased arteries in patient 2. The histological findings with a patchy distribution in patient 2, in combination with markedly affected arteries and arterioles, matches the centrilobular ground-glass opacities that were seen widely on chest CT. Ground-glass opacities, in combination with septal lines and enlarged lymph nodes, are typically seen in patients with PVOD. In our study, patient 1, who had extreme occlusions of interlobular septal veins and clearly dilated septal lymphatics, had more septal lines on chest CT, and he developed pulmonary oedema with vasodilator treatment. Pulmonary oedema is also a typical finding in patients with PVOD, because of the venous obstructions that prevent outflow to the left atrium. It is logical that patient 2 did not develop pulmonary oedema because her venous obstructions were not as extreme as those in patient 1. However, vasodilator treatment was also initiated carefully in patient 2, because the suspicion of PVOD was already high from the start of the study as a result of the typical CT findings.

All the above histological changes have been described in other studies, but often arterial lesions were reported to be less severe, and such a distinct difference between sporadic and hereditary PVOD cases is not usually described⁹⁹. It was first suggested that *EIF2AK4* mutation variant carriers and non-carriers had similar histological and chest CT findings²⁵, but a recent study by Nossent *et al.* found that there was intimal fibrosis and less severe medial hypertrophy in *EIF2AK4* carriers⁶¹, which was more consistent with our findings.

Three-dimensional imaging

Synchrotron-based phase-contrast micro-CT in combination with histology improved the visualisation and confirmed our histological findings (**Figure 22**). It is difficult to distinguish between non-muscularised intra-acinar arterioles and venules in histology. The veins need to be followed to the septa and the arterioles to a bronchiole. With 3D imaging this was possible to do, and we concluded that we did not find any occluded veins in tissue from patient 2, the *EIF2AK4* mutation

carrier. The absence of venous occlusions has been described by others as well¹⁹, but this was the first time it has been imaged in 3D with the advantage of not requiring histological serial sectioning.

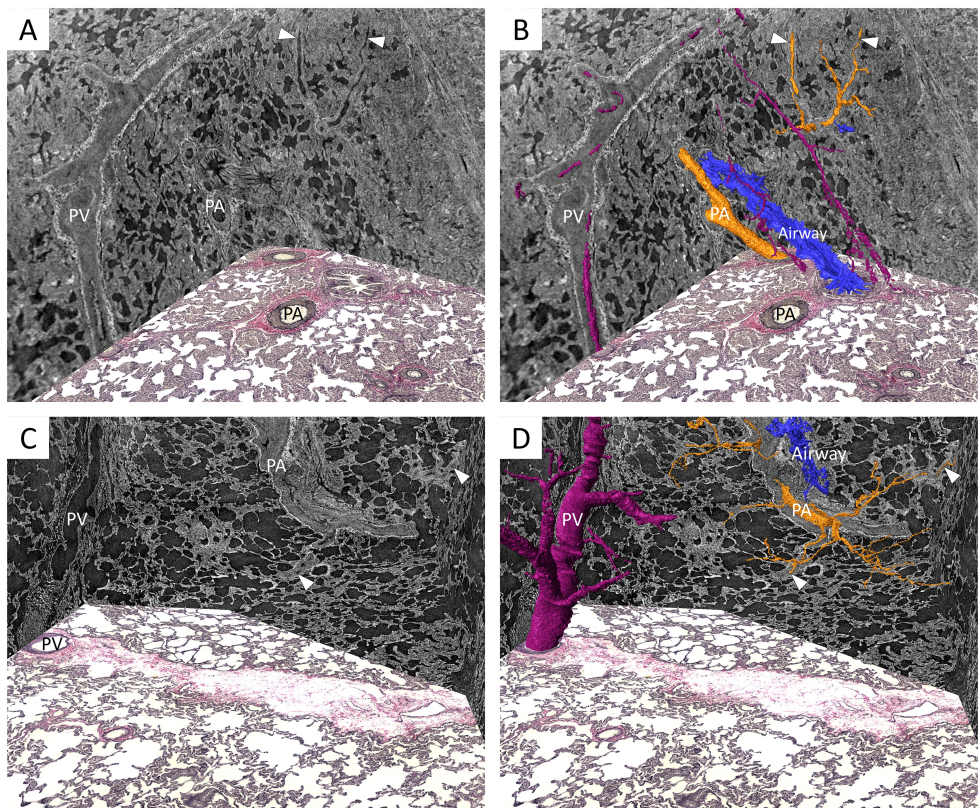


Figure 22

Synchrotron-based phase-contrast micro-computerised tomography with venous (purple) and arterial (yellow) lumens segmented and 3D rendered with 2D histology (elastic van Gieson stain) integrated in the 3D volumes. A-B. Patient 1 with an almost occluded interlobular pulmonary septal vein. Pulmonary arteries with medial hypertrophy, but open and tortuous in the periphery. C-D. Patient 2 with patent interlobular pulmonary vein. Pulmonary arteries are severely hypertrophied. In the periphery, pulmonary arteries remain open but with narrowed lumina and highly tortuous course. White arrowhead, open distal arteries. PA, pulmonary artery. PV, pulmonary vein. *Illustration from Jeremiasen et al., Paper IV; in manuscript, unpublished.*

By 3D imaging, we were also able to demonstrate the presence of intrapulmonary bronchopulmonary anastomoses and highlight tortuous pulmonary arteries with more than one lumen within the same adventitial sheath in patient 2, the mutation carrier. These findings are novel and warrants further investigation. A limitation of the study is the low number of cases, but the findings were distinct and, to avoid bias, three clinically experienced pathologists interpreted the results independently.

Aspects of vasodilator therapy

Papers I-IV describe different aspects of pulmonary vasodilator therapy in patients with PH. Some additional reflections on this subject will now follow.

Aspects of guidelines and WHO classification of paediatric PH

As described in the background section in this thesis, common international consensus guidelines for the classification of paediatric PH were first introduced by the report from the Pulmonary Vascular Research Institute Pediatric Taskforce meeting in Panama in 2011⁷, and were later incorporated into the 2013 adult WHO PH classification⁶³. With improved treatment, children with PH survive until adulthood, and a common classification is an advantage. Adult European guidelines were updated in 2022, and include a shorter section that refers to the paediatric guidelines¹⁰⁰. The most recent revision on the diagnosis and treatment of paediatric PH was made in 2019 by the European Pediatric Pulmonary Vascular Disease Network and was endorsed by European Societies for paediatric cardiology and research and international heart and lung transplantation, with representatives from both Europe and the United States². New definitions from the 2018 World Symposium on PH were included^{3, 4}. An important change was that the criteria for PH were defined as a mean PAP of >20 mmHg in combination with an indexed PVR of >3 WU in pre-capillary PH in all PH groups. Another revision was the incorporation of diagnostics, classifications and treatments of BPD, novel mutations and complex CHD in the classifications system and the inclusion of PVOD/PCH into group 1 PAH. PAH and PVOD/PCH were now considered to represent a spectrum of primary pulmonary vascular disease instead of two distinct conditions. The guidelines were based on paediatric data, or adult studies with at least 10% children included. This is of importance when treating children because, for example, disease spectrum, comorbidities, pharmacokinetics and pharmacodynamics in children are not comparable to those in adults.

WHO group 1, PAH, has, until now, been the most studied group of paediatric PH and the only group with a clear indication for the use of a PDE-5i or an ERA². For all other WHO groups, data are limited. Knowledge about the underlying cause and WHO group of PH is important before treatment is initiated. The treatment recommendations for PAH do not apply to WHO subgroup 1.6, PVOD/PCH, in which pulmonary vasodilators are known to cause pulmonary oedema because of venous occlusions. In Paper IV, pulmonary vasodilators triggered the onset of pulmonary oedema in the child without the *EIF2AK4* mutation, but not in the mutation carrier. Both children were diagnosed with PVOD. We found clear histopathological differences between the two patients, with total occlusions of pulmonary veins in the non-carrier, compared to open but remodelled veins combined with markedly hypertrophied arteries in the mutation carrier. This reflects

the need for individual evaluation and the need for further studies on underlying mechanisms and maybe re-evaluation of the WHO classification.

In PH associated with CHD after shunt closure (WHO group 1), and BPD or chronic lung disease (WHO group 3), guidelines state that monotherapy is reasonable to recommend and that a PDE-5i is mentioned as “commonly used”. However, very few data exist on the efficacy of these drugs in these conditions. In Paper III, we found that sildenafil was the most commonly prescribed pulmonary vasodilator and it was usually used as monotherapy in children with BPD. This is in good agreement with current guidelines citing sildenafil as first-line treatment for PH. A large proportion of the treated children in Paper III were extremely preterm, and there is now a trend towards increased survival in this group. Special care is required when evaluating treatment effects in these patients, and more research is needed, since it is not known how pulmonary vasodilators affect the growth and maturation of the lungs.

Aspects of the new definition of PH

The new cut-off limit of a mean PAP of >20 mmHg for the definition of PH was based on an adult study in which values between 20-24 mmHg represented predictors of poor survival¹⁰¹. The paediatric guidelines accepted this limit, despite the lack of data in children. Our results from Paper I showed that pulmonary vasodilator therapy in children with PH caused by complex CHD (WHO group 5) resulted in good efficacy, with a reduction in the mean PAP from 19 mmHg to 14 mmHg. Even with the new limit for the definition of PH, these children would not have been included as having the condition, but we consider that there are reasonable arguments in favour of them having significant pulmonary vascular disease, even though the pressure was not 20 mmHg. Single ventricle physiology is totally dependent on a passive pulmonary flow with low resistance, and a mean PAP of > 15 mmHg in these patients has been associated with increased risks of failure^{42, 43}. This is one example of the heterogenicity of PH and the need for individual and specific guidelines for each WHO group of the condition.

Aspects of pulmonary vasodilator drugs

New pulmonary vasodilator drugs are launched regularly. In most cases they are tested on adults, and paediatric studies come later, or not at all. It is more difficult to conduct studies in children because of the small number of eligible patients and the heterogenicity of PH. The regulations for studies of medications in children are stricter and the ethical aspects more complicated. To date, sildenafil, bosentan and tadalafil are the only approved pulmonary vasodilator drugs for use in children over 1 year of age. In Papers I-III, we found that sildenafil and bosentan were the most widely used vasodilators, but other vasodilators were also used to some extent, and there was a trend over time of increased prescription of these drugs. The choice and use of pulmonary vasodilators in Paper I, which was conducted in British children,

are comparable with the Swedish prescriptions for these products in Papers II and III. All other pulmonary vasodilator drugs are considered to be off-label use in Sweden and Europe, but studies are ongoing and new data are published continuously.

Riociguat was evaluated recently for paediatric use in the PATENT-CHILD multicentre study¹⁰². The results suggested a reasonable safety profile and positive trends of 6-minute walk test results, but the authors concluded that body weight-adjusted doses of pulmonary vasodilator drugs should always be considered in children in order to obtain pharmacokinetics comparable with those of adults. It is difficult to determine the optimal dose of vasodilator treatment that avoids side effects. We could see neither a better effect, nor more side effects, of higher doses of sildenafil used in Paper I. In Papers II and III, doses of vasodilators were not investigated. High doses of sildenafil have been associated with increased mortality in children weighing > 8 kg or aged > 1 year^{90, 91}. Paediatric studies on the oral prostacyclin agonist selexipag, which were published recently, showed safety of use and favourable effects on pressure measurements, functional class and right ventricular function at a median of 8 months of follow-up^{103, 104}. Selexipag has been approved for the treatment of PAH in adults. These studies on the more novel riociguat and selexipag are examples of ongoing investigations that may affect the next paediatric treatment guidelines.

There is a trend in recent guidelines towards the recommendation of oral combination treatment, either in the form of upfront concomitant, or sequentially added therapy, for children with PAH with moderate or high risk and in functional classes II-III. Perhaps it was the need for better treatment for the most severely ill patients that prompted studies to focus on them in the first place? A potential synergistic effect of addressing different vasodilator pathways is interesting. To date, oral combinations of PDE-5i and ERA are the most studied, and have been shown to improve the WHO functional class and 6-minute walk test in children with PAH¹⁰⁵. In addition, the combination with inhaled, intravenous or subcutaneous prostacyclin can be considered². In Paper II, 13% of patients were on combination treatment, and in Paper III, almost 11% were. Long-term treatment with combination therapy has not been evaluated fully, but may result in better long-term survival in patients with PAH, and our results in Papers II and III did not reveal any severe side effects.

Aspects of new therapeutic agents

Different strategies have been discussed to develop novel therapeutic agents in PH. Pulmonary vasodilators are primarily symptomatic treatments and do not cure the condition. To improve the function of pulmonary vasodilators, objectives may include the development of agents with a longer half-life to reduce dosing frequency in children, or to find agents that act on more specific sites or receptors in an effort to reduce the adverse effects of the medications. The potential synergistic effect of

combinations of pulmonary vasodilator treatment has, to some extent, been studied, but also requires more attention.

Another strategy in the management of patients with PH, besides the use of pulmonary vasodilators, would be to target a potential cause of the condition. Various anti-inflammatory agents, gene modification therapy, drugs that block smooth muscle cell proliferation, or are directed against angiogenesis, are some examples of such an approach³¹. In Paper IV, some degree of inflammation was found in the histological examinations (also a well-known finding in other studies), and steroids exerted a positive temporary effect on symptoms. The effect of steroids may have been a direct effect on the vasculature, but effects on inflammation cannot be excluded. Further studies might focus on different types of inflammatory responses in order to target and impede the vascular remodelling. The novel agent sotatercept, a promising transforming growth factor-beta (TGF- β)-type protein that affects *BMP2* signalling and balances the proliferative processes in PAH patients, is also in clinical trials and has been found to reduce PVR in adults¹⁰⁶. Specific dysregulated gene expressions in PH, that might serve as novel therapeutic targets for pulmonary vascular pathology, were also studied recently¹⁰⁷. Novel therapies in PH could improve short-term well-being, long-term survival and reduce the risk or delay the need for lung transplantation, which is the only cure in many cases.

Aspects of novel imaging

Synchrotron-based phase-contrast micro-CT as a novel imaging technique was shown in Paper IV to facilitate the understanding of how pulmonary veins and arteries are affected differently in patients with PVOD.

Synchrotron imaging is a technique used purely for research because of the high radiation dose and limited availability. It can also be expensive if a project is not considered valuable enough by reviewers of beamtime applications. However, we believe that these aspects are overruled by the positive effects of the technique. One scan takes a couple of minutes and a large amount of data can be collected and compared with the results of traditional histology. It is possible to inject a dye to highlight structures – an approach that was demonstrated in a recent publication by our group⁷². In Paper IV, we visualised that the obstructions in the veins and thickened arteries were present throughout the vessels and not just in certain parts. The collaboration with radiation physicists to examine human tissue in detail was encouraging and educative. Their contribution to advanced imaging helped us to visualise complex structures.

Since synchrotron imaging is non-destructive, the tissue can be subsequently sectioned and analysed by histology. The use of traditional histology has not been replaced by synchrotron imaging, but the combination of the techniques improves

our understanding of pulmonary pathology. It would be interesting to visualise the other causes of PH studied in this thesis using synchrotron imaging as well, but the need for tissue makes this more complicated. Lung biopsy is not part of the routine investigation for PH, but collection of tissue from lung transplantation or autopsies for analyses is possible and could be used more widely in the future for this purpose. In future studies, synchrotron imaging could be used to evaluate the effects of novel treatments, but preferably first in animal models because of the need for tissue and the possibilities of conducting time course experiments.

Aspects of pulmonary vascular disease which are challenging to investigate are presence and distribution of angiogenesis as well as involvement of the bronchial circulation. In Paper IV, some evidence was found for vascular divisions with a double lumen in severely hypertrophied arteries. Intussusceptive angiogenesis is a specific form of angiogenesis with the formation of two new vessels by the splitting of one existing one^{20, 108}. The condition was studied recently in adults with PH arising from alveolar capillary dysplasia and chronic lung disease^{21, 109}. With traditional histology, the formation of the two new vessels is very difficult to visualise, but with the ability to reconstruct the vessels in 3D imaging, our understanding could be improved. The dual lumens within the same adventitia could potentially be explained by an expansion of the vasa vasorum or by splitting of the vessels. This will be further investigated.

Conclusions

The four papers in this thesis together add information on pulmonary vasodilator therapy in children with pulmonary hypertension (PH) of different aetiologies. Despite the lack of evidence-based paediatric guidelines, pulmonary vasodilator therapy is often used in children with PH, as described in all studies.

Children with complex congenital heart defects (CHD) and single ventricle physiology had improved saturation and decreased mean PAP after treatment with pulmonary vasodilator therapy. Long-term treatment showed no serious side effects (*Paper I*).

Pulmonary vasodilator therapy was prescribed widely to Swedish children with all types of PH. The most common underlying aetiologies were PH associated with CHD or BPD. Sildenafil as a single therapy was the most prescribed drug, but combinations with other pulmonary vasodilators were also used to some extent (*Paper II*).

Preterm children in Sweden, diagnosed with BPD, were often prescribed pulmonary vasodilator therapy. Sildenafil as a single therapy was the most commonly prescribed drug. A large proportion of the children in our study were born extremely preterm, and treatment was begun early in life. Treatment was often initiated without prior cardiac catheterisation, and intracardiac shunts were common (*Paper III*).

Clear differences in vascular remodelling were observed between two cases of pulmonary veno-occlusive disease (PVOD). The *EIF2AK4* mutation carrier had markedly hypertrophied arteries and responded positively to low dose pulmonary vasodilator therapy, in comparison to the non-carrier who had complete occlusions of interlobular septal veins and who developed pulmonary oedema on treatment. Different types of PVOD may be more common than that which has been believed previously. The combination of traditional histology, immunohistochemistry and *in situ* hybridisation with synchrotron-based phase-contrast micro-CT provided a better understanding through 3D visualisation of the underlying pathology (*Paper IV*).

Future perspectives

The papers included in this thesis have contributed to the current knowledge on treatment patterns and effects, as well as to the possibilities of studying vascular changes in paediatric PH in 3D. They will hopefully provide the inspiration for further research in this field, such as:

To conduct studies in collaboration with large international centres that treat children with complex CHD and PH with pulmonary vasodilator therapy, to be able to collect data from a larger cohort and gain further knowledge on the positive effects that were observed in Paper I.

To conduct studies by using additional national registries as, for example, the SWEDish registry of CONgenital heart disease (SWEDCON) or the Swedish Neonatal Quality Register (SNQ), to enable an in-depth analysis of pulmonary vasodilator therapy in children and supplement our findings by including a control group to Papers II and III.

To conduct studies by using medical records from children with BPD that were collected in Paper III, to include information on dosing, clinical symptoms (for example functional class) and measurable effects (for example saturation and haemodynamic evaluation) of pulmonary vasodilator therapy to investigate whether such treatment is advantageous in this group of children.

To include more patients with PVOD, both with and without *EIF2AK4* mutations, for histopathological examination and synchrotron imaging to confirm our findings of clear vascular differences in Paper IV.

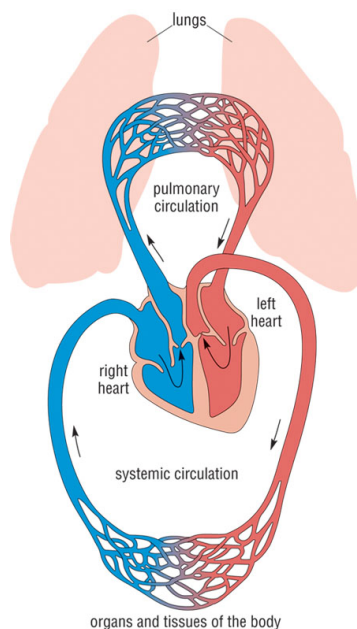
To use synchrotron-based phase-contrast micro-CT in other types of paediatric PH besides PVOD. Possibly this could also be combined with electron microscopy and molecular biology to explore further the role of for example intussusceptive angiogenesis.

Populärvetenskaplig sammanfattning

Pulmonell hypertension betyder att trycket i lungans blodkärl är för högt. Lungcirkulationen tar emot blod från hjärtats högra sida för att syresätta det (**Figur 23**). Syrgasutbytet sker mellan de allra minsta blodkärlen, kapillärerna, och de minsta luftförande luftvägarna och lungblåsorna, de så kallade alveolerna. Syrerikt blod återvänder sedan till hjärtat för att pumpas ut i systemcirkulationen. Vid för högt lungkärlstryck kan blodet inte flöda fram som önskat och denna process kommer att påverkas. Också hjärtat kommer att påverkas av högt lungkärlstryck, eftersom systemen är sammankopplade.

Varför får man högt tryck i lungorna? Det finns flera olika mekanismer och bakomliggande orsaker. Hos barn är medfödda hjärtfel en vanlig orsak, men också sjukdomar som primärt uppstår i lungkärlen förekommer. Kärlen omvandlas och blir förtjockade och ofta blir det en ond cirkel av kompensationsmekanismer som i sig ökar trycket ännu mer. I många fall är det en kronisk sjukdom utan bot. Särskilt hos barn är erfarenheten av hur man ska behandla pulmonell hypertension begränsad.

Syftet med denna avhandling var att fördjupa kunskapen inom pulmonell hypertension hos barn, med fokus på vilken effekt lungkärlsvidgande läkemedel har och hur läkemedlen idag används samt att få en djupare förståelse för olika kärlförändringar som sker vid en ovanlig typ av svår pulmonell hypertension.



Figur 23

Kroppens blodomlopp. Lungpulsådern med syrefattigt blod (blått) från höger hjärthalva till lungorna där syrgasutbytet sker mellan kapillärer och lungans alveoler. Det syrerika blodet återvänder till hjärtats vänstra sida för att sedan pumpas ut i kroppen via stora kroppspulsådern (rött). *Illustration från www.open.edu*

Studie I var en journalstudie som inkluderade 36 barn som alla hade komplicerade medfödda hjärtfel, så kallade enkammarhjärtan, och förhöjt motstånd i lungans blodkärl som en följd av det svåra hjärtfelet. Barnen fick behandling med lungkärtsvidgande läkemedel. Effekten utvärderades bland annat genom att mäta syrgasmättnad i blodet och trycket i lungorna före och efter insatt behandling. Resultatet visade på en positiv effekt av läkemedel och få biverkningar över tid.

Studie II var en registerbaserad studie som syftade till att ta reda på hur mycket lungkärtsvidgande läkemedel som förskrivs på recept i Sverige och till vilken typ av patienter. Data från Socialstyrelsens register användes. Totalt hade 233 barn under en tioårsperiod fått behandling insatt före sju års ålder. Medfödda hjärtfel och lungsjukdomen bronkopulmonell dysplasi (BPD) var vanliga orsaker till behandling. Läkemedlet sildenafil användes mest, men kombination med andra lungkärtsvidgande läkemedel förekom också.

Studie III var en fortsättning av studie II där antalet för tidigt födda barn med BPD specifikt undersöktes. Förskrivning av läkemedel, patientkaraktäristika, utredning och uppföljning av dessa totalt 74 barn bedömdes med hjälp av registerdata. En stor andel var födda extremt tidigt, i graviditetsvecka v.22-27. Många fick behandling med sildenafil vid bara några månaders ålder och behandlingen avslutades sedan ofta inom ett år. Det finns i nuläget inga tydliga riktlinjer för hur lungkärtsvidgande läkemedel ska användas till barn och framför allt inte till för tidigt födda barn. Fortsatta studier av behandlingseffekter och säkerhetsaspekter behövs.

Studie IV baserades på journaluppgifter och vävnadsprover från två barn med den ovanliga lungsjukdomen pulmonell veno-okklusiv sjukdom (PVOD). Det är en sjukdom som främst drabbar lungvenerna och ger upphov till svår pulmonell hypertension. Lungtransplantation är den enda möjligheten till bot. Vävnadsprover från båda patienterna undersöktes med klassisk mikroskopi och med synkrotronbaserad mikro-CT-teknik, som ger möjlighet till 3D-visualisering av lungkärl på mikrometernivå. Det ena barnet hade en för sjukdomen typisk genmutation, som den andra saknade. En oväntad skillnad i lungkärtsförändringarna kunde, bland annat med hjälp av 3D-tekniken, tydligt visualiseras.

De fyra studierna tillsammans visar att barn förskrivs en hel del lungkärtsvidgande läkemedel till alla typer av pulmonell hypertension, trots avsaknad av tydliga bevis för behandlingseffekter på barn. Positiva effekter av behandling kunde dock mätas och framför allt framkom inga allvarliga negativa bieffekter. Lungkärtsförändringar kunde också tydligare framställas med synkrotronbaserad mikro-CT-teknik. Resultaten ger värdefull information för att framöver kunna ge rätt behandling till varje unikt barn.

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