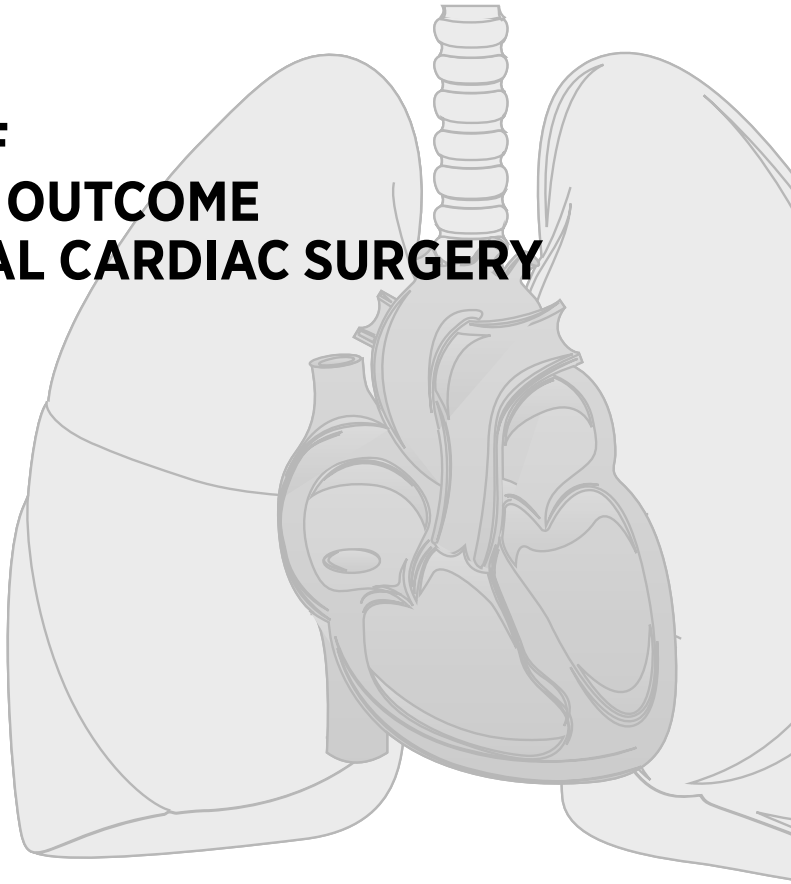
UNIVERSITY OF HELSINKI
FACULTY OF MEDICINE

MEASUREMENT OF LUNG LIQUID AND OUTCOME AFTER CONGENITAL CARDIAC SURGERY

ANU KASKINEN



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Anu Kaskinen

ACADEMIC DISSERTATION

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To all children with congenital heart defects.

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List of original publications

I **Kaskinen AK**, Helve O, Andersson S, Kirjavainen T, Martelius L, Mattila IP, Rautiainen P, Pitkänen OM. Chronic Hypoxemia in Children With Congenital Heart Defect Impairs Airway Epithelial Sodium Transport. Pediatr Crit Care Med. 2016 Jan; 17(1): 45-52.

II **Kaskinen AK**, Martelius L, Kirjavainen T, Rautiainen P, Andersson S, Pitkänen O. Assessment of extravascular lung water by ultrasound after congenital cardiac surgery. Pediatr Pulmonol. Oct 14. doi: 10.1002/ppul.23531. [Epub ahead of print]

III **Kaskinen AK**, Kirjavainen T, Rautiainen P, Martelius L, Andersson S, Pitkänen O. Ventilator-derived dynamic lung compliance measurement: usefulness in children under mechanical ventilation. Submitted.

IV **Kaskinen AK**, Happonen JM, Mattila IP, Pitkänen OM. Long-term outcome after treatment of pulmonary atresia with ventricular septal defect: nationwide study of 109 patients born in 1970-2007. Eur J Cardiothorac Surg. 2016 May; 49(5): 1411-8.

The publications are referred to in the text by their roman numerals and are reprinted here with the permission of the publishers. In addition, this thesis includes unpublished results.

Publication II has also been used in the thesis of Laura Martelius, M.D., entitled "Ultrasound in estimating lung liquid and parenchyma in children" (ISBN:978-951-52-2042-7).

Abstract

Congenital heart defects (CHD) are classified as acyanotic and cyanotic. In cyanotic CHD, a mixing of deoxygenated in oxygenated blood reduces arterial oxygenation and the child may be cyanotic, i.e., bluish. Many children with CHD need invasive treatment, either catheter procedures or cardiac surgery. Congenital cardiac surgery often aims to restore normal circulation and correct the defect as seen in vast majority of pulmonary atresia with ventricular septal defect (PA+VSD), but palliative surgery may also be needed or may be the only possible treatment strategy.

Noxious trauma to the lung, such as cardiopulmonary bypass (CPB) and the reperfusion phase after congenital cardiac surgery, may promote excessive extravascular lung water (EVLW). In the mammalian lung, effective clearance of EVLW is essential in maintaining only a minimal layer of the epithelial lining liquid and warding off lung edema. In humans and animals, this clearance rests on active airway epithelial Na^+ transport. Amiloride-sensitive epithelial Na^+ channel (ENaC), together with basolateral Na-K-ATPase, allow the transcellular movement of Na^+, which is followed by parallel osmotic movement of water. Airway epithelial Na^+ ion and liquid transport is reduced by ambient hypoxia. Furthermore, arterial oxygen saturation level has correlated with airway epithelial Na^+ transport in ambient hypoxia. Postoperative lung edema after congenital cardiac surgery has principally been assessed by chest radiography (CXR), which may be inaccurate and causes irradiation. Excessive EVLW promotes appearance of artifacts called B-lines in lung ultrasound (US), whereas lung compliance associates negatively with increased EVLW.

The first aim of this thesis was to study the effect of chronic hypoxemia in ambient normoxia on lung liquid transport in children with CHD. We measured airway epithelial Na^+ transport activity by nasal transepithelial potential difference (NPD) and Na^+ transporter mRNA levels by quantitative reverse-transcriptase polymerase chain reaction (RT-qPCR). Second, feasibility of lung US and lung compliance in assessment of EVLW and in predicting short-term clinical outcome was tested after congenital cardiac surgery. EVLW was assessed with both CXR and lung US. Lung compliance was quantified as static compliance and as ventilator-derived dynamic compliance. Third, the long-term survival of a cyanotic CHD was retrospectively evaluated in patients with PA+VSD.

According to our findings, the airway epithelial Na^+ transport was impaired in profoundly hypoxemic children with cyanotic CHD. After congenital cardiac surgery, lung US B-line score and static lung compliance correlated with CXR lung edema assessment, unlike ventilator-derived dynamic lung compliance. The dynamic lung compliance values differed clearly from the static ones but these compliance values showed a moderate correlation with each other. However, ventilator-derived dynamic lung compliance may not reflect the state of lung parenchyma similar to static compliance. Furthermore, both early postoperative lung US B-line and CXR lung

edema scorings predicted short-term outcome interpreted as length of postoperative mechanical ventilation and intensive care. Among factors affecting the long-term survival of PA+VSD the primary anatomy of pulmonary circulation and achievement of repair were most important.

In summary, our results emphasize the effect of postoperative pulmonary complications on short-term outcome after congenital cardiac surgery. Our data suggests that hypoxemia may attenuate the constitutional mechanism of the lung to prevent excessive lung liquid accumulation. To detect this, lung US can be used to complement CXR when assessing EVLW in children undergoing cardiac surgery. This may be particularly useful in profoundly hypoxemic children with cyanotic CHD and may promote early recognition of postoperative pulmonary complications. Although primary anatomical factors affect long-term outcome of PA+VSD, an important form of cyanotic heart disease, the treatment should aim for corrective surgery in all PA+VSD patients.

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Tiivistelmä

Synnynnäiset sydämen rakenneviat voivat olla syanoottisia, joissa vähähappinen laskimoveri ja hapekas valtimoveri pääsevät sekoittumaan aiheuttaen valtimoveren happipitoisuuden alenemisen (hypoksemia). Tai viat voivat asyanoottisia, jolloin valtimoveren happipitoisuus on normaali. Merkittävä osa synnynnäisistä sydänvioista vaatii kajoavaa hoitoa joko kirurgisesti tai katetriteitse. Hoidon tavoitteena on usein normaalin verenkierron palauttaminen kuten on tässä kirjassa tarkemmin käsiteltävän syanoottisen synnynnäisen sydänvian, pulmonaaliatresia yhdistettynä kammioväliseinäaukkoon (PA+VSD), tapauksessakin. Kuitenkin osassa synnynnäisistä sydänvioista verenkierto voidaan korjata vain osittain palliatiivisen kirurgian keinoin.

Normaalisti kaasuja vaihtavat ilmatiet sisältävät vain pienen määrän nestettä. Synnynnäisen sydänvian leikkaushoidon jälkeen hengitysteihin voi kertyä liiallista nestettä eli keuhkoödeemaa, joka hankaloittaa keuhkojen pääasiallista tehtävää eli kaasujen vaihtoa. Ylimääräisen keuhkonesteen kuljetus pois ilmatilasta perustuu hengitysteiden pintasolukon (epiteelin) aktiivisen Na^+-ionien kuljetuksen aikaansaamaan osmoottiseen veden siirtymiseen. Aiemmin on kokeellisesti osoitettu, että ilman matala happipitoisuus (hypoksia) heikentää hengitystie-epiteliaalista Na^+-ionien kuljetusta ja keuhkonesteen poistumista. Lisäksi hypoksemian on osoitettu korreloivan hengitystie-epiteliaalisen Na^+-ionien kuljetuksen kanssa korkeassa vähähappisessa ilmanalassa. Sydänleikkauksen jälkeen keuhkoödeeman kuvantaminen perustuu sydän-keuhkokuvaan (thorax-kuva), joka aiheuttaa säteilyä ja voi olla epätarkka. Keuhkojen ultraäänitutkimuksella todettavien ns. B-viivojen on todettu olevan merkki keuhkojen lisääntyneestä nestemäärästä. Ja toisaalta keuhkojen venyvyyttä kuvaavan keuhkokomplianssin on todettu heikentyvän keuhkojen nestemäärän lisääntyessä.

Tutkimme sydänleikkaukseen saapuvilla lapsilla kroonisen hypoksemian vaikutusta hengitystie-epiteliaaliseen nesteen kuljetukseen ja mahdollisen sydänleikkauksen jälkeisen keuhkoödeeman kehittymiseen. Mittasimme nenäepiteelin ionien kuljetusaktiviteettia transepiteliaalisena potentiaalierona (NPD) ja Na^+-ionikanavien lähetti-RNAn määriä RT-qPCR-tekniikalla. Sydänleikkauksen jälkeen teho-osastolla tutkittiin keuhkojen ultraäänen ja keuhkokomplianssin mahdollisuuksia keuhkoödeeman ja toisaalta leikkauksen jälkeisen lyhytaikaisennusteen arvioimisessa. Syanoottisen synnynnäisen sydänvian pitkäaikaisennustetta arvioitiin retrospektiivisesti kattavan PA+VSD-potilaiden pitkäaikaisseurannan perusteella.

Osoitimme hengitystie-epiteliaalisen Na^+-ionien kuljetuksen olevan heikentynyt hypoksemisilla syanoottista sydänvikaa sairastavilla lapsilla. Sydänleikkauksen jälkeinen keuhkojen ultraäänilöydös ja staattinen keuhkokomplianssi korreloivat thorax-kuvan nesteisyysarvion kanssa. Hengityskoneen automaattisesti määrittämä dynaaminen keuhkokomplianssi erosi staattisesta komplianssista huolimatta korrelaatiosta näiden komplianssiarvojen välillä, eikä korreloinut thorax-kuvan

nesteisyysarvion kanssa. Dynaaminen keuhkokomplianssi vaikuttaakin kuvaavan eri asiaa kuin staattinen keuhkokomplianssi, eikä sellaisenaan sovellu keuhkonesteen arvioimiseen. Leikkauksen jälkeinen thorax-kuvasta tai keuhkojen ultraäänestä tehty arvio keuhkojen nesteisyydestä oli itsenäinen leikkauksen jälkeiseen lyhytaikaisennusteen vaikuttava tekijä, päinvastoin kuin keuhkokomplianssi. PA+VSD –potilaiden pitkäaikaisennusteeseen puolestaan tärkeimpinä tekijöinä vaikuttivat alkuvaiheen keuhkoverenkierron anatomia ja onnistunut kirurginen korjaus.

Tulokset korostavat sydänleikkauksen jälkeisten keuhkopulmien vaikutusta sydänleikkauksesta toipumiseen. Havaintomme perusteella syanoottista synnynnäistä sydänvikaa sairastavilla lapsilla voi olla suurentunut riski sydänleikkauksen jälkeiselle keuhkoödeemalle ja keuhkonesteen määrää voidaan sydänleikatuilla lapsilla arvioida thorax-kuvan ohella myös keuhkojen ultraäänitutkimuksella. Keuhkovaurion aktiivinen kuvantaminen sydänleikkauksen jälkeen voi olla hyödyksi potilaan lyhytaikaisennusteen parantamisessa ja tehohoidon keston minimoimisessa. Vaikka keuhkoverenkierron anatomia vaikuttaa PA+VSD potilaiden ennusteeseen, on kirurgiseen korjaukseen pyrkiminen ensiarvoisen tärkeää ennusteen kannalta.

Abbreviations

ACC	Aristotle comprehensive complexity
ALI	acute lung injury
AQP	aquaporin
(A)RDS	(acute/adult) respiratory distress syndrome
AUC	area under the curve
CFTR	cystic fibrosis transmembrane conductance regulator
CHD	congenital heart defect
CK18	cytokeratin 18
CPB	cardiopulmonary bypass
Crs	respiratory system compliance
CXR	chest radiography/ X-ray
e.g.	exempli gratia
ENaC	epithelial sodium channel
EVLW	extravascular lung water
HAPE	high altitude pulmonary edema
HLHS	hypoplastic left heart syndrome
HR	heart rate
i.e.	id est
L-R	left to right
LV	left ventricle
MAPCA	major aortopulmonary collateral artery
mRNA	messenger RNA
NKCC	Na-K-Cl cotransporter
NPD	nasal transepithelial potential difference
NYHA	New York Heart Association
PA+VSD	pulmonary atresia with ventricular septal defect
PICU	pediatric intensive care unit
POD	postoperative day
R-L	right to left
ROC	receiver operating characteristic
RR	respiratory rate
RT-qPCR	quantitative reverse-transcription polymerase chain reaction
RV	right ventricle
SpO_2	arterial blood oxygen saturation level measured by pulse oximeter
TGA	transposition of the great arteries
TNPAI	total neopulmonary arterial index
TOF	tetralogy of Fallot
TPDD	transpulmonary double indicator dilution
TPTD	transpulmonary thermodilution
US	ultrasound
UVH	univentricular heart
VSD	ventricular septal defect

1 Introduction

The gas exchange in human bodies occurs in air-filled alveoli, which are surrounded by capillary vessels carrying oxygen bound to hemoglobin towards the heart and then to the entire body. The human fetus, however, receives oxygen through the umbilical vein, and the placenta serves as the organ for gas exchange. Nevertheless, the fetal lungs, through liquid and surfactant secretion, contribute significantly to fetal development of the respiratory system (Alcorn et al. 1977, Strang 1991).

Adaptation to extrauterine life requires major cardiorespiratory adjustments at birth (Alvaro and Rigatto 2005). Furthermore, clearance of fetal lung liquid requires efficient airway epithelial liquid absorption induced by cathecolamines, glucocorticoids, and increased ambient oxygen level (Strang 1991). Postnatally, low ambient oxygen level (hypoxia) at high altitude and low arterial blood oxygen level (hypoxemia), on the contrary, associate with reduced airway epithelial liquid removal (Sartori et al. 2004, Su et al. 2016).

Arterial blood oxygen levels rise to adult levels within several minutes after birth (Kamlin et al. 2006, Toth et al. 2002). However, in newborns facing problems in cardiorespiratory adjustments or clearance of fetal lung liquid, arterial blood oxygen saturation level measured by pulse oximeter (SpO_2) may remain low. Newborns with congenital heart defects (CHD) may also have low SpO_2, and without corrective surgery the growing child with cyanotic CHD may suffer from long-lasting hypoxemia and cyanosis, i.e., blueness.

Since the cardiovascular and pulmonary systems closely interrelate, children with CHD may be especially sensitive to pulmonary pathologies, and respiratory-related complications are common after congenital cardiac surgery (Sata et al. 2012, Kanter et al. 1986). In particular, congenital cardiac surgery with cardiopulmonary bypass (CPB) causes an ischemia-reperfusion injury and inflammatory response leading to endothelial injury and increased capillary permeability and further to increased amounts of extravascular lung water (EVLW) (Apostolakis et al. 2010, Asimakopoulos et al. 1999a).

This thesis hypothesized that chronic hypoxemia, similar to ambient hypoxia, may impair airway epithelial lung liquid clearance and may predispose children with cyanotic CHD to excessive EVLW after cardiac surgery. Furthermore, the thesis aimed to evaluate feasibility of postoperative lung ultrasound and lung compliance after congenital cardiac surgery through detection of abundance of EVLW (Barnas et al. 1992, Jambrik et al. 2010). Whether early postoperative sonographic and radiographic scorings of EVLW as well as lung compliance predict short-term outcome after congenital cardiac surgery was studied prospectively in children with CHD. Long-term outcome of cyanotic CHD requiring surgical treatment, instead, was studied retrospectively in patients with pulmonary atresia with ventricular septal defect (PA+VSD).

2 Review of the literature

2.1 Congenital heart defect

A congenital heart defect (CHD), considered as a structural abnormality of the heart and/or great vessels present from birth, forms the most common class of birth defect with an estimated incidence of 1% (Dolk et al. 2011, Hoffman and Kaplan 2002). Etiology of CHD is traditionally defined by interaction of multiple genes and environmental factors (Nora 1968). Both noninherited fetal exposures as well as specific genes essential for heart formation contribute to the etiology of CHD (Garg et al. 2003, Jenkins et al. 2007, Schott et al. 1998). However, chromosomal aneuploidies as well as single-gene defect associated with noncardiac malformations account for 10%–15% of CHDs (van der Bom et al. 2011).

Modern prenatal screening allows antenatal diagnosing of CHD. In Finland, prenatal screening for CHD is performed during the second trimester (Eik-Nes 2006, Autti-Rämö et al. 2005). In reports from the last 15 years, prenatal CHD diagnosis has been possible in only one-third of cases, but advances in antenatal screenings have increased the antenatal diagnosis rates in recent years (Quartermain et al. 2015, Ojala et al. 2013). For example, in 2011, the antenatal diagnosis rate of univentricular heart (UVH) was 87% in Finland (Ojala et al. 2013). Furthermore, postnatal pulse oximetry screening used in addition to clinical examination in all Finnish childbirth hospitals has improved early diagnosis especially in critical duct-dependent CHD needing invasive treatment during the neonatal period (de-Wahl Granelli et al. 2009, Valmari 2007, Ojala et al. 2015). Postnatally, echocardiography remains as the basis of CHD diagnostics, although other noninvasive diagnostic modalities and angiography are sometimes necessary for thorough evaluation.

CHDs are classified as acyanotic and cyanotic. In cyanotic CHD, arterial oxygen levels are reduced due to mixing of deoxygenated and oxygenated blood and the child may be cyanotic, i.e., bluish. In acyanotic CHD, instead, arterial oxygen levels are normal, and the defect may consist of an abnormal left to right (L-R) shunt within the heart or great vessels, or narrowed structures diminishing the systemic circulation, or regurgitations of the atrioventricular or semilunar valves (Table 1). However, without treatment, acyanotic CHD may transform to cyanotic due to excessive pulmonary blood flow leading to pulmonary hypertension evolving to right to left (R-L) shunting (i.e., Eisenmenger syndrome).

Table 1. Examples of congenital heart defects

		Duct-dependent	Non-duct-dependent
Acyanotic	L-R shunting		Atrial septal defect Atrioventricular septal defect Patent ductus arteriosus VSD
	Obstructive defect	Critical aortic stenosis [a] Critical coarctation of the aorta [a] Interrupted Aortic arch [a]	Aortic stenosis Coarctation of the aorta Pulmonary stenosis
	Other		Vascular rings
Cyanotic	Reduced pulmonary blood flow	PA+VSD [b] PA+intact ventricular septum [b]	PA+VSD+MAPCAs TOF
	R-L shunting	Critical Ebsteins anomaly [b] Critical pulmonary stenosis [b] HLHS [a] Tricuspid atresia [b]	Ebsteins anomaly
	Separate circulations	TGA [a, b]	TGA+ASD/VSD
	Other	UVH+ventricular outflow tract obstruction [a or b]	Total anomalous pulmonary venous return Truncus arteriosus

[a] duct-dependent systemic circulation
[b] duct-dependent pulmonary circulation

2.1.1 Hypoxemia in cyanotic congenital heart defect

In cyanotic CHD, reduced pulmonary blood flow capability causes drainage of deoxygenated venous blood to oxygenated systemic circulation through septal defects. Also, in transposition of the great arteries (TGA), mixture of parallel deoxygenated and oxygenated circulations by shunting is crucial for survival (Table 1). After birth, the cyanotic CHDs without any concomitant shunts remain dependent on fetal shunts, namely ductus arteriosus and foramen ovale. Thus, closure of these fetal routes may be incompatible with life in some cyanotic CHDs.

The level of systemic hypoxemia between different cyanotic CHDs varies from almost normal to profound (Figure 1). As newborns with tetralogy of fallot (TOF) may have SpO_2 levels over 90% without repair, the ones with TGA may have SpO_2 below 60% prior to initial invasive intervention. Furthermore, there are a group of various CHDs that share the feature of only one ventricle being of adequate functional size, namely UVH. These children may remain profoundly hypoxemic until the age of 2 to 3 years when the final stage of palliative surgery is performed and SpO_2 levels usually rise over 90% (Jolley et al. 2015).

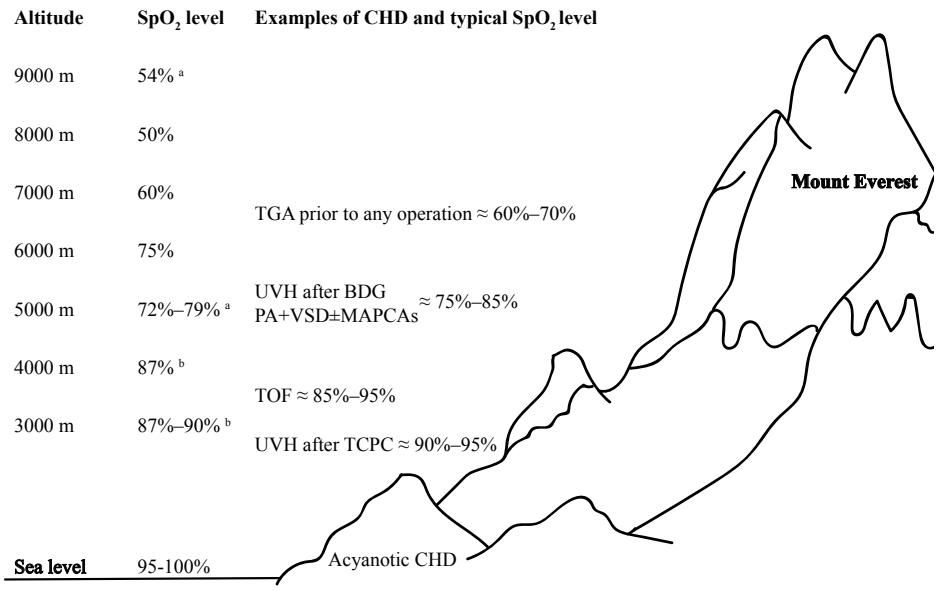

Figure 1 *SpO_2 levels at high altitude measured in adults[a] and infants[b] compared with SpO_2 values typically seen in CHDs. Values between 6000m and 8000m are estimated SpO_2 levels. (Gamponia et al. 1998, Grocott et al. 2009, Hackett and Roach 1995, Niermeyer et al. 1993, Sartori et al. 2004).*

A progressive sudden fall in arterial oxygen level can cause severe symptoms as seen in critically ill patients as well as in mountain sickness at high altitude (Grocott et al. 2007). But, in the case of chronic hypoxemia the human body may adapt. Delivery of oxygen to cells improves in response to hypoxia (Zhou et al. 2008). Most healthy humans living at high altitude can adapt nicely to their hypoxic environment, whereas some subjects may develop chronic mountain sickness with excessive hemopoiesis and polycythemia (Hainsworth and Drinkhill 2007). Similarly in chronically hypoxemic children with uncorrected cyanotic CHD, hemoglobin levels increase. In patients with cyanotic CHD, remarkable neovascularization may also develop to improve oxygenation (Duncan et al. 1999). Moreover, the vascular endothelial growth factor stimulating angiogenesis has been shown to be elevated and to correlate positively with SpO_2 level in profoundly hypoxemic children with cyanotic CHD (Baghdady et al. 2010, Starnes et al. 2000).

2.1.2 Pulmonary atresia with ventricular septal defect

Pulmonary atresia with ventricular septal defect (PA+VSD) is an example of cyanotic CHD, and these patients often present with a profound hypoxemia. PA+VSD results from an error in the infundibular septum alignment during embryonic conotruncal heart development (Van Praagh et al. 1970) and is characterized by complete obstruction of the pulmonary RV outflow tract resulting in an absence of connection between right ventricle (RV) and pulmonary arteries. A ventricular septal defect (VSD) allos R-L shunting (Samanek and Voriskova 1999) (Figure 2).

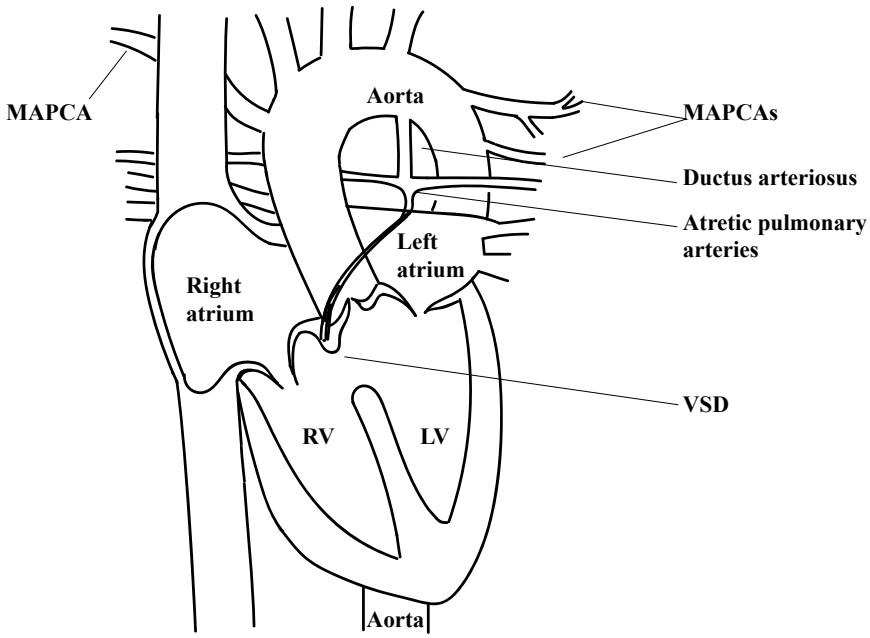

Figure 2 *PA+VSD is a cyanotic congenital heart disease characterized by atresia of the pulmonary valve and artery, and a large VSD. The pulmonary blood supply is dependent on ductus arteriosus or MAPCAs or both.*

In PA+VSD, the anatomy and extent of pulmonary vasculature varies greatly, is determined during embryological development, and depends on timing of termination of antegrade pulmonary blood flow. In addition to echocardiography, a cardiac catheterization is often needed to clearly evaluate the pulmonary vasculature prior to determination of surgical strategy. Nowadays, three-dimensional magnetic resonance angiography and computed tomography are both comparable with cardiac catheterization when identifying pulmonary blood flow (Geva et al. 2002, Lin et al. 2012).

Pulmonary blood flow in PA+VSD derives from systemic circulation, either from uni- or bilateral ductus arteriosus, major aortopulmonary collateral arteries (MAPCAs), or from both (Figure 2). MAPCAs exist in 31%–38% of PA+VASD patients and are developed to compensate for the insufficient antenatal anterograde pulmonary blood flow and their embryologic origin varies (Hofbeck et al. 1991, Leonard et al. 2000, Rabinovitch et al. 1981). More recently, a study by Norgaard and colleagues suggested that all the MAPCAs are dilated bronchial arteries (Norgaard et al. 2006). The descending thoracic aorta serves as the origin for most MAPCAs, but they may originate also from the aortic arch, subclavian artery, distal thoracic aorta, internal mammary artery, and coronary arteries (Liao et al. 1985). In the presence of diminutive central pulmonary arteries and clinically significant MAPCAs, intrapulmonary arteries often become stenotic and hypoplastic due to decreased pulmonary blood flow (Amark et al. 2004, Haworth et al. 1981). However, when the pulmonary blood flow derives wholly from ductus arteriosus, the peripheral pulmonary blood flow and the distribution of the intrapulmonary arteries are usually normal (Amark et al. 2004).

2.2 Treatment of congenital heart defect

Although the mildest forms of CHD may need only to be observed and followed by a cardiologist, many children with CHD need invasive treatment, either catheter procedures or cardiac surgery. The type of CHD determines whether the invasive treatment requires surgery or catheter intervention, whether the procedure is corrective or palliative, whether neonatal procedures are needed, and whether only one procedure or a series of procedures is needed. Critical duct-dependent CHD needs invasive treatment during the first days of life (Table 1).

Congenital cardiac surgery aims either to restore the normal circulation and correct the defect or to make circulation more appropriate by palliation. For instance, the majority of patients with PA+VSD, even with MAPCAs, are nowadays repaired (Amark et al. 2006, Cho et al. 2002). However, patients with UVH such as hypoplastic left heart syndrome (HLHS), generally go through a three-staged palliation in early childhood resulting in Fontan circulation, where the central and hepatic veins are directly connected to the pulmonary arteries (Jolley et al. 2015). The type of first operation in the newborn period varies and depends on the specific type of UVH defect. The first operation aims to complete a mixing of pulmonary and systemic circulations, avoidance of pulmonary venous congestion, unobstructed outflow to the systemic circulation, and a reliable but controlled source of pulmonary blood flow. The second operation, i.e., Glenn operation, is normally performed between the ages of three to six months, and aims to reduce volume load from a single ventricle by connecting the superior vena cava to the pulmonary artery. During the third and final stage, the inferior vena cava is connected directly to the pulmonary arteries by total cavopulmonary connection (TCPC), allowing all venous blood to complete pulmonary circulation through direct blood vessel connections.

Since more complex CHDs are operated on these days and survival of CHD has significantly improved, more children with CHD grow and achieve adulthood. This means the number of reoperations for residual defects, replacement of conduits, and late complications needing reoperations have also increased (Ong et al. 2013, Vida et al. 2007, Erikssen et al. 2015).

2.2.1 Surgery of PA+VSD

The aim in treating PA+VSD patients is to restore normal circulation with separated pulmonary and systemic circulations in series. Thus, the repair of PA+VSD comprises closure of VSD as well as extracardiac pulmonary blood supply and creation of a connection between the RV and pulmonary arteries. The first successful repair was reported in 1955 (Lillehei et al. 1955), after which a variety of surgical techniques have served to treat patients with PA+VSD.

When ductus arteriosus solely supplies pulmonary blood flow, depending on the size of central pulmonary arteries, patients undergo either primary or staged repair. If central pulmonary arteries are considered diminutive and primary repair impossible, often a systemic-pulmonary artery shunt is created to improve the pulmonary circulation, native pulmonary vascular bed, and oxygenation.

In the presence of MAPCAs and scanty central pulmonary arteries, surgical strategies are more complicated and the surgical treatment of PA+VSD with MAPCAs is a more debated topic. Traditionally, a staged repair with unifocalization of MAPCAs into pulmonary circulation has been a widely used surgical strategy (Duncan et al. 2003, Reddy et al. 1997, Song et al. 2009, Iyer and Mee 1991). Also a strategy of primary repair with unifocalization has revealed excellent short-term results (Davies et al. 2009, Lofland 2000, Carrillo et al. 2015). However, a study from Melbourne reported that the majority of unifocalized MAPCAs may thrombose, develop stenosis, or fail to grow (dUdekem et al. 2005). Therefore, unifocalization has become a subject of controversy (Brizard et al. 2009, Malhotra and Hanley 2009), and a strategy focusing on augmenting blood flow within the native pulmonary arteries by systemic-pulmonary artery shunting instead of unifocalizing MAPCAs has also been introduced (Brizard et al. 2009, Liavaa et al. 2012, Mumtaz et al. 2008, dUdekem et al. 2005).

2.2.2 Grading the risk of morbidity after cardiac surgery

Initially high postoperative mortality has dramatically decreased due to advances in surgical techniques, cardiopulmonary bypass (CPB), and postoperative intensive care. However, mortality and morbidity after heart surgery still exist (Erikssen et al. 2015). For some simple defects (e.g., atrial septal defect), surgery is relatively routine and risk-free, whereas surgery for other defects (e.g. UVH) includes a high risk for postoperative morbidity (Erikssen et al. 2015, Nieminen et al. 2001). In addition to the complexity of the operative method, depending on diagnosis and surgical

technique, procedure-independent factors such as neonatal age also contribute to the postoperative mortality and morbidity (Kang et al. 2004).

Since many factors affect morbidity after congenital cardiac surgery, complexity-adjusted scoring methods have been created to evaluate postoperative morbidity and to compare surgical results between surgical centers (Jenkins et al. 2002, Lacour-Gayet et al. 2004). Aristotle basic score is a sum of three procedure-related factors: the potential for postoperative mortality, the potential for postoperative morbidity, and the technical difficulty of the procedure (Lacour-Gayet et al. 2004). Aristotle comprehensive complexity (ACC) scoring also takes into account patient characteristics such as prematurity and neurological impairment and has shown to strongly correlate with observed mortality and to predict postoperative outcome (Bojan et al. 2011a, Sata et al. 2012). Moreover, ACC scoring has predicted operative mortality and length of postoperative intensive care unit stay better than another risk scoring proposed for complexity assessment (Bojan et al. 2011b).

2.3 Lungs and congenital heart defect

The cardiovascular and pulmonary systems closely interrelate, which is clearly demonstrated at birth when the start of breathing and exposure to the ambient O_2 cause a significant decrease in pulmonary vascular resistance (Alvaro and Rigatto 2005). Changes in intrathoracal pressure but also the transpulmonary pressure gradient (alveolar pressure–intrapleural pressure) and the resulting change in alveolar volume influence cardiovascular performance. While ventilation affects cardiovascular performance mainly through changes in RV and LV preload as well as afterload, the reverse is also true, as the pulmonary and systemic circulations in series impact respiratory function (Da Cruz et al. 2014). The interactions are clinically significant when treating children with CHD, particularly those who are mechanically ventilated.

2.3.1 Respiratory morbidity in congenital heart defect

Pulmonary pathology may occur in CHD patients for numerous reasons, particularly if CHD has been left uncorrected (Healy et al. 2012). Airway compression can be caused by massive cardiomegaly, dilated pulmonary arteries, left atrium enlargement, anomalous relation between tracheobronchial tree and vasculature, or by MAPCAs (Kussman et al. 2004). Excessive pulmonary blood flow due to L-R shunting on the one hand, and obstruction of pulmonary venous drainage on the other, elevate hydrostatic forces within the pulmonary capillaries, which may cause interstitial liquid accumulation and lung edema (Healy et al. 2012). Furthermore, decreased flow capacity of the pulmonary lymphatic system may predispose patients to lung edema (Healy et al. 2012). Permanently excessive pulmonary blood flow in various CHDs

may cause pulmonary hypertension (De Wolf 2009). In addition, children with CHD may be especially susceptible to respiratory tract infections (Healy et al. 2012).

During the early postoperative phase after surgery for CHD, respiratory-related complications are common (Sata et al. 2012, Kanter et al. 1986). CPB-activated inflammatory response, anesthesia, ischemia-reperfusion injury, hypothermia, as well as hemodynamic instability, all associate with postoperative pulmonary dysfunction (Apostolakis et al. 2010). Pathophysiology behind pulmonary dysfunction includes increased pulmonary vascular resistance, decreased lung compliance, decreased functional residual capacity, increased ventilation-perfusion mismatch, interstitial edema, and reduced surfactant activity (Griese et al. 1999, Kozik and Tweddell 2006). Postoperative mechanical ventilation may also be prolonged due to both nosocomial pneumonia, reported in 10%–22% of children after heart surgery, and respiratory complications caused by surgical trauma such as chylothorax and diaphragmatic paralysis (Chan et al. 2005, Fischer et al. 2000, Joho-Arreola et al. 2005, Tan et al. 2004). Furthermore, both anesthesia and CPB predispose patients to atelectasis, which further reduces lung compliance and causes ventilation/perfusion mismatch (Lundquist et al. 1995, Magnusson et al. 1997).

2.3.2 Postoperative lung injury and lung edema

Normally, the alveoli are coated with a thin film of liquid, which ensures optimal gas exchange through diffusion. The excessive accumulation of EVLW may result from increased capillary permeability, capillary hydrostatic pressure, or from both (Ware and Matthay 2005).

Surgery on intracardiac defects requires usage of CPB. Inflammatory response to CPB causes the release of various inflammatory mediators and endotoxins, which lead to endothelial injury and increased capillary permeability (Asimakopoulos et al. 1999a). These pathological changes further induce leakage of liquid and proteins from capillaries into interstitium and increased amounts of EVLW (Apostolakis et al. 2010, Asimakopoulos et al. 1999a). Furthermore, CPB causes lung ischemia-reperfusion process, which may further promote postoperative lung injury and edema (Apostolakis et al. 2010). Elevated hydrostatic forces within the pulmonary capillaries may further increase accumulation of EVLW (Healy et al. 2012, Vincent et al. 1984). In addition, particularly in PA+VSD, a development of postoperative pulmonary reperfusion injury after unifocalization, presenting often unilaterally, has also been suggested to associate with severity of stenosis and bilateral unifocalization (Maskatia et al. 2012).

Modern CPB and attempts to limit the trauma caused by cardiac surgery aim to limit the inflammatory process by improving biocompatibility of the extracorporeal circuit, hemodynamic stability by adequate perfusion and hemofiltration, and suppression of inflammatory response with corticosteroids (Apostolakis et al. 2010, Huang et al. 2003, Keski-Nisula et al. 2013, Maharaj and Laffey 2004). In addition, a delayed

sternal closure improves postoperative hemodynamic and respiratory stability in neonates, who are especially susceptible to CPB (Odim et al. 1989).

Despite attempts to limit inflammatory response, it occurs to some extent in all patients predisposing to respiratory impairment, which is still a recognized postoperative complication after heart surgery and CPB (Taggart et al. 1993, Apostolakis et al. 2010). However, only a minority of patients suffer from lung edema and acute (or adult) respiratory distress syndrome (ARDS), which is the most severe form of pulmonary injury (Asimakopoulos et al. 1999a). ARDS has been reported in 1% of patients after CPB (Asimakopoulos et al. 1999b, Christenson et al. 1996, Messent et al. 1992).

2.4 Lung liquid and edema clearance

The optimal balance between drive of liquid toward the interstitium and removal mechanisms of EVLW is necessary for maintenance of an optimal amount of alveolar liquid. In healthy lungs, accumulation of excessive EVLW is prevented by notable capacitance of interstitium, tight alveolar epithelial barrier preventing liquid leakage from interstitium, removal of liquid from alveolar spaces airway by active epithelial ion transport, and an efficient pulmonary lymphatic system (Miserocchi 2009, Bronicki and Penny 2014).

2.4.1 Ion transport and osmotically driven lung edema clearance

Effective lung liquid absorption depends on ion transport, and especially on transport of sodium ions (Na^+) (Matthay et al. 1982). Osmotically driven movement of water follows positively charged Na^+ (Eaton et al. 2009, Matalon et al. 2015) (Figure 3).

The amiloride-sensitive epithelial sodium channel (ENaC) is a crucial apical route for Na^+ (Figure 3). In addition to ENaC, amiloride-insensitive Na^+ channels exist and they contribute 20%–40% of airway epithelial Na^+ transport (Folkesson and Matthay 2006, OBrodovich et al. 2008). Basolateral Na-K-ATPase plays an essential role in creating an electrochemical gradient resulting in Na^+ entry into the cells (Matthay et al. 2002, Folkesson and Matthay 2006) (Figure 3).

Transport of chloride ions is required to maintain electrochemical balance at the airway epithelium (Eaton et al. 2009, Matalon et al. 2015) (Figure 3). Diversity of Cl^- channels present at airway epithelium, such as Na-K-Cl-cotransporter (NKCC), HCO_3^-/ Cl^- exchangers, and apically situated Ca^{2+}-activated ion channels (Hollenhorst et al. 2011). Chloride secretion, however, is largely mediated by the apically located cAMP-dependent cystic fibrosis transmembrane conductance regulator (CFTR) channel (Mall and Galietta 2015). Through negative regulation of ENaC by CFTR, and vice versa, the CFTR channel also may have a role in lung liquid clearance

(Donaldson et al. 2002, Fang et al. 2006, Mall et al. 2004). Moreover, airway epithelial Cl^- secretion may contribute to cardiogenic hydrostatic lung edema (Solymosi et al. 2013).

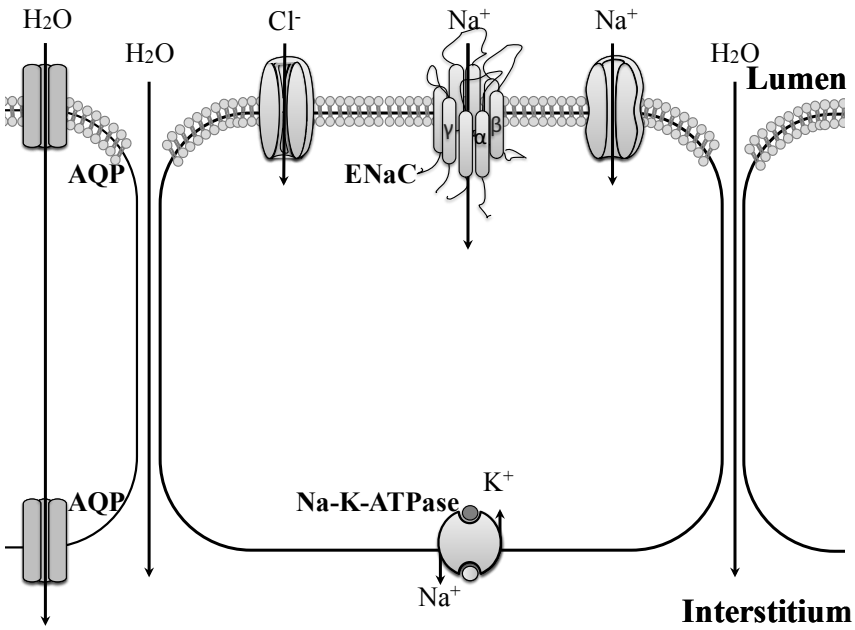

Figure 3 *Airway epithelial liquid transport rests on active Na^+ transport through amiloride-sensitive ENaC channels and amiloride-insensitive Na^+ channels, followed by osmotically driven movement of water through paracellular pores and aquaporin channels (AQP). Gradient formed by basolateral Na-K-ATPase activates apical Na^+ transport, whereas Cl^- transport maintains electroneutrality.*

Na^+ transport-driven liquid absorption maintains EVLW at a minimal level in a healthy state, but the role of effective liquid absorption increases when excessive EVLW arises (Ware and Matthay 2001, Berthiaume and Matthay 2007). The role of EVLW absorption is vital in the lungs of newborns at birth when respiration begins and fetal lung liquid has to be removed (Strang 1991). Furthermore, deficiency in lung liquid absorption associates with two main entities of neonatal lung disease, namely respiratory distress syndrome (RDS) and transient tachypnea of a newborn (TTN) (Helve et al. 2009). Defective airway epithelial ion and liquid transport also contributes to ARDS, acute lung injury (ALI), high altitude pulmonary edema (HAPE), and systemic inflammatory response (Eisenhut and Wallace 2011, Mac Sweeney et al. 2011, Ware and Matthay 2001). Furthermore, in patients with severe hydrostatic pulmonary edema, intact alveolar liquid clearance has been associated with improved short-term outcome interpreted as length of mechanical ventilation and hospital mortality (Verghese et al. 1999). An analogous observation in patients with

post-lung transplant reperfusion injury showed association between intact alveolar liquid clearance and clinical outcomes (Ware et al. 1999).

In studying airway epithelial Na^+ transport and EVLW absorption in humans, proximal airway epithelium is commonly used to assess phenomena in distal airways (Barker et al. 1997, Fajac et al. 1998, Mac Sweeney et al. 2011). In humans, nasal epithelial potential difference (NPD) has served as a measure of airway epithelial ion transport activity and is widely used when studying airway epithelial ion transport in pulmonary diseases such as cystic fibrosis (Knowles et al. 1981, Sermet-Gaudelus 2010).

2.4.2 ENaC

ENaC is expressed at the apical membrane of various Na^+ transporting epithelia including lung, kidney, and colon (Rossier et al. 1994). In the airways, ENaC is expressed throughout and on the alveolar level on both alveolar type I and type II cells (Eaton et al. 2009, Johnson et al. 2006). Four ENaC subunits do exist (α, β, γ, and δ) (Canessa et al. 1994, Ji et al. 2012). According to prevailing views, three homologous subunits (α, β, and γ) make up the most essential and highly Na^+ ion selective ENaC channel in the airway epithelium, whereas other combinations of subunits form channels with reduced selectivity for Na^+ ions (Ji et al. 2006, McNicholas and Canessa 1997, Canessa et al. 1994, Fyfe and Canessa 1998).

The pore-forming α-ENaC has been shown to be the most crucial subunit. In contrast to β- and γ-ENaC knockout mice showing only decreased airway liquid absorption, α-ENaC knockout mice are unable to clear their lungs from liquid and die soon after birth (Hummler et al. 1996, Barker et al. 1998, McDonald et al. 1999). Furthermore, the role of α- and β-ENaC in lung liquid removal has also been shown in the lungs of mature rodents (Li and Folkesson 2006). All three subunits are needed to achieve maximal selectivity for Na^+ over other cations, as well as required for maximal lung liquid absorption (Barker et al. 1998, Fyfe and Canessa 1998, Hummler et al. 1996, McDonald et al. 1999).

The rate-limiting role of ENaC for lung liquid transport has been explicitly demonstrated in newborn guinea pigs, which presented with respiratory distress and excessive accumulation of EVLW after instillation of amiloride into the airways (OBrodovich et al. 1990). Also, in preterm infants with RDS, impaired airway epithelial ENaC activity as well as decrease in β-ENaC protein in tracheal aspirates has been demonstrated (Barker et al. 1997, Li et al. 2009). In addition, at least two genetic polyformisms of α-ENaC might increase susceptibility to RDS (Li et al. 2015).

2.4.3 Na-K-ATPase

Airway epithelial Na-K-ATPase consists of α- and β-subunits in a 1:1 ratio. Both subunits have several isoforms and α1-, α2-, and β1-subunits have been demonstrated to exist in the airway epithelium (Johnson et al. 2002, Li et al. 2009, Sznajder et al. 2002). In the epithelial cell basal membrane, the α-subunit forms a channel pore exchanging intracellular Na^+ for extracellular K^+ in a 3:2 ratio, whereas the role of β-subunit is more regulatory (Chow and Forte 1995, Sznajder et al. 2002). The essential role of Na-K-ATPase on airway liquid transport has been demonstrated in resected human lung, where Na-K-ATPase-blockage caused almost 50% decrease in alveolar liquid clearance (Sakuma et al. 1994).

2.4.4 Regulation of airway epithelial Na^+ transport

The regulation of ion transport and thus airway liquid reabsorption is diverse. Circulating hormones such as glucocorticoids, inflammatory mediators, oxygen level, transmitters interacting with G-protein coupled receptors (e.g. adrenergic, dopaminergic, and purinergic agents), as well as reactive oxygen and nitrogen species regulate Na^+ transport in the airways (Eaton et al. 2009). Glucocorticoids and β_2-agonist, however, are the regulators with the most potential in enhancing airway epithelial Na^+ transport during pathological liquid accumulation (Berthiaume and Matthay 2007, Helve et al. 2009).

Research showing that antenatal glucocorticoids reduce the incidence of RDS in preterm infants underlines the effect of glucocorticoids on airway epithelial Na^+ and liquid transport during pathological lung liquid removal (Roberts and Dalziel 2006). The use of dexamethasone to reduce the incidence of HAPE in HAPE-prone adults, instead, is an example of glucocorticoids potential in treating both pathological liquid accumulation and removal (Maggiorini et al. 2006). Glucocorticoids enhance airway epithelial Na^+ transport on transcriptional, translational, and posttranslational levels (Eaton et al. 2009, Helve et al. 2009). Diversity of in vitro studies have suggested both ENaC and Na-K-ATPase to be influenced at all three levels of regulation (Champigny et al. 1994, Itani et al. 2002, Lazrak et al. 2000, Barquin et al. 1997). The effects of glucocorticoids are mediated through cytosolic glucocorticoid receptor complexes, which by binding on glucocorticoid response elements of genetic DNA, alter gene transcription and translation of various steroid-induced proteins (Eaton et al. 2009, Ma and Eaton 2005, Pochynyuk et al. 2006). For instance, serum- and glucocorticoid-inducible kinase 1 (SGK1) mediates increase in the number of ENaC and Na-K-ATPase channels on the plasma membrane and activate individual channels through activation of upstream activators and inactivation of downstream effectors such as the neural precursor cell expressed, developmentally down-regulated 4-2 (Nedd4-2) (Loffing et al. 2006, Snyder et al. 2002).

β_2-agonists accelerate airway Na^+ transport in vitro (Planes et al. 2002), elevate lung liquid clearance in adult sheep (Berthiaume et al. 1987), raise alveolar liquid

clearance ex vivo in resected human lung (Sakuma et al. 1997), and enhance the reabsorption of lung edema in animals predisposed to hypoxia or lung injury (Saldias et al. 2000, Vivona et al. 2001). However, in humans the potential of β_2-agonists in enhancing airway epithelial Na^+ and liquid transport during pathological liquid accumulation has proved contradictory (Perkins et al. 2014, Sartori et al. 2002). Although intravenous salbutamol has reduced the amount of EVLW in patients with ALI/ARDS, in randomized clinical trials on patients with ALI/ARDS, neither aerosolized nor intravenous β_2-agonists improved clinical outcomes (Gao Smith et al. 2012, Matthay et al. 2011, Perkins et al. 2006). On the contrary, in the BALTI-2 study, intravenous salbutamol impaired outcome (Gao Smith et al. 2012). These contradictory findings may result from difficulty in identifying patients with impaired airway epithelial Na^+ transport potentially benefitting from β_2-agonists but also from variable etiology of ARDS between the studies (Uhlig et al. 2014, Mac Sweeney et al. 2011). The putative influence of β_2-agonists on ENaC and Na-K-ATPase is transcriptional, translational, as well as posttranslational (Dagenais et al. 2001, Looney et al. 2005, Rahman et al. 2010, Sznajder et al. 2002, Thomas et al. 2004). Thus the mechanisms for how the β_2-agonists improve airway epithelial Na^+ transport are various and include diversity of intracellular signaling pathways (Sznajder et al. 2002).

2.4.5 Oxygen and lung liquid clearance

The airway epithelial ion transport and EVLW absorption respond to both increasing and decreasing oxygen levels. The rise in ambient oxygen level enhances ENaC and Na-K-ATPase activity in the in vitro studies mimicking the substantial increase in alveolar oxygen concentration at birth (Pitkanen et al. 1996, Ramminger et al. 2002, Thome et al. 2003, Baines et al. 2001).

As for the decreased oxygen levels, hypoxia attenuates activity of ENaC and Na-K-ATPase both in vitro and in animals in vivo (Carpenter et al. 2003, Mairbaurl et al. 2002, Planes et al. 2002, Tomlinson et al. 1999, Zhou et al. 2008). However, in vitro effects of hypoxia on mRNA and protein levels of Na^+ transporters depend on degree of hypoxia as well as length of exposure (Planes et al. 1997, Planes et al. 2002, Wodopia et al. 2000). The decrease in mRNA and protein levels may need prolonged exposure to hypoxia (Folkesson and Matthay 2006).

Based on in vivo studies on rodents, the putative effect of hypoxia is also related to decreased activity of Na^+ transporters and not only to reduced transcription or translation of Na^+ transporters (Carpenter et al. 2003, Vivona et al. 2001). However, the mechanisms of how hypoxia regulates ENaC and Na-K-ATPase are considered mainly transcriptional (Matthay et al. 2002). Rafii and colleagues have suggested nuclear factor κB with transcription sites in the α-ENaC promoter region and superoxide scavenger to have a role in this regulation (Rafii et al. 1998). Furthermore, there may be other O_2-responsive genes affecting ENaC transcription through their metabolic products. Posttranslational effects of hypoxia, instead, result from

internalization and recycling of both ENaC and Na-K-ATPase channels from the cell membrane (Rotin et al. 2001, Carpenter et al. 2003, Planes et al. 2002, Vivona et al. 2001). Furthermore, reactive oxygen species (ROS) and increased intracellular Ca^{2+} have a role in endocytosis of Na-K-ATPase (Dada et al. 2003, Gusarova et al. 2011, Planes et al. 1996).

In humans, and particularly in HAPE-prone subjects, exposure to ambient hypoxia at high altitude decreases airway epithelial Na^+ transport measured by NPD and Na-K-ATPase but not ENaC mRNA levels (Mairbaurl et al. 2003a, Sartori et al. 2004). Although a decrease in NPD at high altitude also relate to the profound arterial hypoxemia in HAPE-prone subjects (Sartori et al. 2004), the effects of chronic, long-lasting hypoxemia on airway epithelial Na^+ transport remain unknown.

Hypoxia also affects airway epithelial Cl^- transport by reducing NKCC activity and protein levels in vitro and CFTR mRNA levels in humans (Mairbaurl et al. 2003a, Mairbaurl et al. 1997, Wodopia et al. 2000). However, increased Cl^- secretion has been observed by NPD measurement in humans exposed to hypoxia at high altitude (Mairbaurl et al. 2003b, Mason et al. 2003).

2.5 Lung edema assessment after cardiac surgery in children

After congenital cardiac surgery, evaluation of the pulmonary system rests mainly on physical examination, assessment of oxygenation and tissue perfusion, and on repeated chest radiographs. All these methods, however, assess EVLW indirectly and inaccurately (Lange and Schuster 1999). In critically ill patients, an abundance of EVLW has been related to outcome (Eisenberg et al. 1987, Kor et al. 2015, Phillips et al. 2008, Sakka et al. 2002). Thus, precise measuring of EVLW could be useful in pediatric intensive care (PICU) after congenital cardiac surgery.

The methods for measuring EVLW with the best repeatability and accuracy are the most difficult and most expensive to apply in clinical practice (Lange and Schuster 1999). The gold standard for measuring EVLW accurately is gravimetry (Collins et al. 1985, Julien et al. 1984, Nusmeier et al. 2014). However, the gravimetric technique comparing the wet and dry weight of the lungs is only possible postmortem. In clinical use, invasive transpulmonary double indicator dilution (TPDD) and transpulmonary thermodilution (TPTD) techniques are precise methods to measure EVLW and both have been validated against the gravimetric technique (Katzenelson et al. 2004, Mihm et al. 1987, Nusmeier et al. 2014). However, in CHD patients with intracardiac shunt, the methods based on the dilution techniques are not reliable (Giraud et al. 2010, Keller et al. 2011).

The degree of EVLW can be assessed by diversity of imaging techniques. Computed tomography, nuclear magnetic resonance, positron emission tomography, radiography, and ultrasound may all serve as tools in assessing EVLW (Jambrik et al.

2010, Lange and Schuster 1999). However, only ultrasound and chest radiographs (CXR) are practical in daily bedside evaluation.

2.5.1 Chest radiograph

In clinical practice, CXR is a principal method to assess postoperative EVLW and lung edema after surgery for CHD. In CXR, septal lines, peribronchial cuffing, ground glass attenuation, or consolidation of airspaces are signs of excessive EVLW (Gluecker et al. 1999). Various scoring systems to assess EVLW from CXR have been introduced (Anderson et al. 1995, Lemson et al. 2010, Maskatia et al. 2012, Ware et al. 2012, Sibbald et al. 1983). However, the use of CXR to assess EVLW may be inaccurate in intensive care units where portable radiographs are used (Halperin et al. 1985).

CXR scorings assessing EVLW have correlated moderately with total excised lung weight (Ware et al. 2012), and with TPDD and TPTD measurements in adults (Brown et al. 2013, Halperin et al. 1985). In critically ill children, however, CXR showed no correlation with EVLW measured with the TPTD method (Lemson et al. 2010). Moreover, growing children are especially susceptible to radiation, and excessive radiation should be restricted in children with CHD, who are exposed to numerous X-rays through the years of follow-up (Ait-Ali et al. 2010).

2.5.2 Lung ultrasound

Traditionally, the lungs have not been imaged with ultrasound (US), since ultrasound signals from medical US devices cannot reflect from aerated lung tissue to form a realistic image. In the lung, US waveform signals are reflected from air-filled lung parenchyma creating artifacts, and from the superficial structures of the chest wall creating a lucid image (Targhetta et al. 1994). Normal lung parenchyma generates horizontal multiple artifacts (A-lines), which have been suggested to be multiplicative echoes of visceral pleura, whereas vertical artifacts (B-lines) associate with interstitial lung pathology (Lichtenstein et al. 1997, Lichtenstein et al. 2009) (Figure 4). However, sporadic B-lines may also appear in healthy lungs (Caiulo et al. 2011, Reissig and Kroegel 2003).

The B-lines have been suggested to originate from the interfaces formed by liquid-filled and expanded interstitial alveolar septae as well as tissue with reduced aeration by reverberation or ring-down mechanism (Lichtenstein et al. 1997, Soldati et al. 2009, Volpicelli et al. 2012).

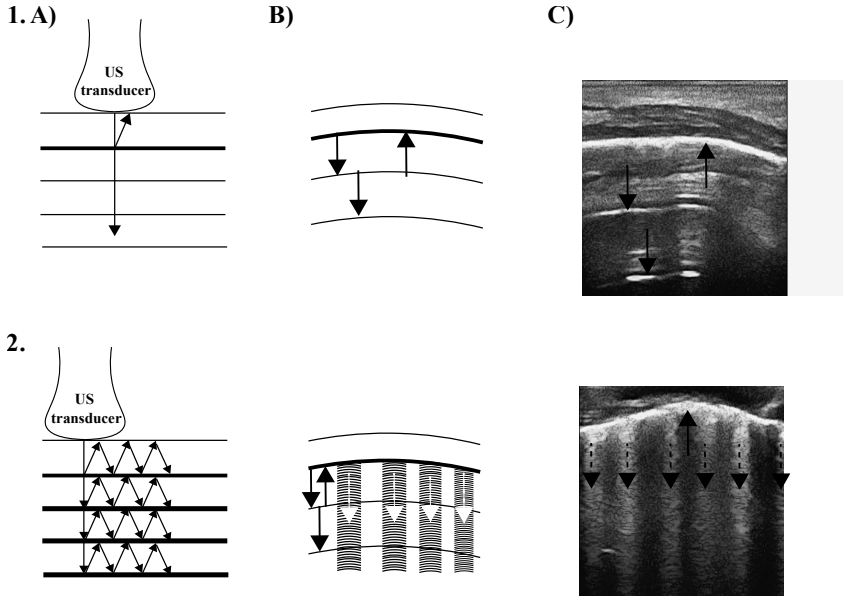

Figure 4 *Schematic explanations (A, B) of the formation of US image (C) as longitudinal scans of normal lung (1.), and of lung with interstitial pathology such as lung edema (2.). US of normal lung shows multiplicative echoes of visceral pleura (upward arrow) as horizontal multiple artifacts (A-lines, downward arrows), whereas B-lines (dashed downward arrow associate with interstitial lung pathology such as lung edema).*

The number of B-lines correlates strongly with the amount of EVLW determined by gravimetry in animals (Jambrik et al. 2010). In humans, B-lines correlate moderately or strongly (r^2 varying from 0.18 to 0.83) with EVLW measured by the TPTD method (Agricola et al. 2005, Bataille et al. 2015, Volpicelli et al. 2013, Enghard et al. 2015). In comparison to other imaging methods in adults with lung edema, B-lines correlate moderately with EVLW findings of CXR and strongly with EVLW findings of CT (Agricola et al. 2005, Baldi et al. 2013, Jambrik et al. 2004, Volpicelli et al. 2008, Volpicelli et al. 2006). In addition, lung US has successfully assessed change in EVLW in decompensated heart failure patients undergoing treatment, in patients undergoing hemodialysis, and in patients developing or recovering from high altitude pulmonary edema (HAPE) (Fagenholz et al. 2007, Noble et al. 2009, Pratali et al. 2010, Vitturi et al. 2014, Volpicelli et al. 2008). However, in children with CHD, only a case report of sonographic assessment of lung injury after a congenital cardiac surgery has been published (Biasucci et al. 2014).

In addition to lung edema, B-lines have been demonstrated in a diversity of interstitial lung pathologies. Although the B-lines may not differentiate between causes of interstitial lung pathologies (Lichtenstein et al. 1997, Martelius et al. 2015a, Soldati et

al. 2009, Volpicelli et al. 2012), interstitial pathologies other than lung edema, such as interstitial pneumonia and sarcoidosis, rarely exist after surgery for CHD. Focal B-lines may be seen in other pulmonary pathologies occasionally present after cardiac surgery, namely atelectasis and pneumonia (Acosta et al. 2014, Caiulo et al. 2013, Reissig and Copetti 2014, Volpicelli et al. 2012). However, in contrast to lung edema in atelectasis and pneumonia, subpleural consolidations typically occur instead of, or in addition to, B-lines (Acosta et al. 2014, Copetti and Cattarossi 2008, Gehmacher et al. 1995). Pneumothorax, instead, creates quite opposite US findings than lung edema, such as absence of B-lines and disappearance of lung sliding (Lichtenstein et al. 2000, Lichtenstein and Menu 1995).

2.6 Lung compliance after cardiac surgery in children

Lung compliance is a sign of the elasticity of the lungs, which is calculated as the ratio of change in volume to change in pressure i.e. inverse of the elastance. The higher the compliance, the better the ability of lungs to stretch and expand. Low compliance instead indicates stiffness of lungs due to various causes such as lung edema (Barnas et al. 1992, Barnas et al. 1994). Since lung compliance demonstrates hysteresis, compliance is affected by its previous value and thus varies between inspiration and expiration (Escolar and Escolar 2004). At moderate lung volumes lung compliance is higher than at very low or very high volumes. Accordingly, ideal mechanical ventilation attempts to keep the lung volume above functional residual capacity all the time and to optimize gas-exchange with the least disturbance to hemodynamics by controlling positive-end expiratory pressure (PEEP), peak airway pressures, and tidal volumes.

In children with CHD, lung compliance may be reduced due to increased pulmonary blood flow and pulmonary arterial pressure (Matthews et al. 2009, Matthews et al. 2007, Yau et al. 1996). Furthermore, cardiac surgery and CPB may reduce lung compliance by causing edema and inadequate lung aeration (Barnas et al. 1994, Lanteri et al. 1995, Polese et al. 1999, Su et al. 2003). This seems to take place in children with normal or reduced pulmonary blood flow, in whom CPB primarily reduces lung compliance (Habre et al. 2004, Lanteri et al. 1995, Stayer et al. 2004). However, in children with increased pulmonary blood flow, the beneficial effects of corrective cardiac surgery may surpass the negative effects of CPB on lung mechanics (Habre et al. 2004, Lanteri et al. 1995).

2.6.1 Methods to measure lung compliance

Since measuring real lung compliance requires esophagus pressure measurement as an estimate of pleural pressure, respiratory system compliance (Crs) is often measured instead. Although the Crs also includes compliance of the chest wall, Crs equals lung compliance in small children with highly elastic chest wall (Papastamelos et al. 1995).

Static compliance is measured in static airways without airflow. For discontinuing airflow, airways are occluded for a time that allows the equilibration of pressure throughout the airways. For single occlusion technique (SOT), airways are occluded once at the end of inspiration and Crs is calculated based on pressure level measured at the occlusion and volume extrapolated from passive flow-volume curve during the next expiration (Stocks et al. 1996). For multiple occlusion technique (MOT), airways are occluded several times at different points of expiration and Crs is calculated from volume-pressure plot (Gappa et al. 2001). In double occlusion technique (DOT), breathing is twice interrupted within the same expiration and Crs is calculated as the difference between the two pressure-volume pairs (Goetz et al. 2001). Compared with other occlusion techniques, DOT is not affected by airway resistance like SOT or by end-expiratory level like MOT (Goetz et al. 2001).

Dynamic compliance instead is measured during airflow. The gold standard method for dynamic lung compliance measurement requires estimation of pleural pressure from esophagus pressure changes (Gerhardt et al. 1989, Stocks et al. 1996).

Neither dynamic lung compliance measurement requiring esophagus pressure measurement nor static Crs measurements with occlusions are suitable for clinical practice in pediatric intensive care after cardiac surgery. Nowadays, however, modern ventilators without esophageal pressure monitoring continuously measure expiratory dynamic Crs, but these data are not routinely used in pediatric intensive care (Macnaughton 2006). Ventilator-derived dynamic compliance measurement shows a strong correlation with static compliance in neonates and adults with severe respiratory failure (Kugelman et al. 1995, Ranieri et al. 1994, Storme et al. 1992). Furthermore, dynamic lung mechanics may be useful in optimizing ventilator management in critically ill patients (Macnaughton 2006, Stenqvist et al. 2008).

2.7 Outcome of congenital heart defect

Natural survival of CHD depends on severity of a CHD (Samanek 1992). Before congenital cardiac surgery began, survival of complex CHD was poor and only patients with mild lesions did survive. In a study from 1950ies 60% of children with CHD died by the end of the first year of life without surgery (Macmahon et al. 1953). However, this material may have lacked surviving children with undiagnosed milder lesions (Macmahon et al. 1953). In fact, the natural survival of simple CHD may be difficult to define.

As for PA+VSD patients without surgery, 1-year, 10-year, and 30-year survival rates have been demonstrated to be 50%, 8%, and 3%, respectively (Bertranou et al. 1978). Natural risk of PA+VSD patients dying soon after birth may be explained by the physiologic closure of ductus arteriosus (Bertranou et al. 1978). However, in rare

cases optimal collateral pulmonary circulation may have resulted in survival even into adulthood (Smitherman et al. 1975).

Advances in diagnostics, treatment, and follow-up of CHD patients have decreased the mortality rates significantly (Erikssen et al. 2015, Izukawa et al. 1979, Raissadati et al. 2016). Significant improvement has been detected even in the 21st century based on data from United States showing that mortality in children with CHD decreased a further 21% from 1999 to 2006 (Gilboa et al. 2010). Although survival of patients with CHD has improved, their survival, apart from successfully repaired patients with simpler defects, is still poorer compared with the general population (Nieminen et al. 2007, Raissadati et al. 2016, Sairanen et al. 2005). Moreover, the children with more complex defects and in need of surgery early in their life still have increased risk for mortality and morbidity (Padley et al. 2011, Bojan et al. 2011a). A relatively recent study with extensive follow-up, also including patients born in the 21st century, estimated 10-year survival of PA+VSD patients to be 71% from first operation (Amark et al. 2006).

3 Aims of the study

This study aimed to find factors affecting lung edema clearance as well as evaluate factors predicting survival in CHD and especially in cyanotic CHD. Furthermore, we aimed to find new methods to assess lung edema and pulmonary recovery after congenital cardiac surgery. The general purpose of this thesis was, by increasing knowledge on an important aspect of pulmonary physiology, to improve evaluation of respiratory system in patients with CHD in need of cardiac surgery.

Specifically, the aims were to study:

1) The effect of chronic hypoxemia on airway epithelial Na^+ transport in children with CHD (I).
2) The feasibility and usefulness of lung US in estimating EVLW in children after congenital cardiac surgery (II).
3) The role of ventilator-derived dynamic Crs in assessing lung mechanics early after congenital cardiac surgery (III).
4) The role of early postoperative sonographic and radiographic scorings of EVLW as well as lung compliance in predicting short-term outcome after congenital cardiac surgery (II, III).
5) The long-term outcome and treatment of patients with a severe cyanotic CHD, PA+VSD, born in Finland between 1970 and 2007, as well as the factors affecting outcome and treatment of these patients (IV).

4 Patients and methods

The ethics committee of the Helsinki University Central Hospital approved the studies. For Studies I–III, the parents of the children provided their written informed consent.

4.1 Patients

4.1.1 Studies I–III

We recruited 137 children previously scheduled for cardiac catheterization or surgery due to different types of CHD or acquired heart disease between December 2010 and March 2013 (Table 2). None of the children had signs or symptoms of respiratory tract infection within the last two weeks, symptomatic asthma, cystic fibrosis, or other primary pulmonary disease.

Table 2. Features of the study patients

	Study I n=99	Study II n=61	Study III n=50
Type of congenital heart defect			
Acyanotic	38 (38%) [a]	22 (36%)	22 (44%)
Cyanotic	55 (56%) [b]	39 (64%)	28 (56%)
Cyanotic after repair	6 (6%)	0 (0%)	0 (0%)
Procedures			
Open-heart surgery	58 (58%)	60 (98%)	48 (96%)
Surgery through thoracotomy	1 (1%)	1 (2%)	2 (4%)
Diagnostic/electrophysiologic/ interventional catheterization	40 (40%)	0 (0%)	0 (0%)

[a] Including 3 patients with arrhythmia and 2 with acquired heart disease [Kawasaki (n=1), Hypertrophic cardiomyopathy (n=1)]
[b] 39 patients with profound hypoxemia ($SpO_2 \leq 85\%$)

4.1.2 Study IV

The study population comprised 109 children with PA+VSD with or without MAPCAs. PA+VSD patients with other major cardiac abnormalities were not included in the study. The patients were born in Finland from 1970 to 2007 and

treated at the Children's Hospital, Helsinki University Hospital. Since 1995, all pediatric cardiac surgery in Finland has been centralized to the Children's Hospital, and even before this, all corrective surgeries and the vast majority of palliative surgeries for children with CHD were performed at this hospital. Thus, all PA+VSD patients born in Finland between 1995 and 2007 and the majority of patients born between 1970 and 1994 were included in our study.

4.2 Clinical data collection

The medical records and operative reports of all studies were retrospectively reviewed to collect the required clinical data.

For Studies I–III, the complexity of care and potential for postoperative morbidity was defined according to the Aristotle scoring (Lacour-Gayet et al. 2004). In addition, the 24-hour fluid balance for the first three postoperative days was collected for Study II.

For Study IV, the last follow-up of all study patients was obtained from their patient records, and the causes and dates of death were obtained from Statistics Finland through December 2011. In Study IV, repair was defined as closure of septal defects, reconstruction of a connection between the right ventricle and pulmonary arteries, and elimination of extracardiac pulmonary blood supply.

4.2.1 Angiograms

For Study IV, the first angiograms and preoperative angiograms prior to repair attempt were analyzed when available. Analysis of a total of 119 angiograms was either from nitrocellulose film (n=55), videotape (n=36), or from digital data (n=28).

From each angiogram, presence of pulmonary artery confluence, blood flow to each lung segment, and the McGoon index were evaluated. To calculate the McGoon index, the combined diameter of the left and right pulmonary arteries at hilar level was divided by the diameter of the descending aorta just above the diaphragm (McGoon et al. 1975). In addition, for patients with MAPCAs, we calculated from the first angiogram a total neopulmonary arterial index (TNPAI). TNPAI was calculated as follows: TNPAI=combined cross-sectional area of left and right pulmonary arteries at hilar level and of MAPCAs / the body surface area. All four authors of Study IV independently measured the vessel sizes for calculating McGoon and TNPAI indexes, and the average of these four measurements was used as a single item of data.

4.3 Expression of epithelial sodium transporters

4.3.1 Sample collection and quantification of mRNA

Nasal epithelial scrape samples for Study I were collected from patients under general anesthesia before the cardiac procedure. A Rhino-Probe (Arlington Scientific, Springville, UT, USA) served to gather the samples by abrasion of the epithelium of the nostril free of endotracheal or nasogastric tubes. Samples were immediately mixed into a lysis buffer (RNeasy kit, Qiagen, Valencia, CA, USA) containing beta-mercaptoethanol. Samples were stored at -80 °C until RNA was purified with an RNeasy Kit. The sample RNA content and purity were determined spectrophotometrically with a NanoDrop (Thermo Fisher Scientific Inc, Wilmington, DE, USA).

4.3.2 Quantitative reverse-transcriptase PCR

In Study I, we performed reverse transcription of 60 ng RNA to cDNA in 20 µl triplicate reactions with a TATAA GrandScript cDNA Synthesis Kit (TATAA Biocenter, Gothenburg, Sweden) according to the manufacturers instructions. We analyzed 20-µl real-time polymerase chain reactions (PCR) with 2 µl of reverse-transcribed RNA as a template using the ABI Prism 7900 Sequence Detection System according to the TaqMan Universal PCR Master Mix protocol (Applied Biosystems, Foster City, CA, USA). The primer concentration was 900 nM for TaqMan pre-developed assays (ENaC subunits: SCNN1A, SCNN1B, SCNN1G, and α1-Na-K-ATPase: ATP1A1), and 400 nM for cytokeratin 18 (CK18) and β1-Na-K-ATPase (ATP1B1). The probe concentration was 250 nM for SCNN1A, SCNN1B, SCNN1G, and ATP1A1, and 200 nM for CK18 and ATP1B1. The primers and probes for CK18 and β1-Na-K-ATPase were designed with Primer Blast (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) and Beacon designer (PREMIER Biosoft) software and validated in compliance with standard recommendations by TATAA Biocenter (Gothenburg, Sweden) (Bustin et al. 2009). The sequences for CK18 primers were ACTGGAGCCATTACTTCAAGATCA (Fw) and GCAAGACGGGCATT-GTCAA (Rv), and for the probe, ACCTGAGGGCTCAGATCTTCGCAAAT. The sequences for ATP1B1 primers were CCATCAGTGAATTTAAGCCCACATA (Fw) and TGGGATCATTAGGACGAAAGGAAA (Rv), and for the probe, TGGCCCCGCCAGGATTAACACAGA.

The dilution series of the pooled nasal epithelium cell sample served to validate the efficiencies of qPCR-reactions. Any possible contamination of samples with genomic DNA was assessed with a minus-RT control. Furthermore, a no template control (NTC) was used as a control for extraneous nucleic acid contamination. We omitted samples that contained less than 5 ng/µl RNA in the scrape samples or were contaminated with genomic DNA. Gene expressions were quantified relatively with

the -ΔΔCq method, where the gene expression ratio of a target gene to a reference gene in a test sample was calculated relative to the calibrator sample. One nasal scrape sample served as calibrator sample throughout the experiments, and epithelially expressed CK18 served as an endogenous reference gene.

4.4 Transepithelial nasal potential difference

For Study I, we used the nasal respiratory epithelium as a surrogate for more distal airways and measured NPD, which represents the electrochemical gradient formed by ion transport across the cell membrane (Mac Sweeney et al. 2011). A single operator (A.K.) performed the measurements before the cardiac procedure, according to the method established first by Alton and colleagues, with some modifications (Alton et al. 1990, Helve et al. 2005, Sermet-Gaudelus et al. 2010). Prior to the study, 30 measurements were performed on healthy adult volunteers to ensure the safety of the method.

During the measurement, the patients lay supine under general anesthesia and were monitored according to routine anesthetic care. A high input impedance (108-1012 ohm), low resistance voltmeter (Logan-Sinclair, Rochester, UK) linked to a computer served to measure the NPD between the two Ag/AgCl electrodes. The reference electrode was placed on slightly abraded skin facilitating electrical contact with the subepithelial (basolateral) space. The probing electrode was connected to the nasal epithelium via a sterile, double-lumen nasal catheter (Marquat Génie Médical, Boissy Saint Léger, France) filled with diluted electrode gel.

Prior to the NPD measurement, reliability of the system was assessed by confirming that the palm skin potential was more negative than -30 mV and circuit offset less than ± 5 mV. The NPD was measured along the floor of the nostril free of endotracheal or nasogastric tubes, and the catheter was secured at the point of maximal NPD. Ringers solution was perfused through the other lumen of the catheter, and basal NPD was recorded. The perfusion of Ringers solution with amiloride (10^{-4} M) later served to determine the portion of amiloride-sensitive NPD (ΔAmi) and the residual NPD after amiloride perfusion equaled amiloride-insensitive NPD. Finally, the perfusion of low Cl^- solution with amiloride (10^{-4} M) and isoproterenol (10^{-5} M) stimulated Cl^- secretion (ΔLowCl^--Iso) (Sermet-Gaudelus et al. 2010) (Figure 5). The Ringers solution contained 140 mmol/L NaCl, 6 mmol/L KCl, 1 mmol/L $MgCl_2$, 2 mmol/L $CaCl_2$, and 10 mmol/L HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid). The low Cl^- solution contained 140 mmol/L Na^+-gluconate, 6 mmol/L K^+-gluconate, 1 mmol/L $MgCl_2$, 2 mmol/L $CaCl_2$, and 10 mmol/L HEPES. Solutions pH 7.4 was accomplished with NaOH. Each perfusion at a rate of 1 ml/min was continued for a minimum two minutes and until steady NPD was achieved for 30 seconds.

A measurement was discarded if the amiloride response was negative or technical problems occurred (drift, unstable N-PD values, problems with the device). Of 95 NPD measurements, 77 (81%) succeeded.

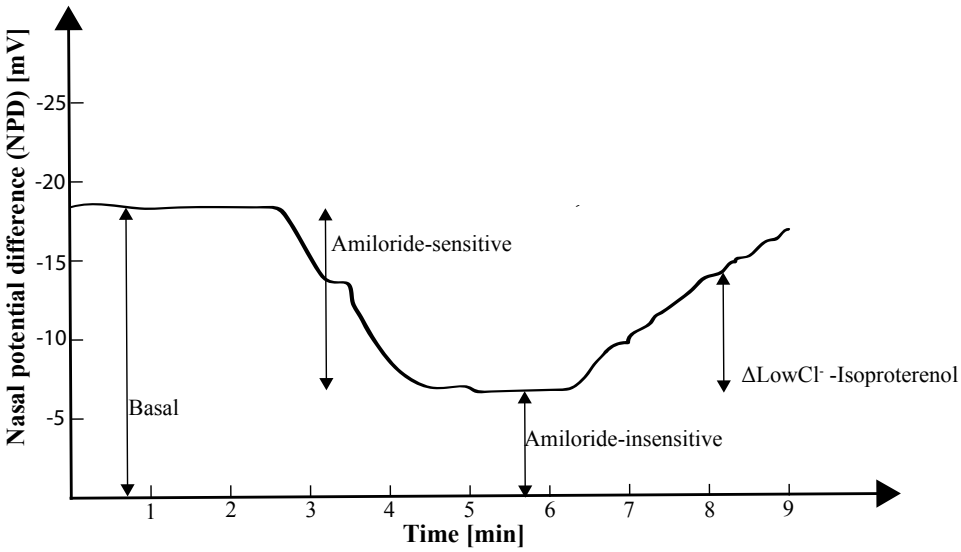

Figure 5 *Diagram of NPD measurement. The final individual value for each solution was determined as a mean NPD value for the last 30 seconds of the perfusion. The measurements had to be interrupted at the onset of surgery. Therefore, the value of ΔLowCl⁻-Iso was calculated as a mean NPD value of 1.5 to 2 minutes from the beginning of perfusion.*

4.5 Imaging studies

4.5.1 Lung ultrasound

The lung US technique for assessing EVLW content in Study II was adapted from a previous study (Copetti et al. 2008b). We performed lung US using the MyLab30CV device with a 10–18 MHz linear transducer (Esaote, Genoa, Italy). At each US examination, lasting 3–8 minutes, six video clips of 3 seconds were stored. The 6-region lung US was performed along three intercostal spaces at right and left sides (Figure 6).

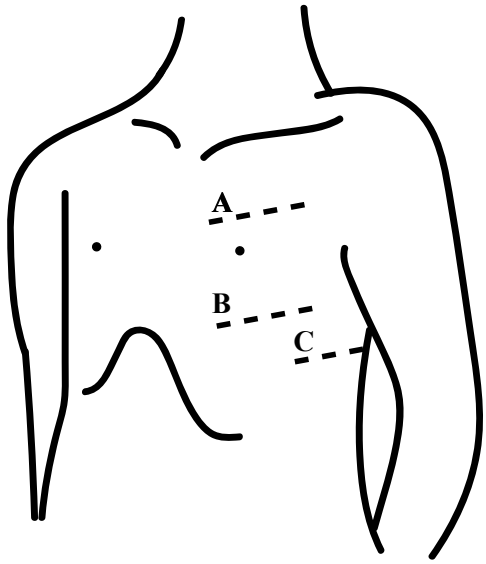

Figure 6 *The lung ultrasound protocol comprised video clips taken horizontally along three intercostal spaces bilaterally [anterior upper chest (A), anterior lower chest (B), and lateral chest approximately halfway between lower costal margin and the axillary pit (C)].*

We defined B-lines in US clips as uninterrupted vertical echogenic artifacts arising from the pleura, traversing the sliding horizontal artifacts, and continuing to the edge of the screen. Each video clip was scored according to a 5-step scale (0=no artifact, 1=B-lines in <25% of surface area, 2=25%–50%, 3=50%–75%, and 4=75%–100%) by a pediatric radiologist blinded to the clinical data. For each patient and time point, a mean score of the six views (the B-line score) was calculated. In addition, 20 video clips were analyzed for interobserver agreement by a second pediatric radiologist.

4.5.2 Chest radiography

CXRs were taken as part of routine follow-up in children undergoing surgery for CHD. For Studies I–III, CXRs taken 1–6 hours postoperatively were retrospectively analyzed to assess EVLW content. In addition, CXRs taken preoperatively and on first postoperative day (POD1) were analyzed for Study I, and CXRs taken daily from first to fifth postoperative day (POD5) were analyzed for Study II.

The technique to estimate EVLW content from CXR was adapted from previous studies (Anderson et al. 1995, Maskatia et al. 2012). A pediatric radiologist, blinded to patient data, scored all CXRs on a 4-step scale. On each CXR, the right upper lobe, right middle lobe, right lower lobe, left upper lobe, lingula, and left lower lobe were

scored separately (0=normal lung, 1=minimal opacity not obscuring lung vessels, 2=opacity partially obscuring lung vessels, 3=opacity totally obscuring lung vessels). For each CXR, a mean lung edema score (CXR LE score) of the areas was calculated. A second pediatric radiologist analyzed 20 CXRs for evaluation of interobserver agreement.

4.6 Lung compliance

During Crs measurements, the patients were mechanically normoventilated under general anesthesia. The use of cuffed tubes (cuff pressure ≥30 cmH_2O) prevented endotracheal tube leak (Main et al. 2001). Both static and dynamic Crs values were reported as values proportional to weight [ml/kPa/kg].

4.6.1 Static respiratory system compliance

For Studies II and III, static expiratory Crs was measured by the DOT with a computerized pulmonary function-testing device (Labmanager 4-521; Erich Jaeger GmbH, Hoechberg, Germany). For the static Crs measurement, breathing was twice interrupted within the same expiration for a time that allowed the equilibration of pressure in the respiratory system (Goetz et al. 2001). A minimum of three double occlusions with a minimum of ten respiratory cycles between each measurement was performed on each patient. Length of occlusions ranged between 100–800 ms, and lengths of acceptable pressure plateaus were ≥70 ms. Plateau was defined as a period with standard deviation (SD) of airway pressure level being below 10 Pa.

During static Crs measurement, all children were under general anesthesia and had stable heart rate (HR) and respiratory rate (RR) without eye, body, or accessory respiratory muscle movement. Thus, we considered a patients physiological state to correspond to deep non-rapid eye movement (NREM) sleep (Helve et al. 2006, Pratl et al. 1999).

4.6.2 Dynamic respiratory system compliance

For Study IV, dynamic Crs was measured by the Servo-i ventilator (Maquet, Rastatt, Germany). The SERVO-i calculated the expiratory dynamic Crs for each breath as follows: dynamic Crs=expiratory tidal volume/ (end inspiratory pressure – PEEP), the formula being comparable to other ventilators. The average value of dynamic Crs for a 15-minute period coinciding with the static Crs measurement was recorded.

In a subset of 12 patients, we retrospectively recorded dynamic Crs, which was stored by the PICU monitoring system in 2-minute intervals. In this subset, we studied the effects of sleep stage (REM vs. NREM) and spontaneous respiratory efforts by

identifying and comparing dynamic Crs of 30-minute periods with stable HR (SD 1.2%) and RR (SD 1.7%), and with irregular HR (SD 4.2%) and RR (SD 14.6%) within 8 hours from static Crs measurement.

4.7 Statistical methods

Statistics were analyzed with SPSS 21.0 and 22.0 (IBM Corp., Armonk, NY, USA) and Prism 5.0 (GraphPad Software, La Jolla, CA, USA). For all analyses, the level of statistical significance was set at 0.05.

Normality of variables on a continuous scale was assessed visually and by Kolmogorov-Smirnov test. Between-groups comparisons of qualitative variables (n with percentages) were performed with the chi square test, and comparisons of continuous variables [median with interquartile range (IQR), or mean ± standard deviation (SD), as appropriate] were performed with *t* test or Mann-Whitney U-test. Comparisons within groups were performed with Wilcoxon matched-pairs test. Associations were examined with Pearsons test and linear regression. Spearmans test was used for studying association between skewed data or data including outliers. Partial correlation was used to study association with a covariate taken into account. Correlations were reported as coefficients of determination (r^2).

In Study II, we performed multivariable linear regression analyses to find independent predictors of short-term outcome. In these multivariable analyses, short-term outcome served as the dependent variable, and length of perfusion, presence of postoperative complications, and B-line score (Model 1) or CXR LE score (Model 2) were the independent variables. In addition, unpublished results of the predictive value of B-line and CXR LE score by receiver operating characteristic (ROC) curve analyses are provided. For these ROC curves, area under curve (AUC) comparisons were performed (Hanley and McNeil 1983). To determine interobserver variability of the B-line scores and CXR LE scores, we calculated the ratio (difference/average) of the scores obtained by both pediatric radiologists and presented these data as percentages. Furthermore, for unpublished comparisons of early postoperative B-line and CXR scorings, we rescaled CXR LE scores to the same scale as the B-line score (0–4).

In Study IV, survival of patients was estimated with the Kaplan-Meier method and compared by log-rank analysis. To further analyze the effect of various variables on survival, we applied univariate Cox proportional hazard model for regression analyses. In the multivariate Cox proportional hazard model, we included all significant variables in the univariate models.

5 Discussion of the results

5.1 Patients and congenital cardiac surgery

5.1.1 Clinical characteristics (I–III)

The patient characteristics for Studies I–III are presented in Table 3. Studies I–III included patients undergoing congenital cardiac surgery. In addition, Study I included 40 patients undergoing cardiac catheterization. None of the study patients died within 30 days of operation or during hospital admission related to the Studies I–III.

Table 3. Patient characteristics in Studies I–III

	Study I n=99	Study II n=61	Study III n=50
Male sex	48 (48%)	35 (57%)	27 (54%)
Age in months	9.9 (3.9–36.3)	4.4 (0.4–21.0)	4.6 (1.4–10.7)
Weight in kg	7.9 (5.4–13.0)	5.3 (3.5–11.2)	5.9 (4.3–9.4)
ACC score	9 (6.8–10) [a]	10 (7–11)	8.8 (6.0–11.0)
Length of cardiopulmonary bypass [min] [b]	93 (57–165) [a]	103 (57–161)	82 (54–148)
Length of aortic cross-clamping [min]	44 (14–95) [a]	43 (17–93)	46 (16–93)
Delayed sternal closure	13 (22%) [a]	15 (25%)	7 (14%)
Days on mechanical ventilation	1 (0.5–4) [a b]	2 (0.5–4) [b]	1 (0.5–3)
Days in PICU postoperatively	4 (2–6) [a b]	4 (2–7) [b]	3 (2–6)

Data presented as n (%) or median with IQR as appropriate
[a] Data of 59 patients with heart surgery
[b] The patient who remained dependent on mechanical ventilation and intensive care for more than 30 days was excluded as an outlier from analysis

In studies of 1400–2300 patients looking at the validity of ACC scoring, the median length of PICU stay has been 3 days (IQR 2–6) and the median ACC scoring 7.9 ±2.7 (Bojan et al. 2011a, Photiadis et al. 2011). Since these values are comparable to our materials (Table 3), Studies I–III may be generalized to other patient populations with CHD undergoing cardiac surgery.

5.1.2 Morphology of PA+VSD (IV)

Of the 109 patients in Study IV, 66 (61%) had simple PA+VSD without MAPCAs, and 97 (89%) had confluent pulmonary arteries (Table 4). Pulmonary artery confluence was more often present in patients with simple PA+VSD (Table 4). This finding corresponds to previous reports showing that in PA+VSD patients confluent central pulmonary arteries exist up to 90%, but in only two-thirds when MAPCAs exist (Liao et al. 1985, Davis et al. 1978). According to another view, a confluence should always exist at birth but the pulmonary arteries continue to atrophy and disappear later in life due to minimal or even absent blood flow, which could be prevented by early creation of a systemic-pulmonary artery shunt (Liavaa et al. 2012).

Table 4. Patient characteristics and operations in Study IV

	Simple n=66	**MAPCAs n=43**	**p**
Male gender	33 (50%)	26 (60%)	0.28
Birth weight in kilograms	2.86 ± 0.83	3.09 ± 0.73	0.15
Associated cardiac findings [a]	27 (41)	17 (40)	0.89
Comorbidities [b]	24 (36)	15 (35)	0.88
Presence of pulmonary artery confluence	66 (100)	31 (72)	<0.0001
Pulmonary blood supply solely from MAPCAs		16 (37%)	
Lung segments supplied by native PAs [c]	19.8 ± 0.9	5.4 ± 6.1	<0.0001
Hypoperfused lung segments [c]	0.3 ± 1.0	1.7 ± 2.2	0.007
Surgery	66 (100%)	40 (93%)	0.06
Repair	42 (64%)	12 (28%)	0.0003
RV-PA connection with septal fenestration	4 (6%)	17 (39%)	<0.0001
Other palliative procedures	20 (30%)	11 (26%)	0.83

Data presented as n (%), median with IQR or mean ±SD, as appropriate
[a] Right-sided aortic arch (n = 27), left superior vena cava (n = 7), atrial septal defect (n = 5), abnormal coronary arteries (n = 4)
[b] 22q11.2 –deletion (n = 12), Extracardiac anomaly (n = 12), Lissencephaly (n = 1), Mayer-Rokitansky-Küster-Hauser syndrome (n=1), Mucolipidosis II (n = 1), other genetic syndromes (n = 4), Trisomy 21 (n = 2), unconfirmed but strongly suspected other syndromes (n = 4), and VACTERL (vertebral defects, anal atresia, cardiac defects, tracheo-esophageal fistula, renal anomalies, and limb anomalies) (n = 2)
[c] Data from 1st available angiograms

Consistent with a previous study describing the initial morphology of PA+VSD (Amark et al. 2006), our results revealed that the patients with simple PA+VSD had a higher McGoon index, more lung segments indicative of perfusion by native pulmonary arteries, and fewer hypoperfused lung segments than did the patients with MAPCAs (Table 4). These differences were found in the primary angiograms prior to any surgery and in the preoperative angiograms prior to repair attempt.

5.1.3 Surgical repair of PA+VSD (IV)

Staged repair has been a primary strategy in Helsinki Children's Hospital. The patients have typically undergone a repair when the McGoon index has been 1.5 or above. At repair, a postoperative RV/LV systolic pressure ratio up to 85% has been accepted in hemodynamically stable patients.

Half of the study patients achieved a repair, and less than 10% of them had a single-stage repair. Although repair rate in Study IV is higher than in studies with earlier follow-up period (Hofbeck et al. 1991, Dinarevic et al. 1995), it is lower than in more recent reports (Amark et al. 2006, Cho et al. 2002) (Figure 7). However, in a report by Cho and colleagues, the repair rate was reduced from 70% to 60% by excluding the repaired patients in whom the VSD had to be reopened (Cho et al. 2002). In addition to some differences in repair definition, the institutional and national differences in the selection criteria for repair may have influenced the disparity in repair rates.

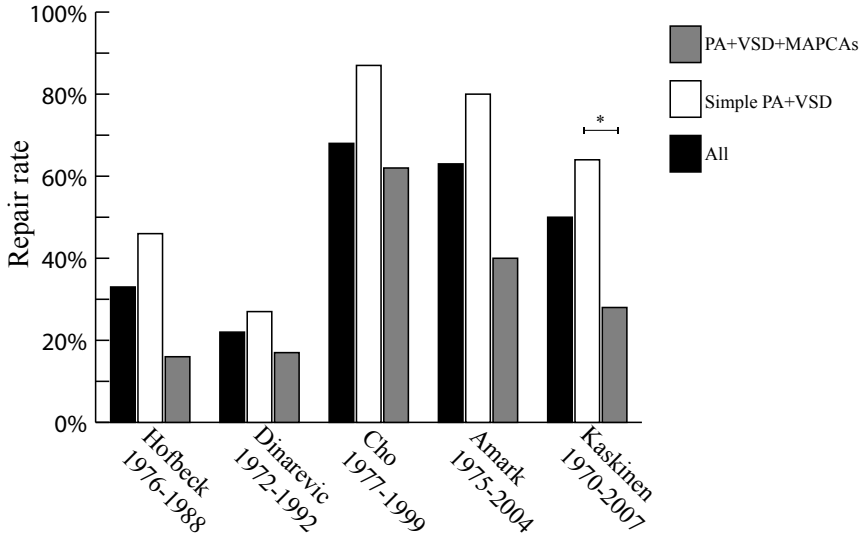

Figure 7 *Repair rates in reports including PA+VSD patients both with and without MAPCAs. * p=0.0003.*

Of the patients with simple PA+VSD, 64% were repaired compared with 28% of the patients with MAPCAs (Figure 7). Some most recent reports of PA+VSD patients with MAPCAs have shown up to 90% repair rates (Brizard et al. 2009, Liavaa et al. 2012, Malthotra et al. 2009). However, no long-term survival data exist yet from the tertiary centers demonstrating these exceptionally high repair rates.

In addition to absence of MAPCAs, other anatomical factors—namely presence of pulmonary artery confluence, higher McGoon index, and higher number of lung segments supplied by true pulmonary arteries—increased the probability of repair in Study IV. These findings support a previous suggestion that a more complex anatomy is a risk factor for remaining palliated (Hofbeck et al. 1991). Although the presence of MAPCAs decreased the probability of repair, the size of MAPCAs assessed by TNPAI index had no effect on achievement of repair. Therefore in the initial assessment of PA+VSD patients, assessing size of native pulmonary arteries and presence of MAPCAs, rather than size of MAPCAs, seems most crucial.

In addition to anatomic factors, consistent with a Canadian report from Amark and colleagues, we found a later birth year to increase the probability of achieving repair (Amark et al. 2006).

5.1.4 Palliative surgery of PA+VSD (IV)

Half of the study patients remained palliated (Table 4). Of these, 38% had repair attempts resulting in the creation of a connection between RV and pulmonary arteries with septal fenestration, 18% were deemed unsuitable for repair, and 44% died before their treatment strategy was chosen or a previously planned repair was achieved.

Even diminutive central PAs have shown to enlarge after placement of systemic-pulmonary artery shunt (dUdekem et al. 2005, Kim et al. 2015, Liavaa et al. 2012, Mumtaz et al. 2008). Accordingly, we evaluated growth of pulmonary arteries by McGoon index from both first and preoperative angiograms prior to repair of 40 patients. Of these patients, 85% received a systemic-pulmonary artery shunt between the angiograms. In the patients receiving a shunt, we could detect enlargement of central pulmonary arteries (Table 5). However, the size of central pulmonary arteries in angiograms may be affected by transient flow and thus an increase in McGoon index may not only reflect the vessel growth.

Table 5. Size of central pulmonary arteries assessed by McGoon index

	McGoon index at angiogram		
	First available	Preoperative prior to repair	p [a]
Simple PA+VSD	1.50 (1.11−1.77)	1.78 (1.39−2.00)	0.001
PA+VSD+MAPCAs	0.75 (0.09−1.09)	1.41 (1.05−1.60)	0.001
p [b]	<0.0001	0.005	
Shunt between angiograms	1.12 (0.76−1.47)	1.58 (1.25−2.01)	<0.0001
No shunt	0.87 (0.31−1.42)	1.06 (0.36−1.54)	0.23

[a] Paired comparison between first available angiogram and preoperative angiogram prior to repair attempt
[b] Comparison between simple PA+VSD and PA+VSD+MAPCAs

Although we could not assess effect of palliative operations on size of MAPCAs, we found patients with MAPCAs to have more hypoperfused lung segments than patients without. This may support previous findings that MAPCAs may have poor growth potential, tendency to stenose, and that MAPCAs may not support development of biologically competent pulmonary circulation (dUdekem et al. 2005, Norgaard et al. 2006).

5.2 Airway epithelial ion transport (I)

The amiloride-sensitive fraction constitutes 60%–80% of airway epithelial Na^+ transport (OBrodovich et al. 2008). In our NPD measurements, 52% of the NPD was amiloride-sensitive, which is consistent with previous NPD measurements in adults exposed to hypoxia at high altitude (Maggiorini et al. 2006, Sartori et al. 2002, Sartori et al. 2004). Furthermore, the basal NPD values of the normoxemic patients ($SpO_2 \geq$ 95%) were comparable to previously reported values in healthy subjects (Su et al. 2016).

The α-ENaC mRNA levels showed a weak to moderate correlation with basal NPD (r^2=0.12, p=0.01) and amiloride-sensitive NPD (r^2=0.10, p=0.01). Since posttranslational alterations crucially influence electrical ENaC activity, these correlations are reasonable. However, in contrast to previous findings in healthy adults, we found no significant correlation between γ-ENaC mRNA levels and NPD values (Otulakowski et al. 1998). The lack of correlation between other than α-ENaC subunit mRNAs and amiloride-sensitive NPD may emphasize the major functional role of α-subunit to function of ENaC (Hummler et al. 1996).

5.2.1 Effect of hypoxemia on airway epithelial ion transport activity

The decrease in airway epithelial Na^+ transport in hypoxic environments has been thought to result from the effects of ambient hypoxia (Zhou et al. 2008). However, the decreased airway epithelial Na^+ transport in rats and humans has been demonstrated not only in ambient hypoxia but also in the presence of systemic hypoxemia (Carpenter et al. 2003, Sartori et al. 2004). However, Study I, was the first study to examine the association between chronic, profound hypoxemia and airway epithelial Na^+ transport in humans.

In Study I, at room air the basal NPD and amiloride-sensitive NPD were lower in the profoundly hypoxemic patients with cyanotic CHD ($SpO_2 \leq 85\%$) than in the patients with acyanotic CHD and normal SpO_2 levels (Figure 8). Furthermore, amiloride-sensitive NPD constituted 36% of the baseline NPD in profoundly hypoxemic patients compared with 57% in normoxemic patients ($p=0.007$). Consistent with a recent meta-analysis demonstrating a correlation between SpO_2 and amiloride-sensitive NPD both in HAPE as well as in RDS (Su et al. 2016), children with CHD showed a moderate correlation between SpO_2 and the amiloride-sensitive NPD ($r^2=0.37$, $p=0.001$). Accordingly, not only hypoxia but also hypoxemia affects airway epithelial Na^+ transport, particularly with ENaC-associated Na^+ transport.

A previous study showed impaired amiloride-insensitive Na^+ transport in HAPE-prone adults in a hypoxic environment (Sartori et al. 2004). In contrast to this finding, in Study I no difference in amiloride-insensitive NPD occurred between profoundly hypoxemic and normoxemic patients. However, in profoundly hypoxemic children, age positively correlated with amiloride-insensitive NPD ($r^2=0.21$, $p=0.01$) and basal NPD ($r^2=0.21$, $p=0.01$). Thus, the longer the children had been hypoxemic the higher the amiloride-insensitive and basal NPD were. Whether this rise compensates for the reduced amiloride-sensitive NPD in prolonged severe hypoxemia remains unclear. However, a heart-failure model has demonstrated a compensatory role of amiloride-insensitive Na transport$^+$ in response to lung edema liquid (Rafii et al. 2002).

Response to a low Cl^- solution was reduced in the profoundly hypoxemic children compared with normoxemic patients in Study I (Figure 8). Also, critically ill children with lung edema due to meningococcal septicemia have shown reduced Cl^- transport (Eisenhut et al. 2006). In contrast to our findings in hypoxemic children, exposure to hypoxia in adults at high altitude has been described to increase Cl^- secretion. However, this increase at high altitude may result from liquid secretion due to epithelial dryness and low temperature (Rennolds et al. 2008, Mairbaurl et al. 2003b). Moreover, the role of Cl^- secretion in airway epithelium is crucial for mucus composition, but on the alveolar level, Cl^- transport contributes also to liquid absorption (Fang et al. 2006, Jiang et al. 1993, Quinton 1990). Thus, the role of Cl^- flux-contributed NPD may be less significant than Na^+ flux in assessing distal airway liquid transport (Su et al. 2016).

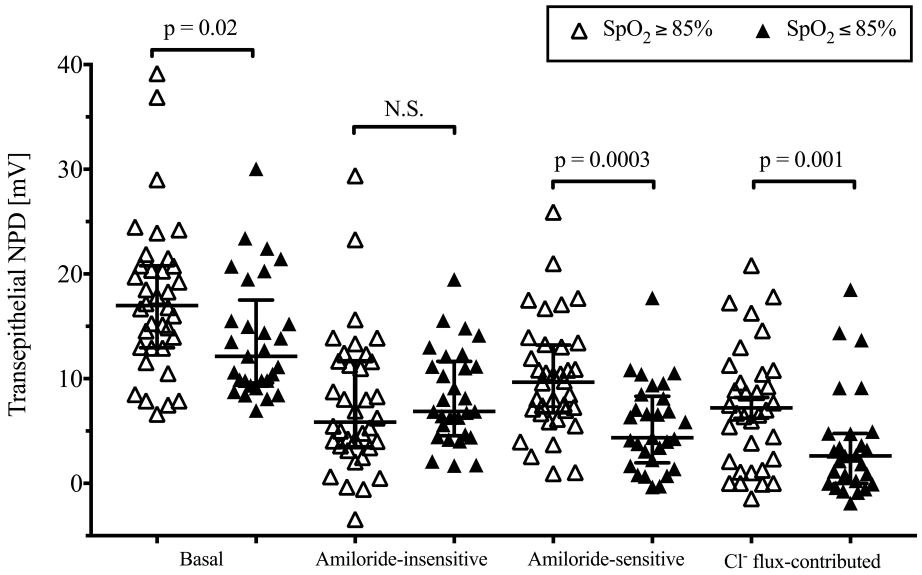

Figure 8 *Comparisons of NPD values between normoxemic patients ($SpO_2 \geq 95\%$) with acyanotic CHD or acquired heart disease and profoundly hypoxemic patients with cyanotic CHD ($SpO_2 \leq 85\%$).*

5.2.2 Effect of hypoxemia on airway epithelial Na^+ transporter expression

Consistent with studies in rats exposed to hypoxia (Carpenter et al. 2003, Vivona et al. 2001), in Study I the β- or γ-ENaC subunit mRNA expressions were not reduced in profoundly hypoxemic ($SpO_2 \leq 85\%$) patients when compared with normoxemic patients (Figure 9). The α-ENaC mRNA levels also did not show a difference between profoundly hypoxemic and normoxemic patients. This finding in Study I, however, contradicts a study in rats reporting increased α-ENaC mRNA levels in response to hypoxia (Vivona et al. 2001). Instead, our observations in cyanotic CHD with chronic hypoxemia support another finding in animals that hypoxia effects are related to decreased Na^+ transport activity rather than to the changes in Na^+ transporter mRNA expressions (Carpenter et al. 2003).

Although hypoxia reduces the activity of Na-K-ATPase both in vitro and in animals in vivo, the effects on mRNA levels have been somewhat contradictory (Carpenter et al. 2003, Mairbaurl et al. 2002, Planes et al. 2002, Tomlinson et al. 1999, Zhou et al. 2008, Vivona et al. 2001). In line with a previous study on rats demonstrating increased β1-Na-K-ATPase mRNA levels as well as a trend toward increased α1-Na-K-ATPase mRNA levels (Carpenter et al. 2003, Vivona et al. 2001), our study revealed significantly higher α1-Na-K-ATPase mRNA expression in the profoundly hypoxemic compared with the normoxemic patients (Figure 9). Based on Study I findings the role of increased Na,K,ATPase expression in profound hypoxemia may

compensate for a reduction in amiloride-sensitive airway epithelial Na^+ transport activity. Our findings, somewhat contradict a previous study showing decreased α1- and β1-Na,K,ATPase mRNA levels in HAPE-prone subjects exposed to hypoxia at high altitude (Mairbaurl et al. 2003a). However, the latter measurements were performed after a short-term exposure to hypoxia and postulates regarding chronic compensatory changes may not be applicable.

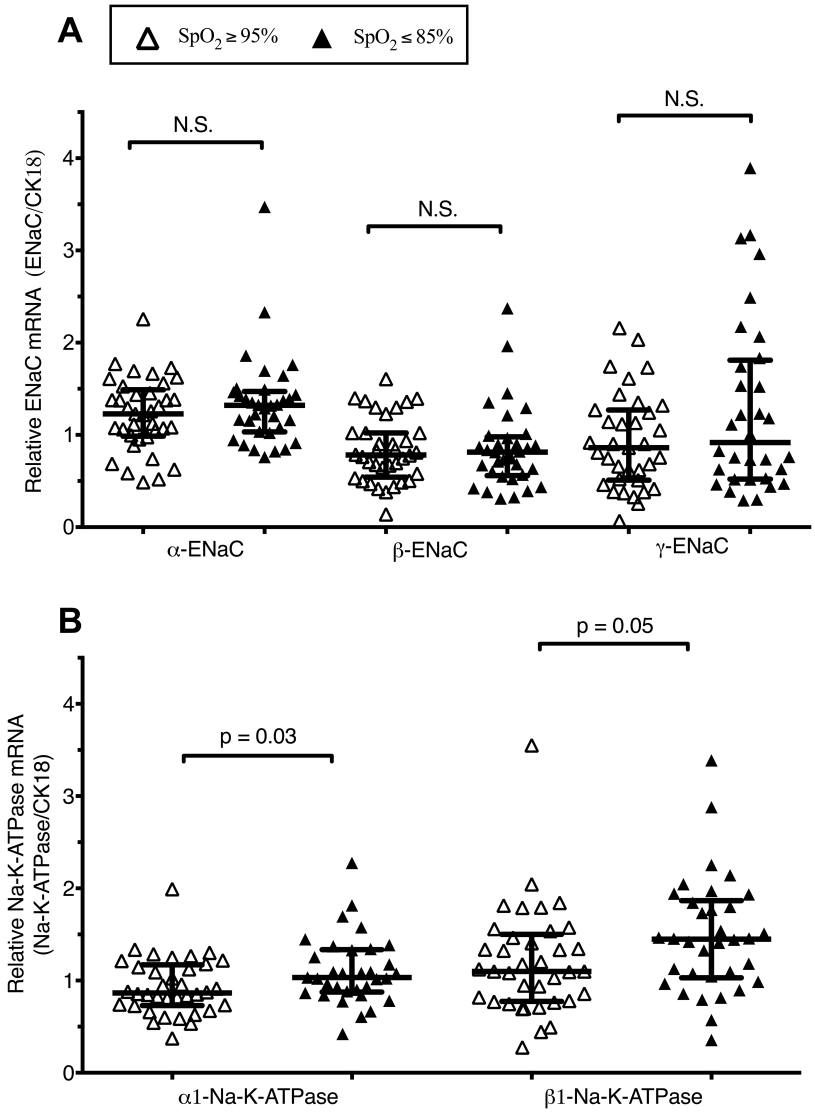

Figure 9 *Comparison of Na^+ transporter subunit mRNA levels between normoxemic patients ($SpO_2 \geq 95\%$) with acyanotic CHD or acquired heart disease and profoundly hypoxemic patients with cyanotic CHD ($SpO_2 \leq 85\%$).*

5.3 Postoperative imaging of EVLW (I-III)

In analyzing EVLW, we focused on early postoperative images because excessive EVLW typically accumulates early after CPB (Asada and Yamaguchi 1971). This assumption is supported by the Study II finding, which shows that early postoperative B-line and CXR LE scorings correlate with perfusion time and aortic cross-clamping time (Table 6). Moreover, this finding emphasizes the importance of CPB in initiating noxious physiologic cascades leading to excessive EVLW and pulmonary dysfunction (Asimakopoulos et al. 1999a). In fact, the complexity of care and potential for postoperative morbidity according to the ACC scoring also correlated with both B-line and CXR LE scores early postoperatively (Table 6).

Table 6. Correlation of early postoperative (1−6 hours postoperatively) B-line score/chest radiography lung edema score and patient-related factors and CPB data

	Early postoperative			
	B-line score		CXR LE score	
	r^2	p	r^2	p
ACC score	0.09	0.03	0.11	0.016
Patient age	0.25 [a]	0.0001	0.17 [a]	0.001
Length of perfusion	0.11	0.016	0.13	0.007
Length of aortic cross-clamping	0.14	0.007	0.16	0.003

[a] a negative correlation

Children with critical CHD are often operated on at a younger age and may thus require more complex surgery. In Study II, patient age correlated negatively with both early postoperative lung US B-line and with CXR LE scorings (Table 6) suggesting that cardiac surgery and CPB may give rise to EVLW especially in younger children. This susceptibility may result from differences in inflammatory profiles in neonates compared with older children as well as from higher vulnerability of infant lung for ischemia-reperfusion (Ashraf et al. 1997, Qiu et al. 2008).

In study I, only in the profoundly hypoxemic patients did CXR LE scoring increase from preoperative to early postoperative ($p=0.0004$) and to CXR taken on the POD1 ($p<0.0001$). Also, the correlation between CXR LE score and length of perfusion was found, in particular, in the profoundly hypoxemic patients ($r^2=0.17$, $p=0.04$). These findings emphasize that after open-heart surgery profoundly hypoxemic patients may be particularly susceptible to excessive EVLW induced by pulmonary reperfusion injury and CPB.

The association between airway epithelial Na^+ transport and postoperative lung edema is emphasized by our finding, which demonstrates a weak negative correlation between early postoperative CXR LE score and preoperative α-ENaC mRNA level

(r^2=0.10, p=0.03, n=48) and α1-Na-K-ATPase mRNA level (r^2=0.12, p=0.02, n=45) (Kaskinen A. et al, unpublished results). However, other Na^+ transporter subunit mRNA levels or any NPD variables showed no correlation with CXR LE score early postoperatively or on the POD1 (data not shown) (Kaskinen A. et al, unpublished results).

5.3.1 Postoperative lung ultrasound

Study II revealed a correlation between B-line scores and CXR LE scores 1−6 hours postoperatively, but also on POD1 and on POD4 (Table 7). The correlations found correspond to previous studies in adults showing coefficients of determinations between 0.38 and 0.61 (Agricola et al. 2005, Jambrik et al. 2004, Volpicelli et al. 2008).

Table 7. Correlation between B-line scores and chest radiography lung edema scores

	n	r^2 (95% CI)	p
1−6 hours postoperatively	55	0.41 (0.21–0.61)	<0.0001
POD1	53	0.15 (0.02–0.36)	0.004
POD2	34	0.02 (-0.04–0.20)	0.44
POD3	21	0.02 (-0.09–0.30)	0.51
POD4	24	0.28 (0.03–0.59)	0.008
POD5	15	0.04 (-0.12–0.42)	0.45

Study II, contrary to other findings on adult patients undergoing hemodialysis (Noble et al. 2009), demonstrated no correlation between B-line scoring and patient fluid balance during POD1, POD2, or POD3. Compared with patients undergoing hemodialysis in need of eliminating extra fluid from the body, in PICU, postoperative fluid management aims at avoiding excessive fluid load. In addition, the patients having longer perfusion and aortic cross-clamping, and thus a greater expected risk for reperfusion-induced lung injury, had postoperatively more abundant B-lines in the lung US (Table 6). Thus, we suggest that the B-lines resulted from increased EVLW due to reasons other than fluid overload. In addition, although both lung US and CXR assess indirectly lung edema, EVLW in adults measured by TPTD technique has showed a stronger correlation with US-based EVLW assessment than with CXR (Brown et al. 2013, Enghard et al. 2015).

Alveolar flooding begins only when EVLW has doubled (Bongard et al. 1984). A clinically valuable imaging method would detect changes below this threshold. CXR densitometry in dogs has invariably recognized a 35% increase in EVLW as definitive

edema (Snashall et al. 1981). B-lines, however, may appear early and precede the radiologic signs of increased EVLW (Jambrik et al. 2004, Lichtenstein et al. 1997). Consistent with these suggestions, the B-line score was significantly higher than the rescaled CXR lung edema score 1–6 hours postoperatively [1.00 (0.50−1.33) vs. 0.63 (0.42−1.04), p=0.004)] (Kaskinen A. et al, unpublished results).

Although lung US protocols with 28 scans have been used in adults, in neonates 6-region scans have been implemented for practical reasons (Agricola et al. 2005, Copetti et al. 2008a, Jambrik et al. 2004, Martelius et al. 2013). We found all six scanning windows of lung US accessible in children of different sizes and also in patients with delayed sternal closure. We also found lung US easy to learn. This observation is in line with a study showing lung US as equally reliable whether done by experienced echocardiographer or a beginner in the field of US (Bedetti et al. 2006). Accordingly, the 6-region lung US is practical in PICU after congenital cardiac surgery and simple to use by a clinician, who may use US findings while determining treatment decisions.

To study the repeatability of 6-region lung US B-line score, we studied the interobserver agreement of B-line scoring. The interobserver correlation was strong for B-line scores (r^2=0.73) but only moderate for CXR LE scores (r^2=0.33). Also, previously reported interobserver correlations of lung US scorings have been shown to be strong, and coefficients of determinations as high as 0.86−0.92 have been reported (Bedetti et al. 2006, Martelius et al. 2013).

5.4 Postoperative lung compliance (II, III)

The repeated static Crs measurements in each patient showed high consistency with standard deviation of 4.1% in Study II and 5.6% in Study III. This excellent consistency between repeated measurements emphasizes the accuracy and validity of the static Crs values and further justifies their comparison with ventilator-derived dynamic Crs (Gappa et al. 2001). Furthermore, Crs did not differ between patients with open or closed sternum. This finding supports the assumption that in young children the naturally elastic chest wall has only minimal effect on Crs (Papastamelos et al. 1995). Proportioning Crs values by weight may have resulted in patient age showing only a weak correlation with static Crs (r^2=0.12, p=0.01) but not with dynamic Crs (r^2=0.03, p=0.23) (Kaskinen et al. unpublished results).

A positive correlation between dynamic and static Crs was moderate (r^2=0.32, p<0.0001) and less remarkable than in previous studies on patients with respiratory failure or test animals predisposed to lung injury (Kugelman et al. 1995, Ranieri et al. 1994, Storme et al. 1992, Suarez-Sipmann et al. 2007). This disparity in correlations may result partly from differences in patient materials or Crs measurement methods. Compared with the previous human studies, our patients after congenital cardiac surgery had no other reason for respiratory failure, major inflammatory disease, or

primary pulmonary disease, and thus were more homogenous in terms of their pulmonary state. Furthermore, we used the double-occlusion method rather than the SOT, which is affected by airway and tube resistance (Kugelman et al. 1995, Ranieri et al. 1994, Storme et al. 1992). Despite the correlation, static compliance was 48% higher than dynamic ($p<0.0001$), which is consistent with previous studies and may result from the effect of airway and tube resistance on dynamic lung mechanics (Kugelman et al. 1995, Stenqvist et al. 2008). Accordingly, dynamic Crs may reflect different phenomena than static Crs.

In Study III, early postoperative static Crs was 7.4 ± 2.4 ml/kPa/kg, which is lower than reported in sleeping healthy full-term infants (Katier et al. 2005, Lodrup Carlsen et al. 1994). Since age has some effect on Crs, values were compared with previous studies also in a subset of patients younger than one year of age, who also had lower static Crs (6.9 ml/kPa/kg) than in the previous studies (Katier et al. 2005, Lodrup Carlsen et al. 1994). In patients with L-R shunt defects, early postoperative static Crs showed no difference from other CHD (7.6 ± 2.7 vs. 7.3 ± 2.2 ml/kPa/kg, respectively, $p=0.89$) (Kaskinen et al. unpublished results). Similarly, no difference occurred in dynamic Crs between patients with L-R shunt and other CHD (5.1 ± 2.4 vs. 5.0 ± 1.3 ml/kPa/kg, respectively, $p=0.62$) (Kaskinen et al. unpublished results). Nor did length of perfusion, aortic cross-clamping, or ACC score correlate with dynamic or static Crs in Study III. These findings contrast with previous studies showing that both CPB and cardiac surgery as well as increased pulmonary blood flow may reduce lung compliance (Lanteri et al. 1995, Matthews et al. 2009, Matthews et al. 2007). However, in children with increased pulmonary blood flow, the beneficial effects of corrective cardiac surgery may surpass the harmful effects of CPB on lung mechanics, whereas in children with normal or reduced pulmonary blood flow CPB primarily reduces Crs (Habre et al. 2004, Lanteri et al. 1995, Stayer et al. 2004).

Based on previous findings demonstrating that a decrease in Crs reflects an increase in EVLW in pigs and that lung compliance correlates with EVLW measured by TPTD in mechanically ventilated adults postoperatively, we hypothesized that a correlation would also exist between the Crs and B-line scorings as well as CXR LE scorings (Gargani et al. 2007, Oshima et al. 2008). In Study III, static Crs, unlike dynamic, showed a negative correlation with CXR lung edema scoring ($r^2=0.25$, $p=0.0002$) (Figure 10). The association between CXR findings and lung compliance in Study III is in line with previous studies showing negative correlation between CXR vasculature gradings and compliance (Howlett 1972, Matthews et al. 2007). Since only static Crs correlated with CXR LE scoring, static Crs may reflect the state of lung parenchyma better than ventilator-derived dynamic Crs. This also may result from the fact that airway resistance affects dynamic Crs unlike static Crs measured by DOT. Disappointingly, but consistent with our previous study on healthy neonates (Martelius et al. 2015b), we found no correlation between postoperative static Crs and the B-line score in Study II (Figure 10). However, a finding that early postoperative static Crs was 26% lower ($p=0.02$) in patients with a B-line score at or above the median demonstrates some association between B-line score and static Crs.

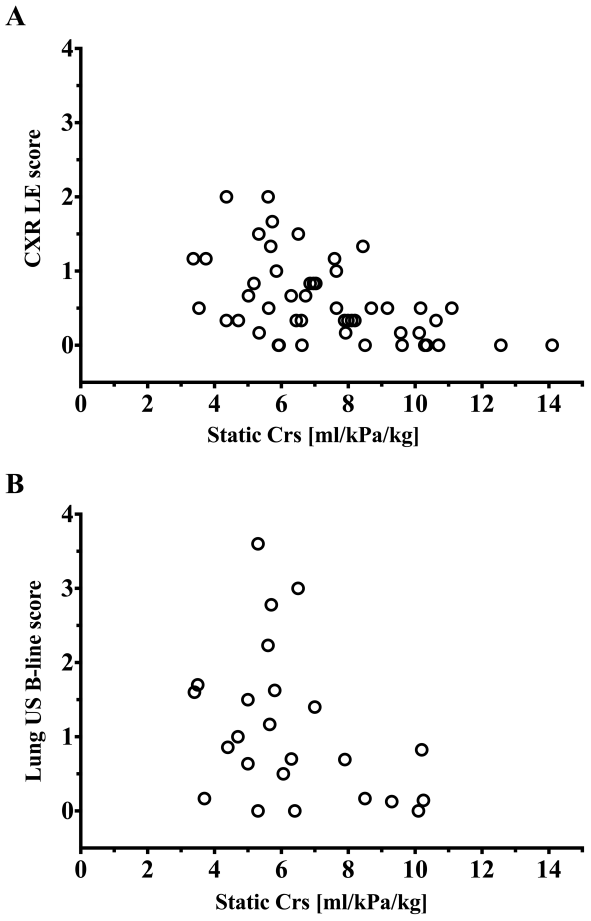

Figure 10 *Early postoperative static Crs correlated with CXR LE scoring (r^2=0.25, p=0.0002, n=50) (A) but not with lung US B-line scoring (r^2=0.13, p=0.08, n=24) (B).*

5.5 Predicting short-term outcome after cardiac surgery (II, III)

In line with previous studies on children undergoing CPB and congenital cardiac surgery (Brown et al. 2003, Bojan et al. 2011b, Bojan et al. 2011a), in Study II the higher operative complexity assessed by ACC scoring, increased perfusion time, aortic cross-clamp time, and postoperative complications was associated with a longer stay at PICU postoperatively (Table 8). We also found these factors to associate with the length of mechanical ventilation (Table 8). Also in accordance with previous studies (Brown et al. 2003, Fischer et al. 2000, Padley et al. 2011), younger age was associated with both a longer need for mechanical ventilation and a longer postoperative PICU stay (Table 8).

Table 8. Correlation between perioperative factors, B-line score, CXR LE score, and short-term outcome

	Length of	
	Mechanical ventilation	PICU stay
	$r^{2,}$, p	$r^{2,}$, p
ACC score	0.24, <0.0001	0.28, <0.0001
Patient age	0.11 [a], 0.008	0.12 [a], 0.007
Perfusion time	0.49, <0.0001	0.49, <0.0001
Aortic cross-clamp time	0.45, 0.49, <0.0001	0.43, 0.49, <0.0001
Major postop. complications [a]	p=0.02 [b]	p=0.001 [b]
Lung US B-line score	0.29, <0.0001	0.22, 0.0003
CXR LE score	0.26, <0.0001	0.21, 0.0004

[a] a negative correlation
[b] Length of mechanical ventilation and PICU stay in patients with major postoperative complications was 4 (1–7) days and 6 (4–11) days compared with 1 (0.5–4) days and 3 (2–6) days in patients without major postoperative complications, respectively.

Abundance of EVLW determined by TPTD method has shown to predict risk for clinically significant lung edema and short-term outcome in ARDS (Kor et al. 2015, Phillips et al. 2008, Sakka et al. 2002). Also, a case report of a child with lung injury after congenital cardiac surgery showed a decrease in B-lines synchronous with recovery (Biasucci et al. 2014). Furthermore, pulmonary complications in general delay recovery of children after congenital cardiac surgery (Bandla et al. 1999, Fischer et al. 2000). We found early postoperative B-line and CXR scoring to correlate with length of mechanical ventilation and PICU stay (Table 8). In harmony with this, the patients with a B-line score or a CXR LE score at or above the median had a longer time on mechanical ventilation and stayed postoperatively longer in PICU. Accordingly, early postoperative B-line score as well as CXR LE score play a role in predicting short-term outcome after heart surgery for CHD.

Lung compliance in predicting outcome has been shown to be inconsistent. In children treated at PICU for various reasons and in adults with acute lung injury, Crs has associated with short-term outcome (Greenough et al. 1999, Nuckton et al. 2002, Seeley et al. 2011). In preterm infants, however, dynamic compliance by esophagus method has not predicted successful extubation (Veness-Meehan et al. 1990). In Study III, neither dynamic nor static Crs predicted the length of mechanical ventilation and PICU stay after congenital cardiac surgery.

To find prognostic factors independently predicting short-term outcome, we performed a multivariable analysis (Table 9). In addition to B-line or CXR LE scores, the length of perfusion and presence of postoperative complications were included as

independent variables, since they have been shown predictive after congenital cardiac surgery (Brown et al. 2003). Length of perfusion independently predicted the short-term outcomes, whereas postoperative complications predicted only length of PICU stay (Table 9). The lung US B-line score independently predicted both length of mechanical ventilation and PICU stay, as did the CXR LE score (Table 9). Consequently, lungs in general, and particularly excessive EVLW, are a potential factor in complicating postoperative recovery after congenital cardiac surgery.

Table 9. Multivariable linear regression analyses predicting short-term outcome after congenital cardiac surgery.

Length of mechanical ventilation						
		Length of perfusion	Major postop. complications	B-line score	CXR LE score	r^2, p [a]
Model 1 [b]	Beta[d]	0.57	0.08	0.36		0.60, <0.0001
	p[e]	<0.0001	0.43	0.001		
Model 2 [c]	Beta[d]	0.54	0.15		0.32	0.59, <0.0001
	p[e]	<0.0001	0.13		0.002	
Length of PICU stay						
		Length of perfusion	Major postop. complications	B-line score	CXR LE score	r^2, p [a]
Model 1 [b]	Beta[d]	0.55	0.20	0.24		0.59, <0.0001
	p[e]	<0.0001	0.04	0.02		
Model 2 [c]	Beta[d]	0.52	0.26		0.26	0.60, <0.0001
	p[e]	<0.0001	0.009		0.009	

[a] coefficient of determination and p-value for a model
[b] Model 1 includes length of perfusion, presence of major postoperative complications and early postoperative B-line score as dependent variables
[c] Model 2 includes length of perfusion, presence of major postoperative complications and early postoperative CXR LE score as dependent variables
[d] standardized beta
[e] p-value for a variable in a model

Based on our statistical multivariable analyses, both the model with B-line score and with CXR LE score could be used in predicting short-term outcome after congenital cardiac surgery. Both models had equal statistical significance in predicting length of mechanical ventilation and length of postoperative PICU stay (Table 9). We further analyzed and compared the predictive value of B-line and CXR LE scores on short-term outcome by using ROC curves (Kaskinen A. et al., unpublished results). The AUC of B-line score and CXR LE scores showed no difference in determining PICU stay or mechanical ventilation lasting over median (Figure 11). The great variability

of CHDs and patient characteristics prevent straightforward prediction of short-term outcome such as length of PICU stay by statistical models. However, to assess postoperative risks influencing resource management, it would be important to identify easily measurable factors predicting postoperative morbidity.

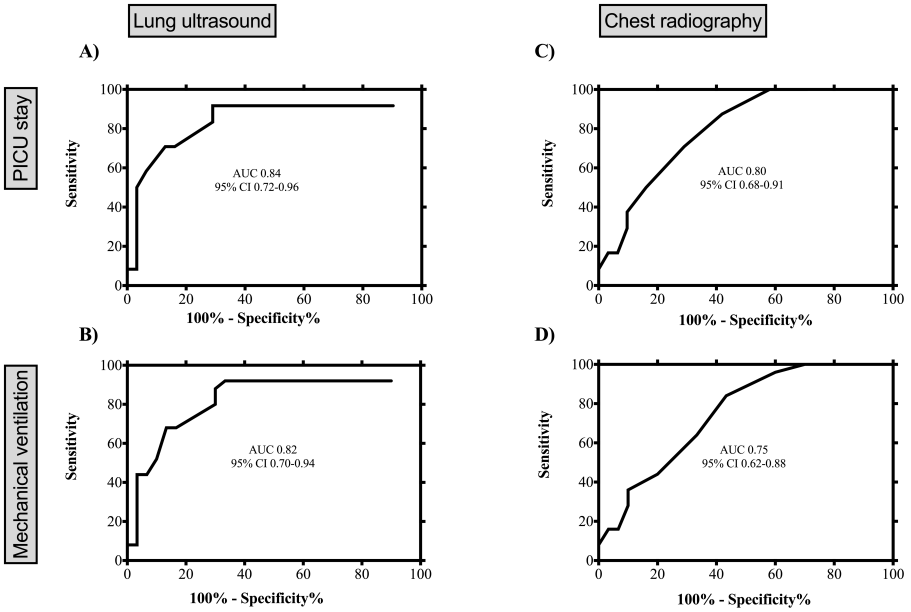

Figure 11 *ROC curves of B-line score and CXR LE score in determining longer than median PICU stay (A, C) and mechanical ventilation (B, D). The area under curve (AUC) of B-line score and CXR LE score showed no difference in determining PICU stay (p=0.48) or mechanical ventilation (p=0.25) lasting over median.*

The early postoperative B-line score was higher in the patients with delayed sternal closure than those with primary sternal closure (p=0.002). As for CXR, early postoperative CXR LE scoring was also higher in patients with delayed sternal closure (p=0.005). Although a higher amount of EVLW postoperatively was associated with delayed sternal closure, our retrospective comparisons did not reveal the value of either B-line or CXR LE scorings in predicting the optimal timing for the sternal closure.

5.6 Long-term outcome of PA+VSD (IV)

The follow-up of PA+VSD patients extended 41 years from 1970 and the median follow-up time was 11.4 years (range 0.01–41.77 years). This long follow-up is an important merit of our study because previous studies have either had a shorter follow-up (Amark et al. 2006, Cho et al. 2002) or have focused on only PA+VSD patients with MAPCAs (dUdekem et al. 2005).

5.6.1 Incidence and diagnosis of PA+VSD

The incidence of PA+VSD in Finland was 6.1 per 100 000 live births, which is in line with previously reported incidence values of 4.2 to 10 per 100 000 live births (Fyler 1980, Leonard et al. 2000, Samanek and Voriskova 1999).

Consistent with a previous report (Dinarevic et al. 1995), no difference in age at postnatal diagnosis occurred between patients with or without MAPCAs in Study IV. However, 95% of patients born between 1995 and 2007 were diagnosed before the age of two weeks compared with 72% of patients born between 1970 and 1994 (p=0.004). This finding may indicate that the support and consult services of the childbirth hospitals from the tertiary care have significantly improved, resulting in earlier diagnosis of PA+VSD in more recent years. The antenatal CHD diagnostics in Finland have further improved after 2010 when our own domestic guidelines for fetal morphology screening took effect and made the national Fetal Cardiology Program (established in 1999) more effective. In Study IV, antenatal diagnosis occurred in only 5% of the patients and thus the effect of antenatal diagnosis on treatment and survival of PA+VSD patient could not be studied. However, other recently published results have shown a high degree of accuracy for prenatal echocardiographic diagnosis of PA+VSD, although the correct assessment of central pulmonary arteries and MAPCAs is still challenging (Vesel et al. 2006). The improvements in prenatal diagnostics may affect the incidence of PA+VSD in the future since, according to a recent meta-analysis, 46% of PA+VSD pregnancies are terminated (Zhao et al. 2016).

5.6.2 Overall outcome of PA+VSD

The Kaplan-Meier estimated overall survival in Study IV corresponded to previous reports with comparable follow-up periods (Amark et al. 2006, Dinarevic et al. 1995, Leonard et al. 2000) (Figure 12).

The factors significantly affecting survival were the primary size of central pulmonary arteries (HR 0.60, 95% CI 0.45–0.82 per 0.5 McGoon units, p=0.001) and achievement of successful repair (HR 0.07, 95% CI 0.03–0.17, p<0.0001) (IV, Table 3 & Figure 2).

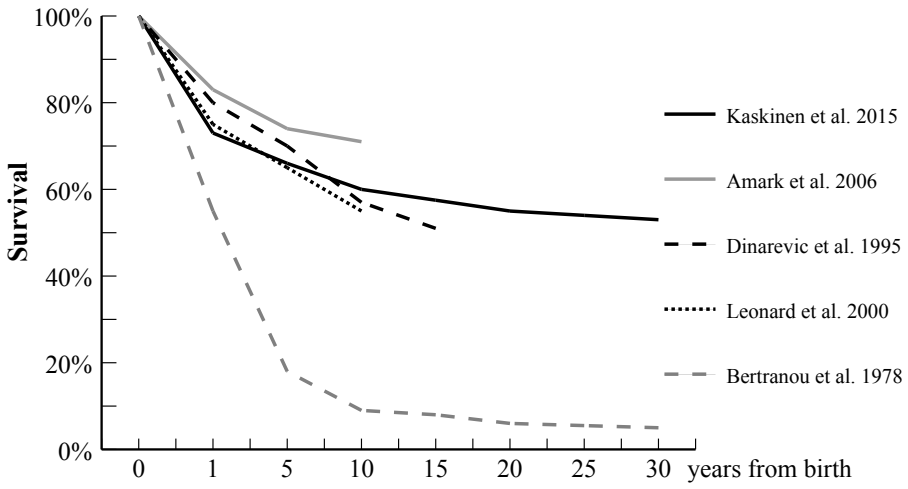

Figure 12 *The modern long-term survival of PA+VSD is significantly better than natural survival reported by Bertranou and colleagues in 1978 (Bertranou et al. 1978).*

5.6.3 Outcome of PA+VSD after repair

After successful repair, the survival of patients was excellent and was comparable to previous series (Amark et al. 2006, Chen et al. 2012, Cho et al. 2002, Dinarevic et al. 1995) (Figure 13). The NYHA class in surviving repaired patients was 1.3 ± 0.5 at the latest follow-up visit, which was a median 18.6 (10.7–23.7) years after repair. Also, the postoperative RV/LV systolic pressure ratios remained at acceptable levels. Kaplan-Meier estimated freedom from reintervention after repair was 86%, 57%, and 33% at 1, 10, and 20 years after repair, respectively. A perioperative RV/LV systolic pressure ratio above 50% increased the probability of surgery or catheter-based reintervention in multivariable Cox regression analysis (IV, Table 4).

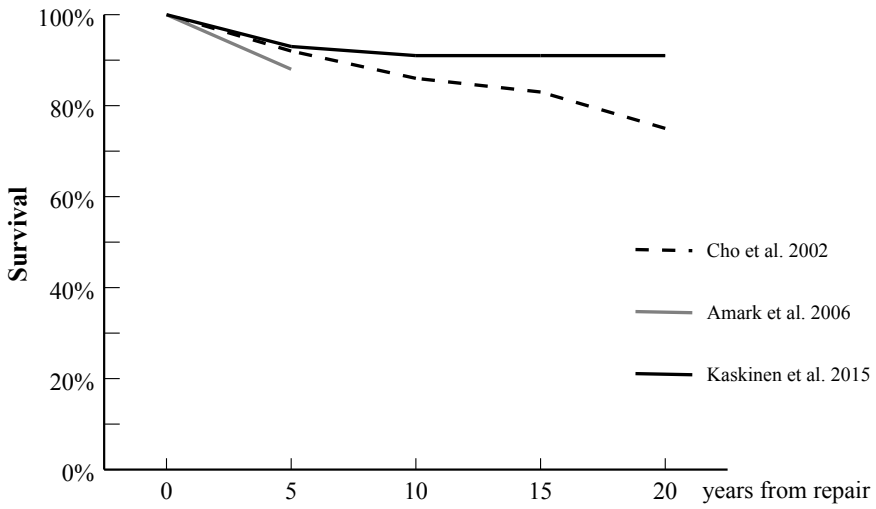

Figure 13 *Survival after repair compared with previous reports (Amark et al. 2006, Cho et al. 2002).*

5.6.4 Outcome of palliated PA+VSD patients

The palliated patients had Kaplan-Meier estimated survival rates of 55%, 34%, 20%, and 15% at 1, 10, 20, and 30 years of age, respectively. Compared with previous reports, these survival rates of Study IV are somewhat disappointing (Cho et al. 2002). However, in our series the palliated patients who underwent incomplete repair attempts with a connection between RV and pulmonary arteries but who had to be left with septal fenestration had better survival than the rest of the palliated patients (IV, Figure 2). Therefore, despite failure to close VSD at repair being considered as a risk factor for mortality (Carotti et al. 2010, Davies et al. 2009), repair attempts resulting in creation of a connection between RV and pulmonary arteries with septal fenestration should be considered as a partial success when treating PA+VSD. For the patients with MAPCAs, reconstruction of RV-outflow tract with septal fenestration was performed more often than for patients without MAPCAs. This may lead to the result that the survival of palliated patients in our series was better in the presence of MAPCAs, a finding contradicting the report of Cho and colleagues (Cho et al. 2002).

5.7 Methodological considerations

Study I lacked airway epithelial ion transport activity measurements and Na^+ transporter mRNA levels from distal airway epithelium, since distal airway measurements would have been invasive. However, in humans, proximal airway epithelium is commonly used to assess phenomena in distal airways (Barker et al. 1997, Fajac et al. 1998, Mac Sweeney et al. 2011). That epithelial ion transport of the proximal airway associates with Crs in newborns and ENaC mRNA expression in adults further justifies the usage of proximal airway as a surrogate for more distal airways (Helve et al. 2005, Otulakowski et al. 1998). From nasal epithelial cell samples only mRNA levels were measured. Thus, whether protein translation of Na^+ transporters is affected by chronic hypoxemia in CHD remains unknown.

In Study II, improvement of postoperative clinical condition after POD1 caused a natural decrease in number of lung US examinations available for analysis (Table 7). This may have contributed to a lack of correlation between B-line and CXR LE scores during POD2, POD3, and POD5. In addition, there are certain limitations in comparing lung US B-line score with CXR LE score in assessment of EVLW. First, although CXR scorings in adults have correlated moderately with EVLW measured with dilution techniques (Brown et al. 2013, Halperin et al. 1985), similar correlation has lacked in critically ill children (Lemson et al. 2010). Second, in Study II the interobserver correlation of CXR LE score was only moderate (r^2=0.33), which emphasizes that interpretation of CXR despite standardization may remain, at least partly, subjective in nature. Third, intravascular filling may interfere with EVLW grading of CXR, unlike with lung US (Lange and Schuster 1999). Despite these limitations, comparison of lung US B-line score with CXR was important, since CXR is still used as the principal imaging method in clinical practice when assessing EVLW and lung edema after congenital cardiac surgery.

In Studies II and III, only sporadic postoperative Crs values were measured. Therefore, the interesting comparison of the preoperative and postoperative values as well as analysis of postoperative variation in Crs was impossible. In Study II, the number of postoperative static Crs measurements was limited due to early postoperative extubations and was thus smaller than in Study III. This may have resulted in no correlation existing between static Crs and CXR LE score in Study II.

No power calculations were made to study the factors predicting short-term outcome after congenital cardiac surgery. Since the length of mechanical ventilation and PICU stay are related to multiple factors during the postoperative course after congenital cardiac surgery (Brown et al. 2003), higher number of Crs values in Study III may have been needed to reach statistical significance in predicting short-term outcome.

Limitations of Study IV were related to the retrospective nature of the study. Changes and progress in pre-, peri-, and postoperative care of PA+VSD during the extensive follow-up of Study IV may have complicated precise analysis of factors affecting survival. Furthermore, many of the factors affecting survival and achievement of

repair are interrelated. For example, the presence of MAPCAs and birth year affected probability of achieving repair but lacked an effect on survival. In addition, the limited number of angiograms available may have affected the analyses concerning McGoon and TNPAI indexes as well as perfusion of lung segments. Angiograms of 79 (72%) patients were available, but angiograms existed for only 54% of the patients with MAPCAs. Furthermore, there was no possibility to evaluate peripheral intrapulmonary perfusion reliably. Thus, despite beneficial for the central pulmonary arteries, the systemic–pulmonary artery shunts ability to promote growth of peripheral intrapulmonary vessels remained undocumented.

5.8 Future perspectives

The Study II findings indicating that the early postoperative assessment of EVLW both by lung US and CXR predicted short-term outcome emphasize the role of lungs as a potential source of complications after congenital cardiac surgery. Thus, postoperative pulmonary pathology, and lung edema in particular, is an interesting area of future studies in children with CHD.

In Study I, we showed that profoundly hypoxemic children with cyanotic CHD have impaired airway epithelial Na^+ transport, which may result from posttranscriptional mechanisms of hypoxemia. However, the specific posttranscriptional and posttranslational mechanisms causing this impairment warrant further studies. Since β_2-agonists have shown to enhance airway epithelial Na^+ transport, these drugs may have potential in promoting postoperative lung liquid removal after congenital cardiac surgery, especially in those with impaired airway epithelial Na^+ transport. Furthermore, the role of glucocorticoids, which are often combined with open-heart surgery to suppress inflammatory response, is largely unknown in postoperative lung liquid removal after congenital cardiac surgery.

Based on the Study II findings, lung US may complement CXR in assessment of EVLW and lung edema in children after congenital cardiac surgery. Whether lung US could potentially replace some postoperative CXRs or treatment decisions could be based on lung US needs further systematic studies. Since CXR has limitations in assessment of EVLW, comparison of lung US B-line score with precise measurement of EVLW such as TPTD in children undergoing congenital cardiac surgery is needed. However, the effect of intracardiac shunts on reliability of TPTD method should be carefully taken into account (Keller et al. 2011). In addition to blinded analysis of lung US, open comparison with other imaging techniques would also provide more knowledge on the significance of lung ultrasound as a diagnostic tool after congenital cardiac surgery. Other postoperative pulmonary complications such as pneumonia, pneumothorax, and atelectasis have also been detected and assessed by lung US. Thus, lung US may also have potential for wider use than congenital cardiac surgery in children. Finally, the postoperative dynamic variation of B-line score in various CHDs is also an important and interesting area of future investigation.

Study III demonstrated that ventilator-derived dynamic lung compliance reflects different phenomena than static lung compliance and may not be ideal tool to reflect the state of lung parenchyma, unlike static Crs. However, it remains to be shown whether continuous ventilator-derived dynamic compliance proves useful in monitoring of the patient after congenital cardiac surgery. In addition, the postoperative variation of dynamic Crs and its association with short-term outcome needs systematic investigation. The role of Crs values, later during the postoperative course, in predicting short-term outcome would be interesting. Another important area for future research would be to study whether postoperative treatment decisions such as timing of sternal closure or ventilator management optimization could be based on ventilator-derived dynamic Crs or on lung US findings.

6 Conclusions

This thesis focused on factors affecting EVLW clearance and methods to assess EVLW after congenital cardiac surgery in children. In addition, we studied factors affecting postoperative short-term outcome in CHD in general and long-term outcome in a particular cyanotic CHD, namely PA+VSD. The main findings include:

1. The airway epithelial Na^+ transport and particularly amiloride-sensitive Na^+ transport was impaired in profoundly hypoxemic children with cyanotic CHD. Posttranscriptional mechanisms may cause this impairment, since no ENaC-subunit mRNA expressions were attenuated in profoundly hypoxemic patients. After open-heart surgery, profoundly hypoxemic patients may be particularly susceptible to excessive EVLW induced by CPB and pulmonary reperfusion injury.

2. Lung US B-line score correlated with radiographic EVLW assessment, and interpretation of lung US had less interobserver variation than CXR. Lung US as a radiation-free and easy bedside tool may complement chest radiography in assessment of EVLW in children after congenital cardiac surgery.

3. Static Crs associates with US and CXR assessment of EVLW early after congenital cardiac surgery. Despite reasonable correlation between the dynamic and static Crs, the values do differ and these measurements reflect different phenomena. Ventilator-derived dynamic lung compliance may not reflect the state of lung parenchyma similar to static compliance.

4. After congenital cardiac surgery, both early postoperative lung US B-line and CXR lung edema scorings independently predicted short-term outcome interpreted as length of postoperative mechanical ventilation and intensive care. Identifying measurable factors predicting postoperative short-term outcome and morbidity may promote resource management and early recognition of postoperative complications.

5. The most significant factors affecting long-term survival of PA+VSD were the primary anatomy of pulmonary circulation and achievement of repair. Thus the initial evaluation of pulmonary circulation has a crucial role in determining the treatment strategy of PA+VSD, which should actively aim at surgical correction. However, palliative surgery also has a role in treating PA+VSD, since systemic-pulmonary artery shunt is advantageous for the growth of the central pulmonary arteries, and reconstruction of RV-outflow tract with septal fenestration improves survival.

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“It always seems impossible until it’s done” – Nelson Mandela

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Anu Kaskinen

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