Left-sided obstructive cardiac lesions in the fetus and the neonate

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Every cloud has a silver lining

ABSTRACT

Introduction Hypoplastic left heart syndrome (HLHS) is a severe cardiac malformation, fatal in the neonatal period in the absence of immediate care. Palliative surgery for HLHS has been available in Sweden since 1993. The outcome has improved over time, but there is still significant mortality. It has been suggested that fetal valvuloplasty in fetal aortic stenosis may prevent progression to HLHS. Home monitoring of oxygen saturation has been suggested as a method to improve survival after the initial surgery.

Aims The aims were to investigate the survival rate of patients born with HLHS in Sweden from1990 to 2010, and to evaluate fetal valvuloplasty of the aortic valve as a method of preventing HLHS. A third aim was to evaluate the importance of home monitoring as a method to improve survival after the initial surgery.

Methods The complete national cohort of patients with HLHS was identified through national databases. Changes in incidence and transplantation-free survival were calculated and analyzed in relation to risk factors for death. The natural history of fetal aortic stenosis and the efficacy of a fetal intervention were investigated in two retrospective multi-center studies. Home monitoring was evaluated in an experimental study and survival was compared with a historical cohort.

Results and conclusions The overall 10-year transplantation-free survival of patients with HLHS increased from 40 % 1993–2000 to 63 % 2001–2010. Female gender was identified as a significant risk factor. The incidence at birth decreased from 15.4 to 8.4 per 100,000. The proportion of liveborn neonates with HLHS undergoing surgery increased from 50 % to 70 %. Fetal intervention with balloon dilatation of the aortic valve improved postnatal survival but did not prevent progression to HLHS. Home monitoring of oxygen saturation was considered lifesaving in a number of individuals but there was no statistical difference in survival compared to a historical cohort.

Keywords: Hypoplastic left heart syndrome, aortic valve stenosis, fetal heart, fetal therapies, outcome studies, survival analysis, epidemiology, incidence, prenatal diagnosis, pregnancy outcome.

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LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Öhman A, Strömvall-Larsson E, Nilsson B and Mellander M. Pulse oximetry home monitoring in infants with single ventricle physiology and a surgical shunt as the only source of pulmonary blood flow. *Cardiology in the Young*. 2013;23:75–81.

II. Gardiner H, Kovacevic A, Tulzer G, Sarkola T, Herberg U, Dangel J, Öhman A, Bartrons J, Carvalho J, Jicinska H, Fesslova V, Averiss I, Mellander M and Fetal Working Group of the AEPC. Natural history of 107 cases of fetal aortic stenosis from a European multicenter retrospective study. *Ultrasound in Obstetrics and Gynecology*. 2016;48:373-381.

III. Kovacevic A, Öhman A, Tulzer G, Herberg U, Dangel J, Carvalho JS, Fesslova V, Jicinska H, Sarkola T, Pedroza C, Averiss I, Mellander M and Gardiner HM. Fetal hemodynamic response to aortic valvuloplasty and postnatal outcome: a European multicenter study. *Ultrasound in Obstetrics and Gynecology*. 2017. doi: 10.1002/uog.18913

IV. Öhman A, El-Segaier M, Bergman B, Hanseus K, Malm T, Nilsson B, Pivodic A, Rydberg A, Sonesson SE, Mellander M. The changing epidemiology of hypoplastic left heart syndrome. Results of a national Swedish cohort study. (Submitted).

V. Öhman A, El-Segaier M, Bergman B, Hanseus K, Malm T, Nilsson B, Pivodic A, Rydberg A, Sonesson S, Mellander M. Transplantation-free survival and risk factors for death or heart transplantation after Norwood surgery in a complete national cohort of patients with HLHS in Sweden 1993–2010. (Submitted).

LIST OF CONTENT

ABBREVIATIONS

AA	Aortic atresia
AEPC	Association for European Pediatric and Congenital Cardiology
AoA	Aortic arch
AoS, AS	Aortic stenosis
AV-valve	Atrioventricular valve
BCPC	Bidirectional cavopulmonary connection
BDG	Bidirectional Glenn operation
BT-shunt	Blalock-Taussig shunt
BV	Biventricular
CC	Comfort care
EFE	Endocardial fibroelastosis
eHLHS	Evolving hypoplastic left heart syndrome
FO	Foramen ovale
FV	Fetal valvuloplasty
HLHS	Hypolastic left heart syndrome
HR	Hazard ratio
HTX	Heart transplantation
ICD-9,10	International Statistical Classification of Diseases and Related Health Problems
IPTW	Inverse probability of treatment weighting
LV	Left ventricle
MA	Mitral atresia
MS	Mitral stenosis
NH	Natural history
NYHA	New York Heart Association
OR	Odds ratio
PS	Propensity score
RCT	Randomized controlled trial
sIUD	Spontaneous intra uterine death
TCPC	Total cavopulmonary connection
ToP	Termination of pregnancy
UV	Univentricular
US	United States of America
VSD	Ventricular septal defect
z-score	Standard score

DEFINITIONS

30-day surgical mortality	Death occurring within 30 days after surgery.
Anatomy and morphology	The study of the structure and development of organisms.
Bias	The result of a systematic error in the design or conduct of a study.
Confounder	A confounding factor influences both the exposure and the outcome.
Congenital	Referring to conditions that are present at birth, regardless of their causation.
Embryology	The study of fetal development. Three major parts; the first three weeks, the embryonic period (third to eighth week) and the fetal period (third month to birth).
Hypoplasia	Incomplete development or underdevelopment of an organ or tissue.
Information bias	Information bias results from either imperfect definition of study variables or flawed data collection procedures.
Interstage mortality	Death occurring between stage I and stage II in the single ventricle palliation.
Interstage mortality (Paper I)	Death occurring between stage I and stage II after discharge from hospital.
Interstage mortality (Paper V)	Death occurring between 30 days after stage I before stage II
Outcome	The result of an event or process.
Physiology	The study of function of living organisms.
Selection bias	A systematic error in recruitment or retention of study subjects.
Stage I	Norwood or hybrid surgery
Stage II	BDG
Stage III	TCPC or Fontan completion
Syndrome	A combination of symptoms resulting from a single cause or commonly occurring together as to constitute a distinct clinical picture.

INTRODUCTION

Left-sided obstructive lesions include single or multiple obstructions on one or more levels of the left side of the heart. This thesis will present research related to fetal aortic stenosis and hypoplastic left heart syndrome. The natural history of fetal aortic stenosis and the potential of fetal cardiac intervention to prevent from evolution of hypoplastic left heart syndrome will be discussed. The epidemiology and outcome for fetuses and neonates with hypoplastic left heart syndrome will be elucidated. The efficacy of home monitoring of oxygen saturation in a cohort of different single ventricle lesions was observed and will be presented.

The PhD project started with the study reported in Paper I, evaluating home monitoring of oxygen saturation in patients with single-ventricle physiology and a shunt as the only source of pulmonary blood flow. The study was an experimental study, aiming to evaluate the influence of the implementation of intensified surveillance of patients between stage I and stage II surgery through home monitoring of oxygen saturation. Papers II and III were the results of a project on fetal aortic stenosis initiated by the fetal working group of AEPC. The study took off during an AEPC meeting in Granada in 2011. At that time, the fetal working group had observed a growing interest in fetal interventions, especially balloon dilation of the aortic valve. There were some promising results, but the scientific evidence was weak. To address this experienced lack of evidence, the fetal working group of AEPC decided to form a research group to study fetal aortic stenosis. The plan, as it was formed at the Granada meeting, was to conduct two parts of the project, a retrospective part and a prospective study. The results of the former would guide the design of the latter. At the time I got involved, there was already a study design and a study protocol in place. I visited London in June 2011 to receive information and to discuss some practical issues. I was assigned to collect data from the Nordic countries, Bonn, and Italy. The procedure to perform fetal cardiac intervention was not practiced in any of the Nordic countries.

At the time of the start of the study, I was working in Stockholm and identified cases of fetal aortic stenosis from the local fetal databases. While collecting the data, I discovered that fetal aortic stenosis was a rare condition and that most of the identified cases were far along the road towards HLHS. The majority of identified cases were counseled by the fetal cardiologist as future univentricular hearts and many chose to terminate the pregnancy. I also

observed that the clinic had neonates with critical aortic stenosis who underwent biventricular repair and none of them had a prenatal diagnosis. This observation made me realize that cases with fetal aortic stenosis and a normal or dilated left ventricle with a likely UV circulatory outcome but with BV potential was rare in our population of fetuses while a postnatal diagnosis of critical aortic stenosis and BV outcome was more common.

The primary aim of performing fetal valvuloplasty is to improve postnatal survival by achieving BV circulation. To investigate the survival probability for liveborn neonates with UV circulation due to left-sided hypoplasia, we decided to identify all cases with HLHS born in Sweden and observe their outcome. We chose to limit the inclusion criteria of the national cohort to patients with the morphology of aortic atresia versus aortic stenosis. The reason to do so was the aim to compare outcomes and exposure factors for patients with as similar morphology and physiology as possible. We feared that including AS/MS would make the comparison more difficult and the morphology (AS/MS) would possibly be a factor more important influencing outcome than other risk factors aimed to study.

This thesis includes chapters on the specified cardiac malformations HLHS/AA and fetal aortic stenosis. Included in the chapter on fetal aortic stenosis is a part where details on the technical procedure is described and a part on fetal echocardiography as a predictor of outcome. This is followed by chapters on the methodology and statistical methods. At the end, there is a discussion including the results from all the papers, as well as conclusions and future perspectives.

Tables 1–3 refer to tables in the thesis itself. Figures 1-2 are referred to figures in the thesis while figures included in any of the papers are referred to as the Roman numeral of the paper and the number of the figure in the paper. Tables A–C are found in the Appendix and provide overviews of the included studies.

AIMS

The specified aims for each paper are listed below.

I. The primary aim was to evaluate whether daily measurement of oxygen saturation at home between stage I and stage II would be beneficial to patients through earlier detection of impending shunt occlusion. A secondary aim was to examine parents' experiences with home monitoring.

II. The primary aim was to report the spectrum of fetal left heart morphology and physiology, pregnancy outcome, survival and final circulatory pathways in a natural history cohort of aortic stenosis (NH). A secondary aim was to test previously published criteria for evolving HLHS and identify ideal candidates for fetal valvuloplasty (FV) in this population of fetuses by comparing predicted with observed outcome.

III. The primary aim was to assess FV efficacy by comparing survival and postnatal circulation between FV and NH cohorts. Secondary outcomes were hemodynamic change and left heart growth.

IV. The primary aim was to describe the incidence and evaluate the possible change in incidence of HLHS/AA in Sweden. A secondary aim was to investigate factors influencing whether or not surgery was performed.

V. The primary aim was to describe the outcome for patients with HLHS/AA who underwent surgery and to analyze factors with correlation to outcome.

HYPOPLASTIC LEFT HEART SYNDROME (HLHS)

DEFINITION

Hypoplastic left heart syndrome (HLHS) defines a constellation of findings with severe obstructions and underdevelopment of the left-sided structures of the heart. The cardiac anomaly was first described by Lev [1] and Noonan and Nadas [2] who introduced the term HLHS in 1958. In the original work, a common atrioventricular valve was included as a variant of HLHS while the modern definition excludes a common atrioventricular junction [2, 3].

In the current version of the International Statistical Classification of Diseases (ICD-10), HLHS is defined as "atresia, or marked hypoplasia of the aortic orifice or valve, with hypoplasia of ascending aorta and defective development of left ventricle, with mitral valve stenosis or atresia" [4, 5]. The term "classic HLHS" has been used to describe "left ventricular hypoplasia associated with mitral and aortic valve hypoplasia or atresia and hypoplastic ascending aorta" [6, 7].

The physiology of HLHS is functionally univentricular circulation in which the right ventricle provides for both systemic and pulmonary blood flow while the left ventricle contributes not at all or very little to the cardiac output.

The three surgical palliative stages for HLHS are the Norwood or hybrid procedure during the neonatal period (Stage I) followed by bidirectional Glenn at 4-6 months of age (Stage II) and finally a Fontan procedure at 2-4 years of age (Stage III) [8].

EMBRYOLOGY AND DEVELOPMENT

There are three main theories suggested to explain the origin of HLHS. The embryological theory, the "flow" theory and the theory of fetal aortic stenosis evolving to HLHS. The embryological theory is related to the early stage in development, controlled by the interplay of genetic expression and environmental factors [9, 10]. Normally, the formation of the aortic valve includes the formation of the aortic sac, which is covered with cells from the endocardial cushions derived from the neural crest. When this process fails, the formation of the aortic valve or mitral valve can be incomplete. The atresia of the aortic or mitral valves results in underdevelopment of all left-sided structures including the left atrium, left ventricle, ascending aorta, and aortic

arch, with or without coarctation. A recent report on the genetic background of HLHS states that it is a multigenic and genetically heterogeneous condition where mutations in specific areas of the genome mediate left ventricular hypoplasia and aortic valve abnormalities in early development [11]. Environmental factors such as maternal exposure to toxic agents and seasonal viruses have been described as increasing the incidence of HLHS in the population [12, 13]. The second explanation is related to disrupted flow due to malalignment of the interatrial septum. According to this theory, the consequences of the disturbed flow result in poor growth and hypoplasia of left-sided structures and HLHS will develop [14]. The third theory is related to fetal aortic stenosis as the primary cardiac lesion evolving into HLHS at birth. This development will be further discussed in the following main chapter.

MORPHOLOGY

HLHS can be the constellation of aortic stenosis (AS) with mitral stenosis (AS/MS), or aortic atresia with mitral stenosis (AA/MS) or mitral atresia (AA/MA). The abbreviation HLHS/AA will in this work be used for the two combinations of HLHS with AA. AS/MS is a heterogeneous malformation with variable expressions. The left ventricle can be dilated or normal to hypoplastic in size, with or without endocardial fibroelastosis (EFE). The outcome of the combination AS/MS can be bi- or univentricular depending on the size and function of the left-sided structures. When there is AA, the ventricle is always small, either rounded and thick or slit-like in shape. The typical appearance of AA/MS is of a rounded and thick left ventricle with a lining of EFE and sometimes with ventriculo-coronary arterial connections [15, 16]. AA/MA is more often seen in combination with a slit-like left ventricle without EFE, and rarely with ventriculo-coronary arterial connections [17]. The outcome of HLHS/AA is always univentricular circulation, since the absence of a left outflow tract and left ventricle cannot be compensated for. The presence of a VSD creates a fourth possibility, but this morphology will not be further discussed here.

EFE is a phenomenon seen as a white lining of the inner wall of the left ventricle when examined using echocardiography in fetal and postnatal life [18]. It is seen in conjunction with HLHS and in relation to viral myocarditis and autoantibody-mediated myocardial disease in the fetus [19]. The finding of EFE in HLHS is described as being more frequent in hearts that have

elevated end-diastolic pressure, as in AS or AA, and some patency of the mitral valve rather than in AA/MA [20]. The presence of EFE has been considered a reason for stunted growth in evolving HLHS, leading to attempts to remove it in postnatal surgery to improve diastolic function and growth [21, 22].

The fetus and the neonate with left-sided obstructive lesions may survive if the right side of the heart can support systemic output through the ductus arteriosus. The right ventricle will continue to be the systemic ventricle after birth and after surgical palliation. The right ventricle, which normally handles various amounts of volume-loading in a low-pressure setting, has to cope with both high volume- and high-pressure load. Variations in morphology of the tricuspid valve in conjunction with HLHS have been reported, as well as changes in right ventricular geometry [23, 24].The conditions for the right ventricle are challenging, and the overall function of univentricular circulation is dependent on a well-functioning right ventricle and tricuspid valve [25] and a low enough pulmonary vascular resistance.

SINGLE VENTRICLE PALLIATION

The treatment options for HLHS are a palliative surgical three-stage pathway to a Fontan circulation or neonatal cardiac transplantation. The first stage in single-ventricle palliation is either the Norwood procedure or a hybrid procedure. The surgical technique of the Norwood procedure is described in Paper V. The second stage in the palliative pathway for HLHS is performed by connecting the systemic venous return from the upper part of the body to the pulmonary circulation. This can be done either through the right atrium, as in the Hemi-Fontan procedure, or through a connection between the superior vena cava and the pulmonary artery branches. This results in the bidirectional cavopulmonary connection (BCPC), also referred to as the bidirectional Glenn procedure (BDG) [26]. The hemodynamic advantages include decreased volume load of the right ventricle and improved oxygen saturation. The risk of thrombosis is less compared to the shunt-dependent circulation after the Norwood procedure. The third and final stage in the palliative pathway is connecting the venous return from the lower part of the body to the pulmonary circulation. This can be achieved by either an intracardiac or an extracardiac tunnel with or without fenestration. Fenestration permits unloading of pressure and volume from the pulmonary venous circulation. The surgical procedure is

commonly referred to as “total-cavopulmonary connection” (TCPC) or Fontan procedure after the French surgeon who described it in 1978 [27].

INCIDENCE

The incidence of congenital heart disease in liveborn is reported to be 6.4 to 11.1 per thousand [28-32]. The variation is mainly due to the definition of congenital heart disease and the detection rate. Postnatal detection rates generally increased in the 1990s, likely due to more advanced ultrasound technology [31]. Norway reported variations within the population with an increase of cardiac defects from 1994 to 2000 followed by a per-year decrease of 3.4% for severe cardiac defects noted from 2004 onward. There was an increasing practice of termination of pregnancy (ToP) when severe heart disease was diagnosed during this time, but the authors thought elimination of risk factors and a possible benefit of pre-conceptional folic acid supplementation were more important factors to explain the decreasing incidence [31]. HLHS is the cardiac lesion with the highest prenatal detection rate and in Sweden and many other countries there is a high termination rate when detected in utero. The incidences of HLHS before general prenatal screening, or in populations where the termination rate is low, are reported to be 8 to 27 per 100,000 live births, Table 1, page 14 [28, 30, 33, 34]. The incidence of univentricular hearts (UVH) in relation to an increasing prenatal detection and termination rate was investigated in Denmark where a significant decrease in incidence was reported from 2003. There was an increasing number of terminations from 2003 onward, with a termination rate of 85% for UVH in 2009 [35].

Table 1. *Incidence of HLHS as reported in the literature. HLHS included cases with AS and AA as defined in the table. The incidence per 100,000 liveborn neonates was calculated by the author (AÖ) [28-31, 33, 34, 36-39].*

Author	*Year*	*Geographical area*	*Morphology*	*N*	*Study population*	*Incidence per 100,000*
Carlgren	1959	Sweden	AA and AS	24	58,105	41
Brownell	1976	Canada	AA	64	-	25
Samanek	1989	Bohemia	AA and AS	24	91,823	26
Samanek	1999	Bohemia	AA and AS	172	816,569	21
Hoffman	2004	California, US	HLHS	-	-	23 (28)
McBride	2005	Texas, US	HLHS	166	1,077,574	15 (13–18)
Reller	2008	Atlanta, US	HLHS VSD	91	398,140	23
Moons	2009	Belgium	HLHS	10	111,225	9
Leirgul	2014	Norway	HLHS	154	943,387	16
Qu	2016	China	HLHS	-	-	8 (6–10)

Abbreviations*: AA, aortic atresia, AS, aortic stenosis, HLHS, hypoplastic left heart syndrome, VSD, ventricular septal defect*

INCIDENCE IN RELATION TO GENDER

The distribution between the genders in the general population is a slight male excess with a ratio of 1.04–1.06:1 for male versus female gender. For certain cardiac defects such as transpositions of the great arteries, coarctation of the aorta and HLHS, the male excess is higher than in the general population [40]. A multicenter study, including registry data from the US, Europe and Australia, reported 2,062 cases of HLHS, of which 1,297 were male, resulting in a ratio of 1.7:1 [40]. A study from the Texas Birth Defects Registry of the years 1999–2001 reported 166 cases of HLHS, 110 of them male, resulting in a male-to-female ratio of 2:1 [37, 41]. In the Bohemian population, the ratio was 2.25:1 [42].

PRENATAL DETECTION RATE AND OUTCOME OF PREGNANCY

The increase in the prenatal detection of cardiac malformations after implementation of a general screening program was reported from the County of Stockholm. The detection rate of significant cardiac malformations (cardiac surgery before 1 year of age) was 7.1% in 1997 compared to 41.0% in 2004

[43]. A prenatal diagnosis of HLHS gives the parents option of terminating the pregnancy in countries where this is legal. When the parental wish is to continue the pregnancy, the prenatal diagnosis allows for a safe in-utero transfer to a cardiac surgical center.

The proportion of termination of pregnancy (ToP) after a prenatal diagnosis of HLHS has been reported from Denmark, Sweden and Australia to be 60–85% [35, 43, 44] . The proportion of fetuses with HLHS that will be liveborn depends on the prenatal detection and termination rate. A significant regional difference in prenatal detection and termination rates for single ventricle lesions in 1996-2006 was reported by the Swedish National Board of Health and Welfare. They noted a termination rate of 45% in the region with the highest detection rate and 5% in the region with the lowest detection rate. The difference in termination rates was mainly explained by the variation in prenatal detection rate [45]. Improvements of the population based screening program have resulted in more similar care in all regions but differences still exist and there are no coherent guidelines in Sweden for what cardiac views to include at the routine ultrasound screening at 18-19 weeks of gestation [46].

In continuing pregnancies, a prenatal diagnosis allows for centralized delivery This should potentially facilitate optimal care of the neonate from birth. A population-based study from Texas found that prenatal diagnosis did reduce neonatal mortality if mothers living far from a cardiac surgical center delivered closer to one [47]. In a systematic review from 2016, Thakur et al. evaluated the preoperative mortality in 609 neonates with HLHS, 228 with a prenatal diagnosis and 381 with postnatal diagnosis. There was no statistical difference in preoperative or post-stage I mortality between the groups, but neonates with a prenatal diagnosis were hemodynamically more stable [48]. In Finland, a country with long distances to tertiary care, centralized delivery resulted in improved postnatal right ventricular function and less metabolic acidosis and less end-organ failure in neonates with a prenatal diagnosis of HLHS [49].

OUTCOME

Implementation of surgical programs for palliation of HLHS in relation to improved results and identification of risk factors in the early surgical era

A few decades ago, the only option for children born with HLHS was terminal supportive care (comfort care) until a staged palliative surgical method was suggested by Norwood in 1981 [50, 51] and introduced in Sweden in 1993. The pioneering work of developing the method was mainly done at institutions in the US [50, 52, 53]. In Europe, Birmingham (UK) was one of the first centers to publish the results of a prospective audit in 1993. The general trend in the 1990s was toward improved surgical survival due to continued experience with both operative and postoperative management [6, 52, 53].The causes of death after the modified Norwood procedure was studied in 122 postmortems, showing impairment of coronary perfusion, excessive pulmonary blood flow, obstruction of pulmonary blood flow, neo-aortic obstruction and right ventricular failure as the leading causes [54]. When 10 potential risk factors for first-stage mortality were analyzed, only cardiopulmonary bypass and circulatory arrest times were predictive of total survival, including late deaths [55]. Early experience with the Norwood procedure in Scandinavia was reported from Denmark in 1997, where a surgical program was initiated in April 1993. As of June 1996, 31 patients had been referred. Twelve of them were not considered for surgery, either because of parental wishes or because of hemodynamic instability. Nineteen patients underwent a Norwood procedure. There were 13 hospital survivors, of which 8 (42%) were alive at a mean follow-up time of 19 months. In Norway, health authorities initiated a program in 1987 where they decided to pay all expenses for transportation, examination, and treatment with staged palliation in the US or Europe. The majority of patients were referred to Philadelphia from 1987 through 1998. At a midterm follow-up, 12 of 31 (39%) patients who had undergone at least one palliative procedure abroad were alive [56].

Modification of the Norwood procedure with introduction of the right-ventricle-to-pulmonary-artery-shunt (Sano shunt)

In 2002, Sano and colleagues at the Okoyama University Hospital in Japan presented a modification to the Norwood procedure. They employed a shunt from the right ventricle directly to the pulmonary arteries, and they reported a clear difference in postoperative hemodynamics between the direct shunt and the more traditional systemic-to-pulmonary arterial shunts (BT and modified BT shunt). They suggested an optimal size for the shunt of 5 mm in patients weighing more than 2.0 kg and 4 mm for smaller patients. They thought the direct shunt would be particularly beneficial for small neonates [57]. A

comparison of shunt types was performed in a randomized trial conducted in 15 North American centers, the single-ventricle reconstruction trial (SVR trial) [58]. The primary outcomes were death or cardiac transplantation 12 months after randomization. Transplantation-free survival was higher with the Sano shunt than with the modified BT shunt (74% vs. 64%, p=0.01). At high volume centers, the advantage of a Sano shunt was negated [58].

Intermediate-term transplantation-free survival after Norwood surgery and interaction with shunt type

An intermediate-term evaluation of mortality and transplantation in relation to risk factors and their interaction with shunt type in the same cohort as above was presented in 2012. The cohort included 549 subjects with a mean follow-up of transplantation-free survivals of 2.7 +/- 0.9 years, with a maximum of 4.4 years. Risk factors were categorized as early-phase factors versus constant-phase factors, and as intrinsic, non-modifiable, or modifiable. Modifiable factors were factors that might be subject to practice variations. Early-phase factors associated with death included lower socioeconomic status, obstructed pulmonary venous return, smaller ascending aorta and anatomic subtype, AA/MS having higher risk compared to AA/MA [59, 60]. Constant phase factors associated with death included genetic syndrome and lower gestational age. The Sano shunt was associated with better survival rate in the 51% who were full term with AA. The modified BT shunt was better among the 4% who were preterm (gestational age less than 37 weeks) with a patent aortic valve. Transplantation was used in 3% of subjects following the Norwood procedure. The authors pointed out socioeconomic status and gestational age as potentially modifiable. Although early delivery is sometimes inevitable, they emphasized the increased risk of earlier elective delivery. They concluded that provision of services to compensate for the challenges associated with low socioeconomic status could positively impact outcome [60].

Interstage mortality between stages I and II

After discharge from the hospital, there is a continued significant risk of death before stage II surgery. The interstage mortality was reported to be 12% in a multicenter study in North America [61]. Improved interstage survival has been reported by institutions practicing a home monitoring program with daily monitoring of oxygen saturation and weight [62, 63]. To prevent shunt occlusion caused by the formation of a thrombus, anticoagulation therapy is

widely practiced. Guidelines for antithrombotic therapy in neonates and children, published in 2008, recommend intraoperative unfractioned heparin followed by aspirin (1–5 mg/kg/d) or no further antithrombotic therapy for patients undergoing surgery with a modified BT shunt. The recommended doses of aspirin in neonates and children are empirical [64]. Aspirin (acetylsalicylic acid, ASA) inhibits platelet aggregation by inhibiting cyclooxygenase irreversibility. A drug that could give an additive effect on platelet aggregation by inhibiting ADP in platelets, Clopidogrel, was studied in a randomized controlled study in patients with BT- shunts [65]. The primary end point was death or heart transplantation, shunt thrombosis, or performance of a cardiac procedure due to an event considered to be thrombotic in nature. There was no significant difference in risk in any of the primary end points to occur between the treated group (19%) and the placebo group (21%). This was true also in subgroups defined by shunt type. There is lack of a "gold standard" in thrombo-prophylactic strategies after Norwood surgery [66].

Optimal timing of the second stage in the palliative surgical treatment of HLHS

The optimal timing of stage II surgery has been the subject of several investigations. A recent study investigated the optimal timing of stage II surgery by analyzing the cohort of the Pediatric Heart Network Single Ventricle Reconstruction Trial Dataset of 547 infants with HLHS that underwent Norwood surgery. The optimal timing of stage II was determined by plotting calculated three-year transplantation-free survival versus the Norwood to stage II survival. Calculated transplantation-free survival at three years was stable at 68 +/- 7% during an inter-stage interval of three to six months. Calculated survival decreased rapidly when stage II was performed before three months and then again gradually after six months. Three-year survival decreased in patients defined as "high-risk," with a transplantation-free survival of less than 50%. An early stage II procedure did not rescue ill patients from poor outcome, and the authors questioned such a strategy. Optimal timing of stage II surgery did not differ between patients with modified BT shunts or right-ventricle-to-pulmonary-artery shunts. The authors pointed out the importance of a formal clinical protocol to ensure that operative planning for stage II in infants with low or average risk factors was put in place at discharge after Norwood surgery [67].

Outcome and risk factors for death after stage II surgery

Carlo et al. studied interstage attrition, defined as death or cardiac transplant more than 30 days after bidirectional Glenn (BDG) and before the Fontan procedure. They concluded that moderate or severe tricuspid valve regurgitation and low weight z-score at the time of BDG were important risk factors for subsequent interstage attrition [68]. Alsoufi et al. reported the outcome after BCPC for a number of single-ventricle malformations, including HLHS. Survival rates were analyzed and stratified by underlying condition, showing the lowest 5-year survival (63%) in the HLHS group. In addition to previously mentioned risk factors, AV-valve regurgitation and lower weight, the authors pointed out a significantly higher risk of death in the group of single-ventricle patients with preoperative pulmonary vascular resistance index above 3 WU/m^2 [69].

Outcome after stage III surgery

Early experience using the Fontan procedure in HLHS patients was reported by Farrell in 1992 to have comparable survival rates as patients with other complex cardiac lesions [70]. More recent studies report a higher risk of major events in the HLHS population after Fontan completion compared to patients with other underlying conditions. In a multicenter study, including centers in North America, the UK and Australia, the outcome of patients with HLHS reaching adulthood after Fontan palliation was reported. Five hundred forty-three patients underwent the Norwood procedure before 1996 with a total pre-Fontan mortality of 71% (n=383). Post-Fontan mortality was 5%. Fifty-nine patients reached adulthood (≥ 18 years), of which 60% were in functional class I (NYHA I). The baseline aerobic capacity was < 85% of the prediction in 98% of the patients. There was a high prevalence of major events reported over the first years of follow-up in adulthood. This observation showed that the cohort of young adults with HLHS differed from other patients with Fontan circulation, where the complications were common over decades rather than within a few years of adulthood. The number of patients in the study was considered too small to evaluate specific risk factors of events [71].

SURVIVAL AFTER NORWOOD SURGERY

The overall survival after Norwood surgery has been reported by several authors. Table 2 shows a summary of recent reports.

Table 2. *The results of earlier studies reporting survival. The included studies in the table are others than mentioned in the above sub-chapters. The measurements of survival were not uniform and are explained for each study in the "Survival" column. N represents the number of patients included in each study.* [1] *The survival probability was estimated from graphically depicted K-M curves. [72-78].*

Author	***Year***	***Study period***	***Geographical area***	***Inclusion criteria***	***N***	***Survival***
Graham	2010	2000–2005	South Carolina, US	Norwood procedure (HLHS and non-HLHS)	76	63–78% cumulative survival at 6 years
Rychik	2010	2004–2009	Philadelphia, US	Infant born with HLHS, standard and high risk, prenatal diagnosis	185	Overall Norwood operative survival of 83.8%.
Menon	2012	1995–2010	The state of Utah, US	Infant born with HLHS	245	33% transplantation-free survival rate at 14 years
Hansen	2012	1996–2010	Kiel, Germany	Norwood procedure HLHS	212	68% transplantation-free survival probability at 10 years[1]
Newburger	2014	2005–	Multicenter, US	Norwood procedure (HLHS and non-HLHS)	342	60–64% transplantation-free survival at 5-years
Beroukhim	2015	1995–2008	Boston, US	Infant born with HLHS, standard risk, prenatal diagnosis	150	72% transplantation-free survival probability at 10 years[1]
Orr	2015	2006–2011	New South Wales, Australia	Norwood procedure HLHS	30	67% overall survival at 12 months

ETHICAL CONSIDERATIONS

Intention-to-treat, comfort care or termination of pregnancy (ToP)

The management of fetuses and neonates with HLHS is a medical, surgical and ethical challenge. In the past, when there was no treatment available, mortality was 100% in the neonatal period. The introduction of Norwood surgery changed this perspective, and today survival probability is approximately 75% at 10 years in our country. Despite the improved survival there is still a considerable risk for death and there is co-morbidity and decreased aerobic capacity. The neurocognitive development is a raising concern [79]. These factors together are challenging and they are likely the main reasons why parents chose ToP after prenatal diagnosis or may refrain from treatment of liveborn neonates when ToP is not an option. The decision to terminate a pregnancy or to refrain from active treatment is obviously emotionally and ethically extremely stressful. The psychological consequences of ToP for fetal abnormalities are well described [80-82].

The dilemmas faced in the decision-making process after a fetal or postnatal diagnosis of HLHS are medical, legal, financial, socioeconomic, personal, and ethical. The parents of a fetus or neonate with HLHS have to consider the potential suffering their child may endure over a lifetime, while at the same time considering that survival is superior to certain death [83]. The term "comfort care" has been used to describe the care of a neonate for whom a decision has been made to refrain from surgery.

The choice to opt for ToP, comfort care or active treatment should be the parents' alone, which is possible only after objective and detailed information given by initiated health care professionals. In some countries, legal jurisdiction limits the options, while medical or financial limitations are present in others. The awareness that the underlying condition of HLHS is severe and the treatment is extensive and expensive and carries high risks and also is a burden on the family is acknowledged. Societies where the prenatal detection rate and/or the termination rate is low, tend to favor comfort care as an alternative option to active treatment. There is recent data from Chicago (US) showing that one-third of infants with HLHS did not undergo surgical intervention [84]. A study from South Carolina, US reports that even though most professional caregivers would favor surgery, they believe that parents should have the option to choose comfort care [85]. The option to terminate the pregnancy is well accepted in many countries while it is illegal in others.

The ethical issues are similar for ToP and comfort care, except that comfort care means exposing the pregnant woman for the additional risk of delivering at full term.

FETAL AORTIC STENOSIS

DEFINITION

Aortic stenosis (AS) in fetal life is an echocardiographic diagnosis based on the morphology and function of the aortic valve. It is commonly seen in conjunction with additional left-sided lesions such as mitral valve anomalies and coarctation. In the presence of fetal AS, the left ventricle can be hypoplastic, of normal size, or dilated. The left ventricular function can be normal or decreased. There can be pathological changes to the myocardium and endocardium of the left ventricle known as endocardial fibroelastosis (EFE).

INCIDENCE

The Baltimore–Washington Infant Study reported aortic stenosis as accounting for 2.9% of all cases of congenital heart defects in liveborn neonates (1981–1989). The incidence of aortic valve stenosis was 12.8 per 100,000 when diagnosed up to one year of age in a study by McBride [37]. The incidence of fetal aortic stenosis is not known and relies on a prenatal detection rate that is low for this condition [86]. In a retrospective report from London, 31 cases were identified during a 10-year period including 19,006 exams of pregnant women referred for specialized fetal echocardiography [87]. That would result in approximately three patients per year if the institution handles 1,900 yearly fetal echocardiograms on a tertiary level, (Gothenburg, 400 per year).

NATURAL HISTORY

Due to the low prenatal detection rate, previous reports of natural history in fetal AS involve few cases. In 1997, Simpson and Sharland reported 27 consecutive cases. Gestational age at diagnosis was 18–35 weeks (median 22 weeks). All except 2 had depressed left ventricular function at diagnosis. Left ventricular end-diastolic volume, function, and the aortic root diameter were monitored during pregnancy. Out of 9 cases in continuing pregnancies without fetal intervention (one case had a fetal intervention), 6 had a biventricular (BV) and 3 a univentricular (UV) outcome. At follow-up, 4 patients with BV outcome and one with UV circulation were alive. The authors observed that there frequently was a failure of growth of the left ventricle in the fetuses with

prenatally detected aortic stenosis. The predictive factors of UV outcome suggested were LV end-diastolic volume, ejection fraction and aortic root size [88]. The prenatal detection rate of critical AS in neonates that survive to hospital discharge with BV circulation is 5–9% while the prenatal detection rate for HLHS at the same institution is 60–100% [86]. Unlike other cardiac malformations, there has been no improvement in prenatal detection rate over time. In a report by Freud, 117 neonates with critical AS and BV outcome were studied. Ten had a prenatal diagnosis, 5 of them were diagnosed in mid-gestation (5 were detected later) and had trivial to mild flow acceleration across the aortic valve, which was thickened and/or dysplastic and there was no other sign of depressed LV function. Left ventricular dysfunction developed by a median of 35 gestational weeks (28–35 weeks) in these patients, when signs of left-sided elevated filling pressures also became evident. There was a downward trend in z-score measurements of the aortic valve and mitral valve during pregnancy; however, it was not statistically significant [86].

PATHOPHYSIOLOGY

A significant obstruction of the aortic valve alters the development of the left ventricle. In the presence of an increased end-systolic pressure, a mature myocardium becomes hypertrophied and by doing so the contractile force increases while the wall stress decreases according to Laplace law [89-91].

Laplace law ; $wall\ stress = \frac{P \times r}{2 \times wall\ thickness}$

P=*Pressure*, r= *radius*

The fetal myocardium is programmed for cell hyperplasia rather than hypertrophy and has poor mechanisms to compensate for increased pressure load [92]. In cases with preserved diastolic function, the filling of the left ventricle is normal. The result in cases with obstruction of the aortic valve with preserved diastolic function is usually dilatation of the left ventricle. When the ventricle dilates, the afterload increases even more and the ventricle cannot preserve its systolic or diastolic function. A vicious circle can begin with ischemia, development of EFE, diastolic dysfunction, and halted growth of the left ventricle. Not all cases of AS in fetal life progress to HLHS. The left ventricle can handle mild to moderate obstruction, and most cases of critical aortic stenosis at birth will go unnoticed during fetal life [86].

HEMODYNAMICS

When there is increased pressure in the ventricle, atrial pressure rises as well. The normal fetal shunting from right to left across the interatrial septum gradually decreases, and in severe cases the flow direction through the foramen ovale (FO) changes to bidirectional or even left-to-right. The decreased filling of the left ventricle in combination with the obstruction of the aortic valve will result in decreased stroke volumes. The cardiac output produced by the left side of the fetal heart will not sufficiently supply the head and neck vessels and their end organs with adequate pressure and flow. In this situation, there will be compensatory flow supported by the right side of the heart through retrograde flow in the aortic arch from the arterial duct [93]. In unfortunate cases, the FO closes during pregnancy. Pulmonary venous return depends on low left atrial pressure during ventricular systole and early diastole. With restriction at the atrial level the flow pattern in pulmonary veins becomes reversed during atrial systole, A-wave reversal. In severe cases bidirectional flow develops, also known to as "to-and fro flow". High pressure in the pulmonary veins is a risk factor for developing lymphangiectasia of the fetal lungs [94, 95]. This condition is associated with postnatal pulmonary hypertension and high mortality [96].

Hydrops

Hydrops is a complex fetal process with several different causes. When hydrops is present in cases of congenital heart malformation, the reason is increased pressure in the right atrium, usually due to severe tricuspid regurgitation. Hydrops is rare in conjunction with left-sided obstructive lesions, but can be seen when the right ventricle is unable to handle the increased volume and pressure load [97].

FETAL VALVULOPLASTY

The rationale behind a fetal cardiac intervention on a stenotic aortic valve (FV), is to relieve the left ventricular outflow tract obstruction to enable continued growth of the left-sided cardiac structures in order to aim for a biventricular (BV) circulation at birth. A second indication is to enhance the chances of fetal survival when there are signs of cardiac failure (hydrops) in combination with left-sided obstructions with or without a restrictive atrial communication. The optimal timing of an intervention when the aim is a BV circulation is not

known, but it is likely that an early intervention is preferred to achieve the best effect. The technical aspects of intervening in small hearts and the fact that many cases are detected late in midgestation limits the possibilities of intervening earlier than at about 20 weeks. Most interventions are performed at midgestation between 24-26 gestational weeks (range 19-34 weeks) [98-100]. How to perform a fetal cardiac intervention includes fetal as well as maternal considerations. In most centers, the team performing the fetal cardiac intervention includes maternal and fetal medicine specialists, fetal cardiologists, and cardiac interventionists. Detailed procedural and technical information on how a fetal cardiac intervention is performed has been published [100-108]. The reports include information on maternal and fetal preparations, resuscitation drugs, techniques for reaching and dilating the aortic valve, needle and balloon sizes, and fetal and maternal complications.

Procedural information

The fetal heart can be reached by a small laparotomy in the maternal abdomen or percutaneously, using a needle to traverse the different maternal and fetal layers. In the current era, a percutaneous approach is used [101, 109, 110]. Essential to gain access to the fetus is a favorable fetal position, which can be attained through positioning of the mother or by gentle manipulation of the fetus [101]. The direction of the cannula through the different layers of the maternal abdomen and the fetus is guided by ultrasound. The cannula is directed towards the fetal aortic valve, and when in position, the cardiac interventionist inserts a guidewire with a balloon. The balloon is inflated across the aortic valve, and after repeated inflations the cannula, the guidewire, and the balloon are removed from the patient [100, 101]. Technical success is achieved when the aortic valve is crossed, the balloon is inflated, and color Doppler ultrasonography show increased flow across the aortic valve and/or new aortic regurgitation [100]. Fetal complications are common, most complications occur during or shortly after the intervention. Cases of fetal demise in close relation to the intervention has been reported [98, 100, 101]. Major maternal adverse events are rare [104]. The women are mostly discharged from the hospital the same day, or when general anesthesia is given they stay overnight [101] [104]. The fetal cardiac function is assessed by ultrasound the day after the procedure. Initiation of digoxin therapy after successful intrauterine valvuloplasties is practiced at some institutions [100] [104] .

CHANGES IN PATHOPHYSIOLOGY AND HEMODYNAMICS FOLLOWING FETAL VALVULOPLASTY

The aim of performing a fetal intervention with balloon dilation (valvuloplasty) of the aortic valve is to relieve the obstruction at the aortic valve level, thereby restoring normal pressure and flow through the left ventricle. Previous reports show that hemodynamics and growth can be restored after an intervention but there are no observations so far suggesting that there will be a catch-up of left heart growth [98, 111, 112]. Changes in hemodynamics can be followed with fetal echocardiography, a method that will be discussed more in the following section.

FETAL ECHOCARDIOGRAPHY AS PREDICTOR OF OUTCOME

Association between fetal echocardiographic measurements and outcome has been described for a number of fetal cardiac conditions [113-115] including fetal AS [98, 116]. Mäkikallio et al reported that UV outcome in fetal AS with normal sized left ventricle could be predicted before 30 weeks of gestation by combining functional data of depressed left ventricular function, retrograde flow in the transverse aortic arch, left-to-right flow at the atrial level and monophasic mitral Doppler inflow pattern [116].

The described hemodynamic changes associated with UV outcome were tested by Hunter et al [87]. Their result was comparable with Mäkikallio, except for FO flow left to right that showed poor sensitivity.

The potential for BV circulation and the potential for a technically successful intervention in hearts fulfilling criteria for UV outcome is given by the size of the left ventricle and signs of some preserved function measured by pressure gradients across the aortic forward- or mitral backward flow [98].

The previous and current selection criteria for fetal aortic valvuloplasty as practiced in Boston include three main criteria. The first is a dominant cardiac anomaly of valvar AS, the second is criteria for evolving HLHS [116] and the third is potential for a technically successful procedure and BV outcome postnatally [98]. The criteria for evolving HLHS was modified compared to Mäkikallio by adding bidirectional flow in pulmonary veins.

The “early” or “previous criteria” used to select cases with BV potential were LV long axis z-score ≥ minus 2, depressed LV function but generating at least a 10 mmHg pressure gradient across the aortic valve or 15 mmHg mitral

regurgitation (MR) jet gradient and a mitral valve z-score > minus 3. The criteria were modified based on the findings that interventions were performed in too small hearts with little potential of BV outcome.

The modified criteria stated that the diagnosis should be unequivocal AS (versus AA), LV long axis z-score > minus 2 and a threshold score ≥ 4. The threshold score was designated to give one point for each of five variables; LV long axis z-score > 0, LV short axis z-score > 0, aortic annulus z-score > minus 3.5, MV annulus z-score > minus 2 and a pressure gradient of ≥ 20 mmHg, MR or AS.

The outcome before and after modification of criteria showed an increased number of cases with BV circulation after implementation of the revised criteria [8].

The experience from Linz presented by Arzt et al. [100] was in agreement with the results from Boston. The successful cases had a mean LV long-axis z-score well above zero. The early results from Linz resulted in a modification of their criteria as well. The criteria for fetal AV valvuloplasty after 2014 in Linz are fetal AS as the dominant lesion with retrograde flow in the aortic arch, left-to right shunt across the FO, LV long-axis z-score > minus 2 and a LV:RV ratio > 0.8 [100].

REPEATABILITY (INTRA-OBSERVER VARIABILITY) AND REPRODUCIBILITY (INTER-OBSERVER VARIABILITY) OF FETAL ECHOCARDIOGRAPHIC MEASUREMENTS

Echocardiography is a commonly available tool using ultrasound and the Doppler effect to perform a noninvasive test in patients with known or suspected cardiac disease. The method provides evaluation of cardiac structure, function and hemodynamics in fetuses, neonates, children and adults. Two-dimensional echocardiography is the foundation of an echocardiographic exam demonstrating cardiac structure and function. Motion of cardiac structures can also be displayed with high frame rate in a distance- time graph with high temporal resolution; M-mode. Doppler colour flow imaging visualizes blood flow direction and relative velocity. Spectral Doppler displays the blood flow measurements graphically, showing flow velocities recorded over time.

Fetal echocardiography was introduced for research purposes in the early 1970s using basic M-mode to image fetal cardiac motion. In the 1980s, basic

two-dimensional ultrasound was able to delineate cardiac structures, function, and rhythm [117], creating a use for the method in clinical practice. Today, fetal echocardiography has a high degree of accuracy in diagnosing fetal cardiac malformations and arrhythmias in the hands of specialized fetal cardiologists and obstetricians [118]. Although well developed in clinical practice, little is reported on the repeatability and reproducibility of fetal echocardiographic results in research. In 2002, Simpson et al. reported repeatability of echocardiographic measurements in the human fetus. Thirty-two different variables, including cross-sectional, M-mode, and spectral Doppler, were measured from videotape by two independent observers in 10 normal fetuses at 23 (17–34) weeks. They concluded that the repeatability of most echocardiographic measurements in the fetus was poor. Inter-observer errors were consistently higher than intra-observer errors. Cross-sectional values and some of the spectral Doppler variables, specifically max velocity, performed better than M-mode measurements. The influence of small structures of the fetal heart was a source of potential errors. Color Doppler was not evaluated [119]. Studies on the validity of assessing fetal arrhythmias are more frequent [120], often including the correlation between Doppler flow echocardiography and AV time intervals [121].

PATIENTS AND METHODS

STUDY DESIGN

The studies included in this thesis were all considered to be epidemiological studies. Those in Papers II–V were observational, while the study in Paper I was experimental. All studies included an analytical part. Paper IV presented traditional epidemiology describing incidence of disease. The cohorts in Papers II–V were historical or nonconcurrent, for which data were collected retroactively. The study presented in Paper I included an intervention (home monitoring) that was initiated by the investigator. Patients included in Paper I were consecutively assigned to an intervention without a randomization process.

Traditionally, epidemiology was only descriptive and aimed to study the distribution and determinants of health-related events in populations [122].

Modern epidemiology includes descriptive and analytical studies as illustrated in Figure 1. Descriptive epidemiology uses available data to describe how mortality and morbidity rates vary according to the characteristics of a given population (demographic variables). In the field of analytical epidemiology, studies are designed to allow the assessment of hypotheses that associate exposure factors with a health-related outcome [122]. The hypotheses can be assessed either by experimental or observational studies. In an experimental study, the researcher exposes the subject to an intervention in a randomized or non-randomized way.

Observational studies are those in which a correlation between exposure and outcome is observed, but no intervention is introduced by the researcher. Studies that include individuals as observational units are cohort studies, case-control studies, or cross-sectional studies, Figure 2. The cohort study is a longitudinal study also known as “prospective,” which refers to the basic concept of a cohort study—that the exposure is known before the outcome and that the cohort is followed over time.

Data can be collected retroactively in a cohort study and the cohort will then be a “nonconcurrent” or “historical” cohort. The “nonconcurrent” or “historical” cohort is characterized by the investigators beginning the study at the end of the follow-up time. The main advantage of a concurrent cohort, or “truly prospective” study compared to a nonconcurrent study is that the

baseline exam, follow-up methods, and ascertainment of events are planned and implemented for the purpose of the study [122].

Cohort studies are considered “the gold standard” of observational studies, while case-control and cross-sectional studies are justified primarily by the logistic ease of performing them.

The epidemiological study design with the highest grade of evidence is the randomized controlled trial, a study design with the potential to reduce confounding and bias when performed under strictly controlled conditions.

Figure 1. *Epidemiological studies can be experimental or observational. Experimental studies can include a randomized or a non-randomized assignment process. When an observational study includes an assessment of a hypothesis, for example a comparison between groups, the study is analytical.*

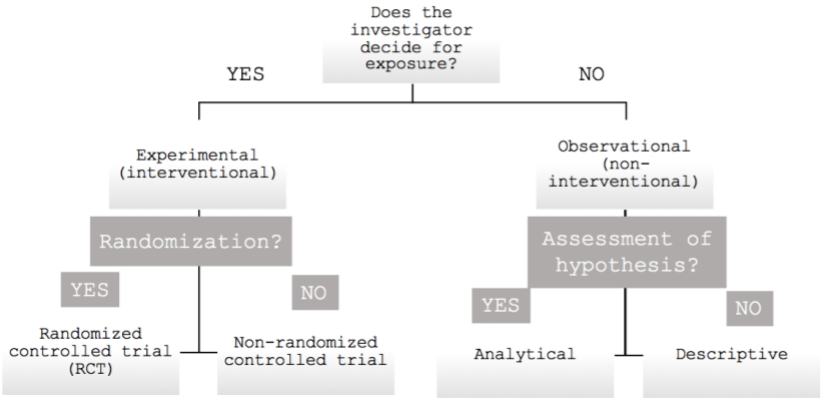
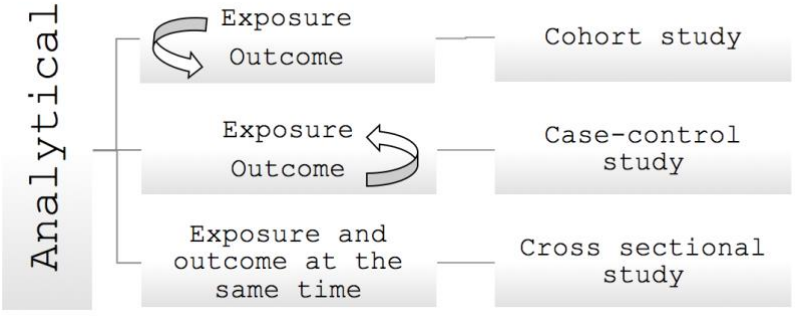


Figure 2. *The analytical approach can be of a prospective nature, when exposure is known before the outcome (cohort study); of retrospective nature, when the outcome is known and exposure is investigated (case-control study); or a cross-sectional study where exposure and outcome are investigated at the same time.*



STUDY POPULATIONS, EXPOSURES AND OUTCOMES

The main components in an epidemiological study are the patients, the exposure variables, and the outcome. The correlation between exposure and outcome is observed and the difference between groups with different exposure can be analyzed. Subjects included in a study population or a cohort should share some health-related characteristics and be included at the same stage in a possible progression towards a health-related outcome. In a cohort study the exposure variables are factors present in the study population at inclusion. The outcome is the result of the exposure on the included subjects over time. Outcomes can be death, morbidity, change in blood pressure, or any other well-defined entity.

Table A in the Appendix of this thesis shows the patient populations, cohorts, and subgroups of cohorts for all studies. Table B shows outcome, exposure and possible confounding and interactive factors in relation to the correlation between exposure and outcome for the cohorts studied in Papers II-III. Table C shows the outcome, exposure, possible confounders, and potential modifiable factors for the cohorts studied in Papers IV and V.

The study subjects in Paper I were patients with single-ventricle physiology and a shunt as the only source of pulmonary blood flow. They were included at the same stage (discharge from hospital after stage I) in a possible progression towards a health-related outcome (survival to stage II surgery). The exposure factor was home monitoring of saturation. Inclusion of study subjects was initiated by the physician on the ward before discharge after informed consent from the parents. The outcomes were queried retrospectively in medical records and compared to a historical cohort without home monitoring.

The patients included in the cohort in Paper II were identified by fetal cardiologists in Europe. The inclusion criterion was fetal diagnosis of aortic stenosis. The studied outcomes reported by the submitting fetal cardiologists were pregnancy outcome, postnatal outcome, and outcome after first intervention. Pregnancy outcomes were ToP, spontaneous intrauterine death (sIUD), and live birth. The postnatal outcomes were intervention (surgery or catheter) or no intervention. Overall survival was estimated for all liveborn with intention-to-treat. For patients who underwent an intervention, 30-day mortality was observed. Changes in morphology and physiology during pregnancy were analyzed and related to the outcomes.

The patients constituting the cohorts in Paper III were identified in the same way as in Paper II, with the addition of a group that underwent fetal valvuloplasty (FV).

The cohort in Paper IV consisted of all liveborn neonates with HLHS/AA born in Sweden from 1990–2010, identified through merged searches of national databases and local surgical and fetal databases. The subjects were included at birth and followed until death or first surgery. The outcome, surgery versus no surgery, was analyzed in relation to exposure factors. A hypothetical cohort consisting of all fetuses with HLHS/AA was also included. The cohort was identified by estimating the fetal incidence from the incidence of liveborn neonates with HLHS/AA before prenatal detection- and termination rate was significant. The outcomes of the hypothetical fetal cohort were ToP, live birth and surgery and live birth and no surgery.

The same cohort as identified in Paper IV was studied in Paper V, but limited to cases that underwent surgery in 1993–2010. The outcome transplantation-free survival was analyzed in relation to multiple variables (exposure factors).

CONDUCTING RESEARCH USING NATIONAL REGISTER DATA

The great strength of conducting research based on national register data is the completeness of the data. Reporting is compulsory and few patients' data are lacking or get lost, so the data are easy to follow up. The basis of research using Swedish national databases is the Swedish personal identity number, which is maintained by the Swedish Tax Agency for all individuals who have resided in Sweden since 1947. The most important use of the identity number in healthcare is to trace patients and their medical records. The number (YY-MM-DD-ABCD) is unique for each individual and is a marker of age and gender [123]. In surveillance of healthcare (national databases), the number is used for vital statistics (date of birth, date of death), but it is also the identifier and the key variable when matching between different registers. The main register holder for national databases regarding healthcare is the National Board of Health and Welfare, while Statistics Sweden keeps track of the number of inhabitants. The surveillance of congenital malformations and chromosomal abnormalities in relation to ante-and perinatal factors was put in place after the Neurosedyne disaster [124]. The "Registry for surveillance of congenital malformations and chromosomal abnormalities" contains one part on maternal

and neonatal data (The Swedish Medical Birth Registry, MBR) and one part reporting congenital malformations and chromosomal abnormalities in fetuses where the pregnancy is aborted. In the latter, there are no personal identity numbers registered. The coverage was low in the "fetal" part of the registry during the study period covered in paper IV and V according to the registry holder and was therefore omitted for data collection (personal reference, The National Board of Health and Welfare, September 2012). A description of each register used as source of information for Paper IV and V follows below.

- The Swedish Medical Birth Register (MBR) was established in 1973, with the purpose of compiling information on ante- and perinatal factors and their importance for the health of the mother and child. The register includes data on practically all deliveries in Sweden. It is compulsory for every healthcare provider to report to the register, and the information in it is collected from medical records from prenatal, delivery, and neonatal care. A content and quality summary is found on the website.[125]
- The Swedish Cause of Death Register (CDS) is a complete register of all deaths in Sweden since 1952, listing cause and time of death. Cause of death is registered according to the diagnosis represented by the international version of ICD codes. A detailed description of CDS was recently published [126].
- The Swedish National Inpatient Register (IPR) was established in 1964. The register has complete national coverage since 1987. Currently, more than 99% of all somatic and psychiatric hospital discharges are registered. It is mandatory for all hospitals to deliver data to the IPR. Variables are divided into patient and medical data, data about the caregiver, and administrative data. An external review and validation of the Swedish National Inpatient Register was published in 2011 [127].

The Swedish Database of Forensic Medicine, Rattsbase, is a register of unnatural deaths that occur outside of hospitals. Rattsbase includes the diagnosis and brief information about clinical details and circumstances of death in free text [128]. The Swedish Registry of Congenital Heart Disease, SWEDCON, started in 2009 with the aim of monitoring patients with congenital heart disease. It includes information on diagnosis, if there was a prenatal diagnosis, performed surgeries, catheter interventions, and inpatient care. All of Sweden's vital statistics are maintained by Statistics Sweden and updated daily with new births, migrations, deaths, and changes in marital status.

ETHICAL CONSIDERATIONS IN RESEARCH STUDIES

There were several ethical considerations regarding the studies included in this thesis.

In Paper I, infants were included as subjects in an experimental study. Informed consent was given by the legal caregivers. Lack of consent by the caregivers excluded the patient. The risks of participation in the study were potentially related to the increased burden of stress on the guardians and the risk of neglecting signs and symptoms of disease if the home monitoring was normal. The questionnaire that was sent out by mail could potentially breach confidentiality. The ethical considerations above were described in an ethical application and the study was approved by the Regional ethical review board in Gothenburg.

The observational cohort studies of patients with HLHS/AA in Papers IV and V included data from several databases, and information from different sources were merged. The main risk ethical concern was loss of privacy. Precautions were taken to protect the subjects with a negligible risk of identifying individual patients. The personal identity number was known to the researchers only for patients who underwent the Norwood procedure; the remaining patients were totally anonymous. Information about the study was announced in the journal *Hjärtebarnet*, which reaches the majority of parents of children with congenital heart disease. Ethical permission to identify the cohort and to investigate exposure and outcome in the study population was applied for and granted by the Regional ethical review board in Gothenburg.

The two studies on fetal aortic stenosis were conducted as multi-center studies in Europe with the principle investigator situated in London, UK. The

potential risk was mainly connected to loss of privacy, but since no patient identification data were submitted to the study, this risk was considered to be very low. The ethics committee at the institution with which the principal investigator was affiliated advised that the study was an audit rather than research, so no ethical permission was needed.

In epidemiological research studies population-based outcomes and consequences of health problems are studied. The same basic ethical principles apply to epidemiologic research as to all research in medicine and are regulated in the Declaration of Helsinki [129]. The general principles of the declaration bind the physician with the words "the health of my patient will be my first consideration" and "a physician shall act in the patient's best interest when providing medical care". The declaration regulates how to apply this in medical research through a number of principles listed below. The list is a shortened version of the complete declaration found on the World Medical Association website citing the ethical principles for medical research involving human subjects [130].

1. Medical research involving human subjects may only be conducted if the benefit outweighs the risks (beneficence and non-maleficence).
2. All vulnerable groups and individuals should receive specifically considered protection.
3. The design and performance of each research study involving human subjects must be clearly described and justified in a research protocol.
4. The research protocol must be submitted to a research ethics committee for consideration, comment, guidance, and approval before study begins.
5. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information.
6. In medical research involving human subjects capable of giving informed consent, each potential subject must be adequately informed and thereafter give informed consent to participation. Special rules apply for incapable subjects and for studies using human material or data contained in biobanks.
7. Researchers have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports.

The risk to the individual is potentially lower in an observational study compared to an experimental study. In an observational study, the main risk is the loss of privacy, while an experimental study poses potential medical risks for the individual. The risk of loss of privacy in observational studies is higher when data are collected and merged from more than one source. The risk should be estimated and balanced against the benefit of the research [131]. Experimental research is not permitted without informed and written consent by the study participants. Special rules apply when informed consent is impossible or difficult to obtain as it is in research involving children or fetuses. In the case of a child under 15 years of age, the legal guardian(s) can give informed consent. The child has the right to be informed according to their developmental stage and age.

Research involving fetuses needs special consideration, since the subject itself has no voice. From a legal perspective, the pregnant woman can give informed consent. The lack of direct consent from children and fetuses should not preclude them from being subjects in research when results cannot be extrapolated from other age groups. As regards observational studies, researchers have discussed the risk of selection bias when informed consent is warranted [132].

The general rule of informed consent in the Declaration of Helsinki has been modified to facilitate observational studies using existing data [133]. The Swedish Medical Ethics Board states that studies using information from existing databases (registries) or medical records normally do not require informed consent [134]. Information to involved individuals and/or populations about ongoing research projects should be given collectively through media, healthcare information sites, or at healthcare institutions.

For projects involving merged searches and data related to individuals, ethical approval is necessary. The recommendations are that informed consent is not required as long as the privacy of each research subject is fully protected and the results are reported by group and not on the individual level. Ludvigsson discussed the need for special precautions in vulnerable groups; he concluded that almost all registry-based research will involve vulnerable individuals and found existing regulations sufficient to protect these subjects as well as those less vulnerable [131]. As a conclusion, our studies satisfied the ethical principles discussed here.

STATISTICAL METHODS

STATISTICAL MEASUREMENTS

The included studies applied a wide range of statistical methods used to describe similarities or differences between groups, to describe and analyze correlation between exposure and outcome, and to describe and analyze incidence.

In testing hypotheses regarding differences or similarities between two groups, the Chi-squared or Fisher's exact tests were used for dichotomous categorical data, the Mantel-Haenszel Chi-squared test for ordered categorical data, and the Mann Whitney U-test for continuous variables. All tests were two-tailed and conducted at an 0.05 significance level. Different regression models were used to measure correlations between exposures and outcomes. Linear regression analysis was used for continuous variables and logistic regression for continuous and categorical variables. Survival analyses were conducted using the Cox proportional hazard regression analysis, taking time-to-event into account and presented as hazard ratios (HR). Survival probability was presented graphically using Kaplan-Meier curves.

The result of the evaluation of the correlation between exposure and risk was presented as "risk" or the probability of an event on the cohort level. For example, the risk of an event was higher for female neonates in the cohort of neonates with HLHS/AA compared to male neonates at HR 2.25 (95% CI 1.35–3.7), Paper V, Table 4. The cohort-level risk gives an indication of the risk to the individual, but is in many situations too general to have a predictive value in the individual case. The more risk factors identified, the more individualized information can be given. The statistical model in Paper V, Figure 6 is a *predictive model* showing the results of a multivariable Cox-regression analysis including several exposure variables found to be important for outcome.

Criteria for fetal intervention are likewise predictive models including more than one risk factor with the aim of predicting the outcome (intervention or no intervention) for the individual.

The validity and reliability of predictive models can be tested for the population using the measurements sensitivity and specificity, and on the individual level as negative and positive predictive values. In this thesis, criteria for fetal intervention were evaluated comparing proportions, but not measuring sensitivity or specificity. The validity and reliability of home

monitoring as a method to predict shunt complications were evaluated in Paper I.

MEASUREMENTS OF INCIDENCE

The incidence of HLHS/AA in Sweden was calculated by dividing the number of liveborn neonates with the disease by the number of neonates born in the country each year. The incidence rate ratio (IRR) was the measurement used to describe the change in incidence over time. The crude IRR was estimated using the Joinpoint Regression Program, a statistical modeling technique that explains the relationship between two variables by means of a segmented linear regression [135]. Adjusted IRR was estimated from a generalized linear equation (GEE) model with Poisson distribution and log-link function.

REDUCING BIAS AND CONFOUNDING IN OBSERVATIONAL STUDIES USING PROPENSITY SCORE

Confounding is the term used when one or more factors influence the exposure and the outcome, making it impossible to investigate the correlation between exposure and outcome without modifications. The term *interaction* is used to describe situations in which two or more risk factors modify each other's effect with regard to outcome. *Bias* can be defined as a systematic error in the design of a study, in the form of selection bias, information bias, or a combination of both. Exposure factors, outcome, and potential interactions, and confounders present in Paper I–V are listed in Appendix Table B and C. When comparing treatment effects, an experimental randomized controlled trial (RCT) is the "gold standard". The RCT study design is superior in handling confounding, but is sometimes difficult or even unethical to perform. Propensity score (PS) is a statistical method used to handle confounding in an observational study [136-138]. The method transforms the observational study into an imaginary RCT using data from the observational study. The basic concept of using PS is "if". The statistical method gives an imaginary experimental study design to allow us to answer the following question:

- *If* cases were similar and *if* they were selected to treatment versus no treatment by chance what would the treatment effect be?

In a randomized controlled trial, the probability of receiving treatment is ½ (0.5). Confounders will randomly be distributed between treated and non-treated cases and confounders will be equally distributed. The outcomes can be evaluated with the knowledge that confounding factors are equally present in both groups they will not be the explanation for the observed outcome. In an observational study, confounders are rarely equally distributed. The assignment to treatment versus no treatment is not random which can disturb the evaluation of the causal effect aimed to study. This bias can be adjusted for in the imaginary model by calculating a score of the probability of being treated for each case. This is the propensity score (PS), which is calculated by using logistic regression models. In these models, the predictors of assignment to treatment are the selected variables and the outcome is treatment or no treatment. This will give each case a probability score of receiving treatment. Through this method, confounders will be "balanced," allowing the causal effect of treatment to be investigated. The propensity score is then used to match and analyze outcome after treatment versus no treatment (the average treatment effect). The hypothesis being statistically tested here is the null-hypothesis, that there is no difference in outcome after treatment compared to no- treatment. When using PS, an assumption is made, called the "Stable Unit Treatment Value Assumption (SUTVA)," that patients in a study should not interfere with each other and there should be no variation in treatment.

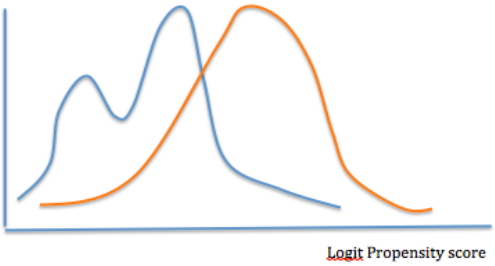


Figure 5. *The distribution of logit propensity score (0–1) for treated cases (orange) and not-treated cases (blue). The probability for treated cases of being treated is normally higher than for the untreated group and they tend to have higher logit propensity scores.*

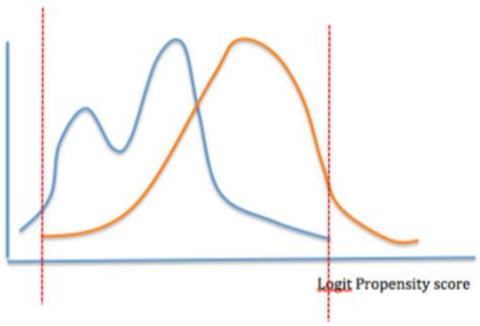


Figure 6. *Cases with propensity scores within the overlapping range can be included in the analysis. When cases without overlap are deleted, propensity scores can be recalculated, a trimming process that can be iterated several times to improve balance. The balance of confounders within the selected cases is checked using the standardized mean difference. To improve the balance in the data and achieve a "better" standardized mean difference that is as close to zero as possible, you can apply inverse probability of treatment weighting (IPTW). This statistical method will "boost" the controls (if they are few) and make them stronger in comparison to the cases.*

In conclusion, the use of propensity score as statistical method in Paper III allowed us to reduce bias when comparing the natural history cohort and the FV cohort.

RESULTS

The main results for each paper are listed below.

I. There was a possible benefit from home monitoring of oxygen saturation on the individual level. There was no observed difference when the result was compared with a historical cohort. The method was well-tolerated by parents.

II. The cohort of subjects with fetal AS demonstrated a spectrum of disease from mild to evolving HLHS. Cases that evolved into HLHS had a statistically significant lower z-score for the mitral valve diameter at the initial exam and a decreased growth velocity of the left ventricle and mitral valve during pregnancy. There was no significant correlation between physiological measurements and outcome. Cases fulfilling criteria for UV outcome had BV outcome in 33% of the cases while cases fulfilling criteria for UV outcome and BV potential had a BV outcome in 42 % of the cases.

III. There was improved fetal hemodynamics and preserved left heart growth after FV compared to NH. There was no effect of FV on BV outcome, but survival was improved for propensity score matched cases after FV regardless of circulation.

IV. The average incidence of HLHS/AA was 11.6 per 100,000 liveborn in Sweden. There was an observed yearly decrease in incidence. The total number of patients born during the surgical era was 208, of whom 121 (58%) underwent surgery. The factors correlated to whether or not surgery was performed were birth period, birth location, prenatal diagnosis, and gestational age ≤ 37 weeks.

V. The result showed a transplantation-free survival of 51% in the entire cohort of patients with HLHS/AA who underwent the Norwood procedure in 1993–2010 in Sweden. Survival probability improved between the early and late birth periods and was 63% at 10 years for those born during the late birth period. Female gender was a risk factor for death. For male patients, there was an increased risk of death when there was a low birth weight in relation to gestational age.

SURVIVAL PAPERS I - V

Survival was the outcome in all the included papers. The short-term survival after first intervention (catheter or surgical) was investigated and described as 30-day mortality or interstage mortality. The definition of interstage mortality was death after discharge from hospital, Paper I, and alternatively referred to as the time period following after 30 days after intervention before next surgical stage in Paper V. The different definitions used were due to the aims of the studies and the quality of the data. The date of discharge from hospital was less reliable for the HLHS/AA cohort, while 30 days after surgery was certain. A comparison of survival between the cohorts in Paper I-V is presented in Table 3.

Table 3. *A comparison of survival of the cohorts presented in Paper I-V.*

Inclusion criteria	***SV and shunt***	***FAS NH***		***FAS FV***		***HLHS/AA***
Paper	I	II	II	III	III	V
Circulatory outcome	UV	UV	BV	UV	BV	UV
Number of cases	28	29	44	12	19	61[2]
30-day mortality (n)(%)	.	8 (28%)	3 (7%)	.	.	7 (11%)
Interstage mortality (n)(%)	4 (14%)	1 of 29	.	.	.	5 (8%)
1-year survival	.	57%[3]	78%[3]	.	.	75%[3]
5-year survival	.	55%[3]	76%[3]	80%[1,3]	70%[1,3]	67%[3]

Abbreviations*: SV = single ventricle; FAS = fetal aortic stenosis; NH = natural history; FV = fetal valvuloplasty; HLHS = hypoplastic left heart syndrome; UV = univentricular circulation; BV = biventricular circulation.*
Indices: *[1] Survival for liveborn neonates. There was a 10% procedure-related loss in the fetal intervention groups, not included. [2] Patients born in 2001–2010. [3]Kaplan-Meier analysis of transplantation-free survival probability.*

DISCUSSION

The aim of the first study was to evaluate the possible effects of home monitoring of oxygen saturation on survival. There were individuals with a possible benefit from home monitoring, but no effect was observed on the cohort level. The clinical experience was optimistic and there was a general agreement to continue with home monitoring of oxygen saturation after the study ended. An observed increase of shunt complications was the reason to initiate the study and even though the effect could not be statistically proven the experience from a clinical point of view was that interstage deaths due to shunt complications decreased. Daily weight, which in previous reports has been reported as an important factor to improve survival [63, 139] has so far not been included which seem to be due to negative experience reported by parents.

A statistical limitation of the study in Paper I was the small sized study population. We calculated that a study to show a 50 % decrease of mortality should take 15 years to complete at a single medium-sized institution like ours. In a country like Sweden, with a population of 10 million inhabitants and two surgical centers, studies like the one presented in Paper I have the risk of being under powered. There might be a difference but the number of patients is too small to reach statistical significance. The study designs in Paper II-V aimed to improve the statistical strength by adding cases from multiple centers as in Paper II and III or to use register data for complete national coverage with high validity data as in Paper IV and V.

The advantage of multicenter studies in collecting a substantial number of cases in fetal cardiology has been discussed [140]. The aim of the study presented in Paper II was to collect cases of fetal aortic stenosis from multiple centers in Europe and to present outcome in relation to morphology and physiology. The condition is rare, and only a multicenter approach was considered when the study was designed. A major disadvantage of collecting data from multiple institutions is the problem of controlling for data quality, which might vary with the experience of the investigator and the technical equipment used. The most reliable predictive factor for univentricular outcome found in the studied cohort presented in Paper II was the size of the mitral valve, while physiological data were inconclusive. The validity and reliability of fetal echocardiography in research is not well studied and the effect of this possible limitation was difficult to fully appreciate.

The aim of the study in Paper III was to evaluate the efficacy of fetal valvuloplasty (FV) to achieve biventricular (BV) circulation and to improve survival. The result showed that there was no higher proportion of BV in the cohort that underwent FV compared to propensity score matched cases that did not undergo an intervention. The early results of FV presented in the literature report that the first case series included hearts with left ventricles too small for a BV outcome also after an intervention [8, 98, 100]. The initial selection of cases could not be compensated for by the propensity score method. The lack of a treatment effect of FV on BV outcome might have been a result of including cases with too small left ventricles. We observed an effect on hemodynamic properties and the growth of left sided structures of the heart. The finding of improved intrinsic cardiac properties could explain the improved survival after FV compared to propensity score matched NH cases regardless of the final circulatory outcome.

The overall survival for patients with fetal aortic stenosis and BV outcome studied in Paper II and III was lower compared to a cohort of patients with critical aortic stenosis with BV outcome in which the reported survival proportion was 89 % at 10 years [141]. A prenatal diagnosis of fetal aortic stenosis might suggest a more severe form of the disease compared to when the condition goes unnoticed during fetal life. The presence of a prenatal diagnosis was a significant risk factor for death (OR = 20, $p = 0.01$) in a cohort of neonates with critical aortic stenosis in which 14% had a prenatal diagnosis[142]. The proportion with BV outcome that survived to 5-years of age was comparably lower in the group of BV cases that underwent FV compared to cases in the NH cohort studied in Paper II. This was an expected finding since the BV cases undergoing FV should represent a more severe form of the disease. The survival was better after FV compared to NH in propensity score matched cases.

The cohorts in Papers IV and V were identified through merged searches in national databases a search and matching of cases that was done manually. The final result, the complete national cohort of liveborn neonates with HLHS/AA, made it possible to observe the total outcome, not just the outcome for patients who underwent surgery. This approach was motivated because of the high pre-surgical mortality in HLHS/AA. The ICD-coding in national databases made it somewhat challenging to select cases with HLHS/AA rather than HLHS/AS since the ICD-code is HLHS with no further information on specific morphology. The selection of cases with AA

rather than AS was based on additional coding of AS or in cases that underwent surgery information in medical records. Some cases that possibly should have been included might have been excluded and vice versa. Overall, the process of selecting appropriate cases was meticulous and a case was excepted or excluded only if data was congruent in a least two databases.

The incidence of HLHS/AA in Sweden in the first years of the study period was comparable with previously published data for populations without important prenatal detection and termination rates. The decrease in incidence was interpreted as the result of increased prenatal detection and termination rates. This assumption was supported by national reports on prenatal screening and terminations of pregnancy [45, 143, 144]. In Paper IV we introduced a model to estimated fetal incidence in order to show the shift from comfort care practiced to some extent in the early birth period to pregnancy terminations more commonly practiced in the late birth period. The model was an estimate, using the highest incidence of liveborn, and was aimed to demonstrate trends, not to calculate exact numbers. The proportions (not the numbers) were the same if an average of the first three years, or the highest incidence, was used. The highest incidence, 19 per 100,000, best corresponded to a detailed population based study performed at our institution covering the Western part of Sweden. The study included every prenatally and postnatally diagnosed case of HLHS/AA 2003-2010 with a fetal incidence of 20 per 100,000 (personal reference, not published).

The finding that birth location influenced outcome (surgery versus no surgery) was first interpreted as different attitudes towards surgery at surgical centers compared to university hospitals without surgery. However, the effect disappeared when adjusting for prenatal diagnosis, a factor that was positively correlated to surgery versus no surgery in liveborn neonates. A prenatal diagnosis resulted most commonly in intrauterine transfer which explained the higher proportion of patients that underwent surgery of those born at a surgical center.

The overall transplantation-free survival for the 121 patients with HLHS/AA who underwent the Norwood procedure 1993-2010 in Sweden was similar to previous reports with some variations. Earlier studies from two single centers reported the survival probability at 10-years to be 68-72 % for the same calendar period as studied in our report [72, 75]. The highest survival probability was observed in a cohort of standard risk neonates with prenatal diagnosis [72]. In a cohort including patients born before 1996 there was a

transplantation-free survival of 14 % at 18 years, which should reflect the earliest international results of the Norwood procedure for HLHS [71]. In our cohort presented in Paper V, the transplantation-free survival probability increased significantly between the two birth periods. The result observed in the later birth period corresponded to what should be expected as discussed above. The finding constitutes an important benchmark when comparing our national surgical programs with single-center experiences at large international centers.

CONCLUSIONS AND FUTURE PERSPECTIVES

HLHS and fetal AS are severe cardiac malformations with high mortality. Major achievements have been made in the past 20 years with improving survival as a result. Despite these improvements there is still a significant mortality and termination of pregnancy is common in Sweden if there is a prenatal diagnosis. Fetal valvuloplasty in aortic stenosis has a potential to promote growth and preserve cardiac function during pregnancy to enhance the chances of successful postnatal treatment and survival in cases with evolving HLHS.

I. The vulnerable period between stage I and stage II in single ventricle palliation continue to be challenging. Improved anticoagulation therapies might be more efficient in preventing shunt occlusions with reduced risks. Optimal timing of stage II is important to ensure that the interstage period is not too short or too long.

II. The improved ultrasound equipment available gives excellent insight into fetal cardiac development and growth. In populations where screening during pregnancy is widely practiced, a wide range of cardiac malformations will be detected. Further studies could give better indications of how to validate fetal echocardiographic data in research. Improved predictive models can be expected as more experience is gained.

III. The future of fetal cardiac interventions for left-sided obstructive cardiac lesions is difficult to predict. So far, success has been limited to interventions performed by highly experienced teams who have treated a substantial number of

cases. In a Swedish perspective, future cases suitable for a fetal cardiac intervention will be referred to any of the larger international centers. The reports of improved hemodynamics and growth of the fetal heart after intervention make the method overall seem promising. If the method becomes more accepted among healthcare professionals, a future randomized controlled trial might be unethical to perform, since it would require a situation with clinical equipoise.

IV. The incidence of HLHS/AA decreased over the study period. However, the trend noted after 2010 contradicts a continued decrease of incidence. The future perspective is that patients with HLHS/AA will continue to be born. A prenatal diagnosis will contribute to well-informed parents who are prepared to provide the best care for their children in cooperation with highly specialized professional teams.

V. The future perspective for the cohort of HLHS/AA is optimistic in the sense that early survival has improved. Possibilities of further improving survival are not easily identified. Future efforts should focus on reducing morbidity and on supporting the patient's neurocognitive development to provide the best possible quality of life for this group of patients.

SAMMANFATTNING PÅ SVENSKA

Bakgrund

Hypoplastiskt vänsterkammarsyndrom (HLHS) är ett allvarligt hjärtfel som är fatalt i nyföddhetsperioden om det nyfödda barnet inte omedelbart omhändertas och behandlas. Palliativ kirurgi har funnits tillgänglig i Sverige sedan 1993. Resultaten har förbättrats över tid men det är fortsatt ett hjärtfel med betydande mortalitet. Det har under senare år publicerats enstaka rapporter om att ballongdilatation hos foster med aortastenos kan förhindra uppkomst av HLHS i vissa utvalda fall. En annan metod för att förbättra överlevnaden hos födda barn är att övervaka dem mer noggrant även efter utskrivningen från sjukhus. Tidigare studier har visat på förbättrad överlevnad om syrgasmättnad och vikt kontrolleras dagligen av föräldrarna.

Syfte med forskningen

Syftet med de ingående arbetena i avhandlingen var att studera incidensen av hjärtfelet samt överlevnad efter kirurgi hos barn födde med HLHS ifrån 1990 - 2010 i Sverige samt att studera om ballongdilatation av aortaklaffen hos foster med aortastenos kan förebygga uppkomsten av HLHS under den fortsatta graviditeten. Ett tredje syfte var att undersöka om hem-monitorering av syremättnaden i blodet en gång dagligen kunde förebygga dödsfall.

Metoder

En komplett nationell kohort med patienter med HLHS identifierades genom sökningar i nationella register. Förändringar av incidensen över tid samt transplantationsfri överlevnad beräknades och riskfaktorer för död analyserades. Naturalförloppet vid aortastenos hos foster samt betydelsen av ballongdilatation av aortaklaffen i fosterlivet studerades i två retrospektiva multicenterstudier i Europa. Hem-monitorering studerades i en experimentell studie och resultatet jämfördes med en historisk kohort.

Resultat och konklusioner

Den totala transplantationsfria överlevnaden för patienter med HLHS som genomgick kirurgi ökade från 40 % under åren 1993-2000 till 63 % under åren 2001-2010. Flickor visade sämre transplantationsfri överlevnad jämfört med pojkar. Incidensen minskade från 15,4 till 8,4 per 100 000 levande födda. Andel av levande födda som genomgick kirurgi ökade från 50 % till 70 % mellan de studerade tidsperioderna. Ballongdilatation av aortaklaffen i fosterlivet förbättrade överlevnaden för levande födda men det kunde inte visas att det var en effekt av en lägre andel som utvecklade HLHS. De vänstersidiga hjärtstrukturernas tillväxt och funktion stimulerades hos foster som genomgick en intervention. Hem-monitorering av syremättnaden i

blodet bedömdes bidra till att rädda liv för ett antal individer men det var inte en statistiskt signifikant förbättrad överlevnad på gruppnivå i interventionsgruppen jämfört med historiska kontroller.

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REFERENCES

1. Lev, M., *Pathologic anatomy and interrelationship of hypoplasia of the aortic tract complexes.* Lab Invest, 1952. **1**(1): p. 61-70.
2. Noonan, J.A. and A.S. Nadas, *The hypoplastic left heart syndrome; an analysis of 101 cases.* Pediatr Clin North Am, 1958. **5**(4): p. 1029-56.
3. Tchervenkov, C.I., et al., *The nomenclature, definition and classification of hypoplastic left heart syndrome.* Cardiol Young, 2006. **16**(4): p. 339-68.
4. Franklin, R.C.G., et al., *Nomenclature for congenital and paediatric cardiac disease: the International Paediatric and Congenital Cardiac Code (IPCCC) and the Eleventh Iteration of the International Classification of Diseases (ICD-11).* Cardiol Young, 2017. **27**(10): p. 1872-1938.
5. WHOandICD-10.Availablefrom: http://www.who.int/classifications/icd/en/.
6. Bove, E.L., *Surgical treatment for hypoplastic left heart syndrome.* Jpn J Thorac Cardiovasc Surg, 1999. **47**(2): p. 47-56.
7. Mellander, M., L. Berntsson, and B. Nilsson, *Quality of life in children with hypoplastic left heart syndrome.* Acta Paediatr, 2007. **96**(1): p. 53-7.
8. Freud, L.R., et al., *Fetal aortic valvuloplasty for evolving hypoplastic left heart syndrome: postnatal outcomes of the first 100 patients.* Circulation, 2014. **130**(8): p. 638-45.
9. Anderson, B., Penny, Redington, Rigby, Wernovsky, *Paediatric Cardiology*. 3rd edition ed.
10. Mei Xin, E.N.O.R.B.-D., *Box 2: Developmental stages and regulation of cardiogenesis.* Nature Reviews Molecular Cell Biology, 2013. **14**: p. 529-541.
11. Liu, X., et al., *The complex genetics of hypoplastic left heart syndrome.* Nat Genet, 2017. **49**(7): p. 1152-1159.
12. Kuehl, K.S. and C.A. Loffredo, *A cluster of hypoplastic left heart malformation in Baltimore, Maryland.* Pediatr Cardiol, 2006. **27**(1): p. 25-31.
13. Eghtesady, P., A. Brar, and M. Hall, *Seasonality of hypoplastic left heart syndrome in the United States: A 10-year time–series analysis.* The Journal of Thoracic and Cardiovascular Surgery, 2011. **141**(2): p. 432-438.
14. Drose, J., *Fetal echocardiograhy*. Vol. 2013.
15. Polimenakos, A.C., et al., *Hypoplastic left heart syndrome and aortic atresia-mitral stenosis variant: role of myocardial protection strategy and impact of ventriculo-coronary connections after stage I palliation.* Pediatr Cardiol, 2011. **32**(7): p. 929-39.

16. Vida, V.L., et al., *Surgical outcome for patients with the mitral stenosis-aortic atresia variant of hypoplastic left heart syndrome.* J Thorac Cardiovasc Surg, 2008. **135**(2): p. 339-46.
17. Anderson, B., M. Pozzi, and S. Hutchinson, *Hypoplastic left heart syndrom*. 2005: Springer. 151.
18. McElhinney, D.B., et al., *Assessment of left ventricular endocardial fibroelastosis in fetuses with aortic stenosis and evolving hypoplastic left heart syndrome.* Am J Cardiol, 2010. **106**(12): p. 1792-7.
19. Clark, E.S., et al., *A mouse model of endocardial fibroelastosis.* Cardiovasc Pathol, 2015. **24**(6): p. 388-94.
20. Axt-Fliedner, R., et al., *Prenatal assessment of ventriculocoronary connections and ventricular endocardial fibroelastosis in hypoplastic left heart.* Ultraschall Med, 2014. **35**(4): p. 357-63.
21. Xu, X., et al., *Endocardial fibroelastosis is caused by aberrant endothelial to mesenchymal transition.* Circ Res, 2015. **116**(5): p. 857-66.
22. Emani, S.M., et al., *Staged left ventricular recruitment after single-ventricle palliation in patients with borderline left heart hypoplasia.* J Am Coll Cardiol, 2012. **60**(19): p. 1966-74.
23. Elmi, M., et al., *Long-term tricuspid valve function after Norwood operation.* J Thorac Cardiovasc Surg, 2011. **142**(6): p. 1341-7.e4.
24. Bharucha, T., et al., *Right ventricular mechanical dyssynchrony and asymmetric contraction in hypoplastic heart syndrome are associated with tricuspid regurgitation.* J Am Soc Echocardiogr, 2013. **26**(10): p. 1214-20.
25. Sugiura, J., et al., *Effects of tricuspid valve surgery on tricuspid regurgitation in patients with hypoplastic left heart syndrome: a non-randomized series comparing surgical and non-surgical cases.* Eur J Cardiothorac Surg, 2014. **46**(1): p. 8-13.
26. Glenn, W.W., *Superior vena cava-pulmonary artery shunt. By William W. L. Glenn, 1958.* Ann Thorac Surg, 1989. **47**(1): p. 62-4.
27. Fontan, F., *Atrio-pulmonary conduit operations for tricuspid atresia and single ventricle.* Nihon Kyobu Geka Gakkai Zasshi, 1978. **26**(3): p. 276-84.
28. Qu, Y., et al., *Incidence of Congenital Heart Disease: The 9-Year Experience of the Guangdong Registry of Congenital Heart Disease, China.* PLoS One, 2016. **11**(7): p. e0159257.
29. Carlgren, L.E., *The incidence of congenital heart disease in children born in Gothenburg 1941-1950.* Br Heart J, 1959. **21**(1): p. 40-50.
30. Hoffman, J.I., S. Kaplan, and R.R. Liberthson, *Prevalence of congenital heart disease.* Am Heart J, 2004. **147**(3): p. 425-39.
31. Leirgul, E., et al., *Birth prevalence of congenital heart defects in Norway 1994-2009--a nationwide study.* Am Heart J, 2014. **168**(6): p. 956-64.

32. Oyen, N., et al., *National time trends in congenital heart defects, Denmark, 1977-2005.* Am Heart J, 2009. **157**(3): p. 467-473 e1.
33. Samanek, M., et al., *Prevalence, treatment, and outcome of heart disease in live-born children: a prospective analysis of 91,823 live-born children.* Pediatr Cardiol, 1989. **10**(4): p. 205-11.
34. Samanek, M. and M. Voriskova, *Congenital heart disease among 815,569 children born between 1980 and 1990 and their 15-year survival: a prospective Bohemia survival study.* Pediatr Cardiol, 1999. **20**(6): p. 411-7.
35. Idorn, L., et al., *Univentricular hearts in Denmark 1977 to 2009: incidence and survival.* Int J Cardiol, 2013. **167**(4): p. 1311-6.
36. Brownell, L.G. and M.H. Shokeir, *Inheritance of hypoplastic left heart syndrome (HLHS): further observations.* Clin Genet, 1976. **9**(2): p. 245-9.
37. McBride, K.L., et al., *Epidemiology of noncomplex left ventricular outflow tract obstruction malformations (aortic valve stenosis, coarctation of the aorta, hypoplastic left heart syndrome) in Texas, 1999-2001.* Birth Defects Res A Clin Mol Teratol, 2005. **73**(8): p. 555-61.
38. Reller, M.D., et al., *Prevalence of congenital heart defects in metropolitan Atlanta, 1998-2005.* J Pediatr, 2008. **153**(6): p. 807-13.
39. Moons, P., et al., *Congenital heart disease in 111 225 births in Belgium: birth prevalence, treatment and survival in the 21st century.* Acta Paediatr, 2009. **98**(3): p. 472-7.
40. Lisi, A., et al., *Sex and congenital malformations: an international perspective.* Am J Med Genet A, 2005. **134A**(1): p. 49-57.
41. Storch, T.G. and E.E. Mannick, *Epidemiology of congenital heart disease in Louisiana: an association between race and sex and the prevalence of specific cardiac malformations.* Teratology, 1992. **46**(3): p. 271-6.
42. Samanek, M., *Boy:girl ratio in children born with different forms of cardiac malformation: a population-based study.* Pediatr Cardiol, 1994. **15**(2): p. 53-7.
43. Bergman, G., et al., *[Improved prenatal diagnosis of congenital heart defects. A follow-up study of prenatal ultrasound screening].* Lakartidningen, 2008. **105**(12-13): p. 899-903.
44. Sivarajan, V., et al., *Impact of antenatal diagnosis of hypoplastic left heart syndrome on the clinical presentation and surgical outcomes: the Australian experience.* J Paediatr Child Health, 2009. **45**(3): p. 112-7.
45. Socialstyrelsen, *The Swedish National Board of Health and Welfare. Birthdefects2014 (*www.socialstyrelsen.se/Lists/Artikelkatalog/Attachments/20093/2016-3-4.pdf, *accessed 2018-01-02)*. 2014.

46. SFOG, *Swedish Society of Obstetrics and Gynecology. Obstetric ultrasound. Report no 73. (In Swedish)*. 2014, Swedish Society of Obstetrics and Gynecology.
47. Morris, S.A., et al., *Prenatal diagnosis, birth location, surgical center, and neonatal mortality in infants with hypoplastic left heart syndrome*. Circulation, 2014. **129**(3): p. 285-92.
48. Thakur, V., et al., *Does prenatal diagnosis of hypoplastic left heart syndrome make a difference? - A systematic review.* Prenat Diagn, 2016. **36**(9): p. 854-63.
49. Markkanen, H.K., et al., *Prenatal diagnosis improves the postnatal cardiac function in a population-based cohort of infants with hypoplastic left heart syndrome*. J Am Soc Echocardiogr, 2013. **26**(9): p. 1073-9.
50. Norwood, W.I., Jr., *Hypoplastic left heart syndrome.* Ann Thorac Surg, 1991. **52**(3): p. 688-95.
51. Norwood, W.I., Jr., M.L. Jacobs, and J.D. Murphy, *Fontan procedure for hypoplastic left heart syndrome.* Ann Thorac Surg, 1992. **54**(6): p. 1025-9; discussion 1029-30.
52. Weldner, P.W., et al., *The Norwood operation and subsequent Fontan operation in infants with complex congenital heart disease.* J Thorac Cardiovasc Surg, 1995. **109**(4): p. 654-62.
53. Bove, E.L., *Current status of staged reconstruction for hypoplastic left heart syndrome.* Pediatr Cardiol, 1998. **19**(4): p. 308-15.
54. Bartram, U., J. Grunenfelder, and R. Van Praagh, *Causes of death after the modified Norwood procedure: a study of 122 postmortem cases.* Ann Thorac Surg, 1997. **64**(6): p. 1795-802.
55. Kern, J.H., et al., *Survival and risk factor analysis for the Norwood procedure for hypoplastic left heart syndrome.* Am J Cardiol, 1997. **80**(2): p. 170-4.
56. Hagemo, P.S., et al., *[Hypoplastic left heart syndrome. Present experiences and future possibilities]*. Tidsskr Nor Laegeforen, 1993. **113**(27): p. 3362-5.
57. Sano, S., et al., *Right ventricle-pulmonary artery shunt in first-stage palliation of hypoplastic left heart syndrome.* J Thorac Cardiovasc Surg, 2003. **126**(2): p. 504-9; discussion 509-10.
58. Ohye, R.G., et al., *Comparison of shunt types in the Norwood procedure for single-ventricle lesions.* N Engl J Med, 2010. **362**(21): p. 1980-92.
59. Siehr, S.L., et al., *Mitral Stenosis and Aortic Atresia--A Risk Factor for Mortality After the Modified Norwood Operation in Hypoplastic Left Heart Syndrome.* Ann Thorac Surg, 2016. **101**(1): p. 162-7.
60. Tweddell, J.S., et al., *Intermediate-term mortality and cardiac transplantation in infants with single-ventricle lesions: risk factors and their interaction with shunt type*. J Thorac Cardiovasc Surg, 2012. **144**(1): p. 152-9.

61. Ghanayem, N.S., et al., *Interstage mortality after the Norwood procedure: Results of the multicenter Single Ventricle Reconstruction trial.* J Thorac Cardiovasc Surg, 2012. **144**(4): p. 896-906.
62. Rudd, N.A., et al., *Improving interstage survival after Norwood operation: outcomes from 10 years of home monitoring.* J Thorac Cardiovasc Surg, 2014. **148**(4): p. 1540-7.
63. Ghanayem, N.S., et al., *Home surveillance program prevents interstage mortality after the Norwood procedure.* J Thorac Cardiovasc Surg, 2003. **126**(5): p. 1367-77.
64. Monagle, P., et al., *Antithrombotic therapy in neonates and children: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines.* Chest, 2012. **141**(2 Suppl): p. e737S-e801S.
65. Wessel, D.L., et al., *Clopidogrel in infants with systemic-to-pulmonary-artery shunts.* N Engl J Med, 2013. **368**(25): p. 2377-84.
66. Agarwal, A., et al., *Incidence and Management of Thrombotic and Thromboembolic Complications Following the Norwood Procedure.* Clin Appl Thromb Hemost, 2016: p. 1076029616679506.
67. Meza, J.M., et al., *The Optimal Timing of Stage-2-Palliation for Hypoplastic Left Heart Syndrome: An analysis of the Pediatric Heart Network Single Ventricle Reconstruction Trial Public Dataset.* Circulation, 2017.
68. Carlo, W.F., et al., *Interstage attrition between bidirectional Glenn and Fontan palliation in children with hypoplastic left heart syndrome.* J Thorac Cardiovasc Surg, 2011. **142**(3): p. 511-6.
69. Alsoufi, B., et al., *Current outcomes of the Glenn bidirectional cavopulmonary connection for single ventricle palliation.* Eur J Cardiothorac Surg, 2012. **42**(1): p. 42-8; discussion 48-9.
70. Farrell, P.E., Jr., et al., *Outcome and assessment after the modified Fontan procedure for hypoplastic left heart syndrome.* Circulation, 1992. **85**(1): p. 116-22.
71. Wilson, W.M., et al., *Outcomes of Patients With Hypoplastic Left Heart Syndrome Reaching Adulthood After Fontan Palliation: Multicenter Study.* Circulation, 2018. **137**(9): p. 978-981.
72. Beroukhim, R.S., et al., *Perinatal outcome after prenatal diagnosis of single-ventricle cardiac defects.* Ultrasound Obstet Gynecol, 2015. **45**(6): p. 657-63.
73. Orr, Y., et al., *Early outcomes from a new regional programme for the surgical management of hypoplastic left heart syndrome.* ANZ J Surg, 2015. **85**(6): p. 466-71.
74. Newburger, J.W., et al., *Transplantation-free survival and interventions at 3 years in the single ventricle reconstruction trial.* Circulation, 2014. **129**(20): p. 2013-20.
75. Hansen, J.H., et al., *Fifteen-year single-center experience with the Norwood operation for complex lesions with single-ventricle*

physiology compared with hypoplastic left heart syndrome. J Thorac Cardiovasc Surg, 2012. **144**(1): p. 166-72.
76. Menon, S.C., et al., *Outcome and resource utilization of infants born with hypoplastic left heart syndrome in the Intermountain West.* Am J Cardiol, 2012. **110**(5): p. 720-7.
77. Rychik, J., et al., *Perinatal and early surgical outcome for the fetus with hypoplastic left heart syndrome: a 5-year single institutional experience.* Ultrasound Obstet Gynecol, 2010. **36**(4): p. 465-70.
78. Graham, E.M., et al., *Comparison of Norwood shunt types: do the outcomes differ 6 years later?* Ann Thorac Surg, 2010. **90**(1): p. 31-5.
79. Marino, B.S., et al., *Neurodevelopmental outcomes in children with congenital heart disease: evaluation and management: a scientific statement from the American Heart Association.* Circulation, 2012. **126**(9): p. 1143-72.
80. Korenromp, M.J., et al., *Long-term psychological consequences of pregnancy termination for fetal abnormality: a cross-sectional study.* Prenat Diagn, 2005. **25**(3): p. 253-60.
81. Korenromp, M.J., et al., *Psychological consequences of termination of pregnancy for fetal anomaly: similarities and differences between partners.* Prenat Diagn, 2005. **25**(13): p. 1226-33.
82. Wallin Lundell, I., et al., *Posttraumatic stress among women after induced abortion: a Swedish multi-centre cohort study.* BMC Womens Health, 2013. **13**: p. 52.
83. Paul, E.A., E.M. Kohlberg, and K. Orfali, *Growing Discomfort With Comfort Care for Hypoplastic Left Heart Syndrome: Why We Should Still Defer to Parental Wishes.* Am J Bioeth, 2017. **17**(7): p. 67-68.
84. Kane, J.M., et al., *Management Options and Outcomes for Neonatal Hypoplastic Left Heart Syndrome in the Early Twenty-First Century.* Pediatr Cardiol, 2016. **37**(2): p. 419-25.
85. Paul, E.A., K. Orfali, and T.J. Starc, *Hypoplastic Left Heart Syndrome: Exploring a Paradigm Shift in Favor of Surgery.* Pediatr Cardiol, 2016. **37**(8): p. 1446-1452.
86. Freud, L.R., et al., *Low rate of prenatal diagnosis among neonates with critical aortic stenosis: insight into the natural history in utero.* Ultrasound Obstet Gynecol, 2015. **45**(3): p. 326-32.
87. Hunter, L.E., et al., *Fetal aortic valve stenosis: a critique of case selection criteria for fetal intervention.* Prenat Diagn, 2015. **35**(12): p. 1176-81.
88. Simpson, J.M. and G.K. Sharland, *Natural history and outcome of aortic stenosis diagnosed prenatally.* Heart, 1997. **77**(3): p. 205-10.
89. Alter, P., et al., *Wall stress determines systolic and diastolic function--Characteristics of heart failure.* Int J Cardiol, 2016. **202**: p. 685-93.
90. Zhong, L., D.N. Ghista, and R.S. Tan, *Left ventricular wall stress compendium.* Comput Methods Biomech Biomed Engin, 2012. **15**(10): p. 1015-41.

91. Schmidt, R. and G. Thews, *Human Physiology*. Second ed. 1989: Spronger-Verlag.
92. Morrison, J.L., *Sheep models of intrauterine growth restriction: fetal adaptations and consequences.* Clin Exp Pharmacol Physiol, 2008. **35**(7): p. 730-43.
93. Rudolph, A.M., *Congenital cardiovascular malformations and the fetal circulation.* Arch Dis Child Fetal Neonatal Ed, 2010. **95**(2): p. F132-6.
94. Lam, C.Z., et al., *Diagnosis of secondary pulmonary lymphangiectasia in congenital heart disease: a novel role for chest ultrasound and prognostic implications.* Pediatr Radiol, 2017. **47**(11): p. 1441-1451.
95. Seed, M., et al., *Antenatal MR imaging of pulmonary lymphangiectasia secondary to hypoplastic left heart syndrome.* Pediatr Radiol, 2009. **39**(7): p. 747-9.
96. Rychik, J., et al., *The hypoplastic left heart syndrome with intact atrial septum: atrial morphology, pulmonary vascular histopathology and outcome.* J Am Coll Cardiol, 1999. **34**(2): p. 554-60.
97. Bellini, C., et al., *Etiology of non-immune hydrops fetalis: An update.* Am J Med Genet A, 2015. **167a**(5): p. 1082-8.
98. McElhinney, D.B., et al., *Predictors of technical success and postnatal biventricular outcome after in utero aortic valvuloplasty for aortic stenosis with evolving hypoplastic left heart syndrome.* Circulation, 2009. **120**(15): p. 1482-90.
99. Moon-Grady, A.J., et al., *International Fetal Cardiac Intervention Registry: A Worldwide Collaborative Description and Preliminary Outcomes.* J Am Coll Cardiol, 2015. **66**(4): p. 388-99.
100. Arzt, W., et al., *Intrauterine aortic valvuloplasty in fetuses with critical aortic stenosis: experience and results of 24 procedures.* Ultrasound Obstet Gynecol, 2011. **37**(6): p. 689-95.
101. Schidlow, D.N., W. Tworetzky, and L.E. Wilkins-Haug, *Percutaneous fetal cardiac interventions for structural heart disease.* Am J Perinatol, 2014. **31**(7): p. 629-36.
102. Tworetzky, W., et al., *Balloon dilation of severe aortic stenosis in the fetus: potential for prevention of hypoplastic left heart syndrome: candidate selection, technique, and results of successful intervention.* Circulation, 2004. **110**(15): p. 2125-31.
103. Schmidt, M., et al., *Percutaneous ultrasound-guided stenting of the atrial septum in fetal sheep.* Ultrasound Obstet Gynecol, 2008. **32**(7): p. 923-8.
104. Wohlmuth, C., et al., *Maternal aspects of fetal cardiac intervention.* Ultrasound Obstet Gynecol, 2014. **44**(5): p. 532-7.
105. Wilkins-Haug, L.E., et al., *In-utero intervention for hypoplastic left heart syndrome--a perinatologist's perspective.* Ultrasound Obstet Gynecol, 2005. **26**(5): p. 481-6.

106. Sekar, P. and L.K. Hornberger, *The role of fetal echocardiography in fetal intervention: a symbiotic relationship.* Clin Perinatol, 2009. **36**(2): p. 301-27, ix.
107. Marantz, P. and S. Grinenco, *Fetal intervention for critical aortic stenosis: advances, research and postnatal follow-up.* Curr Opin Cardiol, 2015. **30**(1): p. 89-94.
108. Emery, S.P., et al., *The learning curve for a fetal cardiac intervention team.* Minim Invasive Surg, 2010. **2010**: p. 674185.
109. Gomez Montes, E., et al., *Fetal intervention in right outflow tract obstructive disease: selection of candidates and results.* Cardiol Res Pract, 2012. **2012**: p. 592403.
110. Tulzer, G. and W. Arzt, *Fetal cardiac interventions: rationale, risk and benefit.* Semin Fetal Neonatal Med, 2013. **18**(5): p. 298-301.
111. Selamet Tierney, E.S., et al., *Changes in left heart hemodynamics after technically successful in-utero aortic valvuloplasty.* Ultrasound Obstet Gynecol, 2007. **30**(5): p. 715-20.
112. Wohlmuth, C., et al., *Tissue Doppler imaging in fetuses with aortic stenosis and evolving hypoplastic left heart syndrome before and after fetal aortic valvuloplasty.* Ultrasound Obstet Gynecol, 2016. **47**(5): p. 608-15.
113. Freud, L.R., et al., *Outcomes and Predictors of Perinatal Mortality in Fetuses With Ebstein Anomaly or Tricuspid Valve Dysplasia in the Current Era: A Multicenter Study.* Circulation, 2015. **132**(6): p. 481-9.
114. Roman, K.S., et al., *Determinants of outcome in fetal pulmonary valve stenosis or atresia with intact ventricular septum.* Am J Cardiol, 2007. **99**(5): p. 699-703.
115. Michelfelder, E., et al., *Predictive value of fetal pulmonary venous flow patterns in identifying the need for atrial septoplasty in the newborn with hypoplastic left ventricle.* Circulation, 2005. **112**(19): p. 2974-9.
116. Makikallio, K., et al., *Fetal aortic valve stenosis and the evolution of hypoplastic left heart syndrome: patient selection for fetal intervention.* Circulation, 2006. **113**(11): p. 1401-5.
117. Allan, L.D., R. Santos, and T. Pexieder, *Anatomical and echocardiographic correlates of normal cardiac morphology in the late first trimester fetus.* Heart, 1997. **77**(1): p. 68-72.
118. Donofrio, M.T., et al., *Diagnosis and treatment of fetal cardiac disease: a scientific statement from the American Heart Association.* Circulation, 2014. **129**(21): p. 2183-242.
119. Simpson, J.M. and A. Cook, *Repeatability of echocardiographic measurements in the human fetus.* Ultrasound Obstet Gynecol, 2002. **20**(4): p. 332-9.

120. Kleinman, C.S., et al., *Fetal echocardiography. A tool for evaluation of in utero cardiac arrhythmias and monitoring of in utero therapy: analysis of 71 patients.* Am J Cardiol, 1983. **51**(2): p. 237-43.
121. Bergman, G., M. Wahren-Herlenius, and S.E. Sonesson, *Diagnostic precision of Doppler flow echocardiography in fetuses at risk for atrioventricular block.* Ultrasound Obstet Gynecol, 2010. **36**(5): p. 561-6.
122. Szklo, M. and J. Nieto, *Epidemiology beyond the basics*. Third edition ed. 2014: Jones and Bartlett Learning.
123. Ludvigsson, J.F., et al., *The Swedish personal identity number: possibilities and pitfalls in healthcare and medical research.* Eur J Epidemiol, 2009. **24**(11): p. 659-67.
124. Asberg, K.H., *[The Neurosedyn disaster became a starting shot. The drug approval process was changed and new era of patient safety was initiated]*. Lakartidningen, 2011. **108**(48): p. 2486-7.
125. National Board of Health and Welfare, S., *The Swedish Medical Birth Register- A summary of content and quality*. 2003.
126. Brooke, H.L., et al., *The Swedish cause of death register.* Eur J Epidemiol, 2017. **32**(9): p. 765-773.
127. Ludvigsson, J.F., et al., *External review and validation of the Swedish national inpatient register.* BMC Public Health, 2011. **11**: p. 450.
128. Wisten, A., et al., *Sudden cardiac death in 15-35-year olds in Sweden during 1992-99.* J Intern Med, 2002. **252**(6): p. 529-36.
129. General Assembly of the World Medical, A., *World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects.* J Am Coll Dent, 2014. **81**(3): p. 14-8.
130. *World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects.* JAMA, 2000. **284**(23): p. 3043-5.
131. Ludvigsson, J.F., et al., *Ethical aspects of registry-based research in the Nordic countries.* Clin Epidemiol, 2015. **7**: p. 491-508.
132. Kho, M.E., et al., *Written informed consent and selection bias in observational studies using medical records: systematic review.* Bmj, 2009. **338**: p. b866.
133. Shephard, D.A., *The 1975 Declaration of Helsinki and consent.* Can Med Assoc J, 1976. **115**(12): p. 1191-2.
134. Medicinska, et al., *Etiska riktlinjer för epidemiologisk forskning. Befolkningens hälsa ett övergripande krav.* . Läkartidningen 1994(91): p. 4783.
135. Martinez-Beneito, M. *A Bayesian Joinpoint Regression model with an unknown number of break-points*. 2009 2018-03-13]; Available from: https://www.uv.es/mamtnez/preprints/joinpoint.pdf.
136. Cohrane W, R.D., *Controlling bias in Observational Studies: A Review.* Sankva A, 1973. **35**: p. 417-446.

137. Rosenbaum, P.R., Rubin D, *Reducing bias in Observational Studies Using Subclassification on the Propensity Score*.
138. Rubin, D.B., *Propensity score methods.* Am J Ophthalmol, 2010. **149**(1): p. 7-9.
139. Ghanayem, N.S., et al., *Home monitoring of infants after stage one palliation for hypoplastic left heart syndrome.* Semin Thorac Cardiovasc Surg Pediatr Card Surg Annu, 2004. **7**: p. 32-8.
140. Donofrio, M.T., *The Power Is in the Numbers: Using Collaboration and a Data Registry to Answer Our Burning Questions Regarding Fetal Cardiac Intervention.* J Am Coll Cardiol, 2015. **66**(4): p. 400-2.
141. Kjellberg Olofsson, C., et al., *Long-term survival and need of reintervention after treatment of critical valvular aortic stenosis in the neonate; a complete national study in Sweden.* 2018.
142. Galoin-Bertail, C., et al., *The mid-term outcome of primary open valvotomy for critical aortic stenosis in early infancy - a retrospective single center study over 18 years.* J Cardiothorac Surg, 2016. **11**(1): p. 116.
143. *Methods of Early Prenatal Diagnosis**.
144. Socialstyrelsen, *The Swedish National Board of Health and Welfare. Statistics on Pregnancies, Deliveries and Newborn Infants 2015 (*www.socialstyrelsen.se/publikationer2017/2017-3-4*, accessed 2018-01-02)*. 2017.

*Routine ultrasound examination during pregnancy. Swedish Council on Health Technology Assessment in Health Care. SBU report no 139 (In Swedish).1998.