A dive into the wondrous world of congenital diaphragmatic hernia

An international multicenter clinical approach

Kitty G. Snoek

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A Dive into the Wondrous World of Congenital Diaphragmatic Hernia

An international multicenter clinical approach

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Een internationale multicenter klinische benadering

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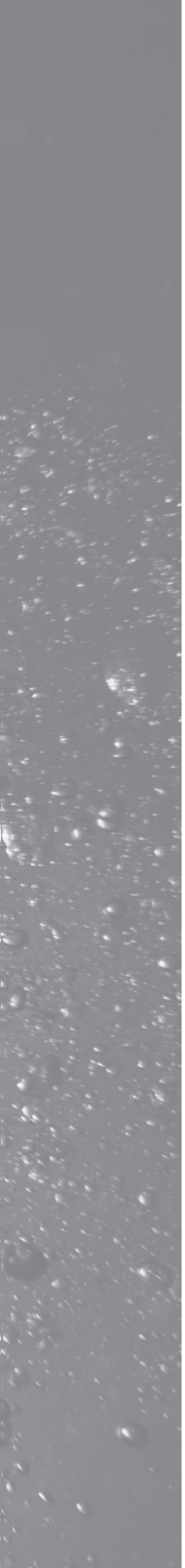
Introduction



Chapter 1

Introduction

Congenital diaphragmatic hernia (CDH) is a congenital anomaly that occurs in about 1 per 2000-3000 live births[1,2]. The reported incidence rates vary worldwide, in part dependent on case selection and source of reporting, since abortions and stillbirths are not always taken into account. In the Netherlands, about 50 to 60 infants with CDH are born alive each year. The mortality rate varies from about 20 to 40%, depending on the presence of associated malformations such as cardiac defects[2-4]. Despite many advances in the treatment in recent decades, CDH remains clinically and scientifically a challenging condition.

BACKGROUND

The first report by Pare of two cases with a traumatic diaphragmatic hernia dates from 1575[5]. In 1672, Riverius described a diaphragm defect as an incidental finding during autopsy in a 24-year-old man[6]. In 1769, Morgagni had classified different types of congenital diaphragmatic defects[7]. Bochdalek in 1848 was the first who described the posterolateral defect and suggested that a surgical correction would be useful in the treatment next to recognizing the role of pulmonary hypoplasia on outcome[8]. The terms 'Bochdalek hernia' and 'Morgagni hernia' are still being used. The Bochdalek hernia on the left posterolateral side is seen in more than 90% of the cases, while a defect in the anterior part of the diaphragm, the Morgagni hernia, is rare[9,10]. In the early 20th century, Heidenhain was the first surgeon who successfully performed a surgical repair in an infant with CDH[11], who survived into adulthood[12]. Despite this successful outcome, surgical correction of CDH remained uncommon for some decades.

In 1921, the importance of pulmonary hypoplasia related to CDH was recognized. Several decades later, pulmonary hypertension was considered the most challenging condition to treat. Because pulmonary hypertension affect the already vulnerable lungs of the neonate with CDH, finding the optimal ventilation management remained difficult[13]. Milestones in the treatment of CDH were the introduction of delayed surgical repair after initial stabilization[14], and the availability of the 'gentle ventilation strategy' with permissive hypercapnia[15,16]. The introduction of prenatal ultrasonography[17] allowed for early recognition of the anomaly, enabling start of optimal treatment as soon as possible in individual cases.

PREDICTION

Antenatal period

A marker, either a diagnostic/ monitoring biomarker or clinical prediction score, should add useful information to the already available information and would ideally have both a high sensitivity and specificity, a high predictive value, and high robustness[18].

When prenatal ultrasound techniques became sufficiently powerful, the second trimester ultrasound screening test was introduced to detect congenital anomalies, including CDH[19]. In the Netherlands, the prenatal ultrasound screening test was introduced relatively late (in 2007) compared with other countries in Europe. In case of a diaphragm defect, abdominal organs can be visualized when obtaining a four-chamber view of the heart. A mediastinal shift caused by herniation of the stomach, intestine or liver into the chest, is often seen. A left-sided diaphragmatic defect is easier to detect than a right-sided defect because in the latter case the liver is most often herniated. The liver is more echogenic than intestine or stomach, and therefore hard to distinguish from lung[20]. Next to recognizing the CDH, it is important to detect any associated anomalies because these can have an impact on prognosis.

Several antenatal predictive markers for CDH outcome in patients have been proposed, such as the lung-to-head circumference ratio (LHR) and the observed-to-expected LHR (O/E LHR). Metkus et al were the first to describe the LHR[21]. It is calculated as the length of the longest axis of the contralateral lung multiplied by the longest diameter perpendicular to it, divided by the head circumference. However, the LHR increases over gestational age since between 12 and 32 weeks of gestation normal lungs increase in size four times more than the head circumference[22]. Ba'ath et al examined the usefulness of the LHR (measured until 32 weeks of gestation) and found no significant difference in LHR between survivors and non-survivors[23].

The O/E LHR is calculated by dividing the observed LHR as explained above by the expected LHR. The expected LHR using the longest diameter method is calculated by different formulas for left and right CDH[23]. Peralta et al found that it was more accurate to trace the lung contours in normal lungs[22]. If this method is chosen for CDH patients, other formulas are used, also different for left and right CDH[24].

Other prenatal measures which have shown prognostic value are the position of the liver (either intra-abdominal or intrathoracic)[25,26], the position of the stomach (either intra-abdominal or intrathoracic)[27], and the side of the defect. Next to prenatal ultrasound measurements, fetal MRI is suitable to accurately measure lung sizes[20]. Still we should be aware that none of these prenatal markers can be used alone as a predictor since the level of postnatal care is the main determinant of outcome of individual patients.

Postnatal period

Because the outcome of CDH patients is highly variable, it would be ideal to be able to predict this as soon as possible. Since in prenatal measurements the transitional phase after birth obviously cannot be taken into account, postnatal clinical parameters might have more accurate predictive value. Many researchers have searched for reliable prediction models, based on several different clinical parameters.

In 2001 the CDH Study Group developed a prediction model containing the 5 minute Apgar score and birth weight[28]. In 2007, Schultz et al developed a prediction model based on blood gas analysis obtained in the first 24 hours of life and found that their model had better predictive abilities than previous models[29]. However, Baird et al could not validate the model of Schultz et al in a different dataset[30]. Based on a study including 2022 infants with CDH, Brindle et al recently developed a validated clinical prediction rule that could identify infants at low, intermediate and high risk of death at the time of the first postnatal echocardiogram[31]. Their model was based on the birth weight, Apgar score at 5 minutes of life, presence of severe pulmonary hypertension, presence of major cardiac anomaly, and the presence of chromosomal anomaly. Their study is limited as many values for at least one of the parameters are missing (most often Apgar score and presence of severe pulmonary hypertension when echocardiography was not performed). Second, patients were born in different centers and not treated according to a standardized treatment protocol. In CDH patients, retrospective studies have shown that SNAP-II scores were higher in patients with a worse outcome[32,33]. Those studies, however, included relatively few patients (47 and 88 patients) and one was performed before the introduction of a gentle ventilation strategy[32].

Instead of clinical parameters, specific tests from blood or other body fluids might serve as better prognostic objective markers. Biomarkers are defined as characteristics that can objectively be measured and evaluated as indicators of normal biological processes, pathogenic processes, or pharmacologic responses to therapeutic interventions[34].

Various biomarkers have been studied in CDH patients in relation to pulmonary hypertension. Kobayashi et al found that levels of ICAM-1, ELAM-1, and VCAM-1 liberated by activated vascular endothelium were higher in CDH patients with pulmonary hypertension than in CDH patients without pulmonary hypertension[35]. Keller et al investigated the predictive role of entohelin-1, which is dysregulated in pulmonary hypertension[36]. It appeared that non-survivors or patients discharged on oxygen had higher plasma ET1 levels. Patel et al prospectively investigated the relationship between plasma vascular endothelial growth factor A (VEGFA) and placental growth factor (PLGF) and measures of pulmonary artery pressure, oxygenation, and cardiac function[37]. They found that increased plasma VEGFA and reduced PLGF correlated with clinical severity of pulmonary vascular disease[37]. Fleck et al found that cord blood levels of epidermal growth factor,

platelet-derived growth factor, and several inflammatory mediators increased with higher severity of pulmonary hypertension[38].

The predictive value of B-type natriuretic peptide (BNP) or N-terminal pro BNP was investigated in several studies. These biomarkers were found useful in predicting the severity of pulmonary hypertension, although those studies were limited in having a single center design with relatively low sample sizes[39,40], or retrospective designs[41].

TREATMENT

Also because findings from randomized clinical trials specifically for CDH patients were still lacking, centers in the past used widely varying treatment protocols, if any. In 1995, the CDH study group was founded[42,43] and started to collect data in a large database enabling to evaluate treatment strategies and enhance further research. However, research based on these data is still limited by the lack of standardized therapy between centers. In 2006, a European cooperative network of tertiary centers with expertise in CDH was founded, named the CDH EURO Consortium. The Consortium acknowledged the need for standardized therapy and therefore a standardized neonatal treatment protocol was implemented in 2008. The protocol was based upon levels of evidence and expert opinion, and consensus was reached during a consensus meeting[44]. Recommendations are given on prenatal management and delivery, management in the delivery room, management in the ICU including management of ventilation and pulmonary hypertension, criteria for extracorporeal membrane oxygenation (ECMO) and surgical repair, sedation and analgesia and fluid management (see appendix 1).

Since 2008, all infants with CDH born in one of the participating centers of the CDH EURO Consortium have been treated according to this protocol. After implementation of the protocol, survival rates significantly increased from 67% to 88% in the high-volume centers in Mannheim and Rotterdam (≥ 10 CDH cases a year[45]). This finding emphasizes that standardized treatment is of paramount importance. Most recommendations in the original protocol, however, were based on expert opinion or on nonanalytic studies such as case reports or case series. An urgent need for randomized clinical trials was acknowledged, therefore, and the consortium designated the issue of the optimal initial ventilation strategy as the most important remaining research question. Consequently, a randomized clinical trial of initial ventilation strategy was set up including inborn neonates with a prenatal diagnosis of CDH and born after more than 34 weeks of gestation[46]. Immediately after delivery, they were randomized for initial ventilation strategy (either conventional mechanical ventilation or high-frequency oscillation).

Nowadays all infants with CDH are routinely intubated immediately after birth. However, ventilator-induced lung injury (VILI) is associated with worse outcome in CDH,

which raises the question whether this practice should be maintained in CDH infants with a good expected prognosis. On the other hand, other strategies such as liquid ventilation might serve as better ventilation modes in the most severe CDH cases.

OUTCOME

As mortality in CDH has decreased, attention for morbidity and long-term follow-up has been increased. Previous studies have shown that about 87% of CDH survivors had a kind of associated morbidity, mainly pulmonary or gastro-intestinally related[47,48]. Gastro-esophageal reflux may be treated both by antireflux medication and by surgical intervention[49]. No prospective studies are available on the specific type of preventive antireflux medication. Several studies have shown that CDH infants are also at risk for impaired growth, mainly at young age[50-52].

Potentially even more importantly is the children's neurodevelopment, which can be assessed in a standardized way. Of the various test instruments, the Bayley Scales of Infant Development (BSID) is often used, and in some countries population normative data are available. Several studies on neurodevelopment have been published in recent years. From 24% to 32% of children had delayed cognition, and from 23% to 73% had delayed motor function[53-56]. Most of these studies, however, were performed in single centers, had small case series and cross-sectional designs.

CONSIDERATION

Despite standardized therapy, survival rates in the various centers still differ. One of the most important differences in treatment between centers is the availability of ECMO[57]. Even in the CDH EURO Consortium, ECMO is not available in all centers. ECMO treatment is indicated for the most severely ill patients, and is initiated most often because of severe respiratory or cardiovascular failure not responding to conventional intensive care treatment due to suprasystemic pulmonary hypertension[58]. Either the venoarterial or venovenous ECMO mode can be used, but the former is still preferred for CDH[59]. The impact of choice of mode on survival, however, is subject of debate.

One of the determinants of outcome next to patient characteristics such as liver position[60], diaphragmatic defect size[61], stomach position[27], and O/E LHR[62], is the expertise of the CDH center. Bucher et al found that high-volume CDH centers achieve better survival rates as compared to low-volume CDH centers[45]. It could be hypothesized that despite the use of a standardized treatment protocol, survival rates still differ between centers because of different patient populations.

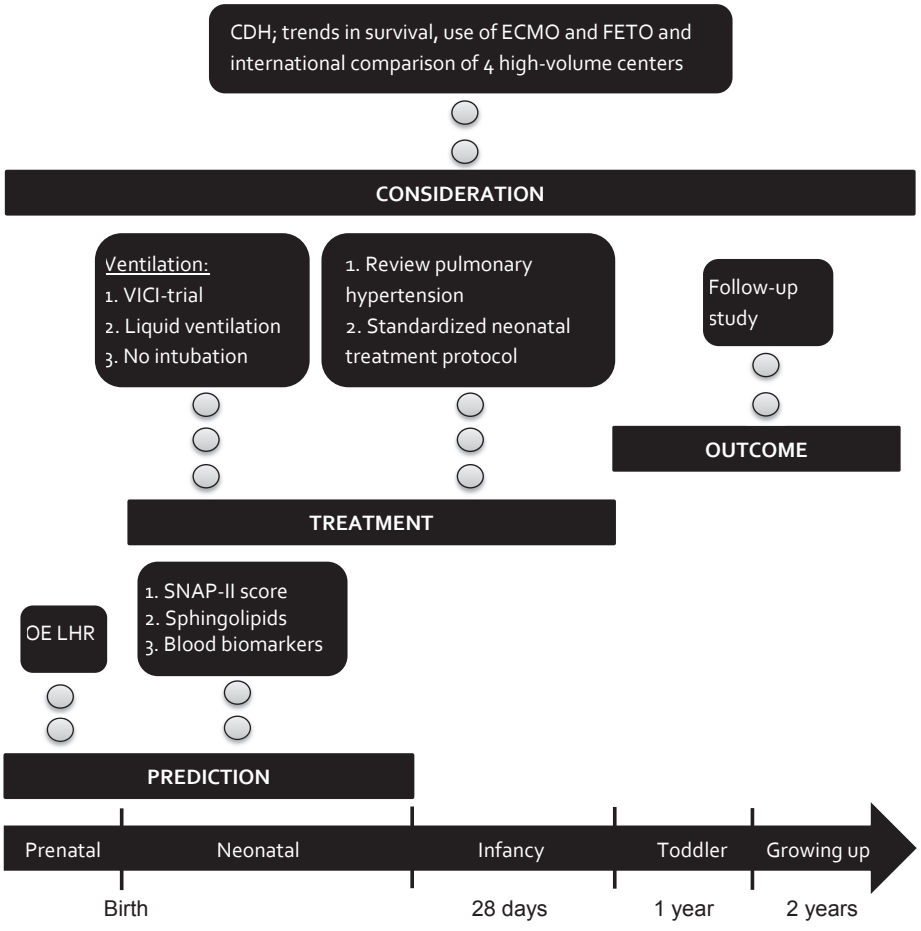
AIMS AND OUTLINE OF THIS THESIS

The aim of this thesis is to evaluate several aspects of CDH after the introduction of the standardized neonatal treatment protocol from the CDH EURO Consortium.

In the first place, clinical parameters and biomarkers with a potential predictive role are investigated. Second, one of the most important remaining questions in treatment, the optimal initial ventilation strategy, is studied. Lastly, differences in outcome such as survival and neurodevelopment between centers are studied. The content is divided into six parts; the figure below illustrates how these are related and what chapters fall under each part.

Part II focuses on the predictive role of both clinical parameters and biochemical parameters. **Chapter 2** deals with the usefulness of a prenatal ultrasound measurement

Figure 1 – Overview of chapters in this thesis



(the O/E LHR) in the prediction of outcome in isolated left CDH. The predictive value of the Score for Neonatal Acute Physiology- II (SNAP-II score) in infants with CDH is investigated in **chapter 3.** The predictive role of sphingolipids, analyzed in tracheal aspirates from CDH infants, is evaluated in **chapter 4**. **Chapter 5** evaluates blood biomarkers (hs-TroponinT and NT-proBNP) associated with impaired outcome in prenatally diagnosed CDH infants.

Part III describes different aspects of the treatment of CDH patients. **Chapter 6** describes the multicenter randomized clinical trial on ventilation strategies (VICI-trial). Prenatally diagnosed CDH infants were randomized to either conventional mechanical ventilation of high-frequency oscillation. In an editorial, the role of liquid ventilation is CDH patients is discussed **(chapter 7)**. The clinical course of five prenatally diagnosed CDH infants with a very good expected prognosis, in whom routine postnatal intubation was not performed, is presented in **chapter 8.** Pharmacodynamic considerations in the treatment of pulmonary hypertension in infants, including CDH infants, are studied in **chapter 9.** In **chapter 10** an update of the standardized postnatal management of infants with CDH is described. Consensus of optimal treatment was reached within the CDH EURO Consortium.

Part IV concerns the neurodevelopmental outcome in high-risk CDH infants at the ages of one and two years, assessed from psychomotor development and mental development as evaluated in two prospective standardized follow-up programs from two high-volume centers (**chapter 11)**.

In **Part V** the influence of patient characteristics and center specific differences on outcome is evaluated **(chapter 12)**.

In **Part VI** results of the studies are placed in a broader perspective, and areas of current and future research projects are described in **chapter 13**. Results of the studies are summarized in **chapter 14**.

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APPENDIX 1

Prenatal diagnosis and delivery

Following prenatal diagnosis, the absolute and O/E LHR and the position of the liver should be evaluated.

Planned vaginal delivery or cesarean section after a gestational age of 37 weeks in a designated tertiary center should be pursued.

In case of preterm labor prior to 34 weeks of gestation, antenatal steroids should be given.

Initial treatment and procedures in the delivery room

After delivery, the infant should be intubated immediately without bag and mask ventilation.

The goal of treatment in the delivery room is achieving acceptable preductal saturations levels between 80 and 95%.

Ventilation in the delivery room may be done by conventional ventilator or ventilation bag with a peak pressure as low as possible, preferably below 25 cm H_2O.

An oro- or nasogastric tube with continuous or intermittent suction should be placed.

Arterial blood pressure has to be maintained at a normal level for gestational age. In case of hypotension and/or poor perfusion, 10-20 ml/kg NaCl 0.9% should be administered 1-2 times and inotropic agents should be considered.

Sedatives and analgesics should be given.

No routine use of surfactant in either term or preterm infants with CDH.

Ventilation management in the Intensive Care Unit

Adapt treatment to reach a preductal saturation between 85 and 95% and a postductal saturation above 70%.

In individual cases, preductal saturation above 80% might be acceptable, as long as organs are well perfused.

The target $PaCO_2$ range should be between 45 and 60 mmHg.

Pressure-controlled ventilation initial settings are a peak inspiratory pressure of 20-25 cm H_2O and a positive end expiratory pressure of 2-5 cm H_2O; ventilator rate of 40- 60/min.

High-frequency oscillation: initial setting are a mean airway pressure 13-17 cm H_2O, frequency 10 Hz, ΔP 30-50 cm H_2O depending on chest wall vibration.

After stabilization, the FiO_2 should be decreased if preductal saturation is above 95%.

Further management in the Intensive Care Unit

If symptoms of poor perfusion and/or blood pressure below the normal level for gestational age occur and are associated with preductal saturation below 80%, echocardiographic assessment should be performed.

In case of hypovolemia, isotonic fluid therapy 10-20 ml/kg NaCl 0.9% up to 3 times during the first 2 hours may be given and inotropics should be considered.

Pulmonary hypertension management

Perform echocardiography within the first 24 hours after birth.

Blood pressure support should be given to maintain arterial blood pressure levels at normal levels for gestational age.

Inhaled nitric oxide should be considered if there is evidence of extrapulmonary right-to-left shunting and the oxygenation index is above 20 and/or the saturation difference is more than 10%.

In case of suprasystemic pulmonary artery pressure and right-to-left shunting through the foramen ovale, intravenous prostaglandin E1 has to be considered.

Extracorporeal membrane oxygenation (ECMO)

Criteria for ECMO :

- Inability to maintain preductal saturations >85% or postductal saturations >70%.
- Increased $PaCO_2$ and respiratory acidosis with pH <7.15 despite optimization of ventilatory management.
- Peak inspiratory pressure >28 cm H_2O or mean airway pressure >17 cm H_2O is required to achieve saturation >85%.
- Inadequate oxygen delivery with metabolic acidosis as measured by elevated lactate ≥5 mmol/l and pH <7.15.
- Systemic hypotension, resistant to fluid and inotropic therapy, resulting in urine output <0.5 ml/kg/h for at least 12- 24 hours.
- Oxygenation index ≥40 consistently present.

Timing of surgical repair and postoperative management

Surgical repair of the defect in the diaphragm should be performed after physiological stabilization, defined as follows:

- Mean arterial blood pressure normal for gestational age;
- Preductal saturation levels of 85- 95% SaO_2 on fractional inspired oxygen below 50%;
- Lactate below 3 mmol/ l;
- Urine output more than 2 ml/kg/h.

No routine chest tube placement.

Repair on ECMO may also be considered.

Sedation and analgesia

Infants should remain sedated and be monitored using validated analgesia and sedation scoring systems.

Neuromuscular blocking agents should be avoided if possible.

Enteral feeding and gastroesophageal reflux

Enteral feeding should be started postoperatively combined with antireflux medication.

Fluid management and parenteral feeding

40 ml/kg/day including medication for the first 24 hours, intake increases thereafter.

Diuretics should be considered in case of a positive fluid balance, aim for diuresis of 1- 2 ml/kg/hour.

Part II

Prediction

Chapter 2

The validity of the observed-to-expected lung-to-head ratio in congenital diaphragmatic hernia in an era of standardized neonatal treatment; a multicenter study

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Submitted for publication

ABSTRACT

Objective: To assess the predictive value of observed-to-expected lung-to-head ratio (O/E LHR) for survival and chronic lung disease (CLD) in survivors of left-sided congenital diaphragmatic hernia (CDH) in an era of standardized neonatal treatment. Furthermore, we evaluated the predictive value for survival of changes of O/E LHR values during gestation.

Methods: This study was performed in two high-volume CDH centers in the Netherlands. Observed-to-expected lung-to-head ratio (O/E LHR) and liver position was determined using 2D ultrasonography at 19+0-24+0 weeks gestational age (GA), between 24+1-29+6 weeks GA, and ≥30 weeks GA in prenatally detected, isolated left-sided CDH patients prenatally detected born between 2008 and 2014. Ultrasound measurements were performed by one single experienced operator. All patients were treated according to a standardized neonatal treatment protocol.

Results: Of the 126 included isolated left-sided CDH cases, 97 patients (77.0%) survived, of which 38 (39.2%) developed CLD. Multivariable logistic regression analysis including the first measured O/E LHR per patient and prenatal liver position showed that the O/E LHR was significantly associated with survival (OR 1.11; 95% CI 1.056- 1.176, $p<0.001$), and development of CLD in survivors (OR 0.96; 95% CI 0.917- 0.995, $p=0.03$). Prenatal liver position was not significantly associated with survival or development of CLD in survivors. Longitudinal analyses of the O/E LHR measurements during gestation showed no significant association of the trajectory of O/E LHR with survival.

Conclusion: In an era of standardized neonatal treatment, the first measured O/E LHR per patient significantly predicts survival and development of CLD in survivors in isolated left-sided CDH infants.

INTRODUCTION

Congenital diaphragmatic hernia (CDH) occurs in approximately 1 in 2200 live births[1]. Although the survival rate has significantly increased in the last decade to about 70-80%[2], CDH is still a life-threatening congenital anomaly[3]. Various prenatal parameters are related to a worse prognosis, such as a right-sided diaphragmatic hernia, (prenatal) herniation of the liver into the thorax, and associated congenital and/or chromosomal malformations[4,5].

Metkus et al. were the first in 1996 who described the predictive value of lung-to-head ratio (LHR) in CDH patients[6]. Since the LHR increases exponentially with gestation in normal fetuses[7], the observed-to-expected LHR (O/E LHR) was introduced in 2007 by Jani et al. in a multicenter study in 354 isolated CDH fetuses[8]. Thereafter, several studies have demonstrated that O/E LHR is a useful predictor of outcome[9-11].

In fetuses with severe CDH *(O/E LHR <25%)* (NCT01240057) and fetuses with moderate CDH *(O/E LHR 25-34.9% or O/E LHR 35-44.9% with intrathoracic liver herniation)* (NCT00763737) the benefit of fetoscopic endotracheal occlusion (FETO) is currently being investigated in randomized controlled studies. Groups were based on survival rates in the study from Jani et al[8], which is the largest study to date. However, in that period there was still a lack of postnatal standardization of treatment, which has proven in the meantime to have influenced postnatal outcome, reaching survival rates up to over 80%[12]. Secondly, in their study each of the participating centers provided the data. Information concerning inter-observer reproducibility was not available and variability in prenatal ultrasound measurements may have influenced the results.

From 2008 onwards, all patients born in one of the centers from the CDH EURO Consortium have been treated according to a standardized neonatal treatment protocol[13]. Subsequent high survival rates might influence the "original" cut-off points and their validity at present for counseling future couples. Therefore, we evaluated the predictive value of O/E LHR established in the two Dutch prenatal CDH centers on survival and development of chronic lung disease (CLD) when neonates are subjected to an optimal standardization of treatment with ECMO availability. Moreover we evaluated the significance of the used cutt-off values for prediction of outcomes.

METHODS

This is an observational retrospective cohort study. All patients with a prenatal diagnosis of CDH, born between January 2008 and December 2014, and treated in the Erasmus MC University Medical Center, Rotterdam, The Netherlands or Radboud University Medical Center, Nijmegen, The Netherlands were included. Since all infants from the Netherlands

with a CDH are referred to one of the two CDH centers, this represents a nationwide cohort. Exclusion criteria were defined as: right-sided CDH, termination of pregnancy, gestational age at birth < 30 weeks, and associated major structural or chromosomal anomalies. Since subjects are not being submitted to any handling, nor are there rules of human behavior being imposed, Institutional Review Board approval was waived by the ethical committee of the Erasmus Medical Center, Rotterdam, The Netherlands.

The original ultrasound image of a transverse plane of the fetal chest at the level of the four-chamber view of the heart was retrieved from patient records and used for measurement of the contralateral (right) lung. The lung area was measured by manual tracing of the limits of the lung (mm^2) Also the head circumference (mm) was determined. The O/E LHR was then calculated as described by Jani et al[14]. Prenatal ultrasound measurements were performed using the GE Voluson E8 or E10 system (GE Medical Systems, Zipf, Austria) at the Department of Obstetrics and Gynecology, Division of Obstetrics and Prenatal Medicine at the Erasmus University Medical Center. The measurements were performed by one single experienced operator (NP) who was unaware of postnatal outcome. Ultrasound measurements were categorized as follows; ultrasound 1: 19+0-24+0 weeks gestational age (GA), ultrasound 2: 24+1-29+6 weeks GA, and ultrasound 3: ≥30+0 weeks GA. Postnatal patient characteristics were retrieved from medical records. Patient demographics included: gestational age at birth, birth weight, gender, associated major structural or chromosomal anomalies, FETO, side of diaphragmatic hernia, liver position (intrathoracic/ intra-abdominal) determined during prenatal ultrasound and surgical repair, diaphragmatic defect size (A/B/C/D), need for ECMO, survival and presence of CLD in survivors. Survival was defined as survival after the first year of life. CLD was defined as oxygen dependency (>0.21) at day 28 of life[15]. Since 2008 all patients have been treated according to a standardized neonatal treatment protocol[13]. ECMO therapy was available for patients born after >34 weeks gestation and birth weight above 2000 grams during the complete study period. Severity of CDH was divided in the same groups as proposed by Deprest et al[16]: Extreme CDH (O/E LHR <15), severe CDH (O/E LHR ≤25%), moderate CDH (O/E LHR 26- 35% or O/E LHR 36- 45% with intrathoracic liver position), and mild CDH (O/E LHR 36-45% and liver down or O/E LHR ≥46).

Statistical analysis

Patient characteristics were described as numbers (%) for categorical data, or median (interquartile range; IQR) for non-normally distributed data. The first measured O/E LHR per patient was selected and used for all analyses, excluding the longitudinal analyses. O/E LHR was compared between survivors and non-survivors, and survivors with and without development of CLD, using Mann-Whitney tests. O/E LHR was compared between centers using the Mann-Whitney test. Independent associations between O/E LHR and mortality, and CLD in survivors were evaluated using univariable logistic re-

gression modelling. Multivariable logistic regression analysis with prenatal liver position and O/E LHR as independent variableswas then used to evaluate their predictive value on survival and development of CLD in survivors. The calibration of the multivariable logistic regression models were assessed using the Hosmer-Lemeshow goodness-of-fit test.

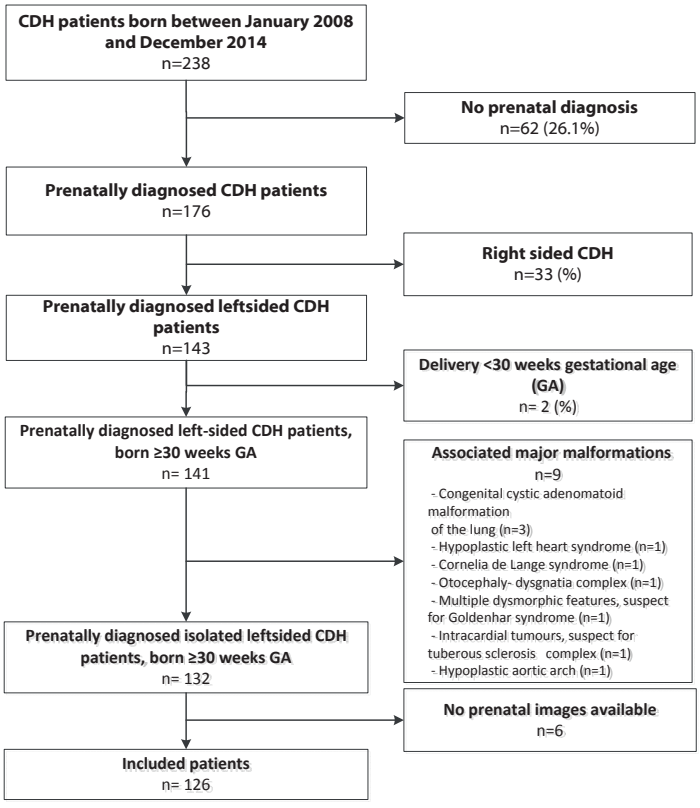
Receiver operating characteristic (ROC) curves were used to evaluate the prognostic value of O/E LHR for mortality, and development of CLD in survivors. Data were presented as areas under the ROC curves (AUC); [95% CI]. Optimal cut-off values were determined for significant AUC by maximizing the Youden index (sensitivity plus specificity minus 1). Univariable logistic regression analysis was repeated in a selection of patients in whom the CDH was detected before 24 weeks GA. Results from this group were compared with the results of the complete study group to assess whether the O/E LHR observations are missing at random. Then, for the evaluation of the predictive value of the trajectory of O/E LHR over time on survival in patients not treated with FETO, missing data of O/E LHR 19+0-24+0 weeks GA (n=64 patients), O/E LHR between 24+1-29+6 weeks GA (n=70), and O/E LHR ≥30+0 weeks GA (n=11 patients), were imputed using multiple imputation by chained equations in SPSS with 100 imputations. Using the multiple imputation dataset, a linear regression of the O/E LHR at the three time points was performed for each patient separately, with GA (coded as a continuous variable) as the only independent variable. The purpose of this analysis was to summarize the longitudinal data of O/E LHR using an estimated level (intercept in the linear regression) and time trend (slope in the linear regression). The resulting estimates of the intercept and slope in the linear regressions were then used as independent variables in logistic regressions for survival and CLD in survivors. The linear regressions were performed using Microsoft Excel 2010, and all other analyses were performed using SPSS version 21.0 for Windows. A two-sided p-value of <0.05 was considered statistically significant.

RESULTS

During the study period, 238 CDH patients were born alive with a CDH in the Netherlands. In176 patients, the CDH was prenatally detected. Reasons for exclusions for the study are summarized in Figure 1. In total, 126 patients with a prenatal diagnosis of an isolated left CDH were included. In the study group, 97 (77.0%) patients survived, and 38 (39.2%) of the survivors developed CLD (Table 1). Four patients were treated with FETO and two of them died postnatally, and of them the first O/E LHR was measured before plugging and ranged from 30.2- 40.9%.

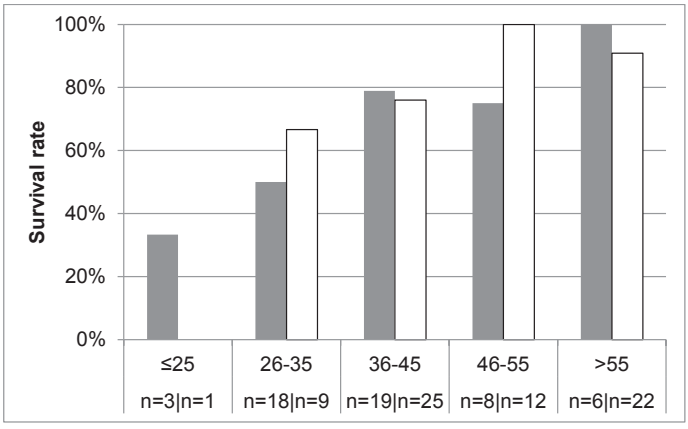
O/E LHR and survival rate of patients with CDH were not significantly different between the two centers (data not shown). The relationship between O/E LHR and survival, strati-

Figure 1 – Flowchart of inclusion



Abbreviations; CDH: congenital diaphragmatic hernia.

Figure 2 – O/E LHR per category for survival



Survival rate according to the fetal observed-to-expected lung area to head circumference ratio (O/E LHR) in fetuses with isolated left-sided diaphragmatic hernia. The filled bars represent fetuses with intrathoracic herniation of the liver and the open bars represent those without herniation.

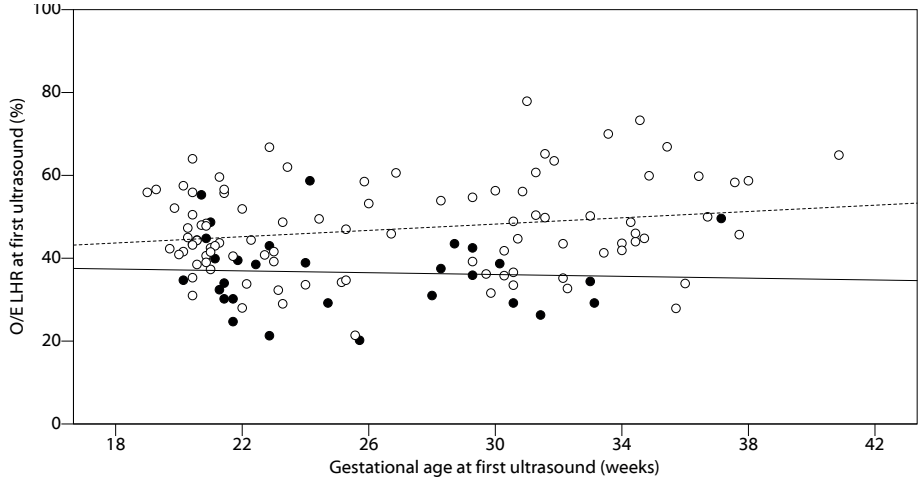
Table 1 – Background characteristics

Variables	CDH patients (n=126)
FETO	4 (3.2%)
Gestational age at delivery (weeks)	38.2 (37.7- 38.7)
Birth before 34 weeks GA	5 (4.0%)
Birth before 37 weeks GA	20 (15.9%)
Birth weight (grams)	3000 (2700- 3200)
Gender: male	65 (51.6%)
Prenatal liver position	
Intra-abdominal	69 (54.8%)
Intrathoracic	54 (42.9%)
Unknown/ missing	3 (2.4%)
Liver position (during surgery)	
Intra-abdominal	66 (52.4%)
Intrathoracic	53 (42.1%)
No repair	5 (4.0%)
Unknown/ missing	2 (1.6%)
Defect size	
A	10 (7.9%)
B	28 (22.2%)
C	59 (46.8%)
D	10 (7.9%)
No repair	5 (4.0%)
Unknown/ missing	14 (11.1%)
ECMO	41 (32.5%)
Survival in first year of life	97 (77.0%)
CLD (in survivors)	38 (39.2%)

Data were presented as numbers (%) or median (interquartile range). Defect size was classified (Pediatrics. 2007 Sep;120(3):e651-7).Abbreviations: FETO: fetoscopic tracheal occlusion; GA: gestational age; ECMO: extracorporeal membrane oxygenation; CLD: chronic lung disease.

fied by prenatal liver position in each group is shown in Figure 2. None of our patients fell in the extreme CDH group *(O/E LHR <15%)*. Only one of four patients (25%) with severe CDH *(O/E LHR ≤25%)* survived. In the moderate group *(O/E LHR 26- 35% or O/E LHR 36- 45% with intrathoracic liver position)* of 47 patients, 31 patients (66.0%) survived. In the mild group *(O/E LHR 36-45% and liver down or O/E LHR ≥46%)*, 65 (86.7%) of the 75 patients survived. In figure 2, 46 patients seem to fall in the moderate group and 73 patients in the mild group. Those differences are explained by the fact that in the moderate group for one patient prenatal liver position was unknown and in the mild group for two patients the prenatal liver position was unknown.

Figure 3 – Relationship between O/E LHR and gestational age.



The closed circles represent the babies who died and the solid line is the regression line is their regression line; the open circles represent the survivors and dashed line is their regression line.

Table 2 – Logistic regression analyses with outcomes survival and chronic lung disease in survivors

	Univariable analyses			Multivariable analyses		
Variable	**OR**	**95% CI**	***p*-value**	**OR**	**95% CI**	***p*-value**
	Survival			**Survival**		
O/E LHR	1.11	1.055- 1.171	<0.001	1.11	1.056- 1.176	<0.001
	CLD in survivors			**CLD in survivors**		
O/E LHR	0.96	0.918- 0.995	0.03	0.96	0.917- 0.995	0.03

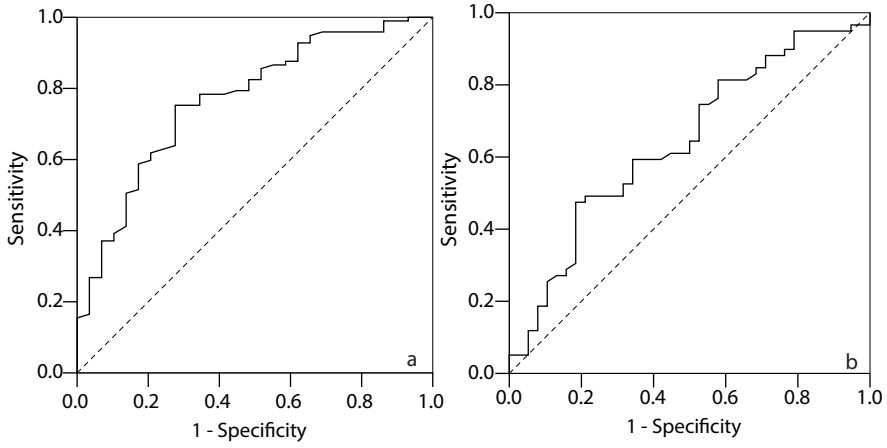
For the multivariable analyses prenatal liver position (intrathoracic versus intra-abdominal) was also included.

Abbreviations: O/E LHR; observed-to-expected lung-to-head ratio. GA: gestational age; OR: odds ratio; CI: confidence interval.

In non-survivors the median O/E LHR was 35.9 (IQR 29.7- 42.8) and 45.0 (IQR 39.9- 55.9) in survivors (p<0.001). The median of the O/E LHR was 43.8 (IQR 35.7- 48.9) in survivors with CLD and 48.0 (IQR 41.6- 57.5) in survivors without CLD (p=0.02). Figure 3 shows for each patient the relationship of the O/E LHR with gestational age, stratified by survivors and non-survivors.

Univariable logistic regression analysis showed that lower O/E LHR was significantly associated with mortality and with the development of CLD in survivors (Table 2). Multivariable logistic regression analysis with correction for prenatal liver position resulted in the same conclusions and showed that liver position was not significantly associated with the outcome (Table 2). P-values of the Hosmer-Lemeshow test were larger than 0.05, indicating an adequate model calibration. Based on ROC analysis, mortality was

Figure 4 – ROC curves for mortality and development of CLD in survivors



Receiver operating characteristics curves for the prediction of survival in fetuses with isolated left-sided congenital diaphragmatic hernia according to cut-off values of observed-to-expected lung area to head circumference ratio (continuous line). The dashed lines are the reference lines. Figure 4a: mortality; 4b: chronic lung disease (CLD).

predicted correctly by the O/E LHR with an optimal cut-off value of 40.2 (sensitivity 0.75 and specificity 0.72, AUC 0.77; [0.673- 0.865], p<0.01) (Figure 4). Development of CLD in survivors was predicted by the O/E LHR (AUC 0.64; [0.528- 0.754], p=0.02) with an optimal cut-off value of 49.9 (sensitivity 0.48 and specificity 0.18).

In non-FETO treated patients, the category of CDH severity based on O/E LHR measurements and prenatal liver position per patient remained stable for 58 patients (79.5%) of the 73 patients with at least two ultrasound measurements during gestation. In the univariable logistic regression analysis, no differences were found in predictive value of the O/E LHR on survival between the selected group of patients in whom the CDH was detected before 24 weeks gestational age and total patient population. Therefore, multiple imputation was performed in the selection of patients who were not treated with FETO. Longitudinal analyses of the trajectory of O/E LHR measurements during gestation showed no significant association with survival (data not shown).

DISCUSSION

In this nationwide study performed for the first time in an era of standardized neonatal treatment, we demonstrated that the first measured O/E LHR can predict survival in isolated left-sided CDH infants. Survival within the different O/E LHR categories was comparable with data from Jani et al., the largest multicenter study concerning the

predictive value of the O/E LHR[8]. A lower O/E LHR was significantly associated with development of CLD in survivors. The O/E LHR remained stable during gestation.

The rationale for the use of O/E LHR is that it provides an indirect assessment of contralateral lung volume, and therefore the likelihood of pulmonary hypoplasia[17]. Adequate prenatal counseling considering the indication for prenatal treatment (FETO) and expected postnatal prognosis, requires accurate prediction tools. Jani et al. retrospectively evaluated the predictive value of O/E LHR in a multicenter study of 354 isolated CDH patients (of whom 329 left-sided), who were treated without a standardized protocol in a large number of centers with both high-volume and low-volume case load on a yearly base. The present survival rates in the different categories of O/E LHR are comparable with their study. Since inclusion criteria in the TOTAL trial (moderate CDH (NCT01240057) and severe CDH (NCT00763737)) were based on that study, it is important that we can conclude that those criteria are still valid in an era of standardized neonatal treatment protocol. A difference between the two studies is, next to different patient numbers and standardized treatment, a different inclusion period (2008 -2014 in our study versus 1996-2005 in theirs). Improvements of neonatal therapy are a moving target thus this may explain the different survival rates between the two study periods (76% in our study versus 65% in their study). In the past a change in postnatal survival has resulted in a negative outcome of the original plug study and even in the US to a temporary moratorium about antenatal plugging of the trachea[18].

We found an AUC for survival of 0.77, which is comparable to previous studies (AUC 0.76 in the study by Jani et al.[8], AUC 0.78 in the study by Ruano et al[19], and AUC 0.84 in the study by Kehl et al[20]). The relevance of the cut-offs of the ROC curves for clinical practice is debatable. Deprest et al.[16] proposed a division of patients in categories (extreme/severe/moderate/mild). Since our data show that 80% of the patients remain in the same O/E LHR category during gestation, those categories seem more suitable for prenatal counseling.

We found that a lower O/E LHR was significantly associated with development of CLD in survivors. The only study that has also evaluated CLD was the multicenter study by Jani et al.[8], who found the same result. Therefore, it is likely that prenatally assessed size of the contralateral lung is not only a predictor of mortality, but also for pulmonary morbidity.

Interestingly, when prenatal liver position and O/E LHR were evaluated in the multivariable logistic regression analysis, only the O/E LHR was significantly associated with survival and development of CLD in survivors, whereas prenatal liver position was neither significantly associated with survival nor with development of CLD in survivors. This is in contrast with previous studies[11,18,21] but is consistent with the study of Jani et al.[8] The volume of intrathoracic liver could be of more value than just the fact that a part of the liver is herniated[22,23].

Since the first O/E LHR measurement for each patient was performed before the FETO procedure in the five FETO-treated patients, we decided to include the FETO-treated patients in all analyses, except for the longitudinal analysis. Because of the small number of patients, it seems not useful to perform separate analyses for this subgroup.

Strengths of our study are that we included a large cohort of isolated left CDH patients in a relatively short inclusion period who were all treated according to the same standardized treatment protocol including the same ECMO protocols, next to standardized prenatal measurements. Since one single experienced operator has performed all measurements, interobserver variability has not influenced our results.Cruz-Martinez et al. have shown that there is a learning curve for performing O/E LHR, which emphasized the importance of an experienced operator[24]. Moreover, we have used the tracing method to calculate the O/E LHR which was shown to be superior to the anteroposterior diameter method in predicting postpartum survival in isolated left-sided CDH[19].

A limitation of our study may be that, although O/E LHR was measured by one observer, measurements were performed from stored ultrasound images which may not have been the perfect section of the cross-sectional view of the fetal thorax at the level of the four-chamber view of the heart. However, if there was no suitable image available a measurement was regarded as missing. In addition, we made sure that all measurements, including outliers, were up to standard for daily clinical practice.

In conclusion, in isolated left-sided CDH patients, O/E LHR still predicts survival and development of CLD in survivors in an era of a standardized neonatal treatment protocol, and the previously established categories of severe, moderate and mild CDH remain valid.

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Chapter 3

Score for Neonatal Acute Physiology-II predicts outcome in congenital diaphragmatic hernia patients

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ABSTRACT

Objective: Accurate and validated predictors of outcome for infants with congenital diaphragmatic hernia are needed. Score for Neonatal Acute Physiology-II has been validated to predict mortality in newborns. We investigated whether Score for Neonatal Acute Physiology-II in congenital diaphragmatic hernia could predict mortality, need for extracorporeal membrane oxygenation (ECMO) (in patients born in a center with extracorporeal membrane oxygenation availability), and development of bronchopulmonary dysplasia (oxygen dependency beyond 28 days after birth) in survivors.

Design, setting: Data were obtained from a prospective, multicenter RCT of initial ventilation strategy carried out by the Congenital Diaphragmatic Hernia EURO Consortium (NTR 1310).

Patients, interventions: Congenital diaphragmatic hernia infants without severe chromosomal anomalies or severe cardiac anomalies born between November 2008 and December 2013 were randomized for initial ventilation strategy (high-frequency oscillation/ conventional mechanical ventilation).

Measurements and main results: Logistic regression analyses were used to evaluate associations between Score for Neonatal Acute Physiology-II and outcome parameters. Of the 171 included patients, 46 died (26.9%), 40 of 108 (37.0%) underwent extracorporeal membrane oxygenation, and 39 of 125 survivors (31.2%) developed bronchopulmonary dysplasia. In nonsurvivors, the median Score for Neonatal Acute Physiology-II was 42.5 (interquartile range 33.5-53.8) and 16.5 (interquartile range 9.0-27.5) in survivors ($p<0.001$). Score for Neonatal Acute Physiology-II also significantly differed between extracorporeal membrane oxygenation and non-extracorporeal membrane oxygenation-treated patients ($p<0.001$), and survivors with and without bronchopulmonary dysplasia ($p<0.001$). Multivariable logistic regression analyses adjusted for hernia side, liver position, ventilation mode, gestational age, center and observed-to-expected lung-to-head-ratio, showed that Score for Neonatal Acute Physiology-II was associated with mortality (OR 1.16 [1.09-1.23], $p<0.001$), and need for ECMO support (odds ratio 1.07 [1.02-1.13], $p=0.01$), but not for the development of bronchopulmonary dysplasia (odds ratio 1.04 [0.99-1.09], $p=0.14$).

Conclusions: The Score for Neonatal Acute Physiology-II predicts not only mortality but also need for extracorporeal membrane oxygenation in congenital diaphragmatic hernia patients. We, therefore, recommend to implement this simple and rapid scoring system in the evaluation of severity of illness in patients with congenital diaphragmatic hernia and thereby have insight into the prognosis within 1 day after birth.

INTRODUCTION

Congenital diaphragmatic hernia (CDH) occurs in approximately 1 in 2,200 live births[1]. The defect in the diaphragm allows abdominal organs to "herniate" into the thoracic cavity, which leads to underdevelopment of both lungs although more pronounced on the ipsilateral side. Lung hypoplasia and pulmonary hypertension are the main causes of respiratory failure and death. The aim of postnatal treatment is to prevent further lung damage by a gentle ventilation strategy and to stabilize the cardiopulmonary status, followed by surgical repair of the defect. If conventional management fails, infants may require extracorporeal membrane oxygenation (ECMO) with a resulting survival of 51%[2].

Although the mortality rate has significantly decreased in the past decade to about 30%[3], CDH is still a life-threatening congenital anomaly[4]. Various prenatal parameters are related to a worse prognosis, such as a low observed-to-expected lung to head ratio (O/E LHR), a right-sided hernia, herniation of the liver or stomach into the chest, and associated congenital malformations[5]. Nevertheless, some neonates with prenatally expected poor outcome survive, whereas neonates with prenatally expected good outcome die; those contradictions may be due to the inability to predict the pulmonary vascular response after birth. Reliable postnatal prediction models of survival and need of different treatment modalities in CDH infants are scarce. Recently, Brindle et al published a prediction model based on postnatal baseline indicators and showed that this model could discriminate between high, intermediate and low risk of death[6].

The Score for Neonatal Acute Physiology, version II (SNAP-II score) is a prediction model used in newborns admitted to neonatal ICUs and is based on six physiological parameters. SNAP-II score is a simplification of the original 28-items SNAP score[7]. Some studies have shown that the SNAP-II is related to worse outcome in prematurely born neonates and infants with persistent pulmonary hypertension of the newborn[8,9]. In CDH patients, retrospective studies have shown that SNAP-II were higher in patients with a worse outcome[10,11]. The studies, however, included relatively low numbers of patients (47 and 88 patients) and one was performed before the introduction of a gentle ventilation strategy[10].

We have evaluated data collected in a multicenter, prospective randomized study comparing initial ventilation strategy[12]. Our aim was to evaluate whether the SNAP-II predicted mortality, need for ECMO, and development of bronchopulmonary dysplasia (BPD) in CDH infants. We hypothesized that higher SNAP-II would have been assigned to nonsurvivors, patients with need for ECMO, and survivors who developed BPD.

MATERIALS AND METHODS

Study design and population

Data collected in a randomized clinical multicenter trial were analysed[12]. From November 2008 to December 2013, inborn neonates with prenatally detected CDH could be included in the trial. All parents gave prenatally written informed consent prior to inclusion in the study. The study was initially approved by the Erasmus MC Ethical Review Board (NTR1310), and the local institutional review boards provided institution-specific approval.

The study was conducted in nine tertiary, perinatal centers that all participated in the CDH EURO Consortium. High-volume centers were defined as more than 10 CDH patients treated in 1 year. Exclusion criteria were gestational age below 34 weeks, severe chromosomal anomalies likely to result in death in the neonatal period, severe cardiac anomalies with expected need of corrective surgery in the first 60 days after birth, renal anomalies associated with oligohydramnios, severe orthopaedic and skeletal deformities likely to influence lung development, severe anomalies of the central nervous system. We excluded patients with a gestational age below 34 weeks, so that the results could not be influenced by severe lung prematurity. Besides, a gestational age below 34 weeks is internationally considered a contraindication for neonatal ECMO treatment due to the high risk of bleeding complications, in particular, intracranial hemorrhage. After birth, children were randomized to either initial conventional mechanical ventilation (CMV) or high-frequency oscillation (HFO). All children were treated according to a standardized neonatal treatment protocol[13].

The SNAP-II score was collected within the first 12 hours after birth, and comprises six physiologic parameters; mean blood pressure, temperature, (PaO_2 (mmHg): FiO_2 (%)), serum pH, presence of multiple seizures, and urine output (Supplemental Digital Content 1)[7]. For each parameter, the worst score within the first 12 hours of life was used to calculate the SNAP-II, independent of possible treatment interventions.

BPD was defined as oxygen dependency at day 28 according to the definition of Jobe and Bancalari[14]. ECMO support (in centers with availability of ECMO only) could be initiated if one or more of the following predetermined failure criteria were met for at least 3 hours at two consecutive measurements: inability to maintain preductal saturations above 85% (± 7 kPa or 52 mmHg) or postductal saturations above 70% (± 5.3 kPa or 40 mmHg); increase in CO_2 greater than 65 torr or mmHg (8.5 kPa) despite optimization of ventilatory management; peak inspiratory pressure greater than 28 cm H_2O or mean arterial pressure greater than 17 cm H_2O; inadequate oxygen delivery with metabolic acidosis defined as lactate greater than or equal to 5 mmol/L and pH less than 7.20 and/or hypotension resistant to fluid therapy and adequate inotropic support, resulting in a urine output less than 0.5 mL/kg/hour; and oxygenation index consistently greater than

or equal to 40[12]. None of the patients was transferred from a non-ECMO to an ECMO center.

Data analysis

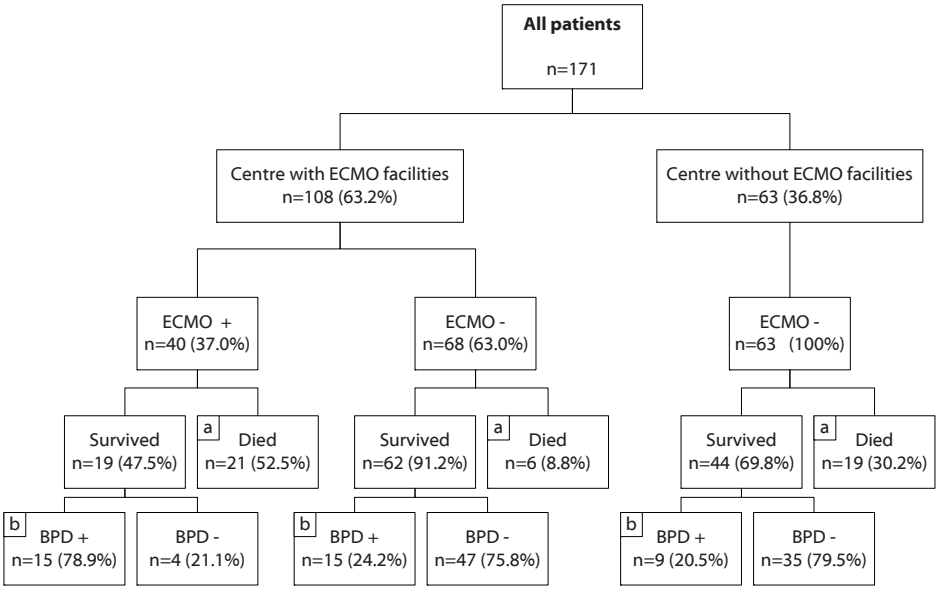
Patient characteristics are described as number (%), mean± SD or median (interquartile range; IQR). Survival rates of patients born in a center with ECMO facilities and patients born in a center without ECMO facilities were compared using the chi-square test. SNAP-II were compared between survivors and nonsurvivors, patients with and without need of ECMO treatment (in patients born in a center with availability of ECMO only), and the presence of BPD (in survivors only) using the Mann-Whitney *U* test. SNAP-II were compared between centers using the Kruskal-Wallis test. Independent associations between SNAP-II and mortality, ECMO support (in patients born in a center with availability of ECMO only), and BPD (in survivors only) were evaluated using univariable logistic regression modeling and were presented as odds ratio (OR) [95% CI], *p* value. In multivariable logistic regression analyses, SNAP-II, gestational age, initial ventilation type, side of defect, center, O/E LHR, and position of the liver were included as independent variables. For BPD, ECMO support was also included as independent variable in the multivariable logistic regression analysis. The calibration of the multivariable logistic regression models were assessed using the Hosmer-Lemeshow goodness-of-fit test. Receiver operating characteristic (ROC) curves were used to evaluate the prognostic value of SNAP-II for mortality, need of ECMO support (in patients with availability of ECMO only), and development of BPD (in survivors only). Those data were presented as areas under the ROC curves (AUC); [95% CI]. All analyses were performed using SPSS version 21.0 for Windows. A two-sided *p* value of <0.05 was considered statistically significant.

RESULTS

One hundred and seventy-one patients were included in the analysis of whom 108 (63.2%) were born in a hospital with ECMO facilities. Forty-six patients (26.9%) died, 40 of the 108 infants born in a center with ECMO facilities received ECMO (37.0%), and 39 of the 125 survivors (31.2%) developed BPD (Figure 1) (Table 1). In six centers, ECMO was available; two centers had no ECMO availability; and in one center ECMO was available from January 1, 2013, onward. Twenty-seven patients (25.0%) born in a center with ECMO facilities died versus 19 patients (30.2%) born in a center without ECMO facilities, p=0.46.

The SNAP-II scores were not significantly different between centers (data not shown). The SNAP-II scores were not significantly different between the two ventilation groups (CMV (median 21.0 (IQR 10.0 to 40.0)) and HFO (median 25.0 (IQR 14.0 to 40.0)), p=0.44. Of the nine centers, five were high-volume centers and four were low-volume centers.

Figure 1 – Flowchart of included patients



Abbrevations: ECMO: extracorporeal membrane oxygenation. BPD: bronchopulmonary dysplasia. a: total of patients that died: n=46/ 171 (26.9%) b: total of survivors with BPD: n=39/ 125 (31.2%).

The median SNAP-II scores were comparable in high-volume centers (median 23.0 (IQR 10.0 to 37.0)) and low-volume centers (median 25.0 (IQR 16.0 to 41.0)), p=0.21.

In nonsurvivors the median SNAP-II score was 42.5 (IQR 33.5 to 53.8) and 16.5 (IQR 9.0 to 27.5) in survivors (p<0.001). In the selection of the 108 patients born in a center with ECMO facilities, the median SNAP-II score was 35.0 (IQR 30.0 to 46.0) in patients treated with ECMO and 16.0 (IQR 10.0 to 26.0) in patients without ECMO treatment (p<0.001). Survivors with BPD had a median SNAP-II score of 25.0 (IQR 21.0 to 35.0) versus 14.0 (IQR 7.0 to 21.0) in those without BPD (p<0.001). In a subgroup of patients who were treated with ECMO (n=40), 19 survived and 21 died. Of the 19 survivors, the median SNAP-II score was 32 (IQR 23 to 44), and the median SNAP-II score was 40 (IQR 32 to 51) for nonsurvivors, p=0.04.

Univariable logistic regression analyses showed that SNAP-II score was significantly associated with mortality (OR 1.11 [1.08- 1.15], p<0.001), need of ECMO support (OR 1.08 [1.05- 1.12], p<0.001) (in patients born in a center with ECMO facilities), and BPD in survivors (OR 1.07 [1.04- 1.11], p<0.001) (Table 2).

Multivariable logistic regression analyses correcting for O/E LHR, defect side, liver position, type of ventilation, center, and gestational age, demonstrated that SNAP-II score was significantly associated with mortality (OR 1.16 [1.09- 1.23], p<0.001), and need for ECMO support in patients born in a center with ECMO facilities (OR 1.07 [1.02- 1.13],

Table 1 – Patient characteristics

Variable	n=171
Gestational age (weeks)	38.0± 1.3
Birth weight (grams)	2923± 460
Fetoscopic endotracheal occlusion	19 (11.1%)
Male gender	84 (49.1%)
Left sided defect	148 (86.5%)
Liver position: intrathoracic	101 (59.1%)
Type of repair	
Patch correction	92 (53.8%)
Primary closure	53 (31.0%)
No repair	26 (15.2%)
Defect size	
A	11 (6.4%)
B	49 (28.7%)
C	68 (39.8%)
D	12 (7.0%)
No repair	26 (15.2%)
Missing	5 (2.9%)
Treatment with nitric oxide	84 (49.1%)
Treatment with inotropics	146 (85.4%)
Initial ventilation mode: HFO	80 (46.8%)
Age at repair (days)	4.0 (3.0- 6.5)
Ventilation time (days)	11.5 (7.0- 20.5)
ICU admission (days)	22.0 (13.0- 40.0)

Results are presented as n (%), mean± SD or median (IQR).
Abbreviations: HFO: high-frequency oscillation. ICU: intensive care unit.

Table 2 – Associations of SNAP-II score with death, need for ECMO, BPD

Outcome	OR	*p*-value	95% CI
Mortality (n=171)	1.11	<0.001	1.08- 1.15
Need for ECMO (n=108)	1.08	<0.001	1.05- 1.12
BPD (n= 125)	1.07	<0.001	1.04- 1.11

Abbreviations: ECMO: extracorporeal membrane oxygenation; BPD: bronchopulmonary dysplasia; OR: odds ratio; CI: confidence interval.

p=0.01). In the multivariable logistic regression analysis with correction for O/E LHR, defect side, liver position, type of ventilation, center, gestational age, and adding the variable ECMO support, we found that the SNAP-II score did not predict the development of BPD in survivors (OR 1.02 [0.97- 1.08], p=0.46) (Table 3). The *p*-values of the Hosmer-Lemeshow tests were larger than 0.05, indicating an adequate model calibration.

Based on ROC analysis, SNAP-II scores predicted mortality (AUC 0.88; [0.82-0.94], p<0.001), need for ECMO support (in patients born in a center with ECMO facilities) (AUC 0.81; [0.72-0.90], p<0.001) and BPD development in survivors (AUC 0.77; [0.68-0.86], p<0.001) (Figure 2).

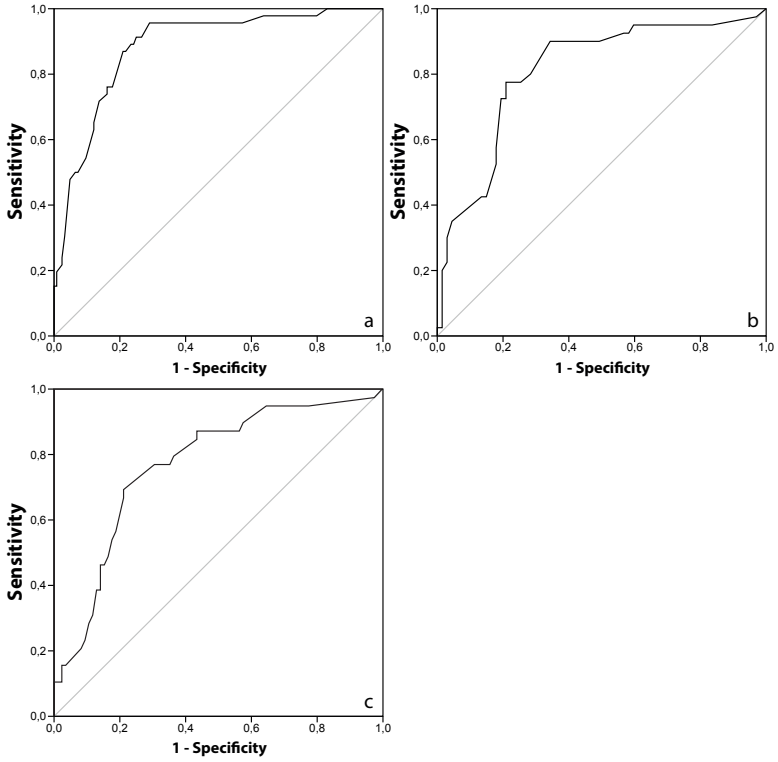
DISCUSSION

We have demonstrated in this prospective study on a large cohort of prenatally detected CDH patients that the SNAP-II score calculated within the first 12 hours of life predicted the outcome of survival, and need for ECMO support in inborn patients with CDH.

Skarsgard et al have assessed the ability of admission SNAP-II score to predict mortality in 88 infants with CDH born between January 1996 and October 1997 in the Canadian Neonatal Network database[10]. This was a retrospective, multicenter study with patients selected based on the International Classification of Diseases, 9th Edition code (756.6) for diaphragmatic anomalies and included patients ventilated before the introduction of the gentle ventilation strategy. They found that SNAP-II predicted mortality with an OR of 1.06 and AUC of 0.76. In our study, we found a comparable OR (1.11) and AUC (0.88). It is possible that the higher predictive value we found may be due to the greater homogeneity of our study population when compared with that of Skarsgard's report that derived from centers with different treatment protocols.

In 2001, The Congenital Diaphragmatic Hernia Study Group estimated disease severity in the first 5 minutes after birth in more than one thousand neonates with CDH[15]. In that retrospective study, a prediction model based on variables obtained at the time of birth such as birth weight and 5-minute Apgar score was designed to estimate risks for populations and they found that those variables were most useful in a predictive equation. It is not, however, clear whether those variables were obtained before or after intubation. More recently, Schultz et al retrospectively investigated the Wilhord Hall/ Santa Rosa prediction formula (pO_2[max]– pCO_2[max]) and showed that mortality in CDH patients could be predicted with that formula[16]. They, however, included a relatively small number of patients, and the infants were not treated according to a standardized treatment protocol. Subsequently, Baird et al compared 3 different prediction models in the same cohort of 94 infants[17]. They showed that the prediction model of the Con-

Figure 2 – ROC curves



Receiver operating characteristic (ROC) curves
a: mortality b: extracorporeal membrane oxygenation (ECMO) c: bronchopulmonary dysplasia (BPD)

genital Diaphragmatic Hernia Study Group predicted mortality best with an AUC of 0.85, followed by SNAP-II score (AUC 0.79). In addition, they concluded that gestational age did not improve the prediction model, which is consistent with our results. A limitation of their study is that not all the components of the SNAP-II score, for example, lowest temperature, were not collected, and the SNAP-II score was not routinely calculated and recorded.

In the study from Brindle et al[6], postnatal variables were collected, including birth weight, 5-minute Apgar score, presence of chromosomal or major cardiac anomaly, and suprasystemic pulmonary hypertension. Their model identified infants at low, intermediate, and high risk of death. The data from the CDH registry, however, were obtained from voluntary participation, which had potential selection bias. In future studies, a combination of the SNAP-II score and the prediction model published by Brindle et al may further improve risk stratification of CDH infants. Chiu et al recently showed in 52 outborn CDH patients that Score for Neonatal Acute Physiology with Perinatal Extension-II (SNAPPE) predicted survival and need for ECMO well[18]. In the SNAPPE score, however, Apgar

Table 3 – Associations of SNAP-II score with death, need for ECMO, BPD

Outcome	OR	*p*-value	95% CI
Mortality			
SNAP-II score	1.16	<0.001	1.09- 1.23
O/E LHR	0.96	0.04	0.92- 1.00
Side: left	0.26	0.18	0.04- 1.83
Liver: intrathoracic	1.37	0.66	0.34- 5.50
Initial ventilation: HFO	2.48	0.16	0.69- 8.86
Gestational age	0.74	0.24	0.44- 1.22
Center	0.85	0.21	0.65- 1.10
Need for ECMO			
SNAP-II score	1.07	0.01	1.02- 1.13
O/E LHR	0.95	0.04	0.91- 1.00
Side: left	0.44	0.41	0.06- 3.10
Liver: intrathoracic	4.38	0.04	1.08- 17.83
Ventilation: HFO	1.78	0.35	0.53- 6.00
Gestational age	0.94	0.83	0.56- 1.60
Center	1.29	0.04	1.01- 1.64
BPD			
SNAP-II score	1.02	0.46	0.97- 1.08
O/E LHR	0.99	0.67	0.97- 1.02
Side: left	1.10	0.92	0.15- 8.18
Liver: intrathoracic	6.71	0.003	1.94- 23.22
Initial ventilation: HFO	1.13	0.83	0.38- 3.38
Gestational age	0.81	0.37	0.51- 1.28
Center	1.04	0.79	0.81- 1.33
Need for ECMO	6.00	0.03	1.15- 31.20

Abbreviations: ECMO: extracorporeal membrane oxygenation; BPD: bronchopulmonary dysplasia; OR: odds ratio; CI: confidence interval; O/E LHR: observed-to-expected lung-to-head ratio; HFO: high-frequency oscillation.

score, birth weight and small for gestational age are also included, which makes this score more time consuming to calculate. Furthermore, in their study, only outborn CDH patients were included, whereas we only included inborn CDH patients.

We investigated the role of the SNAP-II score in the prediction of the need of ECMO. This was recently researched by Coleman et al[11]. In their retrospective study, they found that in outborn CDH patients, the SNAP-II score calculated within the first 24 hours after admission predicted the use of ECMO support with an AUC of 0.76. We calculated SNAP-II score prospectively in the first 12 hours after birth, hence our study population was more homogenous than that of the previous study[11] and found an AUC of 0.81. A

prediction study based on blood gas analyses showed that infants with a $PaCO_2$ greater than 60mmHg on the first arterial blood gas had a higher 90-day mortality rate and were more likely to receive ECMO[19]. They, however, did not report the site of obtaining the blood samples (pre- or postductal) and they did not perform ROC curves so our results cannot be compared with theirs. Survival rates following ECMO found in the current study are comparable with those reported by Stevens et al[20]. On the other hand, Downard et al reported that 12 of the 14 patients treated with ECMO survived, which means a high survival rate of 86%[21]. In the study of Downard et al, however, 28% of the patients were outborn, which is associated with a favorable prognosis[22]. Furthermore, our study population may have been more severely ill. Downard et al regrettably did not report parameters associated with survival such as gestational age, lung-to-head ratio and liver position (intrathoracic or intra-abdominal).

We also investigated whether the SNAP-II score predicted BPD in surviving CDH patients. To the best of our knowledge, this has not been done in previous studies. A large number of patients who were treated with ECMO subsequently died, and many survivors who were treated with ECMO developed BPD (Figure 1). This could explain why SNAP-II score predicted the development of BPD in survivors in the univariable logistic regression analysis, but not in the multivariable logistic regression analysis in which ECMO was added as independent variable.

In various reports of patients with CDH, other nonpatient-related factors were analyzed for association with the outcome. For example, Nasr and Langer showed that in 140 infants location of delivery influenced the outcome[22]. In our study, all patients were inborn so a bias of severity of illness due to location of birth did not influence our results. Grushka et al evaluated the effect of hospital case volume on the outcome in 121 CDH patients[23]. They found that SNAP-II score was not significantly different for high- compared with low-volume centers; this was confirmed in our study.

Our data were collected in a randomized clinical trial, and the difference in initial ventilation strategy could be seen as a study limitation. SNAP-II score, however, was not significantly different between the 2 ventilation groups, and the type of ventilation was not significantly associated with outcome in the multivariable logistic regression modeling. The optimal *initial* ventilation strategy was recently investigated[12]. We have shown that independent of ventilation strategy, SNAP-II score reliably predicts outcome. Therefore we do not think that this has influenced our results. A limitation of the SNAP-II score itself is that one of the items concerns the presence of multiple seizures. It is known, however, that neonatal seizures can be very subtle so possibly some of the studied patients may have experienced unrecognized seizures in the first 12 hours after life[24]. The strengths of the current study are that, besides initial ventilation strategy, all children were treated according to the same study protocol. Furthermore, all children were inborn, which means that information from birth onward was available. A strength

of the SNAP-II score is that data collection of the six scoring items takes only 2-4 minutes[7]. Future studies should address whether the use of a combination of the SNAP-II score and other predictive (biochemical) markers such as lactate[25], troponin T[26], and endothelin-1[27], could further improve prediction of prognosis in CDH patients.

CONCLUSIONS

Determining SNAP-II score in the first 12 hours after birth is a reliable, not time-consuming scoring system to predict outcome in antenatally diagnosed CDH patients with a gestational age of more than 34 weeks. We, therefore, recommend to implement this simple and rapid scoring system in the evaluation of severity of illness in patients with CDH, to thereby obtain insight into the prognosis within 1 day after birth. For research purposes, it can also be used to compare severity of illness to evaluate differences in outcomes between centers.

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Supplemental digital content 1 - Score for Neonatal Acute Physiology-II

Variables	**Values**	**Points**
Mean blood pressure	≥ 30 mmHg	☐ 0 points
	20 – 29 mmHg	☐ 9 points
	< 20 mmHg	☐ 19 points
Lowest temperature	>35.6 °C = > 96°F	☐ 0 points
	35° - 35.6°C = 95° - 96°F	☐ 8 points
	< 35.0°C = < 95°F	☐ 15 points
pO_2 (mmHg) : FiO_2 (%)	> 2,49	☐ 0 points
	1,0 – 2,49	☐ 5 points
	0,3 – 0,99	☐ 16 points
	< 0,3	☐ 28 points
Lowest serum pH	≥ 7,2	☐ 0 points
	7,10 – 7,19	☐ 7 points
	< 7,10	☐ 16 points
Multiple seizures	No	☐ 0 points
	Yes	☐ 19 points
Urine output (ml./kg./hr.)	≥ 1	☐ 0 points
	0,1 – 0,9	☐ 5 points
	< 0,1	☐ 18 points
Total score		**…… points**

Please fill in the worst score within the first 12 hours of life

Chapter 4

Sphingolipids in congenital diaphragmatic hernia; results from an international multicenter study

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ABSTRACT

Background: Congenital diaphragmatic hernia is a severe congenital anomaly with significant mortality and morbidity, for instance chronic lung disease. Sphingolipids have shown to be involved in lung injury, but their role in the pathophysiology of chronic lung disease has not been explored. We hypothesized that sphingolipid profiles in tracheal aspirates could play a role in predicting the mortality/ development of chronic lung disease in congenital diaphragmatic hernia patients. Furthermore, we hypothesized that sphingolipid profiles differ between ventilation modes; conventional mechanical ventilation versus high-frequency oscillation.

Methods: Sphingolipid levels in tracheal aspirates were determined at days 1, 3, 7 and 14 in 72 neonates with congenital diaphragmatic hernia, born after > 34 weeks gestation at four high-volume congenital diaphragmatic hernia centers. Data were collected within a multicenter trial of initial ventilation strategy (NTR 1310).

Results: 36 patients (50.0%) died or developed chronic lung disease, 34 patients (47.2%) by stratification were initially ventilated by conventional mechanical ventilation and 38 patients (52.8%) by high-frequency oscillation. Multivariable logistic regression analysis with correction for side of the defect, liver position and observed-to-expected lung-to-head ratio, showed that none of the changes in sphingolipid levels were significantly associated with mortality/development of chronic lung disease. At day 14, long-chain ceramides 18:1 and 24:0 were significantly elevated in patients initially ventilated by conventional mechanical ventilation compared to high-frequency oscillation.

Conclusions: There is no predictive role of tracheal aspirates sphingolipid levels for mortality/development of chronic lung disease in congenital diaphragmatic hernia infants. Elevated levels of ceramides 18:1 and 24:0 in the conventional mechanical ventilation group when compared to high-frequency oscillation could probably be explained by high peak inspiratory pressures and remodelling of the alveolar membrane.

INTRODUCTION

In patients with congenital diaphragmatic hernia (CDH), lung related problems such as chronic lung disease (CLD) and pulmonary vascular disease including pulmonary hypertension are the primary causes of mortality[1] with ventilator-induced lung injury (VILI) and high concentrations of oxygen predisposing newborns to develop CLD as prime morbidity[2].

Since 2008, CDH neonates are treated according to the same neonatal treatment protocol, developed during a consensus meeting of the CDH EURO Consortium[3]. Prenatally diagnosed CDH infants are intubated after birth and mechanically ventilated. Conventional mechanical ventilation (CMV) and high-frequency oscillation (HFO) are two ventilation modalities that are associated with VILI and thus predispose to developing CLD. In a randomized clinical trial (the VICI-trial) looking at initial ventilation strategy (CMV vs HFO) of prenatally diagnosed CDH infants, 41 of the 91 patients (45.1%) initially ventilated with CMV died or had CLD by day 28 compared to 43 of the 80 patients (53.8%) using HFO[4]. This difference, however, did not reach statistical significance.

The exact mechanism for the development of CLD in CDH remains unknown. In postmortem lung biopsies non-specific features as persistent inflammation, edema, vascular changes and parenchymal fibrosis were observed after mechanical ventilation[5]. Due to changes in treatment modalities over the years in premature born neonates, the so-called 'new BPD' developed, characterized by interrupted septation and abnormal vascularization, leading to fewer and enlarged alveoli[6]. In contrast to premature born neonates, lungs of fetuses with CDH are not surfactant deficient[7], and surfactant replacement therapy has no beneficial effect in term neonates with CDH[8].

Sphingolipids are classically thought to be purely structural elements of the cell membrane, but have been revealed as key bioactive mediators in a variety of pathophysiological processes[9]. They have an important role as messenger molecule in the regulation of proliferation and apoptosis[10]. Sphingolipids are involved in lung development, injury and repair as suggested by elevated sphingolipid levels in brochoalveolar lavage of newborn rats exposed to hyperoxia (injury model of CLD) which declined during subsequent ambient air exposure (repair)[11].

Given the lack of knowledge on the pathogenesis of CLD in CDH, we have analyzed the bronchoalveolar lavage for sphingolipids in tracheal aspirates at specific time-points during the first month of a prospective ventilation study (VICI-trial)[4]. We hypothesized that sphingolipid profiles could have a predictive role for mortality/development of CLD. Secondly, we aimed to determine whether CMV versus HFO leads to different sphingolipid levels in CDH patients. These aims were achieved.

MATERIALS AND METHODS

Patient Population

Inborn neonates born between November 2008 and December 2013, after a gestation of more than 34 weeks with a prenatal diagnosis of CDH, were included in a multicenter RCT of initial ventilation strategy (NTR 1310)[4]. Ethical approval was given by the medical ethics review board of Erasmus MC, Rotterdam, the Netherlands (NTR 1310). Thereafter, all local medical ethical committees gave their approval (Bambino Gesu Children's Hospital, Universitätsklinikum Mannheim, Radboud University Medical Centre). Parents gave written informed consent. The procedures, including obtaining informed consent, were conducted in accord with the ethical standards of the Committee on Human Experimentation of the institution in which the experiments were done. Exclusion criteria were: severe chromosomal anomaly such as trisomy 13 or 18, which may imply a decision to stop or not to start medical treatment; severe cardiac anomalies expected to need corrective surgery in the first 60 days after birth; renal anomalies associated with oligohydramnios; severe orthopedic and skeletal deformities which were likely to influence thoracic or lung development; and severe anomalies of the central nervous system. Patients with a gestational age of less than 34 weeks were excluded so that the results could not be influenced by severe lung prematurity. Written parental informed consent was obtained before birth. All children were treated according to the same standardized protocol[3]. Patients were randomized for initial ventilation strategy (CMV or HFO) within two hours after birth. CLD was defined as oxygen dependency (>21%) at day 28 as described by Jobe and Bancalari[12]. Diaphragmatic defect size was classified according to the definition of the CDH study group and assessed during surgical repair[13].

Tracheal Aspirates

Tracheal aspirates were obtained during routine tracheal suctioning within the first 24 hours of life and at day 3, 7, and 14. Tracheal aspirates were only collected during the period of mechanical ventilation. During tracheal suctioning, flushing with 0.5-1.0 ml saline was performed according to standard practice. Tracheal aspirates were immediately centrifuged at 1500g for 6 minutes at 20 °C and samples were stored at −80°C until analysis.

Sphingolipid measurements

Sphingolipids were measured as previously described[11]. Briefly, lipids were extracted from tracheal aspirate samples and sphingolipids were separated and analyzed using high performance liquid chromatography and tandem mass spectrometry (LC-MS/MS). The analyses were performed at the Analytical Facility for Bioactive Molecules of the Hospital for Sick Children, Toronto, ON, Canada. Sphingolipid levels were presented as ng/mL.

Statistical analysis

Patient characteristics were described as number (%) for categorical variables, mean ± SD for normally distributed variables or median (interquartile range; IQR) for continuous variables that were not normally distributed. Patient characteristics between participants and non-participants were analyzed using independent samples t-test for continuous data or chi square tests for categorized data. Sphingolipid levels were compared between patients who died/developed CLD and patients who survived/did not develop CLD, and between patients who were initially ventilated by CMV and HFO by using the Mann-Whitney U test. All analyses were performed for each of the four time-points separately. Independent associations between sphingolipids levels and mortality/ development of CLD were determined using multivariable logistic regression modelling and were presented as odds ratio (OR) [95% confidence interval], *p*-value. Observed-to-expected lung-to-head ratio (O/E LHR), side of the defect, liver position, centre and ventilation mode were included as independent variables in these models. Sphingolipid levels were logarithmically transformed due to skewed distribution, and non-detectable values were set to the lower detection threshold 0.03 ng/mL. The calibration of the multivariable logistic regression models was assessed using the Hosmer-Lemeshow goodness-of-fit test. All statistical tests, except for the analyses to determine difference in patients characteristics between participants and non-participants, were two-sided and used a Bonferroni-adjusted significance level of 0.0125 to correct for multiple testing at the four time points. All analyses were performed using SPSS version 21.0 for Windows statistical software.

RESULTS

Tracheal aspirates were collected in 69 of the 171 infants who were included in the RCT at the four participating centers. Additionally, in three patients from one center there was only written consent for sample collection, and not for randomization of initial ventilation mode. Thus, tracheal aspirates from 72 patients were collected at various time-points. 179 tracheal aspirates were obtained, of which 49 were collected at day 1, 56 samples at day 3, 46 samples at day 7, and 21 samples at day 14. Patients who had tracheal aspirates collected showed an increased prevalence of left-sided diaphragm defect compared to the patients who had no aspirates collected (Table 1). Of the 72 included patients, 16 patients (22.2%) died in the first year of life, and 20 of the 56 survivors (35.7%) developed CLD; thus, 36 patients (50.0%) died/developed CLD. Thirty-eight patients (52.8%) were initially ventilated by HFO and 34 patients (47.2%) by CMV.

No significant differences in sphingolipid profiles were found at day 1, 3, 7 and 14 between patients who died/developed CLD and patients who survived/did not develop

Table 1 – Patient characteristics

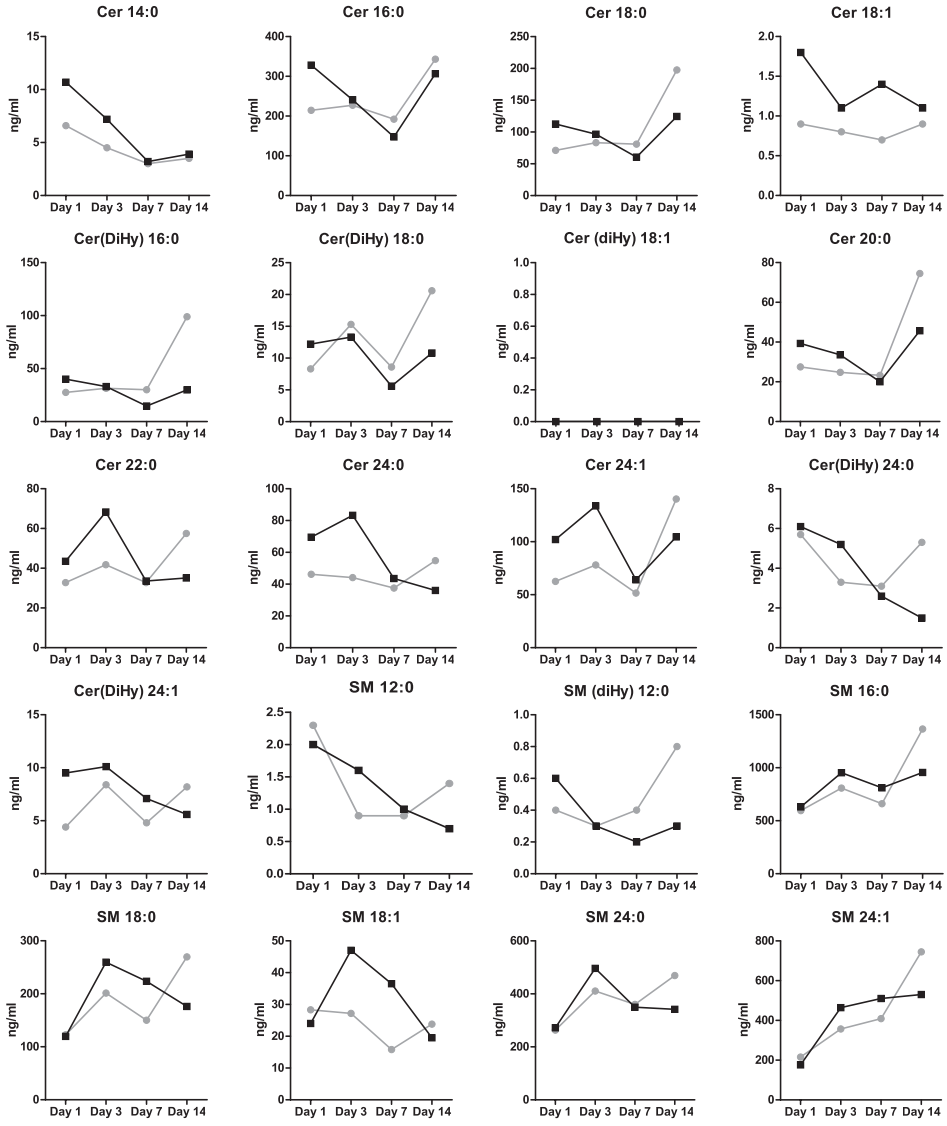
	Included patients n=72	Non-included patients n=102	*p*-value
Gestational age (weeks)	38.0± 1.2	37.9± 1.4	0.66
Birth weight (grams)	2957± 443	2901± 467	0.43
Fetoscopic endotracheal occlusion	5 (6.9%)	14 (13.7%)	0.16
Male gender	33 (45.8%)	51 (50.0%)	0.59
Left sided defect	68 (94.4%)	83 (81.4%)	0.01
Liver position: intrathoracic	37 (51.4%)	66 (64.7%)	0.08
Type of repair			0.06
Patch correction	48 (66.7%)	46 (45.1%)	
Primary closure	19 (26.4%)	35 (34.3%)	
No repair	5 (6.9%)	21 (20.6%)	
Diaphragmatic defect size			0.05
A	5 (6.9%)	7 (6.9%)	
B	5 (6.9%)	28 (27.4%)	
C	21 (29.2%)	34 (33.3%)	
D	35 (48.7%)	10 (9.8%)	
No repair	5 (6.9%)	21 (20.6%)	
Missing	1 (1.4%)	2 (2.0%)	
Treatment with nitric oxide	33 (45.8%)	53 (52.0%)	0.43
ECMO (if ECMO was available)	22/ 56 (39.3%)	20/ 55 (36.4%)	0.76
Treatment with inotropics	60 (83.3%)	89 (87.2%)	0.47
Age at repair (days)	5.0 (3.0- 9.0)	4.0 (3.0- 6.0)	0.28
Ventilation time (days)	15.0 (7.8- 23.0)	10.0 (7.0- 17.5)	0.29
ICU admission (days)	22.5 (15.3- 39.8)	20.5 (13.0- 40.5)	0.99

Data are presented as n (%), mean± SD or median (IQR).
Abbreviations: ECMO: extracorporeal membrane oxygenation. HFO: high-frequency oscillation; ICU: intensive care unit.

CLD (Table 2). Median sphingolipid levels over time for each sphingolipid are presented in Figure 1. Multivariable logistic regression analyses with correction for O/E LHR, side of the defect, liver position, center and ventilation mode showed that none of the sphingolipid levels were associated with mortality/development of CLD (Table 3). The p-values of the Hosmer-Lemeshow test were larger than 0.05, indicating an adequate model calibration.

No significant differences in sphingolipid profiles were found at day 1, 3, and 7 between patients who were initially ventilated by CMV versus HFO (Table 4). At day 14, ceramide-C18:1 and ceramide-C24:0 were increased for patients initially ventilated by CMV (median 1.4 (IQR 1.1- 4.3)) and (median 81.0 (IQR 33.6- 205.2)) respectively compared to HFO (median 0.5 (0.0- 1.1)) (p=0.005) and (median 25.3 (IQR 2.9- 53.0))

Figure 1 – Median values of sphingolipids over time



Black: CLD or died. Grey: No CLD or alive.

respectively (p=0.008). In a selection of patients who died/developed CLD, at day 14 ceramide-C18:1 was increased for patients initially ventilated by CMV (median 1.4 (IQR 1.2- 5.6)) compared to HFO (median 0.5 (0.1- 1.1)) (p= 0.009), but ceramide-C24:0 was significantly increased in patients initially ventilated by CMV (median 102.5 (IQR 31.3- 225.9)) compared to HFO (median 23.3 (IQR 6.4- 33.7)) (p=0.011).

Table 2 – Associations of sphingolipids with outcome mortality/ development of CLD

	Day 1		Day 3		Day 7		Day 14	
Independent variable	Median (IQR)	*p*-value	Median (IQR)	*p*-value	Median (IQR)	*p*-value	Median (IQR)	*p*-value
Cer 14:0		0.37		0.35		0.76		0.76
No CLD or alive	6.6 (3.0- 14.6)		4.5 (2.7- 10.8)		3.0 (1.5- 7.3)		3.5 (0.8- 8.0)	
CLD or died	10.7 (5.1- 20.0)		7.2 (2.6- 14.5)		3.2 (0.9- 13.2)		3.9 (1.9- 8.7)	
Cer 16:0		0.70		0.73		0.43		0.97
No CLD or alive	214.5 (144.1- 487.8)		227.0 (118.4- 380.4)		192.0 (114.0- 730.0)		343.0 (81.6- 649.1)	
CLD or died	328.0 (120.0- 612.0)		240.8 (133.9- 504.8)		147.5 (48.0- 565.0)		306.5 (122.5- 781.9)	
Cer 18:0		0.86		0.90		0.50		0.90
No CLD or alive	71.2 (41.9- 176.4)		83.2 (45.5- 167.1)		81.0 (45.2- 268.0)		197.5 (50.5- 278.8)	
CLD or died	112.5 (33.7- 265.5)		96.5 (43.5- 164.8)		60.5 (15.5- 183.5)		124.5 (37.0- 339.4)	
Cer 18:1		0.57		0.78		0.61		0.64
No CLD or alive	0.9 (0.3- 2.0)		0.8 (0.5- 1.8)		0.7 (0.2- 2.9)		0.9 (0.2- 1.7)	
CLD or died	1.8 (0.1- 3.5)		1.1 (0.4- 2.7)		1.4 (0.1- 3.4)		1.1 (0.4- 2.5)	
Cer(DiHy)16:0		0.61		0.64		0.60		0.93
No CLD or alive	27.6 (14.1- 73.0)		31.4 (15.4- 60.0)		30.1 (12.4- 53.0)		98.9 (1.3- 196.5)	
CLD or died	40.0 (12.5- 104.3)		33.1 (14.7- 92.5)		14.7 (4.5- 84.6)		29.9 (18.8- 97.8)	
Cer(DiHy)18:0		0.67		0.94		0.53		0.52
No CLD or alive	8.3 (3.7- 17.7)		15.3 (7.0- 22.8)		8.6 (3.3- 36.2)		20.6 (5.3- 64.5)	
CLD or died	12.2 (2.8- 31.3)		13.3 (7.3- 31.5)		5.6 (2.1- 26.3)		10.8 (5.7- 29.5)	
Cer(DiHy)18:1		0.35		0.93		0.04		0.97
No CLD or alive	0.03 (0.03- 0.03)		0.03 (0.03- 0.03)		0.03 (0.03- 0.03)		0.03 (0.03- 0.56)	
CLD or died	0.03 (0.03- 0.30)		0.03 (0.03- 0.03)		0.03 (0.03- 1.21)		0.03 (0.03- 0.13)	

Table 2 – (continued)

	Day 1		Day 3		Day 7		Day 14	
Independent variable	Median (IQR)	*p*-value	Median (IQR)	*p*-value	Median (IQR)	*p*-value	Median (IQR)	*p*-value
Cer 20:0		0.69		0.46		0.46		0.90
No CLD or alive	27.5 (15.6- 67.9)		24.8 (14.1- 52.2)		23.2 (15.0- 94.5)		74.5 (16.3- 105.5)	
CLD or died	39.3 (14.1- 108.0)		33.6 (20.2- 58.2)		20.0 (5.4- 66.5)		45.7 (14.0- 122.2)	
Cer 22:0		0.59		0.16		0.65		0.83
No CLD or alive	32.8 (18.0- 88.5)		41.8 (14.6- 67.9)		32.8 (16.0- 137.5)		57.5 (14.7- 109.9)	
CLD or died	43.5 (17.7- 198.8)		68.3 (24.4- 91.9)		33.6 (3.9- 108.5)		35.1 (20.0- 130.5)	
Cer 24:0		0.42		0.16		0.76		0.890
No CLD or alive	46.2 (24.7- 122.5)		44.1 (17.1- 105.0)		37.5 (14.4- 217.5)		54.8 (14.6- 114.6)	
CLD or died	69.5 (25.3- 340.3)		83.3 (24.5- 161.0)		43.5 (4.4- 125.5)		36.0 (23.1- 102.3)	
Cer 24:1		0.52		0.23		0.65		0.90
No CLD or alive	62.5 (36.1- 174.5)		78.0 (27.5- 157.3)		51.5 (29.9- 449.5)		140.3 (32.3- 343.4)	
CLD or died	102.0 (32.7- 352.0)		133.8 (49.3- 196.6)		64.0 (10.0- 203.5)		104.5 (40.7- 306.5)	
Cer(DiHy)24:0		0.96		0.31		0.48		0.41
No CLD or alive	5.7 (2.8- 13.2)		3.3 (1.2- 6.7)		3.1 (0.8- 11.0)		5.3 (2.1- 11.3)	
CLD or died	6.1 (2.5- 22.8)		5.2 (1.6- 12.2)		2.6 (0.3- 11.4)		1.5 (1.0- 8.9)	
Cer(DiHy)24:1		0.42		0.25		0.88		0.90
No CLD or alive	4.4 (1.6- 16.2)		8.4 (2.5- 15.4)		4.8 (1.2- 29.7)		8.2 (1.3- 39.9)	
CLD or died	9.5 (2.0- 20.7)		10.1 (2.4- 25.3)		7.1 (0.8- 25.9)		5.6 (1.6- 16.6)	
SM12:0		0.53		0.06		1.00		0.97
No CLD or alive	2.3 (1.2- 3.7)		0.9 (0.5- 1.8)		0.9 (0.3- 2.2)		1.4 (0.2- 3.2)	
CLD or died	2.0 (1.1- 4.4)		1.6 (0.6- 3.3)		1.0 (0.3- 2.2)		0.7 (0.6- 2.0)	

Table 2 – (continued)

	Day 1		Day 3		Day 7		Day 14	
Independent variable	Median (IQR)	*p*-value	Median (IQR)	*p*-value	Median (IQR)	*p*-value	Median (IQR)	*p*-value
SM(DiHy)12:0		0.59		0.45		0.30		0.36
No CLD or alive	0.4 (0.3- 0.7)		0.3 (0.2- 0.5)		0.4 (0.2- 0.6)		0.8 (0.2- 1.5)	
CLD or died	0.6 (0.3- 1.0)		0.3 (0.2- 0.8)		0.2 (0.1- 0.5)		0.3 (0.1- 0.7)	
SM16:0		0.89		0.69		0.43		0.64
No CLD or alive	595.0 (355.1-1053.8)		808.5 (520.0- 1247.5)		660.0 (497.5-1862.0)		1365.0 (311.9- 1722.5)	
CLD or died	630.0 (300.5- 1352.5)		953.3 (411.6.61- 1701.3)		810.0 (122.5- 1385.0)		955.0 (518.3- 1577.5)	
SM18:0		0.75		0.62		0.82		0.97
No CLD or alive	122.5 (71.4- 259.3)		201.3 (91.8- 300.8)		150.0 (82.0- 325.0)		269.5 (64.5- 346.5)	
CLD or died	119.5 (60.0- 296.5)		259.5 (86.3- 387.5)		223.5 (30.9- 360.0)		176.0 (103.8-409.9)	
SM18:1		0.93		0.30		0.65		0.97
No CLD or alive	28.3 (15.6- 57.7)		27.2 (14.7- 56.3)		15.8 (9.3- 51.5)		23.8 (5.4- 70.5)	
CLD or died	24.0 (12.4- 85.5)		47.0 (14.3- 119.1)		36.5 (4.0- 82.1)		19.5 (8.0- 41.7)	
SM24:0		0.91		0.59		0.73		0.97
No CLD or alive	263.0 (124.6- 585.3)		410.5 (203.0- 661.3)		360.0 (180.5- 982.0)		469.0 (122.8- 571.3)	
CLD or died	272.0 (122.8- 761.0)		496.3 (241.0- 797.0)		349.5 (69.5- 1020.0)		341.5 (232.3- 630.0)	
SM24:1		0.83		0.61		0.51		0.97
No CLD or alive	215.8 (102.4- 410.7)		357.3 (187.1- 680.0)		409.0 (183.5- 1116.0)		745.0 (105.9- 1278.8)	
CLD or died	176.5 (80.8- 535.0)		464.0 (172.6- 877.3)		510.0 (62.0- 1025.0)		530.0 (219.0- 1107.5)	

Sphingolipids were expressed as ng/mL. Abbreviations: IQR: interquartile range; Cer: ceramide; SM: sphingomyelin.; CLD: chronic lung disease.

Table 3 – Multivariable logistic regression analysis for outcome CLD/ died

Independent variable	Day 1			Day 3			Day 7			Day 14		
	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value
Cer 14:0	0.826	0.457- 1.495	0.53	1.718	0.654- 4.516	0.27	1.055	0.581- 1.916	0.86	0.821	0.132- 5.103	0.83
Cer 16:0	0.713	0.364- 1.397	0.32	1.269	0.527- 3.059	0.60	0.969	0.509- 1.846	0.93	0.582	0.090- 3.787	0.57
Cer 18:0	0.709	0.389- 1.294	0.26	0.930	0.384- 2.255	0.87	0.872	0.463- 1.643	0.67	0.500	0.061- 4.086	0.52
Cer 18:1	0.642	0.345- 1.193	0.16	1.447	0.697- 3.005	0.32	1.047	0.667- 1.644	0.84	0.926	0.174- 4.929	0.93
Cer(DiHy)16:0	0.452	0.135- 1.511	0.20	2.854	0.519- 15.694	0.23	0.970	0.470- 2.001	0.93	*-	–	–
Cer(DiHy)18:0	0.453	0.193- 1.061	0.07	1.191	0.451- 3.151	0.72	0.939	0.569- 1.548	0.81	0.173	0.009- 3.179	0.24
Cer(DiHy)18:1	1.433	0.710- 2.893	0.32	1.040	0.469- 2.304	0.92	1.267	0.704- 2.282	0.43	*-	–	–
Cer 20:0	0.692	0.358- 1.337	0.27	1.016	0.428- 2.407	0.97	0.893	0.481- 1.660	0.72	0.330	0.025- 4.336	0.40
Cer 22:0	0.744	0.400- 1.385	0.35	1.290	0.605- 2.750	0.51	0.964	0.568- 1.634	0.89	0.203	0.013- 3.155	0.26
Cer 24:0	0.802	0.456- 1.410	0.44	1.531	0.681- 3.442	0.30	0.957	0.635- 1.442	0.83	0.113	0.004- 3.032	0.19
Cer 24:1	0.801	0.459- 1.399	0.44	1.369	0.645- 2.902	0.41	0.978	0.587- 1.628	0.93	0.169	0.008- 3.628	0.26
Cer(DiHy)24:0	0.650	0.391- 1.080	0.10	1.369	0.699- 2.681	0.36	0.901	0.558- 1.454	0.67	0.041	0.000- 11.813	0.27
Cer(DiHy)24:1	0.787	0.497- 1.245	0.31	1.175	0.653- 2.113	0.59	1.059	0.753- 1.489	0.74	0.526	0.064- 4.316	0.55
SM12:0	0.611	0.270- 1.380	0.24	1.446	0.444- 4.705	0.54	1.116	0.641- 1.943	0.70	0.429	0.033- 5.645	0.52
SM(DiHy)12:0	0.942	0.362- 2.448	0.90	0.936	0.323- 2.717	0.90	0.880	0.401- 1.932	0.75	0.277	0.026- 2.948	0.29
SM16:0	0.444	0.142- 1.382	0.16	0.750	0.212- 2.654	0.66	0.917	0.489- 1.720	0.79	0.326	0.012- 8.848	0.51
SM18:0	0.496	0.186- 1.324	0.16	0.924	0.363- 2.349	0.87	0.934	0.473- 1.846	0.85	0.477	0.042- 5.465	0.55
SM18:1	0.754	0.418- 1.361	0.35	1.019	0.461- 2.252	0.96	1.123	0.639- 1.973	0.69	0.316	0.031- 3.257	0.33
SM24:0	0.542	0.214- 1.374	0.20	0.958	0.369- 2.483	0.93	0.920	0.529- 1.601	0.77	0.372	0.023- 5.928	0.48
SM24:1	0.636	0.311- 1.300	0.22	0.988	0.394- 2.477	0.98	0.919	0.520- 1.624	0.77	0.440	0.022- 8.834	0.59

Abbreviations: CLD: chronic lung disease; OR: odds ratio; CI: confidence interval; Cer: ceramide; SM: sphingomyelin; CMV: conventional mechanical ventilation; HFO: high-frequency oscillation..

Observed-to-expected lung-to-head ratio, side of the defect, liver position, center and ventilation arm were included as independent variables.

*Too limited number of observations to perform logistic regression analysis.

Table 4 – Sphingolipid levels for initial ventilation (CMV or HFO)

	Day 1		Day 3		Day 7		Day 14	
Independent variable	Median (IQR)	*p*-value	Median (IQR)	*p*-value	Median (IQR)	*p*-value	Median (IQR)	*p*-value
Cer 14:0		0.98		0.54		0.90		0.05
CMV	7.8 (3.0- 17.4)		6.2 (2.7- 13.7)		0.3 (1.1- 13.2)		6.9 (2.9- 11.0)	
HFO	8.2 (3.6- 18.0)		5.2 (2.3- 13.7)		3.2 (0.9- 9.4)		2.6 (0.4- 4.6)	
Cer 16:0		0.98		0.44		0.68		0.06
CMV	266.5 (112.0- 534.8)		242.0 (148.4- 485.9)		151.0 (47.8- 695.0)		505.6 (261.0- 931.3)	
HFO	256.5 (147.5- 548.0)		225.0 (109.6- 395.6)		204.5 (66.4- 550.0)		202.5 (17.9- 348.0)	
Cer(DiHy)16:0		0.71		0.64		0.87		0.22
CMV	32.2 (9.3- 83.1)		33.1 (16.5- 71.0)		20.1 (8.3- 78.3)		30.6 (20.0- 175.5)	
HFO	38.3 (16.5- 87.6)		30.8 (11.7- 78.5)		27.5 (4.6- 62.0)		24.2 (2.5- 32.5)	
Cer 18:0		0.92		0.06		0.65		0.07
CMV	86.3 (32.3- 244.8)		120.0 (59.1- 183.6)		50.0 (15.5- 268.0)		253.4 (101.4- 392.6)	
HFO	81.5 (44.9- 189.0)		73.3 (37.5- 145.3)		82.5 (19.9- 192.5)		94.0 (5.4- 195.0)	
Cer 18:1		0.93		0.77		0.91		0.005
CMV	1.0 (0.5- 2.6)		0.8 (0.6- 2.2)		0.7 (0.1- 3.4)		1.4 (1.1- 4.3)	
HFO	0.9 (0.3- 3.4)		1.0 (0.1- 2.1)		0.8 (0.2- 3.0)		0.5 (0.0- 1.1)	
Cer(DiHy)18:0		0.36		0.29		0.72		0.08
CMV	6.0 (2.3- 22.5)		16.9 (7.0- 31.9)		5.0 (2.2- 26.3)		22.0 (9.4- 38.2)	
HFO	9.8 (4.1- 29.4)		12.7 (7.2- 20.0)		9.6 (2.2- 27.6)		9.2 (1.0- 18.8)	
Cer(DiHy)18:1		0.72		0.89		0.58		0.05
CMV	0.0 (0.0- 0.0)		0.0 (0.0- 0.0)		0.0 (0.0- 1.2)		0.1 (0.0- 1.2)	
HFO	0.03 (0.03- 0.03)		0.03 (0.03- 0.03)		0.03 (0.03- 0.55)		9.2 (3.7- 16.3)	

Table 4 – Sphingolipid levels for initial ventilation (CMV or HFO) (continued)

	Day 1		Day 3		Day 7		Day 14	
Independent variable	Median (IQR)	*p*-value	Median (IQR)	*p*-value	Median (IQR)	*p*-value	Median (IQR)	*p*-value
Cer 20:0		0.83		0.26		0.62		0.11
CMV	33.3 (12.2- 89.6)		38.8 (19.3- 56.1)		17.3 (4.7- 94.5)		85.7 (35.9- 144.6)	
HFO	31.6 (20.0- 81.4)		27.7 (12.9- 54.5)		31.2 (7.6- 66.5)		31.8 (1.7- 69.0)	
Cer 22:0		0.80		0.81		0.68		0.04
CMV	46.0 (15.3- 98.1)		41.8 (19.5- 91.6)		25.3 (6.2- 137.5)		79.1 (33.8- 164.6)	
HFO	33.3 (20.9- 108.5)		47.5 (14.6- 86.3)		59.0 (10.5- 108.5)		22.0 (1.9- 55.0)	
Cer 24:0		0.65		0.95		0.64		0.008
CMV	69.0 (20.9- 132.5)		51.5 (18.1- 114.4)		31.1 (5.7- 217.5)		81.0 (33.6- 205.2)	
HFO	46.3 (26.5- 132.0)		54.2 (16.3- 154.1)		53.8 (8.3- 125.5)		25.3 (2.9- 53.0)	
Cer 24:1		0.95		0.81		0.67		0.02
CMV	87.8 (28.0- 201.9)		80.5 (31.6- 177.9)		51.5 (10.7- 397.0)		247.0 (89.0- 437.8)	
HFO	65.5 (40.9- 172.0)		80.5 (27.6- 190.8)		110.0 (14.2- 203.5)		48.3 (5.8- 139.5)	
Cer(DiHy)24:0		0.63		0.97		0.97		0.06
CMV	6.2 (2.5- 18.0)		4.1 (1.6- 8.0)		2.6 (0.8- 10.7)		7.1 (1.4- 13.9)	
HFO	4.8 (2.7- 7.7)		3.9 (1.3- 11.2)		3.1 (0.3- 13.6)		1.1 (1.0- 5.1)	
Cer(DiHy)24:1		0.85		0.91		0.67		0.05
CMV	9.3 (1.6- 17.2)		8.6 (3.4- 15.5)		7.1 (3.5- 25.9)		11.6 (3.3- 42.7)	
HFO	4.7 (1.9- 16.6)		9.9 (2.2- 19.9)		5.7 (0.8- 25.9)		3.8 (0.0- 9.5)	
SM12:0		0.14		0.40		0.95		0.11
CMV	2.7 (1.4- 5.4)		1.2 (0.6- 2.4)		0.9 (0.3- 2.2)		1.2 (0.6- 2.7)	
HFO	2.0 (1.0- 2.8)		1.0 (0.4- 2.1)		1.2 (0.3- 2.2)		0.6 (0.1- 1.2)	
SM(DiHy)12:0		0.67		0.68		0.41		0.22
CMV	0.4 (0.3- 1.1)		0.3 (0.2- 0.6)		0.2 (0.1- 0.4)		0.3 (0.2- 1.4)	

Table 4 – Sphingolipid levels for initial ventilation (CMV or HFO) (continued)

	Day 1		Day 3		Day 7		Day 14	
Independent variable	Median (IQR)	*p*-value	Median (IQR)	*p*-value	Median (IQR)	*p*-value	Median (IQR)	*p*-value
HFO	0.4 (0.3- 0.8)		0.3 (0.2- 0.7)		0.3 (0.1- 0.8)		0.2 (0.1- 0.7)	
SM16:0		0.84		0.69		0.40		0.34
CMV	555.0 (316.3- 1367.5)		808.5 (517.5- 1207.5)		600.0 (173.0- 1385.0)		1115.0 (697.5- 1685.0)	
HFO	760.0 (348.5- 1220.0)		908.3 (378.4- 1733.8)		1290.0 (230.5- 1862.0)		955.0 (260.0- 1670.0)	
SM18:0		0.66		0.45		0.44		0.12
CMV	100.5 (54.2- 297.6)		238.5 (123.4- 373.5)		134.0 (36.5- 325.0)		279.5 (150.3- 512.5)	
HFO	144.5 (83.5- 242.0)		197.4 (80.4- 305.1)		223.5 (42.2- 360.0)		169.0 (18.5- 231.0)	
SM18:1		0.72		0.39		0.84		0.09
CMV	28.8 (14.3- 96.0)		41.9 (18.4- 70.4)		19.0 (5.9- 82.1)		30.6 (14.8- 57.6)	
HFO	26.6 (14.2- 63.0)		38.6 (11.4- 87.9)		36.5 (5.4- 67.2)		16.0 (2.8- 31.9)	
SM24:0		0.88		0.38		0.68		0.07
CMV	244.0 (103.4- 878.8)		494.5 (241.6- 707.5)		325.5 (79.5- 1020.0)		515.0 (339.8- 796.3)	
HFO	304.5 (128.0- 550.0)		410.5 (201.7- 630.0)		575.0 (88.0- 982.0)		321.0 (40.5- 453.0)	
SM24:1		0.70		0.59		0.58		0.05
CMV	178.8 (83.5- 890.0)		445.8 (219.3- 690.0)		299.0 (76.0- 1025.0)		885.0 (444.8- 1472.5)	
HFO	245.5 (115.0- 419.5)		377.3 (142.6- 880.0)		605.0 (96.0- 1036.0)		380.0 (100.0- 775.0)	

Sphingolipids were expressed as ng/ mL. Abbreviations: IQR: interquartile range; Cer: ceramide; SM: sphingomyelin.
*Constant values. The threshold for significance was set at 0.0125.

DISCUSSION

In this prospective international multicentre study, we determined sphingolipid levels in tracheal aspirates of antenatally diagnosed CDH patients in the neonatal period. We found no significant differences in temporal sphingolipid profiles between patients who died/developed CLD compared to patients who survived/did not develop CLD. Furthermore, no significant sphingolipid differences were found between patients initially ventilated by CMV compared to patients initially ventilated by HFO except for ceramide-C18:1 and ceramide-C24:0 at day 14.

Bioactive sphingolipids have been investigated regarding their role in respiratory diseases such as asthma[14] and COPD[15]. Moreover, sphingolipids are important factors in lung development and disease[9], and recently have been shown to play a role in the pathogenesis of CLD in mice[16].

In lungs of preterm infants who were ventilated or received oxygen treatment, epithelial cell apoptosis and proliferation of epithelial, endothelial and smooth muscle cells were observed[17]. Since we did not find any significant difference in sphingolipid levels in tracheal aspirates of CDH patients, it seems that the role of sphingolipids in the pathophysiology of CLD is different in CDH patients when compared to for instance premature born neonates. The finding that lungs of fetuses with CDH are not surfactant deficient[7] supports this idea. No beneficial effect of surfactant replacement therapy has been shown in term neonates with CDH[8], nor in CDH neonates on ECMO[18]. Even in prematurely born neonates with CDH, surfactant replacement therapy did not improve survival rates[19]. The predisposing risk factors for CLD also vary between neonates with CDH and preterm born neonates; for example, chorioamnionitis being associated with premature birth and an increased risk of developing BPD[20] is absent in CDH. Chorioamnionitis is an inflammatory process and sphingolipids being involved in the regulation of inflammation[21], underscore a role for sphingolipids in the pathophysiology of CLD in premature infants.

Another possibility is that the lung hypoplasia seen in CDH leads to less production of ceramides essential for lung development. To solve this problem other causes of pulmonary hypoplasia should be investigated such as obstructive uropathy, but these data are neither available in our biobank nor in the literature. Fetal lung development occurs in a relative hypoxic environment that stimulates vascular development *via* Hypoxia Inducible Factors (HIFs)[22]. HIFs upregulate genes necessary for proper lung vascular and alveolar development[23]. Most deaths among CDH patients are due to severe pulmonary hypertension. It was recently shown that ceramide upregulation was associated with decreased Vascular Endothelial Growth Factor (VEGF) expression via suppression of HIF-1α, suggesting a role for sphingolipids in VEGF regulation[24]. Since VEGF has an important role in pulmonary vascular development, and it was shown that increased

plasma VEGF-A correlates with clinical severity of pulmonary vascular disease in CDH infants[25], this mechanism could be involved in CDH patients. Our study design, however, did not provide for measuring VEGF plasma levels to test this hypothesis.

When we compared tracheal aspirate sphingolipids levels for the initial ventilation strategy, HFO versus CMV, we found no differences between the two ventilation modalities at day 1, 3 and 7. However, at day 14, two long chain ceramides (ceramide-C18:1 and ceramide-C24:0) were significantly elevated in patients initially ventilated by CMV when compared to HFO. It is remarkable that only at day 14 significant differences were observed. One explanation may be that in CMV the use of high peak inspiratory pressures and shear stress over time lead to sphingomyelinase activation and sphingomyelin degradation to ceramides. Alternatively, it is plausible that CMV increases over time *de novo* ceramide synthesis. Unfortunately, the study design did not allow inclusion of a non-ventilated control group and, therefore, we do not know whether ceramide levels were increased by either ventilation modality. Ceramides enhance apoptosis and decrease vascular barrier integrity[26]. Of note, increased apoptosis has been found in epithelial cells of CLD patients, but whether this was triggered by ceramides was not investigated[27]. In the current study, the increase in very long-chain (C24:0) ceramides, known to stimulate cell proliferation and not apoptosis[28], fits with the favorable clinical outcomes in the CMV group when compared to the HFO group[4].

To our knowledge this is the first study investigating lung sphingolipid metabolism in CDH patients. Unique is the prospective multicentre design in a relatively large cohort of patients. Secondly, apart from the initial ventilation strategy, all children were treated according to the same study protocol[3]. A few limitations should be considered. Firstly, patient characteristics between patients of whom samples were collected and patients of whom no samples were collected, were different with respect to the side of the defect, although a recent multicentre study found that morbidity following repair of right-sided CDH was not significantly different from that in left-sided CDH survivors[29]. Therefore we believe that there was no bias in patient inclusion. Secondly, we have corrected for multiple testing for multiple time-points, but no formal testing for multiple testing of different sphingolipids was performed, which could be seen as a possible limitation. The number of TA samples decreases over time which may lead to a selection bias. This decrease is explained by the fact that some CDH infants have died and other infants were not invasively ventilated anymore. However, due to the study design TA sampling was only performed in ventilated infants. Our data were collected in a randomized clinical trial and the difference in ventilation strategy could be seen as a study limitation. However, in the multivariate analyses we adjusted for the possible effect of ventilation strategy. In conclusion, sphingolipids are likely not involved in the pathogenesis of CLD or mortality in antenatally diagnosed CDH infants.

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Chapter 5

High-sensitivity troponin T and N-terminal pro-brain natriuretic peptide in prediction of outcome in congenital diaphragmatic hernia: results from a multicenter, randomized controlled trial

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ABSTRACT

Biomarkers may be helpful in prediction of outcomes of infants with congenital diaphragmatic hernia. The predictive value of high-sensitivity Troponin T and N-terminal pro-brain natriuretic peptide was investigated in 128 infants with congenital diaphragmatic hernia. After correction for multiple testing, those biomarkers did not predict severe pulmonary hypertension, death, need of extracorporeal membrane oxygenation, or bronchopulmonary dysplasia.

INTRODUCTION

Congenital diaphragmatic hernia (CDH) is a severe congenital anomaly characterized by pulmonary hypoplasia and pulmonary vascular abnormalities[1]. The severity of illness is determined mainly by the extent of the pulmonary hypertension (PH)[1]. Some severely ill children may benefit from extracorporeal membrane oxygenation (ECMO), but even in these children, the rate of mortality remains relatively high[2]. Alternatively, a right-sided defect, a lower observed-to-expected lung-to-head ratio (O/E LHR), and the intrathoracic liver position are associated with worse prognosis. A reliable method to predict postnatal outcome is still lacking. Echocardiography is used to estimate the degree of PH, but its application is often not standardized and associated with great interobserver variability. A biomarker that is investigator independent and not reliant on the availability of specialized physicians could improve risk stratification, identify high-risk patients, and guide decision making on the initiation of specific treatment modalities such as ECMO. Two biomarkers that have proven predictive value in cardiovascular diseases are high-sensitivity Troponin T (hsTnT) and N-terminal pro-brain natriuretic peptide (NT-proBNP)[3,4]. HsTnT is released in response to myocardial damage[5]. Increased hsTnT levels were shown to be a marker of poor prognosis in patients with PH[5]; thus, they also may have a prognostic role in CDH. NT-proBNP is a natriuretic peptide that is secreted from the ventricles in response to increased ventricular pressure and volume loads. Four studies that investigated the predictive value of natriuretic peptides in infants with CDH[6-9] found that these markers could predict the severity of PH. The studies, however, were from single centers with relatively small samples (maximum of 28 patients)[6,7,9] or had a retrospective study design[8].

We hypothesized that in infants with antenatally diagnosed CDH, hsTnT and NT-proBNP levels are greater in those with severe PH, nonsurvivors, those requiring ECMO, survivors with bronchopulmonary dysplasia (BPD) and nonsurviving ECMO-treated patients.

METHODS

Inborn patients with a prenatal diagnosis of CDH and gestation >34 weeks were eligible to participate in an international, multicenter prospective randomized controlled trial, named the VICI-trial (supplemental figure 1)[10]. Ethical approval was given by the medical ethics review board of Erasmus MC, Rotterdam, The Netherlands. Thereafter, all local medical ethical committees provided their approval. Written parental informed consent was obtained before birth. One of the exclusion criteria was expected corrective heart surgery within the first 60 days of life[10]. Immediately after birth, infants were randomized to conventional mechanical ventilation or high-frequency oscillation ventilation. Apart

from the initial ventilation strategy, all infants were treated according to the standardized neonatal treatment protocol[11]. Veno-arterial ECMO therapy could be initiated if one or more predetermined failure criteria applied[10]. The severity of PH was dichotomized into two levels[12] (<2/3 systemic pressure versus (mild) vs >2/3 systemic-to-systemic pressure (severe)), measured by echocardiography within the first 24 hours of life. BPD was defined as oxygen dependency (>0.21) at day 28[13]. Defect size was classified according to the definition of the CDH study group[14].

In five centers, 1-mL blood samples were collected within the first 24 hours of life and at days 3, 7 and 14 when a central or peripheral line was present (also in ECMO-treated patients). Samples were immediately centrifuged at 3000 rpm for 6 minutes at 20 °Celsius. Thereafter, plasma was removed and serum from the samples was stored at −20 °C or less. Levels of hsTnT and NT-proBNP levels were measured in the central chemistry laboratory of Erasmus MC (Good Laboratory Practice certified) with the Elecsys electrochemiluminescence immunoassay (Cobas, a division of Roche Diagnostics Limited, Rotkreuz, Switzerland). For hsTnT, for quality control, Elecsys PreciControl Troponin 1 and 2 were used as suggested by the company. HsTnT levels are expressed as pg/mL and the measuring range was from 3 to 10.000 pg/mL. For NT-proBNP, for quality control, Liquichek Cardiac Markers Plus Control LT 1 and 2 were used as suggested by the company, Cobas. NT-proBNP levels are expressed as pmol/L, and the measuring range was from 5 to 35000 pg/mL or 0.6 to 4130 pmol/L. The upper reference limit was defined as the 97.5th percentile[15]. The analyzer automatically calculated the analytic concentration of each sample with a conversion factor of pmol/L x 8.457= pg/mL and pg/mLx 0.118=pmol/L.

Data analysis

Descriptive statistics are shown as number (%), mean± SD or median (interquartile range (IQR)) as appropriate. HsTnT and NT-proBNP values were compared between groups by the use of the Mann-Whitney U tests. Groups were defined based on the severity of PH at day 1, death, need for ECMO (in ECMO centers only), BPD in survivors, and mortality in ECMO-treated patients. Multivariable logistic regression analyses with the independent variables diaphragmatic defect side, liver position (thoracic or abdominal), and O/E LHR as covariates were performed to determine the additional predictive value of hsTnT and NT-proBNP on the predefined outcome parameters. To facilitate the interpretation of the results of the logistic regression, ORs for NT-proBNP are presented with the use of nmol/L as unit of measurement. The logistic regression analyses were repeated by the use of biomarker values at different days (1, 3, 7, or 14 days) for all outcomes except mortality in ECMO-treated patients. For mortality in ECMO-treated patients, the difference between the first biomarker value during ECMO and last biomarker value before ECMO was used as predictor. We considered a correction for multiple testing at different

days of life by using a significance level of 5% divided by the number of time points per outcome.

RESULTS

The inclusion period was from November 2008 until December 2013. Blood samples were collected from 121 of 171 randomized patients. For seven patients, their parents had provided written consent for only sample collection and no randomization. Of the 99 surviving infants (77.3%), 31 (31.3%) developed BPD. Eighty-one infants (63.3%) were born in an ECMO-center; 27 of them (33.3%) were treated with ECMO, and 15 of those (55.6%) died (Table 1). For five infants, the classification of PH was missing; 51 (39.8%) had <2/3 systemic PH (mild) and 72 (56.3%) had >2/3 systemic PH (severe). Sampled infants had significantly greater birth weights, more often a left-sided defect, more often an intra-abdominal liver position, more often diaphragmatic defect size B or C, died later, and had a shorter duration of stay in the intensive care unit than non-sampled infants (Table 2).

At day 1, hsTnT levels were analyzed in 86 patients and NT-proBNP levels in 116 patients. The median age of blood sampling at day 1 was 2 hours (IQR 1.3- 3.9 hours). For the following days, samples were collected less frequently (Figures 2 and 3). Median (IQR) storage time was 3.78 years (IQR 2.59- 4.75 years). HsTnT levels on day 1 were significantly greater in patients with ECMO treatment compared with patients without ECMO treatment (p=0.01), and in survivors with BPD compared with survivors without BPD (p=0.02) (Table 3). Multivariable logistic regression analysis with correction for defect side, liver position, and O/E LHR, but without correction for multiple testing, showed that levels of hsTnT on day 1 were associated with need for ECMO (p=0.03), and BPD in survivors (p=0.03) (Table 4). The estimated OR for ECMO of 1.018 (95% CI 1.002- 1.033, p=0.03) expresses the increase in the probability for the need for ECMO because of a change in hsTnT on day 1 of 1 unit pg/mL. For example, the estimated OR associated with an increase of hsTnT on day 1 from the 25th percentile (58.5 pg/mL) to the 75th percentile (269.8 pg/mL) was an OR=$1.018^{(269.8\text{-}58.5)}$= 43.36. After correction for multiple testing, no significant associations for levels of hsTnT on day one were found. There were no significant differences in the levels tested at the other time points. Levels of NT-proBNP were significantly greater in survivors with BPD compared with survivors without BPD on day 1 (p=0.01) and on day 3 (p=0.04) (Table 3). In multivariable logistic regression analysis with correction for defect side, liver position, and O/E LHR, however, NT-proBNP was not significantly associated with any of the outcomes (Table 4). There were no significant differences in levels tested at the other time points.

Table 1 – Patient characteristics

	Included patients n=128
Gestational age (weeks)	38.1± 1.3
Birth weight (grams)	2981± 467
Fetoscopic endotracheal occlusion	12 (9.4%)
Male sex	60 (46.9%)
Left sided defect	120 (93.8%)
Liver position: intrathoracic	68 (53.1%)
Type of repair	
Patch correction	72 (56.2%)
Primary closure	43 (33.6%)
No repair	13 (10.2%)
Diaphragmatic defect size*	
A	7 (5.5%)
B	43 (33.6%)
C	55 (43.0%)
D	5 (3.9%)
No repair	13 (10.2%)
Missing	5 (3.9%)
Treatment with nitric oxide	60 (46.9%)
Treatment with inotropics	111 (86.7%)
Initial ventilation mode: HFO	64 (50.0%)
Age at repair (days)	4.0 (3.0- 8.0)
Age at death (days)	13.0 (4.8- 25.5)
Ventilation time (days) (in survivors)	10.0 (7.0- 17.5)
ICU admission (days) (in survivors)	19.0 (13.0- 35.5)

Data are presented as n (%), mean± SD or median (IQR).
Abbreviations: HFO: high-frequency oscillation; ICU: intensive care unit.
*Reference: Lally KP, Lasky RE, Lally PA, Bagolan P, Davis CF, Frenckner BP, et al. Standardized reporting for congenital diaphragmatic hernia--an international consensus. J Pediatr Surg. 2013;48:2408-15.

DISCUSSION

In this prospective, multicenter study, the predictive values of hsTnT and NT-proBNP were investigated in patients antenatally diagnosed with CDH. In the univariable analysis, levels of hsTnT on day 1 were significantly different between patients with and without the need for ECMO and between survivors with and without BPD, and NT-proBNP levels on day 1 and 3 were significantly different between survivors with and without BPD. After correction for multiple testing in the multivariable analyses, neither hsTnT nor NT-proBNP levels were significantly related with any of the outcomes.

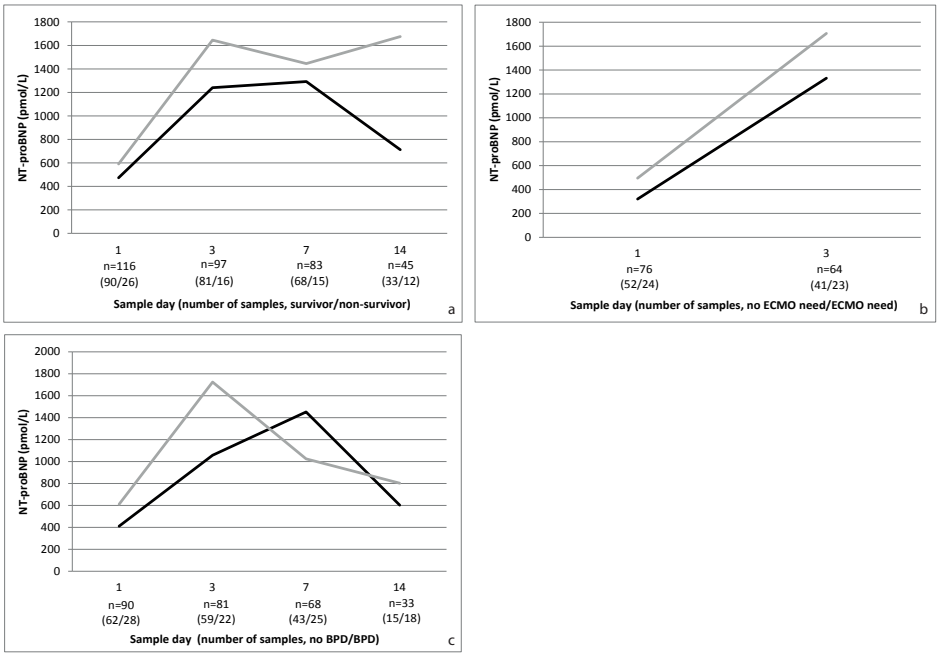
Table 2 – Patient characteristics of included and non-included patients

	Included patients n=128	Non-included patients n=50	*p*-value
Gestational age (weeks)	38.1± 1.3	37.8± 1.4	0.18
Birth weight (grams)	2981± 467	2804± 415	0.02
Fetoscopic endotracheal occlusion	12 (9.4%)	7 (14.0%)	0.37
Male gender	60 (46.9%)	26 (52.0%)	0.54
Left sided defect	120 (93.8%)	35 (70.0%)	<0.001
Liver position: intrathoracic	68 (53.1%)	37 (74.0%)	0.01
Type of repair			0.02
Patch correction	72 (56.2%)	26 (52.0%)	
Primary closure	43 (33.6%)	11 (22.0%)	
No repair	13 (10.2%)	13 (26.0%)	
Diaphragmatic defect size*			<0.001
A	7 (5.5%)	4 (8.0%)	
B	43 (33.6%)	8 (16.0%)	
C	55 (43.0%)	17 (34.0%)	
D	5 (3.9%)	8 (16.0%)	
No repair	13 (10.2%)	13 (26.0%)	
Missing	5 (3.9%)	0 (0%)	
Treatment with nitric oxide	60 (46.9%)	29 (58.0%)	0.18
Treatment with inotropics	111 (86.7%)	41 (82.0%)	0.42
Initial ventilation mode: HFO	64 (50.0%)	22 (44.0%)	0.47
Age at repair (days)	4.0 (3.0- 8.0)	4.0 (3.0- 9.0)	0.90
Age at death (days)	13.0 (4.8- 25.5)	1.5 (0.8- 15.3)	0.02
Ventilation time (days) (in survivors)	10.0 (7.0- 17.5)	13.0 (7.0- 26.8)	0.58
ICU admission (days) (in survivors)	19.0 (13.0- 35.5)	26.5 (19.3- 51.5)	0.03

Data are presented as n (%), mean± SD or median (IQR).
Abbreviations: HFO: high-frequency oscillation; ICU: intensive care unit.
*Reference: Lally KP, Lasky RE, Lally PA, Bagolan P, Davis CF, Frenckner BP, et al. Standardized reporting for congenital diaphragmatic hernia--an international consensus. J Pediatr Surg. 2013;48:2408-15.

Several studies have investigated the prognostic value of Troponin T[16-20] in infants; however, it has never been investigated solely in infants with CDH. Astoria et al. analyzed peak troponin levels from daily samples in 48 neonates (18 of whom had CDH) while they received ECMO as a predictor of death[19], whereas we analyzed the difference between the first hsTnT level during ECMO and the last hsTnT level before ECMO was used. They found that cardiac troponin was an independent marker for nonsurvival. In our study, hsTnT levels did not predict mortality in patients with the need for ECMO. We did find that hsTnT levels on day 1 were significantly different for patients who required ECMO compared with patients without need for ECMO. For the 18 infants with CDH in

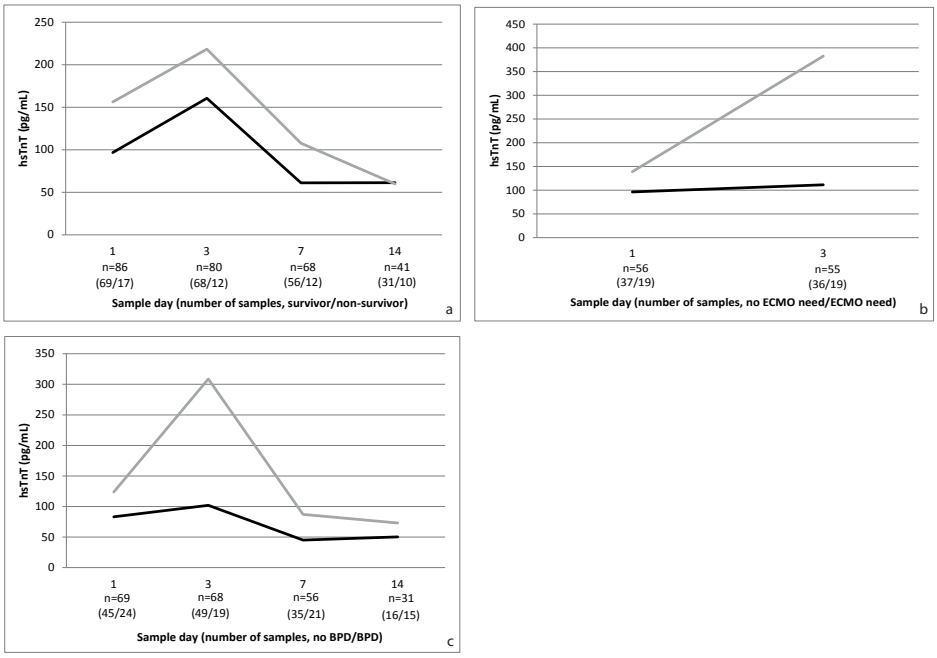
Figure 2 – Mean values of NT-proBNP over time



a) Black: survivors. Grey: non-survivors. b) Black: patients without need for ECMO. Grey: patients with need for ECMO. c) Black: survivors without bronchopulmonary dysplasia. Grey: survivors with bronchopulmonary dysplasia. Abbreviations: ECMO: extracorporeal membrane oxygenation.

the study of Astoria et al., levels of troponin are consistent with the CDH neonates who required ECMO in our study. El-Khuffash et al. investigated Troponin T in prematurely born infants in three studies[16-18]. In the first study, greater levels of cardiac troponin were found in nonsurvivors/ patients with severe impaired neurodevelopment[18]. In another study, troponin T levels were greater in premature infants with persistent ductus arteriosus than controls[17]. In the third study, significantly greater levels of troponin T were found in premature infants with lower Apgar scores at 5 minutes of life with significant correlations of troponin T levels and echocardiographic markers for left ventricular function[16]. Because those patient populations differed from those investigated in our study, results could not be compared. An interesting finding however, was that troponin T was correlated positively with echo measurements of myocardial dysfunction. Ventricular function is impaired in patients with CDH[7,9]. Patel and Moenkemeyer have shown that in response to therapy for PH, echo functional measurements changed[9]. Moreover, Baptista et al. showed that levels of NT-proBNP were correlated with myocardial dysfunction[7]. The right ventricular pressure overload in CDH may lead to a decrease in coronary perfusion gradient and thus impaired right coronary artery flow[21], resulting in troponin T release and immediate troponin T leakage of the right and left ventricle[22]. That speculation may

Figure 3 – Mean values of hsTnT over time



a) Black: survivors. Grey: non-survivors. b) Black: patients without need for ECMO. Grey: patients with need for ECMO. c) Black: survivors without bronchopulmonary dysplasia. Grey: survivors with bronchopulmonary dysplasia. Abbreviations: ECMO: extracorporeal membrane oxygenation.

be supported by the study of Möller et al., who found significantly greater levels of troponin T in a cohort of 47 asphyxiated infants compared with controls[20]. Troponin T may be excreted in periods of asphyxia that cause myocardial damage. Hence, those data support why we found greater levels of troponin T in infants with CDH who required ECMO. This might also explain the substantial elevation of troponin levels in our study as compared to with prematurely born neonates[16-18].

To our knowledge, few studies have investigated the usefulness of NT-proBNP in infants with CDH[6-9]. In contrast to our study, Baptista et al. found in a prospective study that levels of NT-proBNP at 24 hours of life were significantly greater in nonsurvivors than survivors[7] and that NT-proBNP levels correlated with estimated pulmonary artery pressure. We found no significant difference in levels of NT-proBNP between nonsurvivors and survivors. The differences in the results between the two studies may be explained by the different sample sizes and Baptista et al. had estimated the mean pulmonary artery pressure, whereas we distinguished two levels (mild PH vs severe PH) based on the estimated pulmonary artery pressure. Steurer et al. found in 27 infants with CDH that levels of BNP were greater on day 1 in poor-outcome patients (nonsurvivors/ ongoing respiratory support at 56 days of age), but on day 7 there was no longer an association

Table 3 – Mann Whitney U tests results

Day		NT-proBNP	hsTnT
Day 1	**Pulmonary hypertension**	p=0.81	p=0.06
	<2/3 systemic (mild)	181.1 (80.1- 429.7)	56.5 (43.6- 109.6)
	>2/3 systemic (severe)	141.2 (87.5- 461.1)	82.0 (49.4- 195.2)
	Death	p=0.11	p=0.07
	Survivors	95.2 (57.4- 303.6)	65.4 (41.3- 106.4)
	Nonsurvivors	192.1 (118.1- 676.8)	98.0 (47.2- 260.7)
	ECMO need	p=0.09	p=0.01
	No need of ECMO	134.5 (62.9- 451.5)	67.3 (44.3- 111.9)
	Need of ECMO	237.5 (137.8- 422.1)	130.7 (58.5- 269.8)
	BPD in survivors	p=0.01	p=0.02
	No presence of BPD	81.0 (40.4- 232.3)	56.5 (35.0- 77.5)
	Presence of BPD	127.9 (77.3- 1170.8)	80.6 (57.7- 240.5)
Day 3	**Death**	p=0.10	p=0.71
	Survivors	174.9 (122.8- 1235.0)	118.6 (59.4- 164.3)
	Nonsurvivors	654.2 (397.3- 4328.8)	216.8 (149.3- 613.8)
	ECMO need	p=0.23	p=0.22
	No need of ECMO	754.1 (255.9- 1306.0)	80.5 (56.0- 157.8)
	Need of ECMO	654.5 (257.8- 3409.3)	174.4 (81.4- 566.0)
	BPD in survivors	p=0.04	p=0.13
	No presence of BPD	148.0 (129.7- 866.0)	136.4 (61.1- 173.5)
	Presence of BPD	1021.0 (72.6- 1441.8)	91.8 (47.0- 147.2)
Day 7	**Death**	p=0.62	p=0.22
	Survivors	240.9 (145.3- 592.7)	56.7 (28.3- 99.1)
	Nonsurvivors	829.6 (326.4- 3040.3)	102.7 (47.5- 396.8)
	BPD in survivors	p=0.41	p=0.05
	No presence of BPD	297.9 (165.7- 546.6)	56.7 (24.8- 98.1)
	Presence of BPD	208.7 (65.5- 780.9)	50.5 (29.0- 145.4)
Day 14	**Death**	p=0.09	p=0.52
	Survivors	281.9 (154.9- 873.0)	58.2 (37.3- 85.6)
	Nonsurvivors	1709.5 (915.5- 3483.5)	59.5 (34.2- 110.4)
	BPD in survivors	p=0.35	p=0.69
	No presence of BPD	271.5 (152.6- 692.9)	58.2 (42.7- 73.2)
	Presence of BPD	562.4 (151.5- 1390.3)	67.3 (30.1- 152.2)
Mortality in patients with ECMO need*		p=0.89	p=0.85
Survivors		115.5 (−168.5- 2706.0)	24.6 (−134.4- 99.3)
Nonsurvivors		471.7 (−1134.4- 525.1)	−46.4 (−77.5- 157.6)

Data are presented as median (IQR). NT-proBNP was presented in pmol/L and hsTnT in pg/ mL. * The difference of the value of the biomarkers before and after start of ECMO was analyzed. Abbreviations: ECMO: extracorporeal membrane oxygenation; BPD: bronchopulmonary dysplasia.

Table 4 – Multivariable logistic regression analyses
Associations of biomarkers with severity of PH, death, ECMO need, BPD in survivors and death in patients who were placed on ECMO

Independent variable	OR	*p*-value	95% CI	Sign. Correction*
		PH <2/3 systemic vs >2/3 systemic		
NT-proBNP day 1	0.858	0.76	0.325- 2.263	O/E LHR
HsTnT day 1	1.010	0.07	0.999- 1.020	O/E LHR
		Death		
NT-proBNP day 1	1.120	0.82	0.423- 2.963	Liver, O/E LHR
NT-proBNP day 3	0.993	0.97	0.700- 1.407	O/E LHR
NT-proBNP day 7	1.140	0.54	0.749- 1.737	–
NT-proBNP day 14	2.100	0.11	0.843- 5.232	–
HsTnT day 1	1.002	0.58	0.995- 1.009	Liver, O/E LHR
HsTnT day 3	0.999	0.23	0.996- 1.001	O/E LHR
HsTnT day 7	1.004	0.34	0.996- 1.012	–
HsTnT day 14	1.002	0.85	0.982- 1.023	–
		ECMO need		
NT-proBNP day 1	0.457	0.39	0.077- 2.719	Liver, O/E LHR
NT-proBNP day 3	1.015	0.95	0.651- 1.581	O/E LHR
HsTnT day 1	1.018	0.03	1.002- 1.033	Liver, O/E LHR
HsTnT day 3	1.004	0.23	0.997- 1.011	O/E LHR
		BPD in survivors		
NT-proBNP day 1	0.995	0.98	0.662- 1.496	Liver
NT-proBNP day 3	1.145	0.43	0.820- 1.600	Liver
NT-proBNP day 7	0.576	0.10	0.298- 1.113	Liver
NT-proBNP day 14	0.992	0.99	0.257- 3.830	Liver
HsTnT day 1	1.014	0.03	1.002- 1.026	Liver
HsTnT day 3	1.005	0.25	0.996- 1.014	Liver
HsTnT day 7	1.015	0.05	1.000- 1.031	Liver
HsTnT day 14	1.020	0.17	0.992- 1.049	Liver
		Death in patients who were treated with ECMO**		
NT-proBNP	0.626	0.22	0.296- 1.322	–
HsTnT	0.988	0.32	0.964- 1.012	–

*Observed-to-expected lung-to-head ratio, side and liver position were included as covariates. Covariates with significant effects (*p*-value <0.05) are shown in this column.
**The difference between the first biomarker value during ECMO and last biomarker value before ECMO was used as predictor.
NT-proBNP was presented in nmol/L and hsTnT in pg/mL.
Abbreviations: OR: odds ratio; CI: confidence interval. PH: pulmonary hypertension; ECMO: extracorporeal membrane oxygenation; BPD: bronchopulmonary dysplasia.
Significance levels: PH <2/3 systemic vs >2/3 systemic: 0.025; death 0.0125; ECMO need 0.025; BPD in survivors 0.0125; death in patients who were treated with ECMO 0.05.

with worse outcome[6]. This finding is consistent with our study. In contrast to the study from Steurer et al., however, we did not find greater levels of NT-proBNP on day 1 in nonsurvivors. There are a number of differences in the two studies, including the sample sizes. Second, they defined poor outcome as the composite outcome of prolonged respiratory support at 56 days or death, whereas we performed separate tests for survival and second, BPD (defined as oxygen dependency at day 28) in the selection of survivors. Third, they used another method for measuring the BNP levels, which is a different molecule. Because NT-proBNP has a longer half-life than BNP (118 vs 18 minutes) and it is easier to measure[23], we believe that NT-proBNP should be used in future studies, such as studies that focus on biomarker level differences in response to therapy.

Partridge et al. performed a single-center study in a relatively large cohort of infants with CDH[8]. In contrast to our results, they found that BNP measured prerepair was significantly lower in patients without PH compared with patients with PH. Differences may be explained that in the first place a different molecule was measured. Second, in their paper, the timing of the prerepair BNP measurement was not specified, and it may be the peak troponin level during this time period. This may account for differences from our study, next to the fact that they performed a retrospective study without pre-specified time points. Third, to define PH, echocardiographies in their study were performed as clinically indicated, whereas we performed one echocardiography in the first 24 hours. Moreover, they measured the right ventricular systolic pressure estimate to determine the presence of PH, and we categorized patients based on the ratio between the pulmonary and systemic pressure. We believe that a standardized protocol that includes the assessment of ventricular function, and with a correlation of data to biomarkers levels, would be the next step in future studies. Partridge et al. did not find differences in BNP levels in patients who required ECMO or phosphodiesterase type 5 inhibitors (sildenafil) compared with patients managed with inhaled nitric oxide alone. Similarly, we also did not find that levels of NT-proBNP were different between patients with and without need for ECMO.

The strengths of this study are, first, that samples were drawn routinely at predetermined times starting very early after birth and, second, that the outcomes were not dependent on a participating center because all patients were treated according to the same standardized neonatal treatment protocol. The outcome 'need for ECMO' was analyzed in a selection of patients born in an ECMO center. To exclude laboratory differences, samples were analyzed in one laboratory.

A limitation of our study is that included patients had a shorter duration of stay in the intensive care unit, so our results may not be generalizable to all patients with CDH. Another limitation is that effects of therapies might have influenced biomarkers levels. Thus, in the interpretation of biomarker levels, the current treatment and clinical setting should always be taken into account. Moreover, the severity of PH was estimated on

the basis of echocardiographic measurements instead of right heart catheterization, which is seen as the golden standard but unrealistic in these patients. PH was only assessed within the first 24 hours of life. In future studies, it would be better to assess the persistence of PH into the first weeks of life, based on a standardized protocol including assessment of ventricular function, and correlate the data to biomarkers levels.

In conclusion, in inborn patients with CDH, the biomarkers hsTnT and NT-proBNP had no significant predictive value when measured at predetermined specific time points for the specific outcomes tested. Since routine based measurements of biomarkers do not seem useful, future research should study whether biomarker levels determined before and after the start of clinical interventions (such as start of iNO or initiation of an ECMO procedure) could better predict outcomes.

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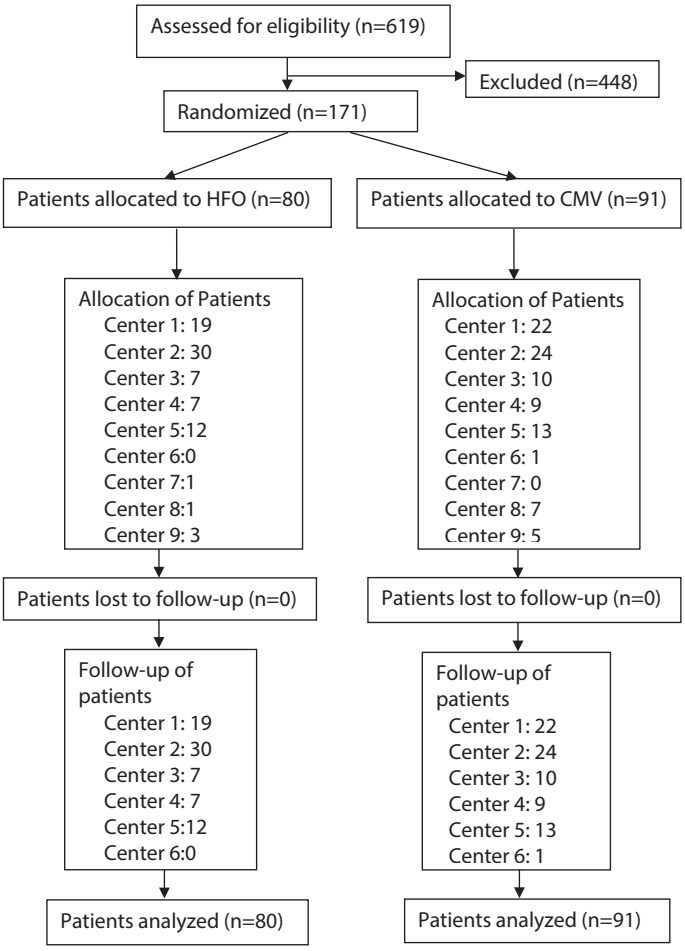
SUPPLEMENTAL

Supplemental figure 1 – Participant flow diagram for multicenter trial



Abbreviations: CMV: conventional mechanical ventilation; HFO: high-frequency oscillation.

Part III

Treatment

Chapter 6

Conventional mechanical ventilation versus high-frequency oscillatory ventilation for congenital diaphragmatic hernia. *A randomized clinical trial (the VICI-trial)*

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ABSTRACT

Objectives: To determine the optimal initial ventilation mode in congenital diaphragmatic hernia.

Summary background data: Congenital diaphragmatic hernia is a life-threatening anomaly with significant mortality and morbidity. The maldeveloped lungs have a high susceptibility for oxygen and ventilation damage resulting in a high incidence of bronchopulmonary dysplasia (BPD) and chronic respiratory morbidity.

Methods: An international, multicenter study (NTR 1310), the VICI-trial was performed in prenatally diagnosed congenital diaphragmatic hernia infants (n=171) born between November 2008 and December 2013, who were randomized for initial ventilation strategy.

Results: Ninety-one (53.2%) patients initially received conventional mechanical ventilation and 80 (46.8%) high-frequency oscillation. Forty-one patients (45.1%) randomized to conventional mechanical ventilation died/ had BPD compared with 43 patients (53.8%) in the high-frequency oscillation group. An odds ratio of 0.62 [95% confidence interval (95% CI) 0.25-1.55] (P=0.31) for death/ BPD for conventional mechanical ventilation vs high-frequency oscillation was demonstrated, after adjustment for center, head-lung ratio, side of the defect, and liver position. Patients initially ventilated by conventional mechanical ventilation were ventilated for fewer days (P=0.03), less often needed extracorporeal membrane oxygenation support (P=0.007), inhaled nitric oxide (P=0.045), sildenafil (P=0.004), had a shorter duration of vasoactive drugs (P=0.02), and less often failed treatment (P=0.01) as compared with infants initially ventilated by high-frequency oscillation.

Conclusions: Our results show no statistically significant difference in the combined outcome of mortality or BPD between the 2 ventilation groups in prenatally diagnosed congenital diaphragmatic hernia infants. Other outcomes, including shorter ventilation time and lesser need of extracorporeal membrane oxygenation, favored conventional ventilation.

INTRODUCTION

Congenital diaphragmatic hernia (CDH) is a developmental defect of the diaphragm that occurs in approximately 1 in 3000 live births[1]. It is characterized by maldevelopment of both the ipsilateral and contralateral lung and abnormal prenatal pulmonary vascular growth. Although pulmonary hypoplasia and pulmonary hypertension are the main causes of mortality, ventilator-induced lung injury and oxygen toxicity may result in prolonged oxygen dependency, or bronchopulmonary dysplasia (BPD)[2]. Logan et al[3] have suggested that 25% of the mortality is due to potentially treatable aspects of the underlying pathophysiology and that ventilator-induced lung injury could be one of these aspects.

Since 2008, all CDH patients born in European countries represented in the CDH EURO consortium have been treated according to a standardized neonatal treatment protocol that was developed at a consensus meeting[4]. After implementation of this protocol, mortality decreased from 33% to 12%[5]. This decrease in mortality rate should be interpreted against of the year upon year variability in mortality rates. Nevertheless, standardization of care is an ideal backcloth to undertake multicenter randomized controlled trials (RCTs).

The optimal initial invasive ventilation strategy in antenatal diagnosed CDH patients, however, is still unknown. Certain studies with retrospective or observational study designs have shown improved survival and lower incidence of BPD with the use of high-frequency oscillation (HFO) ventilation[6-11]. They reported that HFO may result in favorable outcome in CDH because of better oxygenation and higher mean airway pressure without increasing the incidence of barotrauma. In another study, however, based on the CDH EURO consortium registry, HFO as initial ventilation mode was associated with increased rates of mortality and BPD[2]. We, therefore, performed the first prospective multicenter study (VICI-trial) of conventional mechanical ventilation (CMV) or HFO as the initial ventilation strategy[12]. The primary outcome measure was death or BPD[13].

METHODS

A prospective, randomized clinical trial was performed. All participating centers were members of the CDH EURO consortium[4]. Prenatally diagnosed CDH infants, born at a gestational age of more than 34 weeks between November 2008 and December 2013 in 1 of the 9 centers were eligible for inclusion. Exclusion criteria were severe chromosomal anomaly such as trisomy 13 or 18, which may imply a decision to stop or not to start medical treatment; severe cardiac anomalies expected to need corrective surgery in the first 60 days after birth; renal anomalies associated with oligohydramnios; severe

orthopedic and skeletal deformities that were likely to influence thoracic or lung development; and severe anomalies of the central nervous system. We excluded patients with a gestational age of less than 34 weeks so that the results could not be influenced by lung prematurity. Besides, neonates with a gestational age below 34 weeks cannot be placed on extracorporeal membrane oxygenation (ECMO). Ethical approval was given by the medical ethics review board of Erasmus MC, Rotterdam, the Netherlands (NTR 1310). Thereafter, all local medical ethical committees gave their approval. Parents gave written informed consent. The procedures, including obtaining informed consent, were conducted in accord with the ethical standards of the Committee on Human Experimentation of the institution in which the experiments were done. The centers' duration of experience with HFO before initiation of the VICI-trial ranged from 8 years to 19 years.

To achieve equal distribution of the 2 ventilation modes among the participants, block randomization stratified per center was carried out using a computer-generated randomization schedule for each centre by a 24-hour interactive web response system. After birth, infants were centrally randomized to either CMV or HFO. The allocated ventilation mode was started within 2 hours after birth. Patients were monitored up to 1 year of life or until discharge whichever came first. CMV was provided by a neonatal ventilator capable of positive pressure ventilation or triggered modes. Initial settings were a positive inspiratory pressure (PIP) of 20 to 25 cmH_2O and a positive end-expiratory pressure (PEEP) of 3 to 5 cmH_2O, with a ventilator rate of 40 to 60/min. According to clinical practice, PIP was increased in the case of ventilation problems and the PEEP or FiO_2 were adjusted if oxygenation problems occurred. Weaning from ventilation was preferentially by means of decreasing PIP or frequency to achieve $PaCO_2$ levels above 45mm Hg. HFO was provided by a high-frequency oscillatory ventilator. Initial settings were mean airway pressure 13 to 17 cmH_2O, frequency 10 to 12 Hz, delta P 30 to 50 cmH_2O depending on chest wall vibration. According to clinical practice, the frequency was reduced and/or delta P was increased in the case of ventilation problems and the MAP and/or FiO_2 were adjusted if oxygenation problems occurred. All patients were treated according to the same standardized CDH EURO Consortium neonatal treatment protocol.[4] The initially allocated ventilation mode could be switched if one or more of the following predetermined failure criteria were met at 2 consecutive time points for at least 3 hours: inability to maintain preductal saturations above 85% (±52mm Hg or 7 kPa) or postductal saturations above 70% (±5.3 kPa or 40 mm Hg); increase in CO_2 >65 mm Hg or 8.5 kPa despite optimization of ventilatory management; PIP > 28 cmH_2O; mean airway pressure > 17 cmH_2O; inadequate oxygen delivery with metabolic acidosis defined as lactate ≥ 5 mmol/ L and pH < 7.20; hypotension resistant to fluid therapy and inotropic support resulting in a urine output < 0.5 ml/kg/hour; oxygenation index of longitudinal evaluation ≥ 40. Patients could experience one or more criteria for treatment failures. If a patient born in an ECMO center should meet one of these failure criteria, either an ECMO

procedure was considered to be initiated or the initially allocated ventilation mode was considered to be switched. If subsequently there was no improvement, the infant was placed on ECMO. If a patient born in a center without ECMO availability should meet one of these failure criteria, only the ventilation mode was considered to be switched. None of the patients was transferred from a non-ECMO center to an ECMO-center. Arterial blood pressure was to be maintained at a normal level for gestational age. In case of hypotension and/or poor perfusion, 10 to 20 ml/kg NaCl 0.9% was to be administered 1 to 2 times and inotropic agents were considered according to the local practice. If there was suprasystemic pulmonary artery pressure and right-to-left shunting through the foramen ovale, administration of intravenous phosphodiesterase type-5 inhibitor E5 (PDE-5) (Sildenafil) was to be considered. According to the protocol, paralysis in the delivery room was to be avoided if possible. Antenatal data, neonatal characteristics as well as data on the clinical course, and treatment were collected. Defect size was classified intraoperatively according to the CDH study group[14]. Liver position was determined during surgery or if there was no surgical repair from prenatal echographic data. Pulmonary hypertension was categorized as none, <2/3 systemic, 2/3 systemic to systemic, or suprasystemic. The latter 3 categories are according to the definition of Keller et al[15].

Sample size calculation

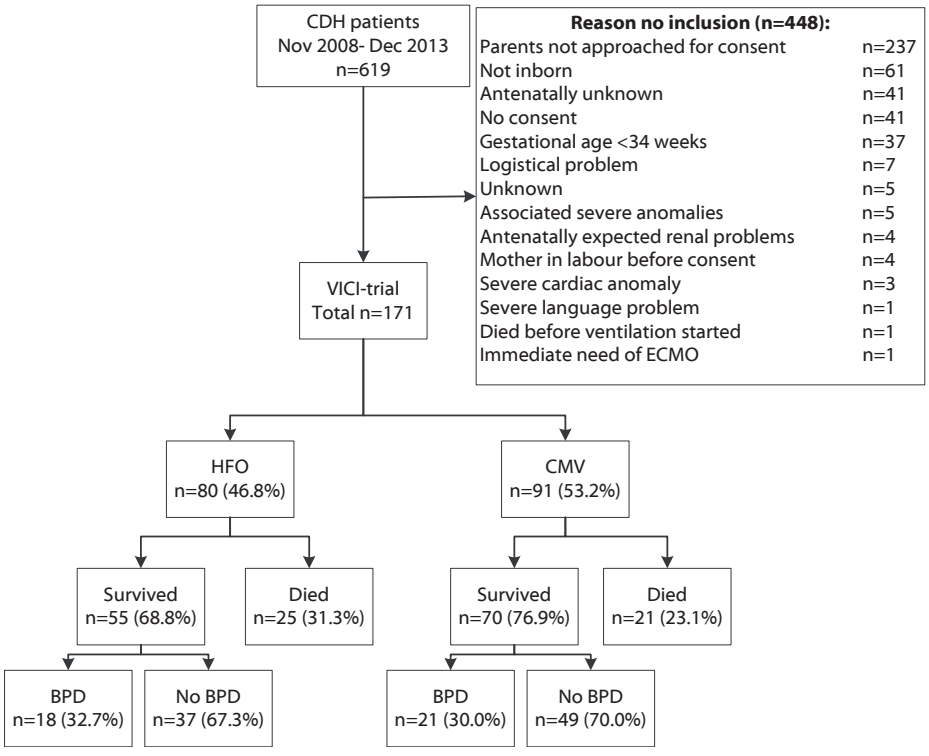
In each ventilation category, 187 infants were to be randomized to give 80% power using a 5% significance level to detect a 15% difference in the combined primary outcome of death or BPD. BPD was defined as oxygen dependency beyond 28 days after birth[13]. To allow for some nonevaluable patients and dropouts, inclusion of 200 patients per group was planned.

Statistical analysis

To determine whether differences in the demographics of the 2 treatment arms were statistically significant, independent samples *t* tests for continuous variables that were normally distributed, χ^2 tests for categorical variables, and Mann-Whitney *U* tests for continuous variables that were not normally distributed, were used. Multiple logistic regression analysis using treatment arm, center, lung-to-head ratio, position of the liver, and side of the defect as independent variables served to evaluate the primary outcome. A subgroup analysis was performed for infants who underwent surgical repair, with defect size added to the independent variables to evaluate the primary outcome. The subgroup analysis and the independent variables for the logistic regression model were selected a priori. However, the 4 centers that each treated fewer than 10 patients in total, were evaluated as a single center in all analyses. Missing data of lung-to-head ratio (n=23 patients) and diaphragmatic defect size (n=5) were imputed by automatic multiple imputation in SPSS with 100 imputations. The predictors in the imputation models

consisted of BPD or death, as well as all independent variables (except initial ventilation mode) in the logistic regression models. The goodness-of-fit of the logistic regression models was assessed using the Hosmer-Lemeshow test. Patients were analyzed in the group of randomization, even after switching of ventilator mode. Overall mortality within first 60 days of life was calculated using Kaplan-Meier curves and compared between treatment arms using a log-rank test stratified by center. The Mantel-Haenszel test with stratification by center was used to compare overall mortality in the first year of life, presence of pulmonary hypertension, iNO, vasoactive medication, phosphodiesterase inhibitor 5 (Sildenafil, Pfizer, Basel, Switzerland), and requirement of ECMO (in ECMO centers only) between treatment arms. The Van Elteren test with stratification by center was used to compare the difference of severity of BPD, number of days of ventilation, the number of treatment failures, severity of pulmonary hypertension, and number of days treated with vasoactive medication between the 2 treatment arms. Treatment failures were only recorded (ie, those infants whose initial ventilation mode was switched or who were placed on ECMO) for patients whose ventilator mode was switched and those

Figure 1 – Flowchart of patient inclusion



BPD indicates bronchopulmonary dysplasia; CDH, congenital diaphragmatic hernia; CMV, conventional mechanical ventilation; ECMO, extracorporeal membrane oxygenation; HFO, high-frequency oscillation.

who were placed on ECMO. All statistical tests were 2-sided, and a *P* value of <0.05 was considered statistically significant. The analyses were conducted using IBM SPSS 21.0 for Windows (IBM Corp., Amonk, NY) for the primary outcome and STATA 13.0 for Windows (StataCorp LP, College Station, TX) for secondary outcomes.

RESULTS

Between November 2008 and December 2013, 171 antenatally diagnosed CDH patients from 9 European centers were included (Figure 1). The predicted severity of illness (SNAP-II score and O/E LHR) did not significantly differ between the 2 groups (Table 1). The characteristics of the 448 nonincluded patients were not significantly different for the prevalence of death as compared with the included patients (Table 2). None of the included patients was withdrawn from the study and the primary outcome was observed for all included patients. ECMO was available in 6 of the 9 centers (involving

Table 1 – Baseline Characteristics by Randomized Ventilation Mode

Variable	HFO (n= 80)	CMV (n= 91)	*P*-value
FETO	12 (15.0%)	7 (7.7%)	0.15
LHR	1.51 (1.07- 1.92)	1.53 (1.13-2.00)	0.67
O/ E LHR	47% (15%-141%)	48% (21%- 100%)	0.46
Gestational age	38 (37.3- 39.0)	38.1 (37.4- 38.9)	0.39
Birth weight (kg)	2.89 (0.47)	2.95 (0.46)	0.38
Male sex	36 (45.0%)	48 (52.7%)	0.36
SNAP-II score	25.0 (14.0- 40.0)	21.0 (10.0- 40.0)	0.44
Left side CDH	73 (91.3%)	75 (82.4%)	0.12
Liver			0.76
Intrathoracic	46 (57.5%)	55 (60.4%)	
Abdominal	34 (42.5%)	36 (39.6%)	
Type of repair			0.73
Primary closure	26 (32.5%)	27 (29.7%)	
Patch repair	42 (52.5%)	50 (54.9%)	
No repair	12 (15.0%)	14 (15.4%)	
Diaphragmatic defect size			0.10
A	6 (7.5%)	5 (5.5%)	
B	21 (26.3%)	28 (30.8%)	
C	28 (35.0%)	40 (44.0%)	
D	10 (12.5%)	2 (2.2%)	
No repair	12 (15.0%)	14 (15.4%)	
Unknown	3 (3.8%)	2 (2.2%)	

Table 1 – (continued)

Variable	HFO (n= 80)	CMV (n= 91)	*P*-value
Major cardiac anomaly			0.42
Aortic hypoplasia	2 (2.6%)	0 (0%)	
ASD and VSD	1 (1.3%)	0 (0%)	
HLHS variant	0 (0%)	1 (1.1%)	
Aortic stenosis	0 (0%)	1 (1.1%)	
No cardiac anomaly	77 (96.3%)	89 (97.8%)	
Age at repair (days)	5.0 (3.0- 9.0)	4.0 (3.0- 5.0)	0.005
Centers			0.43
1: (ECMO)	19 (46.3%)	22 (53.7%)	
2: (ECMO)	7 (41.2%)	10 (53.8%)	
3: (no ECMO)	7 (43.8%)	9 (56.2%)	
4: (ECMO since 01-01-2013)	30 (55.6%)	24 (44.4%)	
5: (ECMO)	12 (48.0%)	13 (52.0%)	
6: (ECMO)	3 (37.5%)	5 (62.5%)	
7: (ECMO)	1 (12.5%)	7 (87.5%)	
8: (no ECMO)	1 (100%)	0 (0%)	
9: (ECMO)	0 (0%)	1 (100%)	

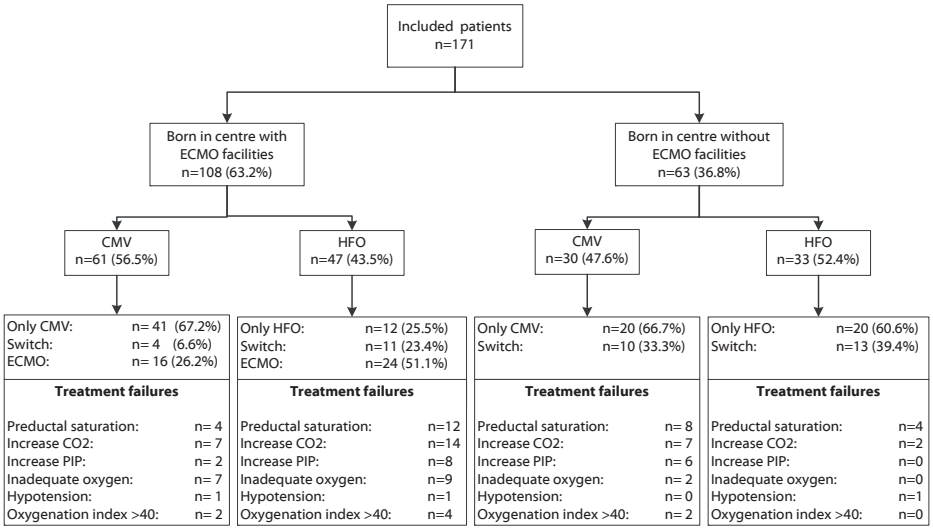
Results presented as n (%), mean (SD) or median (IQR). ASD, atrial septal defect; FETO, fetoscopic endotracheal occlusion; HLHS, hypoplastic left heart syndrome; LHR, lung-to-head ratio; O/E LHR, observed to expected lung-to-head ratio; PH, pulmonary hypertension; SNAP-II score, score for neonatal acute physiology-II; VSD, ventricular septal defect.

Table 2 – Background characteristics nonparticipants

Variable	n =448
Sex: female	167/ 411 (40.6%)
Unknown	37 (8.3%)
Gestational age (weeks)	36.8 (3.0)
Birth weight (kg)	2.76 (0.68)
Side	
Left	345/ 421 (81.9%)
Right	73/421 (17.3%)
Bilateral	3/421 (0.7%)
Unknown	27 (6.0%)
Inborn	320/425 (75.3%)
Unknown	23 (5.1%)
Death	105/ 420 (25.0%)
Unknown	28 (6.3%)

Results are presented as n (%) or mean (SD).

Figure 2 – Flowchart treatment failures



CMV indicates conventional mechanical ventilation; ECMO, extracorporeal membrane oxygenation; HFO, high-frequency oscillation; PIP, peak inspiratory pressure. Preductal saturation: inability to maintain preductal saturations above 85% (±52 mmHg or 7kPa) or postductal saturations above 70 % (±5.3 kPa or 40 mmHg). Increase CO_2: increase in CO_2 >65 mmHg or 8.5 kPa despite optimization of ventilatory management. Increase PIP: peak inspiratory pressure >28 cm H_2O; mean airway pressure >17 cm H_2O. Inadequate oxygen: inadequate oxygen delivery with metabolic acidosis defined as lactate ≥5 mmol/ L and pH <7.20. Hypotension: hypotension resistant to fluid therapy and inotropic support resulting in a urine output <0.5 ml/kg/hour. Oxygenation index >40: oxygenation index of longitudinal evaluation ≥40.

100 patients), in 2 centers it was not (17 patients), and in 1 center ECMO was introduced in 2013 (46 patients before ECMO and 8 patients after ECMO). Forty of the 108 (37%) patients born in a center with ECMO availability were subsequently supported by ECMO (Figure 2). Although the protocol dictated that the allocated ventilator mode should be started within 2 hours after birth, in all children, it was started within 1 hour after delivery.

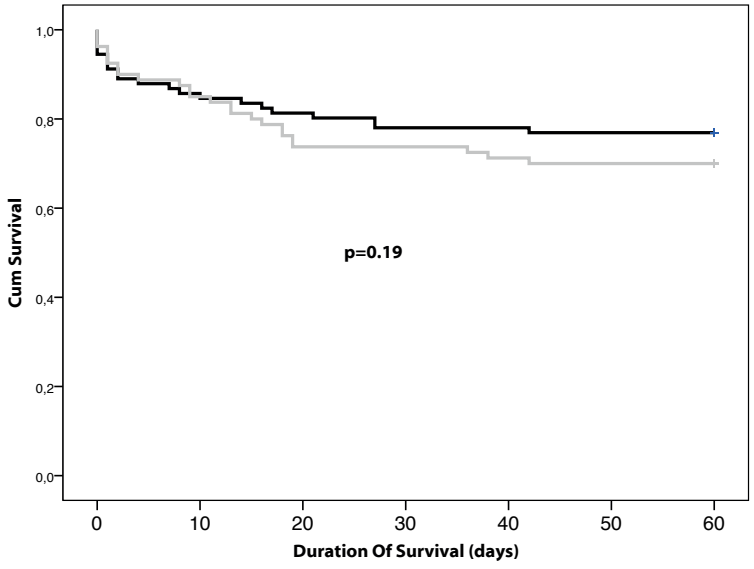
Forty-one of the 91 patients (45.1%) initially ventilated with CMV died or had BPD by day 28 compared with 43 of the 80 patients (53.8%) in HFO. That difference was not statistically significant after taking into account of center, lung-to-head ratio, side of the defect, and position of the liver, with an odds ratio (OR) of 0.62 [95% confidence interval (95% CI) 0.25- 1.55] (*P*=0.31) comparing CMV with HFO (Table 3). LHR (OR 0.164, *P* <0.001, 95% CI 0.064- 0.420) and liver position (OR 9.47, *P* <0.001, 95% CI 3.402- 26.359) were significantly associated with a worse outcome. The primary outcome results without pooling of the centers are shown in Supplemental Digital Content 1. A subgroup analysis of the 145 operated infants taking into account center, lung-to-head ratio, side of the defect, position of the liver, and defect size, demonstrated that 27 of 77 patients

Table 3 – Primary outcome: All patients (n=171)

Variable	OR	95% CI	*P*
Ventilation			
HFO	Ref		
CMV	0.620	0.249- 1.548	0.306
LHR	0.166	0.067- 0.413	0.000
Liver			
Down	Ref		
Up	10.574	4.006-27.911	0.000
Side			
Left	Ref		
Right	0.986	0.235- 4.139	0.985
Centers			
Center(1)	Ref		
Center(2)	0.221	0.034- 1.453	0.116
Center(3)	0.114	0.019- 0.683	0.017
Center(4)	0.210	0.038- 1.152	0.072
Center(5)	0.440	0.133- 1.455	0.179
Center(6)	2.310	0.461- 11.585	0.309

95% CI indicates 95% confidence interval; CMV, conventional mechanical ventilation; HFO, high-frequency oscillation; OR, odds ratio; Ref: reference category.

Figure 3 – Kaplan-Meier curve of all included patients comparing CMV and HFO



Black: Conventional mechanical ventilation. Grey: High-frequency oscillation.

(35.1%) in CMV and 31 of 68 patients (45.6%) in HFO died or had BPD by day 28; OR of 0.76 (95% CI 0.24- 2.41) (*P* =0.64) (Table 4). Median (interquartile range, IQR) values in the Hosmer Lemeshow test in the primary outcome of all patients were 0.306 (0.178 to 0.481) and in the subgroup analyses 0.653 (0.547to 0.779).

The duration of survival within first 60 days after birth did not differ between the 2 groups (*P*=0.19). (Figure 3). The overall mortality in the first year after birth was 21 patients (23.1%) in the CMV group and 25 patients (31.3%) in the HFO group (*P*=0.26). The median duration of ventilation was 10.0 days (IQR 6.0 to 18.0 days) in the CMV group and 13.0 days (IQR 8.0 to 23.0 days) in the HFO group (*P*=0.03) (Table 5). A subgroup analysis of the 108 patients born in a center with ECMO availability showed that 16 patients (26.2%) initially ventilated by CMV received ECMO vs 24 patients (51.1%) ventilated by HFO (*P*=0.007). The number of treatment failures was significantly different between the 2 treatment groups, 27 patients (33.8%) in the HFO group vs 20 patients (22.0%)

Table 4 – Subgroup analyses in operated patients (n=145)

Variable	OR	95% CI	*P*
Ventilation			
HFO	Ref		
CMV	0.755	0.237- 2.412	0.636
LHR	0.157	0.043- 0.578	0.005
Liver			
Down	Ref		
Up	8.234	2.230- 30.400	0.002
Side			
Left	Ref		
Right	0.764	0.096- 6.065	0.799
Defect size			
Defect size A	Ref		
Defect size B	6.087	0.145- 255.322	0.343
Defect size C	9.915	0.260- 378.726	0.217
Defect size D	49.831	0.588- 4222.363	0.084
Centers			
Center(1)	Ref	Ref	Ref
Center(2)	0.110	0.010- 1.213	0.071
Center(3)	0.087	0.008- 0.896	0.040
Center(4)	0.048	0.005- 0.480	0.010
Center(5)	0.155	0.032- 0.750	0.020
Center(6)	2.386	0.424- 13.426	0.324

95% CI indicates 95% confidence interval; CMV, conventional mechanical ventilation; HFO, high-frequency oscillation; OR, odds ratio; Ref: reference category.

Table 5 – Secondary Outcomes by Ventilation Group Corrected for Center

Variable	HFO (n= 80)	CMV (n= 91)	*P*
Overall mortality in first year of life	25 (31.3%)	21 (23.1%)	0.26
Length of ventilation (days)	13.0 (8.0- 23.0)	10.0 (6.0- 18.0)	0.03
Severity BPD			0.13
No BPD	37 (46.3%)	50 (54.9%)	
Mild BPD	7 (8.8%)	13 (14.3%)	
Moderate BPD	2 (2.5%)	1 (1.1%)	
Severe BPD	9 (11.3%)	6 (6.6%)	
Died	25 (31.3%)	21 (23.1%)	
ECMO (in ECMO centers only)	24/ 47 (51.1%)	16/ 61 (26.2%)	0.007
Inhaled nitric oxide	45 (56.2%)	39 (42.9%)	0.045
Phosphodiesterase inhibitor 5 (Sildenafil)	25 (31.2%)	11 (12.1%)	0.004
Vasoactive medication	73 (91.2%)	73 (80.2%)	0.08
Duration vasoactive medication (days) (in survivors only)	8.0 (4.3- 19.0)	6.0 (3.3- 11.8)	0.02
Number of treatment failures	27 (33.8%)	20 (22.0%)	0.01
Presence pulmonary hypertension	57 (71.3%)	59 (64.8%)	0.16
	Missing: n=3	Missing: n=4	
Severity pulmonary hypertension			0.59
None	20 (25.0%)	29 (31.9%)	
<2/3 systemic	9 (11.3%)	10 (11.0%)	
2/3 systemic- systemic	26 (32.5%)	26 (28.6%)	
> systemic	22 (27.5%)	21 (23.1%)	
Missing	3 (3.8%)	5 (4.4%)	

Results presented as n (%) or median (IQR). BPD indicates bronchopulmonary dysplasia; ECMO, extracorporeal membrane oxygenation.

in the CMV group (*P*=0.01). Of the 22 infants who were initially ventilated by CMV and due to treatment failures that switched to HFO, 14 (63.6%) died, 18 (81.8%) died or had BPD by day 28, and 8 (36.4%) also received ECMO treatment. In the CMV group, 39 patients (42.9%) received iNO vs 45 patients of the 80 patients (56.2%) in the HFO group (*P*=0.045). Eleven patients (12.1%) initially ventilated by CMV received a phosphodiesterase 5 inhibitor vs 25 patients (31.2%) initially ventilated by HFO (*P*=0.004). The median duration of vasoactive medication was 6.0 days (IQR 3.3 to 11.8 days) in the CMV group and 8.0 days (IQR 4.3 to 19.0 days) in HFO (*P*=0.02). The median age at repair was 5.0 days (IQR 3.0 to 9.0 days) in the CMV group and 4.0 days (IQR 3.0- 5.0 days) in the HFO group (*P*=0.005). The median length of ICU stay of the survivors in the CMV group was 23.0 days (IQR 23.8 to 35.3 days) and that of the survivors in the HFO group 20.0 days (IQR 13.0 to 54.0 days), *P*=0.99. Patients born in a center with ECMO facilities as

compared with patients born in a center without ECMO facilities were not significantly different for the primary outcome (died or BPD), overall mortality in the first year of life, length of ventilation, severity of BPD, frequency of iNO, vasoactive medication, number of treatment failures, and switching of ventilation mode (Supplemental Digital Content 2). In Supplemental Digital Content 3a and 3b, secondary outcomes per ventilation group and differences between ECMO centers and non-ECMO centers are shown. In the sensitivity analysis, there was no significant interaction between ECMO availability and type of initial ventilation (OR 2.05, *P*= 0.44).

The trial was stopped early after enrolment of 171 participants in an inclusion period of 5 years because of lower than anticipated recruitment rates and due to a lack of financial resources and a lack of research infrastructure in 1 high-volume center.

DISCUSSION

In this first RCT comparing HFO and CMV in infants prenatally diagnosed with CDH, we have demonstrated no statistically significant difference in the combined outcome of mortality or BPD between the 2 ventilation groups. The infants initially supported by CMV required a significantly shorter duration of ventilation and inotrope support, however, and were less likely to receive vasoactive medication or phosphodiesterase type 5 inhibitors or be placed on ECMO.

Previous animal studies as well as observational and retrospective human studies about the optimal mechanical ventilation strategy in CDH have shown contradictory results[2, 8, 9]. On the one hand, animal studies have suggested that HFO could improve pulmonary gas exchange, minimize barotrauma, and decrease the presence of inflammatory mediators[16, 17]. The models used in those studies are not comparable, however, to the clinical situation, as in the animal models, the CDH was induced by interfering with development of the lungs in a normally programmed lung antenatally, which is quite different from the pathophysiology of the abnormal lung development in CDH. On the other hand, Wilson et al[18] showed in infants that there was no difference in outcome between HFO and CMV ventilation. That study, however, was performed before the introduction of a gentle ventilation strategy with permissive hypercapnia. We found that infants initially ventilated by HFO needed mechanical ventilation for a longer time despite no significant difference in markers of severity such as SNAP-II score and O/E LHR between the 2 ventilation groups. A possible explanation is the process by which HFO causes overinflation of the terminal lung units, and especially of the ipsilateral lung, which leads to disruption of the epithelium and thereby to retained secretions and debris. Due to that process, the alveoli could possibly be more vulnerable to inflammation[19].

Moreover, the intubation and endotracheal suctioning procedures in mechanical ventilation can lead to injury of the tracheobronchial tree and – possibly even more important – to damage to the ciliated cells of the tracheal epithelium and mucociliary transport system[19]. We did not document the frequency of endotracheal suctioning and it is debatable whether this is more often needed in HFO than CMV, but the longer duration of HFO may have been associated with more endotracheal suctioning procedures. Although in HFO tidal volumes are very low, high levels of PEEP with constant tidal volumes may also exacerbate VILI[20]. Schultz et al[21] found that prolonged mechanical ventilation induced pulmonary inflammation in preterm infants by increasing pulmonary edema. As infants with HFO were ventilated longer, this could have contributed to more pulmonary inflammation[21]. In future studies, tracheal aspirates of CDH patients examining sphingolipids levels may possibly give further insight in the exact mechanism of VILI[22].

Infants in the HFO group received vasoactive medication for a longer period and were significantly more likely to receive iNO and a phosphodiesterase type 5 inhibitor. Those treatment modalities are used in the treatment of pulmonary hypertension[15]. We also found that infants initially ventilated by CMV were significantly less likely to receive ECMO treatment. The above results suggest that in the CMV group, the occurrence of pulmonary hypertension was less frequent and that it was less severe. The protocol dictated echocardiography only in the first 24 hours after birth, in which period we documented no significant difference in the occurrence and severity of pulmonary hypertension. However, subsequent echocardiographies were undertaken that influenced management of the patients.

Some limitations of this study should be considered. Firstly, we did not achieve the calculated sample size. Secondly, we excluded patients born before 34 weeks of gestation, as these infants could additionally have respiratory distress syndrome and the results of surfactant administration in this group are poor[23]. Moreover, very poor survival has been reported in CDH infants born before 33 weeks of gestation treated by FETO[24]. Almost 10% of the parents refused to participate in the study. Due to limited financial resources and a lack of research infrastructure in 1 high-volume center, inclusion was stopped after 1 year and 8 months. We had extended the inclusion period from 3 to 5 years and we calculated that the study would need to continue for a total period of 10 years to achieve the sample size. At that time, we had no expectation of an improving inclusion rate and saw no reason to further extend the inclusion period and therefore stopped the study. The data were not analyzed until after this decision and thus the decision was not influenced by preliminary results.

Using the assumptions of the original power calculation, the attained sample size (n=80 for the HFO group and n=91 for the CMV group) yields a power of at least 44% to detect a difference of 15% in primary outcome between the 2 ventilation groups. After a

child's condition had stabilized situation in the delivery room, the child was transferred to the ICU and received the allocated ventilation strategy. In all children, the allocated ventilator support was started within 1 hour after birth, but it cannot be excluded that receiving the other type of ventilation in the delivery room before that has influenced our results.

The major strengths of the current study was the fact that it was carried out in as many as 9 European centers in which all CDH patients are treated according to a standardized neonatal treatment protocol[4]. All centers had at least a period of more than 10 years of experience with HFO so we do not think that the factor "experience" has influenced our results. Secondly, central randomization stratified per center was carried out. We have reported size of the defect, major cardiac anomalies, and the outcome of patients who did not receive surgical therapy.

A meta-analysis of HFOV and prevention of BPD in prematurely born infants has shown a significant, although modest reduction in BPD[25], but a subsequent more detailed analysis did not confirm that effect[26]. A recently reported follow-up study of one of the RCTs included in that meta-analysis showed superior lung function at 11 to 14 years, despite no reduction in BPD[27]. It would thus be important to re-assess our patients at school age. It is acknowledged that the lung morphology of very prematurely born infants is very different from the dysplastic lungs of CDH infants, so we cannot extrapolate those results to our study.

In conclusion, although secondary analyses seem to suggest some benefit to CMV, based on the primary outcome of interest, we must conclude that there is no difference in effect between CMV and HFO as a primary mode of ventilation in infants with antenatally diagnosed CDH. Infants with CDH initially ventilated by CMV compared with those who received HFO required a shorter duration of ventilation and vasoactive medication and were less likely to require other medication to treat pulmonary hypertension or ECMO.

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Supplemental digital content 1 – Primary outcome without pooling of the centers

Variable	OR	95% CI	p-value
Ventilation(1)	0.615	0.245- 1.545	0.301
LHR	0.164	0.064- 0.420	0.000
Liver(1)	9.989	3.757- 26.564	0.000
Side(1)	0.999	0.220- 4.545	0.999
Center(1)	0.362	0.033- 3.948	0.404
Center(2)	0.110	0.018- 0.659	0.016
Center(3)	0.213	0.007- 6.532	0.375
Center(4)	0.208	0.038- 1.142	0.071
Center(5)	0.436	0.133- 1.428	0.170
Center(6)	2.258	0.455- 11.210	0.319
Center(7)	0.000	0.000- 0.000	1.000
Center(8)	0.000	0.000- 0.000	1.000
Constant	10.469	1.342- 81.651	0.025

Supplemental digital content 2 – Primary and secondary outcomes by ECMO availability

Variable	No ECMO available (n=63)	ECMO available (n=108)	p-value
BPD or died	28 (44.4%)	56 (51.9%)	0.35
Overall mortality in first year of life	19 (30.2%)	27 (25.0%)	0.46
Length of ventilation (days)	9.0 (7.0- 16.0)	12.0 (7.0- 21.0)	0.25
Severity BPD			0.42
No BPD	35 (55.6%)	52 (48.1%)	
Mild BPD	5 (7.9%)	15 (13.9%)	
Moderate BPD	1 (1.6%)	2 (1.9%)	
Severe BPD	3 (4.8%)	12 (11.1%)	
Died	19 (30.2%)	27 (25.0%)	
Inhaled nitric oxide	27 (42.9%)	57 (52.8%)	0.21
Phosphodiesterase inhibitor 5 (Sildenafil®)	11 (17.5%)	25 (23.1%)	0.38
Vasoactive medication	54 (85.7%)	92 (85.2%)	0.93
Duration vasoactive medication (days) (in survivors only)	6.0 (4.0- 8.0)	8.0 (4.5- 13.0)	<0.001
Number of treatment failures	1.0 (1.0-2.0)	1.0 (0.0- 2.0)	0.39
Presence pulmonary hypertension	32 (53.3%)	83 (79.8%)	<0.001
Severity pulmonary hypertension			0.004
None	28 (44.4%)	21 (19.4%)	
<2/3 systemic	7 (11.1%)	12 (11.1%)	
2/3 systemic- systemic	11 (17.5%)	41 (38.0%)	
> systemic	14 (22.2%)	29 (26.9%)	

Supplemental digital content 2 – (continued)

Variable	No ECMO available (n=63)	ECMO available (n=108)	p-value
Missing	3 (4.8%)	5 (4.6%)	
Switching ventilation mode	23 (36.5%)	38 (35.2%)	0.86

Results presented as n (%) or median (IQR). Abbreviations: BPD: bronchopulmonary dysplasia; ECMO: extracorporeal membrane oxygenation.

Supplemental digital content 3a – CMV group: Outcomes by ECMO availability

Variable	No ECMO available (n=30)	ECMO available (n=61)	p-value
BPD or died	12 (40.0%)	29 (47.5%)	0.50
Overall mortality in first year of life	9 (30.0%)	12 (19.7%)	0.27
Length of ventilation (days)	9.0 (7.0- 13.0)	11.0 (6.0- 19.5)	0.25
Severity BPD			0.38
No BPD	18 (60.0%)	32 (52.5%)	
Mild BPD	2 (6.7%)	11 (18.0%)	
Moderate BPD	0 (0.0%)	1 (1.6%)	
Severe BPD	1 (3.3%)	5 (8.2%)	
Died	9 (30.0%)	12 (19.7%)	
Inhaled nitric oxide	11 (36.7%)	28 (45.9%)	0.40
Phosphodiesterase inhibitor 5 (Sildenafil®)	2 (6.7%)	9 (14.8%)	0.27
Vasoactive medication	23 (76.7%)	50 (82.0%)	0.55
Duration vasoactive medication (days) (in survivors only)	6.0 (4.0- 8.0)	8.0 (4.0- 12.3)	0.005
Number of treatment failures	2.5 (1.8- 3.0)	1.5 (1.0- 3.0)	0.22
Presence pulmonary hypertension	13 (44.8%)	45 (77.6%)	0.002
Severity pulmonary hypertension			0.03
None	16 (53.3%)	13 (21.3%)	
<2/3 systemic	3 (10.0%)	7 (11.5%)	
2/3 systemic- systemic	4 (13.3%)	22 (36.1%)	
> systemic	6 (20.0%)	15 (24.6%)	
Missing	1 (3.3%)	4 (6.5%)	
Switching ventilation mode	10 (33.3%)	12 (19.7%)	0.15

Results presented as n (%) or median (IQR). Abbreviations: BPD: bronchopulmonary dysplasia; ECMO: extracorporeal membrane oxygenation.

Supplemental digital content 3b – HFO group: Outcomes by ECMO availability

Variable	No ECMO available (n=33)	ECMO available (n=47)	p-value
BPD or died	16 (48.5%)	27 (57.4%)	0.43
Overall mortality in first year of life	10 (30.3%)	15 (31.9%)	0.88
Length of ventilation (days)	12.0 (7.0- 21.0)	13.5 (8.0- 23.0)	0.43
Severity BPD			0.78
No BPD	17 (51.5%)	20 (42.6%)	
Mild BPD	3 (9.1%)	4 (8.5%)	
Moderate BPD	1 (3.0%)	1 (2.1%)	
Severe BPD	2 (6.1%)	7 (14.9%)	
Died	10 (30.3%)	15 (31.9%)	
Inhaled nitric oxide	16 (48.5%)	29 (61.7%)	0.24
Phosphodiesterase inhibitor 5 (Sildenafil®)	9 (27.3%)	16 (34.0%)	0.52
Vasoactive medication	31 (93.9%)	42 (89.4%)	0.48
Duration vasoactive medication (days) (in survivors only)	7.0 (4.0- 13.5)	8.0 (6.0- 14.0)	0.005
Number of treatment failures	1.0 (0.5- 1.0)	1.0 (0.0- 2.0)	0.58
Presence pulmonary hypertension	19 (61.3%)	38 (82.6%)	0.04
Severity pulmonary hypertension			0.15
None	12 (36.4%)	8 (17.0%)	
<2/3 systemic	4 (12.1%)	5 (10.7%)	
2/3 systemic- systemic	7 (21.2%)	19 (40.4%)	
> systemic	8 (24.2%)	14 (29.8%)	
Missing	2 (6.1%)	1 (2.1%)	
Switching ventilation mode	13 (39.4%)	26 (55.3%)	0.16

Results presented as n (%) or median (IQR). Abbreviations: BPD: bronchopulmonary dysplasia; ECMO: extra-corporeal membrane oxygenation.

Chapter 7

Liquid ventilation in congenital diaphragmatic hernia: back on stage?!

Kitty G. Snoek, Robert Jan Houmes, Dick Tibboel

Pediatr Crit Care Med. 2014 Nov;15(9):914-5.

In congenital diaphragmatic hernia (CDH), lung hypoplasia and pulmonary hypertension are the main causes of neonatal mortality[1]. Mortality significantly decreased during the past 10 years, after the introduction of the gentle ventilation strategy and the development of international standards for postnatal therapy[2]. Still, ventilator-induced lung injury is largely responsible for the development of chronic lung disease in children with CDH[3].

In this issue of *Pediatric Critical Care Medicine*, Herber-Jonat et al present the results of a well- performed laboratory study in rabbits with induced CDH[4]. CDH was induced by fetal surgery and 5 days later perfluorooctylbromide, a perfluorocarbon, was instilled into the lungs of randomly selected fetal rabbits; other fetal rabbits received saline. A third group were non-operated fetuses who served as controls. Fetal instillation of perfluorooctylbromide was associated with improvement of lung-to-body weight ratio, total lung capacity, and lung compliance when compared with fetal instillation of saline. Secondly, at messenger RNA (mRNA) level only, expression of genes involved in extracellular matrix formation and remodeling in the hypoplastic lung was increased. However, surfactant protein expression, distal airway size, mean linear intercept, and airspace and tissue fractions were similar between the two groups and also similar to fetuses who were not operated upon. The authors concluded that fetal perfluorooctylbromide treatment resulted in improved lung growth, lung mechanics and extracellular matrix remodeling. Extrapulmonary effects of perfluorooctylbromide, such as effects on neuronal cell alteration and effects in the brain, should be determined in future studies before this therapy can be studied in human prenatal studies.

A ventilation technique known as liquid ventilation stems from the year 1929, when Von Neergard incidentally found that filling the lungs with saline solution dramatically improved the static pulmonary compliance in cats[5]. After further investigation of different types of liquids, Clark and Gollan received fame for their experiments of liquid ventilation using perfluorocarbon in mice for the first time[6]. In 1989, liquid ventilation showed its potential in a first trial in prematurely born neonates[7]. In CDH, Hirschl et al conducted a randomized trial in sheep[8] and concluded that partial liquid ventilation (PLV) during extracorporeal membrane oxygenation may have beneficial effects on pulmonary function and gas exchange. Pranikoff et al applied PLV with the use of perflubron in four CDH patients who required extracorporeal life support postnatally[9]. They concluded that this therapy was possibly associated with improvement in gas exchange and lung compliance. Later on Hirschl et al conducted a randomized trial in 13 CDH infants who were randomized to either PLV perfluorocarbon-induced lung growth or conventional mechanical ventilation[10]. They found that perfluorocarbon-induced lung growth can be performed safely. However, when this trial was still ongoing, in 2001 the Food and Drug Administration decided that all clinical trials with perflubron had to be discontinued until safety data were available. That decision was based on findings that

adults with acute respiratory distress syndrome randomized to PLV had no improved outcome and experienced more adverse events such as more pneumothoraces, hypoxic episodes, and hypotensive episodes[11]. Nevertheless in China, adults with acute respiratory distress syndrome are currently recruited in a randomized controlled trial of perfluorocarbon instillation (NCT01391481).

In normal fetal lung development, the lungs are liquid-filled, and fluid secretion and fetal breathing movements are necessary for lung maturation[12]. In abnormal situations such as in prematurely born neonates in which transition from liquid-breathing to an air-breathing situation takes place prematurely, and in fetuses with an amniotic fluid-deficient environment, lung development is likely to be immature resulting in lung-related problems postnatally. Instillation of perfluorooctylbromide in the trachea approximately to functional residual capacity can simulate the antenatal situation of liquid-filled airway branches. Thereafter, gas tidal volumes are delivered using a mechanical conventional ventilator. This is called PLV. In total liquid ventilation the lungs are completely filled with a liquid, whereas in PLV the lungs are filled until functional residual capacity. Perfluorocarbons have a high solubility for respiratory gases[6]. By eliminating the air-liquid interface, lung compliance can be improved[13]. Because of their dense characteristics, perfluorooctylbromide gravitate to dependent part of the lungs, and collapsed regions can be re-opened and ventilation/perfusion ratio may improve[13]. Next to these advantages, pulmonary inflammation and injury may be reduced as a result of decreased cytokine production. Moreover, in pigs receiving PLV, a redistribution of pulmonary blood flow away from the dependent region of the lung was found, as well as increased vascular resistance and pulmonary artery pressure[14].

In line with the experiments of the article from Herber-Jonat et al, we know that a complete obstruction of the fetal airways results in massive lung distension and a poly-alveolar lung the so-called congenital high airway obstruction syndrome (CHAOS)[15]. Taking this concept, the Tracheal Occlusion To Accelerate Lung Growth trial (NCT01240057) is an ongoing trial of tracheal occlusion to accelerate lung growth in prenatally diagnosed high-risk CDH infants stratified according to observed to expected lung-to-head ratio. Another study is planning to include patients for early tracheal occlusion (NCT01731509). Moreover, a trial known as the HFO versus conventional Ventilation in Infants with Congenital Diaphragmatic hernia: an International randomized clinical trial (NTR 1310) was performed in nine European centers to identify the optimal ventilation strategy in antenatally diagnosed CDH infants. These studies might solve some of the challenges that stand in the way of further improvement in the treatment of CDH infants. Herber-Jonat et al conducted a randomized laboratory study in animals with a unique study design. Instead of only obstructing the fetal airway, they antenatally filled the lungs with perfluorooctylbromide, thus simulating the situation in normal lung development. However, the authors focused on mRNA expression and protein

analyses were not performed, which should be a serious limitation for the interpretation of the results. Second, in this study diaphragmatic hernia was induced by fetal surgery of normal programmed lungs, which makes the pathophysiology of developing CDH potentially different when compared to humans. Moreover, in rabbits, term birth occurs in the early saccular stage of lung development, whereas in humans the alveolarization process has taken place already during gestation. Therefore a different respons on perfluorooctylbromide may be found in humans.

Although the prognosis of CDH has improved during the past years, it is still a life-threatening disease and ventilator-induced lung injury remains a significant problem. Conclusive findings from randomized clinical trials may enable us to improve the outcome of CDH further. Once adverse long-term effects of PLV have been excluded, setting up a randomized clinical trial of antenatal or postnatal instillation of perfluorooctylbromide in carefully selected patients might be a promising tool to investigate alternative ways of supporting the vulnerable lungs in high-risk newborns with CDH further.

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Chapter 8

Routine intubation in the newborn with congenital diaphragmatic hernia; resetting our minds

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ABSTRACT

Due to the high risk of respiratory insufficiency in congenital diaphragmatic hernia (CDH), international guidelines suggest that neonates are routinely intubated after birth. We encountered five prenatally diagnosed CDH cases, in which we considered to forgo routine intubation after birth. Three patients were ventilated during surgical repair only as they did not experience respiratory insufficiency. One patient received continuous positive airway pressure for several minutes after birth followed by oxygen through a nasal cannula, and received mechanical ventilation only during surgical repair. The fifth patient developed a bradycardia directly after birth and was therefore intubated several minutes after birth. Extubation was feasible after 30 minutes, and oxygen was provided by a nasal cannula until the surgical repair. Our experience indicate that dispensing with routine intubation dependent of the transitional phase of the neonate in a selected subgroup of CDH infants (LHR >2.5 or O/E LHR >50%, liver down) was found feasible.

INTRODUCTION

Congenital diaphragmatic hernia (CDH) is a severe congenital anomaly with still a high mortality rate of about 25%. In the 1980s a shift in treatment occurred after the introduction of delayed surgical repair[1], i.e. waiting until the infant's condition has stabilized, and a new 'gentle' ventilation strategy was introduced[2]. The consensus guidelines published by the CDH EURO Consortium dictate routine intubation and mechanical ventilation after birth[3]. However, ventilator-induced lung injury is a risk factor of chronic lung disease, which is seen in about 56% of infants with CDH[4]. We describe five cases in which we considered to forgo routine intubation after birth. For each patient, this decision was prenatally discussed with the parents and discussed during regular multidisciplinary perinatal meetings between all involved clinicians.

CASE REPORT

In these five cases, CDH was antenatally detected between 25 weeks and 38.5 weeks of gestation, with the following characteristics: lung-to-head ratio (LHR) from 2.5 to 4.2, observed-to-expected LHR (O/E LHR) between 50 to 80%, liver position intra-abdominal, and gastric position in all but one case intra-abdominal. Prenatal ultrasound was performed using the GE Voluson E8/E10 system (GE Medical Systems, Zipf, Austria) at the Department of Obstetrics and Gynecology, Division of Obstetrics and Prenatal Medicine by experienced investigators.

Between the gestational ages of 36+1 and 38+6 weeks, four patients were delivered by vaginal delivery, and one patient was delivered by caesarean section. The Apgar score at 5 minutes was 8 or higher, lowest umbilical arterial cord blood pH was 7.27, and birth weight was between 2470 and 3580 grams. After birth, a nasogastric tube was inserted and continuous suctioning was started to prevent dilatation of bowel loops in the ipsilateral thoracic cavity compromising lung expansion. In all patients, preductal saturations in the transitional phase were in the normal range[5].

Three patients were ventilated during the surgical repair only and postoperatively received oxygen by nasal cannula for a maximum of 14 days. They did not experience respiratory insufficiency after birth. A fourth patient received continuous positive air pressure (CPAP) with PEEP of 5 cm H2O and 80% O_2 for several minutes after birth followed by oxygen through a nasal cannula, and received mechanical ventilation only during surgical repair. Postoperatively this patient received supplemental oxygen by a nasal cannula for 26 days. The fifth patient developed a bradycardia directly after birth (60 bpm) and was therefore intubated several minutes after birth. Extubation was feasible after 30 minutes, and oxygen was provided by a nasal cannula until the surgical repair.

In all but one cases a thoracoscopic approach and a primary closure could be performed. In one case conversion to a laparotomy with patch repair was necessary because the anterior part of the diaphragm could not be attached with the weak tissue of the posterior part of the diaphragm. Length of stay at the intensive care unit ranged from four to ten days.

DISCUSSION

In conclusion, dispensing with routine intubation dependent of the transitional phase of the neonate in a selected subgroup of CDH infants (LHR >2.5 or O/E LHR >50%, liver down) was found feasible. It is even preferable to prevent consequences of ventilation such as fluctuations of blood pressure, sedation and ventilator-induced lung injury. Larger studies should corroborate this finding and perhaps bring about a resetting of the dogma of endotracheal intubation in all prenatally diagnosed CDH patients.

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Chapter 9

Pharmacodynamic considerations in the treatment of pulmonary hypertension in infants: challenges and future perspectives

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ABSTRACT

Introduction: Pulmonary hypertension (PH) in infants is a life-threatening disease with a high mortality. It is treated with different drugs that act upon the three different pathways involved in its development. Studies on the drug pharmacodynamics are sparse, however.

Areas covered: This review reports a search on the currently available literature in English on drug pharmacodynamics in infants with PH. The search yielded 2499 citations in the EMBASE, MEDLINE, COCHRANE, Web of Science, PubMed Publisher and Google Scholar databases since 1961. Of these, 1691 did not meet the research question. Eventually, 655 articles were of interest, including 44 randomized controlled trials on PH in infants. These articles cover all PH medications used in infancy.

Expert opinion: Mortality of PH in infancy has dropped considerably over the past years. iNO is widely used, followed by sildenafil – both orally and intravenously in contrast to the exclusively oral use in adults. In adults, the pharmacodynamic effects of the different medications are tested using the 6-minute walking test, changes in the NYHA classification, or by invasive measurement of pulmonary pressure. Reliable data of pharmacodynamics tested in adequate series or in randomized controlled trials in children are lacking, however, for most of these medications.

ARTICLE HIGHLIGHTS:

- All medications influencing the three major pathways of PH are also used in infants. Only for iNO there is sufficient evidence for its positive effect in PPHN and a favorable effect in post- cardiac surgery patients.
- Sildenafil is used increasingly even though only a few RCTs showed a decrease in OI and mortality in resource limited settings. This effect needs to be validated in comparison to for example iNO.
- To date, Bosentan cannot be recommended due to a lack of valid data. Availability of an intravenous formulation might make (selective) endothelin receptor blockers drugs of choice in the future.
- The lack of noninvasive and continuous methods to monitor actual changes of PH in infants complicates pharmacodynamic research. Advanced echocardiography including tissue Doppler imaging, strain and strain rate imaging and new plasma biomarkers will hopefully solve this problem in the future.
- Only large international RCTs can include sufficient numbers of patients and yield valid data to further improve treatment of infants with PH.

1 INTRODUCTION

Pulmonary hypertension (PH) in children is a life-threatening cardio-respiratory disorder with considerable mortality and morbidity[1]. Like in adults, it is defined as the mean pulmonary artery pressure (mPAP) exceeding 25 mmHg with a pulmonary capillary wedge pressure of minimal 15 mmHg evaluated by right heart catheterization[2]. However, the pulmonary vascular transition of the neonate after birth takes time to achieve normal values of mPAP. During fetal life, the placenta delivers oxygenated blood to the organs. Due to the high resistance in the pulmonary circulation, most of the blood flow bypasses the lungs through the foramen ovale and ductus arteriosus. Immediately after birth when the newborn takes the first breaths, the blood flow to the lungs significantly increases and the pulmonary vascular resistance drops due to multiple factors. Normal values of mPAP are usually reached around two months after birth.

Although the etiology of PH is heterogeneous, the elevated mPAP and increased pulmonary vascular resistance (PVR) will lead to right ventricular hypertrophy and dilation, which can result in right ventricular failure and eventually in death. General mechanisms that cause PH include increased vascular tone and reactivity, vessel wall structural remodeling and thrombosis, and impaired vascular growth[3]. Typical histopathological changes are adventitial thickening, medial hypertrophy, intima proliferation and fibrosis, and more complex lesions such as plexogenic arteriopathy[4]. Different molecular path-

ways are involved in the development of abnormal pulmonary vasculature, but the roles of these pathways in human pulmonary vascular development are not understood in great detail. *In vitro* studies in adults and children with PH have shown increased expression of endogenous vasoconstrictors such as thromboxane and endothelin-1 (ET-1), and decreased expression of vasodilators[5]. Increased ET-1 plasma concentrations have been found in newborn infants with PPHN and in CDH infants but also in patients with PH due to congenital heart defects[6,7]. *In vitro*, ET-1 activated Rho-kinase in the lungs of fetal lambs with PPHN, which decreased tube formation and the number of branch points. The latter events were prevented by treatment with bosentan, suggesting that bosentan stimulates vascular growth in PPHN[8]. Increased ET-1 plasma concentrations have also been found in piglets with PH due to overcirculation. Bosentan completely prevented arteriolar thickening and thereby the development of PH[9]. Sildenafil is thought to be able to increase cGMP in the pulmonary artery smooth muscle cells but also seems to have a role in the treatment of right ventricle hypertrophy (RVH). PDE5 is not expressed in the normal human heart nor in the rat heart, but is markedly upregulated in the hypertrophied right ventricle. In a rat model with RVH, cGMP and cAMP increased after treatment with sildenafil but this was not the case in the normal right ventricle. Moreover, contractility increased significantly, and sildenafil therefore seems promising for the treatment of PH[10].

The first classifications of PH were designed mainly to address the different causes of PH in adults, but the latest adaption also includes classic neonatal and pediatric causes of PH. A new pediatric classification has been developed in 2011, which differentiates five subcategories (Table 1). Persistent pulmonary hypertension of the newborn (PPHN) is listed in a separate subcategory in Group 1. Other conditions added are congenital and acquired left heart inflow and outflow tract obstruction (Group 2) as well as developmental lung diseases associated with PH, such as congenital diaphragmatic hernia and bronchopulmonary dysplasia (Group 3)[11].

Epidemiological data on pediatric PH are sparse. Recently, however, a few national and international registries have published incidence and prevalence figures. The majority of children with progressive PH suffer from idiopathic/ heritable PH or from PH due to a congenital heart disease, but the number of children with lung disease, including lung hypoplasia or bronchopulmonary dysplasia, is growing[12]. In the United Kingdom, the prevalence of idiopathic PH is 2.1 per million children[13], and in France it is 2.2 per million[14]. PPHN has an incidence of 2 per 1000 live births[15]. In this transient form of PH the physiological decrease of pulmonary vascular resistance after birth does not occur.

Targeted PH therapies in infants and children have improved outcomes in general, but are mainly based on data from adult studies. Three main pathways are known to influence PH: the nitric oxide-cGMP pathway, the endothelin pathway, and the prostacyclin pathway (Figure 1)[16]. Targeted pharmacological therapy includes three classes of drugs

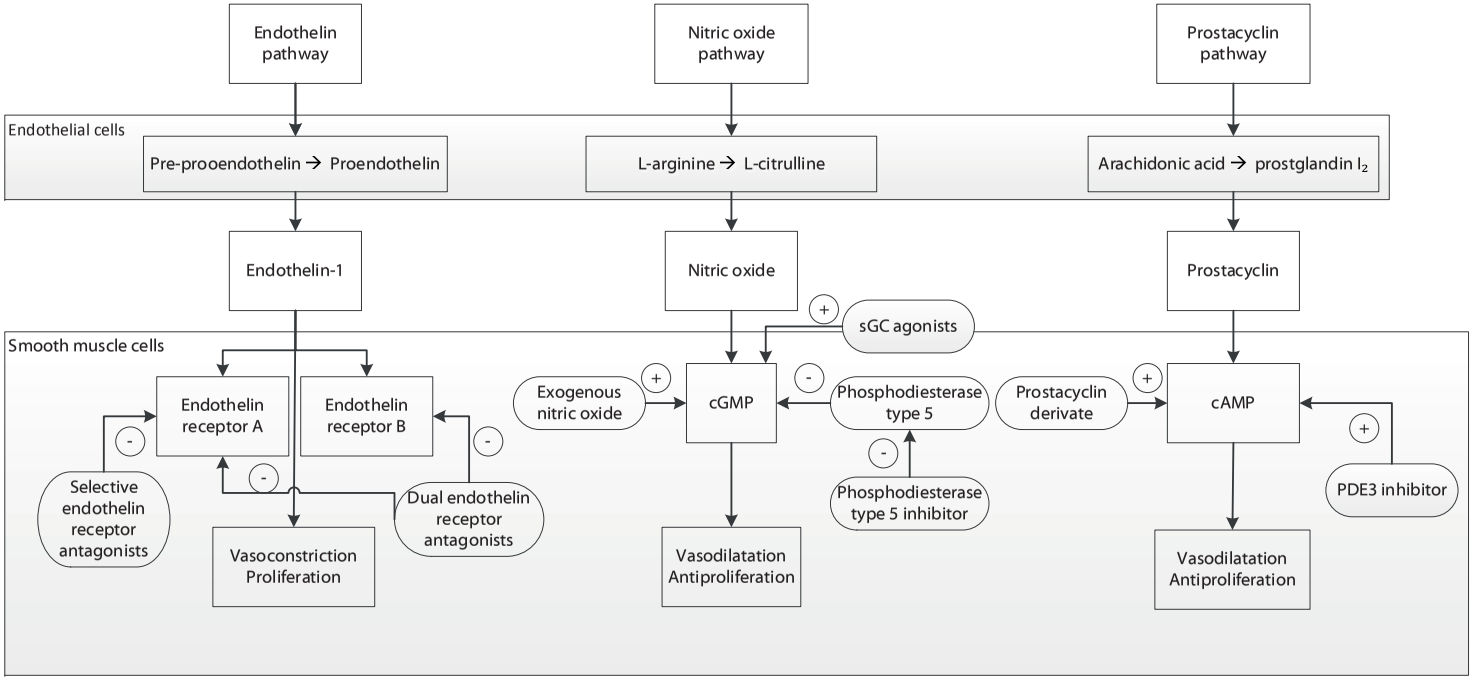
Table 1 – Updated Classification of Pulmonary Hypertension*

1. Pulmonary arterial hypertension
1.1 Idiopathic PAH
1.2 Heritable PAH 1.2.1 BMPR2 1.2.2 ALK-1, ENG, SMAD9, CAV1, KCNK3 1.2.3 Unknown
1.3 Drug and toxin induced
1.4 Associated with: 1.4.1 Connective tissue disease 1.4.2 HIV infection 1.4.3 Portal hypertension 1.4.4 Congenital heart diseases 1.4.5 Schistosomiasis
1' Pulmonary veno-occlusive disease and/or pulmonary capillary hemangiomatosis
1" Persistent pulmonary hypertension of the newborn (PPHN)
2. Pulmonary hypertension due to left heart disease
2.1 Left ventricular systolic dysfunction
2.2 Left ventricular diastolic dysfunction
2.3 Valvular disease
2.4 Congenital/acquired left heart inflow/outflow tract obstruction and congenital cardiomyopathies
3. Pulmonary hypertension due to lung diseases and/or hypoxia
3.1 Chronic obstructive pulmonary disease
3.2 Interstitial lung disease
3.3 Other pulmonary diseases with mixed restrictive and obstructive pattern
3.4 Sleep-disordered breathing
3.5 Alveolar hypoventilation disorders
3.6 Chronic exposure to high altitude
3.7 Developmental lung diseases
4. Chronic thromboembolic pulmonary hypertension (CTEPH)
5. Pulmonary hypertension with unclear multifactorial mechanisms
5.1 Hematologic disorders: chronic hemolytic anemia, myeloproliferative disorders, splenectomy
5.2 Systemic disorders: sarcoidosis, pulmonary histiocytosis, lymphangioleiomyomatosis
5.3 Metabolic disorders: glycogenstorage disease, Gaucher disease, thyroiddisorders
5.4 Others: tumoral obstruction, fibrosing mediastinitis, chronic renal failure, segmental PH

Notes: *5th WSPH Nice 2013.
BMPR: bone morphogenic protein receptor type II; CAV1: caveolin-1; ENG: endoglin; HIV: human immunodeficiency virus; PAH: pulmonary arterial hypertension.

based on these pathways. Adults also receive combinations of these medications even though there is no consistent evidence that an upfront combination therapy (use of two or more therapies in treatment-naïve patients) might be beneficial to long-term outcome[17]. Inhaled, oral and intravenous therapies are being used. Most of these drugs,

Figure 1 – The three major pathways influencing pulmonary hypertension



however, not only dilate the pulmonary vasculature, but also have systemic effects such as hypotension.

There is a lack of knowledge of the pathophysiology of the different types of PH and lack of well-designed trials for this specific group of patients. Hence, an extensive overview of the level of evidence for the different medications used in infancy can help clinicians optimize their therapies.

2 MEDICATION INFLUENCING THE NITRIC OXIDE-CGMP PATHWAY

2.1 Inhaled nitric oxide (iNO):

2.1.1 Introduction to iNO:

In 1987, nitric oxide (NO) was recognized as a key endothelial-derived vasodilator and endothelial derived growth factor[18]. The therapeutic potential of inhaled NO (iNO) as a selective vasodilator in patients with PH was first described in 1991[19]. The inhalation gas INOmax (Linde Gas Therapeutics GmbH, Oberschleissheim, Germany) was the first to be accepted as a medical product – in 1999 by the Food and Drug Administration (FDA) for the USA and in 2001 by the European Commission for the EU for the treatment of ARDS and PH in neonates.

2.1.2 Pharmacokinetics and metabolism of iNO

After inhalation, iNO diffuses rapidly across the alveolar-capillary membrane into the smooth muscle of pulmonary vessels and activates soluble guanylate cyclase. This enzyme mediates many of the biological effects of iNO and is responsible for the conversion of GTP to cGMP. The increase of intracellular concentrations of cGMP relaxes smooth muscle via several mechanisms. iNO also has some other effects in the lung such as bronchodilation, as well as anti-inflammatory and antiproliferative effects[20]. iNO diffuses into the bloodstream and reacts with oxyhemoglobin to form methemoglobin and nitrate, and reacts with deoxyhemoglobine to form iron-nitrosyl Hb. Within 48 hours, 70% of the iNO is excreted as nitrate in the urine.

In a normal lung, low oxygen tension leads to constriction of the vessels in the hypoxic region and the blood flow is directed toward lung regions with better ventilation. iNO enhances this mechanism by increasing blood flow to well ventilated lung areas that, in some diseases, have an elevated vascular tone. This is the advantage of iNO over intravenous vasodilators. The latter usually lead to a general vasodilation of the pulmonary vasculature, also including areas with less or no ventilation, resulting in increased intrapulmonary shunting and reduced PaO_2[20].

2.1.3 Clinical efficacy and role of iNO in current PH management

Over 20 years, iNO has been frequently used in the treatment of PH in children, especially in neonates suffering from PPHN. In a Cochrane analysis from 2006[21], fourteen randomized controlled trials (RCTs) were included. The authors concluded that with the present evidence it appears reasonable to use iNO in an initial concentration of 20 ppm for term and near term infants with hypoxic respiratory failure. Outcome appeared to be improved in terms of a reduced incidence of the combined endpoint of death or need for extracorporeal membrane oxygenation (ECMO). There was less need for ECMO, but the mortality stayed the same. In 50% of the infants oxygenation was improved while receiving iNO. Interestingly, the outcome did not seem to be affected by the presence or absence of echocardiographic signs of PH. iNO could not be recommended for patients with congenital diaphragmatic hernia (CDH) because in the one available RCT in patients with CDH[22] outcome had not improved, and was even slightly worse.

In 2010, Golomek *et al.* published a retrospective pooled analysis of three pivotal clinical trials comparing patients treated with iNO with control patients[23]. All three trials were part of the Cochrane analysis and included a total of 524 patients (260 iNO, 264 control). This combined analysis was performed to assess the effects of iNO on oxygenation and the efficacy across a range of disease severities, and days of ventilation. Golomek *et al*. defined disease severity in four categories depending on the baseline oxygenation index (OI): ≤ 15 = mild, >15 - ≤25 = moderate, >25 - ≤ 40 = severe, and > 40 = very severe. They studied the effect of iNO after 30 minutes. In all four categories the PaO_2 significantly improved. Furthermore, the median duration of mechanical ventilation was significantly shorter (11 days versus 14 days).

In contrast to what is the case in the US, iNO is standard of care in infants with CDH in Europe if PH is evident, even though positive pharmacodynamic effects in these infants seem to be weaker than in infants with PPHN[24]. In the US there is an increasing use of iNO; however, in view of its high costs and the absence of a decrease in mortality, iNO is used less frequently than in Europe[25,26]. It is not clear why iNO is less effective in infants with CDH than in infants with other underlying causes of PH. The actual pathophysiological mechanism is not completely understood. Shehata *et al*. found reduced vascular expression of NO synthase in postmortem lung specimens of CDH-patients not treated with ECMO, which might explain the lack of effect of iNO[27]. Such reduction was found earlier in a CDH rat model as well[28].

There is some evidence that premature infants with prolonged preterm rupture of the membranes (PPRM) might respond favorable to iNO. Part of the evidence comes from a non-RCT on the antenatal risk factors and inflammatory response during hypoxic respiratory failure (HRF) in infants ≤ 32 weeks[29]. The 17 premature infants who developed HRF were also exposed to PPROM and responded well to iNO. They showed a transient suppression in nitrite and nitrate and inflammatory cytokines obtained from airway

specimens. The authors concluded therefore that these infants may have a transient deficiency in the inflammatory response, including a defect in nitric oxide generation in airspace. Further research is needed to support these findings. Furthermore, one Cochrane analysis has evaluated the effects of iNO in premature infants with respiratory distress on death, bronchopulmonary dysplasia (BPD), intraventricular hemorrhage (IVH), and neurodevelopmental disability[30]. A total of 14 RCT's were identified and grouped into three categories: entry in the first three days of life based on oxygenation criteria, routine use in preterm infants, and later enrolment based on increased risk of BPD. An evaluation of the absence or presence of PH was mostly not part of the study design. The authors of the Cochrane analysis found no positive effect of iNO used in the early period, but suggested that the later use of iNO might be effective to prevent BPD. However, this needs to be proven by further studies, especially with the focus on development of PH in this age group. Other factors such as maternal ethnicity have been discussed; but so far there is no proof for a possible beneficial response of iNO related to maternal ethnicity.

iNO is also often used in patients with PH due to congenital heart disease before and after surgery. This application was subject of a Cochrane analysis in 2005, which was updated in 2014[31,32]. The first analysis included four RCTs with a total of 215 infants and children and found no difference in mortality, changes of pulmonary hypertensive crises (PHTC), changes in mPAP, heart rate or PaO_2:FiO_2 ratio. The updated analysis included another three studies and again concluded that outcomes did not differ between the use of iNO or not. One study, including 124 patients with a median age of three months, had a low risk of bias[33]. This was a trial investigating the effect of iNO on PHTC, time on study gas, and length of intensive care stay when given routinely to children with high pulmonary flow or pressure, or both, after corrective heart surgery[33]. The pharmacodynamic effect of iNO was monitored by pulmonary artery, systemic artery, and right and left atrium pressure measurements. In the iNO group there were significant fewer PHTC's and the time to extubation was also shorter. The PVR index was significantly lower in the iNO group after surgery. As expected, the time to wean a patient from the study gas was longer in the iNO group. Even though the Cochrane analyses stated that it is impossible to validate positive effects of iNO on outcome after cardiac surgery, iNO is widely used for this indication[34].

Because iNO inhibits platelet aggregation, adhesion, and agglutination, it may lead to bleeding disorders. Further side effects of iNO are elevation of nitrogen dioxide levels and methemoglobinemia. All of these side effects seem to be insignificant, however, when using a low dose iNO. In all the above studies iNO doses up to 20 ppm did not cause toxic effects. In view of the low risk of methemoglobinemia, infants are usually not monitored in general practice, even though monitoring is mostly recommended. Furthermore, iNO can lead to rebound PH which can complicate weaning of iNO. Namachivayam *et al.*

published a RCT on the effect of a single dose of sildenafil in 30 ventilated infants and children with iNO and showed that sildenafil given one hour before withdrawal of iNO prevented rebound PH and reduced the duration of ventilation[35]. As mentioned above, iNO is expensive since it has been accepted as a medicinal product.

One RCT of 40 children investigated the effect of oral citrulline as a precursor to NO synthesis in congenital heart surgery. A total of 40 patients received either citrulline or placebo just before the start of surgery. Oral citrulline supplementation proved safe and patients with elevated citrulline concentration had a lower risk of postoperative PH[36]. So far, the supplementation of NO precursors has not yet been implemented in standard of care.

iNO has been investigated broadly and has been well established in every day practice. It is effective in PPHN and seems to be effective in post cardiac surgery in increasing oxygenation and decreasing PVR but there have been no changes in mortality documented. Not all studies discussed included a control group. The validity of comparing the effects of a "proven" therapy like iNO with those of an untested therapy such as iloprost is debatable. But in this review we did not specifically focus on the study designs. All referred trials and studies have been summarized in Table 2.

2.2 Sildenafil

2.2.1 Introduction to sildenafil

Sildenafil citrate is a selective phosphodiesterase type 5 (PDE5) inhibitor. PDE5 is an enzyme that specifically degrades cGMP. With the inhibitory effect of sildenafil on PDE5, it increases cGMP and enhances NO-mediated vasodilatation of the smooth muscles in vessels, viscera, and corpus cavernosum.

Sildenafil was approved by the FDA in 1998 for the treatment of erectile dysfunction; in 2005, it was also approved for its use in PH in adults. In 2012, the FDA issued a warning against its use in children based on the STARTS-2 study, which showed an increased risk of death in the group of children over 20 kg receiving a high dose of sildenafil. STARTS-2, the extension phase of the STARTS-1 study, found increased mortality in the high-dose group after two years compared to the medium- and low-dose groups. There was no placebo group. However, when corrected for idiopathic PH etiology, high PVR index and high right atrial pressures at baseline, the hazard ratios were reduced in the high-dose group. These findings raise uncertainty about the strength of the relationship between sildenafil dose groups and survival[53,54]. Therefore, the EMA did approve the use of low dose sildenafil in children.

Table 2 – Trials concerning the nitric oxide-cGMP pathway: iNO

Study	Number of patients	Indication	Methods	Age of patients	Outcome parameter	Pharmaco-dynamics	Outcome
Loukanov *et al.* 2011[37]	15	Left-to-right shunt and pulmonary hypertension after weaning from cardiopulmonary bypass	iNO versus Iloprost	77-257 days	Occurrence of PHTC, duration of mechanical ventilation, safety of administration, in-hospital mortality	mPAP PAP/SAP PaO2:FiO2	No difference, Iloprost with a favorable safety profile
Gorenflo *et al.* 2010[38]	15	Postoperative management of PH in infants and children with CHD	iNO versus inhaled iloprost (postcardiac surgery)	Median 3 months	Occurrence of major or minor PHTC, duration of mechanical ventilation	mPAP Cardiac index PVR	No difference
Konduri *et al.* 2004[39]	299	Severe hypoxic respiratory failure	Early iNO versus stimulated initiation of iNO	Neonates born after >34 weeks	Death, ECMO, combined incidence of death and ECMO	PaO_2	iNO improves oxygenation. No difference of death or ECMO
Sadiq *et al.* 2003[40]	87	Moderate PH	iNO versus control	Infants >35 weeks	Improvement of arterial paO2, Prevention of progression of PPHN, improvement of clinical outcome	Increase of arterial paO2, decrease of OI and alveolar-arterial OI	Improvement of paO2 and ventilatory support, prevents progression to severe PPHN
Stocker *et al.* 2003[41]	15	PH after cardiac surgery	iNo and sildenafil	139 days	Vascular pressures, cardiac output and a blood gas at 0, 20 and 40 min	PVRI, systemic blood pressure, sytemic vascular resistance	Sildenafil augmented the pulmonary vasodilator effects of iNO. Sildenafil produced systemic hypotension and impaired oxygenation, which was not improved by iNO.
Finer *et al* 2001[42]	36	Hypoxic respiratory failure	Low-dose iNO versus high-dose iNO	34.2 hours	Improvement of oxygenation	Increase of arterial PaO2, decrease of OI	No difference between both groups

Table 2 – (continued)

Study	Number of patients	Indication	Methods	Age of patients	Outcome parameter	Pharmaco-dynamics	Outcome
Clark *et al.* 2000[43]	248	PH	iNO versus control	0- 4 days	ECMO, mortality, chronic lung disease	Ratio of arterial oxygen to alveolar oxygen	iNO group had less need for ECMO, and less chronic lung disease. Mortality was similar between the groups
Day *et al.* 2000[44]	20+20 control	PH during early postoperative period in CHD	iNo versus conventional therapy (postcardiac surgery)	Median 6 months	Occurrence of PHTC	PAP Systemic blood pressure RA presuure	No difference
Miller *et al.* 2000[33]	124	High pulmonary flow, pressure or both and undergoing corrective surgery for CHD.	Low-dose iNO versus placebo (postcardiac surgery)	Median 3 months	Occurrence of PHTC, time on study gas, hours spent in IC	PAP Systemic blood pressure PVR SVR	Fewer PHTC in iNO group ($p<0.001$) Shorter times until criteria for extubation ($p=0.019$)
Morris *et al.* 2000[45]	12	PAP> 25 mmHg after biventricular repair of CHD	iNO versus hyperventilation (postcardiac surgery)	Between 2 months and 18 years	Cardiac output and derived hemodynamimc parameters	PAP PVR	No difference between iNO and HV
Cornfield *et al.* 1999[46]	38	PPHN	iNO versus control	< 1day	Improvement of oxygenation, clinical deterioration (ECMO or death)	Decrease of OI	No improvement at 2 ppm iNO, acutely improvement of oxygenation at 20ppm
Franco-Belgium Collaborative NO Trial Group, 1999	204	Preterm and near-term neonates with OI 12.5- 30.0 and 15- 40.	iNO versus control	Preterm<33 weeks Term >33 weeks	Decrease of OI at 2 h, number of days on mechanical ventilation, stay on ICU, incidence of BPD, severe intracranial hemorrhage, cystic leukomalacia, death	OI	No benefit in preterm neonates. Improvement of oxygenation, shorter time on ventilation and length of stay on ICU in term neonates.

Table 2 – (continued)

Study	Number of patients	Indication	Methods	Age of patients	Outcome parameter	Pharmaco-dynamics	Outcome
Wood *et al.* 1999[47]	29	PPHN	Low dose iNO versus high dose iNO	17 hours	Improvement of oxygenation, mortality and need of ECMO	Decrease of OI	No difference.
Davidson *et al.* 1998[48]	155	PPHN	iNO versus control	26 hours	Risk of death, need of ECMO, neurologic injury, BPD	Increase of arterial PaO2, decrease of OI, SAP	Improvement of oxygenation for 24 hours, no other differences
NINOS trial Ehrenkranz *et al.* 1997[22]	235	Severe hypoxic respiratory failure	iNO versus O2	< 14 days	Risk of death by day 120, need of ECMO	Increase of arterial PaO2, decrease of OI and alveolar-arterial OI	Lower rate of need of ECMO (p=0.006), no difference in mortality
Kinsella *et al.* 1997[49]	205	Severe PPHN	iNO versus HFO	Neonates	PaO2>60mmHg	Increase of arterial PaO2, decrease of OI and alveolar-arterial OI	
Roberts *et al.* 1997[50]	58	Severe hypoxemia and PPHN	iNO versus control	Neonates	Improvement of oxygenation, need of ECMO	Increase of arterial PaO2, decrease of OI	Improvement of oxygenation, lower need of ECMO (p=0.02). No difference in mortality.
Wessel *et al.* 1997[51]	49	Severe PPHN	iNo versus control	Neonates	Improvement of oxygenation, mortality and need of ECMO	Increase of arterial PaO2, decrease of OI and alveolar-arterial oxygenation index	Improvement of oxygenation. No difference in mortality or need of ECMO
Goldman *et al.* 1995[52]	13	Severe PH	iNO versus prostacyclin iv	3 days - 12 months	Decrease of mPAP	mPAP PAP/SAP paO2	mPAP significantly lower in iNO group

Notes: PGE1: prostaglandin E1. OI: oxygenation index . SAP: systolic arterial blood pressure. iNO: inhaled nitric oxide. PHTC: pulmonary hypertensive crisis. mPAP: mean pulmonary arterial pressure. PH: pulmonary hypertension. CHD: congenital heart disease. PVR: pulmonary vascular resistance. PPHN: persistent pulmonary hypertension of the newborn. PVRI: pulmonary vascular resistance index. PAP: pulmonary artery pressure. RA: right atrium. SVR: systemic vascular resistance. HV: hyperventilation. ECMO: extracorporeal membrane oxygenation. ICU: intensive care unit. BPD: bronchopulmonary dysplasia.

2.2.2 Pharmacokinetics and metabolism of sildenafil

Sildenafil is available for oral and intravenous administration. Nebulized sildenafil has been studied in animal models, but data on human use are not available at this point in time[55].

Sildenafil is metabolized in the liver via CYP3A4 and CYP2C9 into its active metabolite desmethylsildenafil (DMS). The tissue half-time is approximately 2 - 4 hours for both intravenous and oral preparations. In severe renal or hepatic failure, clearance can be significantly reduced. Pharmacokinetics of oral sildenafil in children over one year of age is similar to that in adults[53,56]. The plasma concentration for sildenafil derived from *in vitro* studies is approximately 50 - 400 ng/mL. Desmethylsildenafil concentrations can be measured too; however, in most studies only sildenafil concentrations are determined. Three studies in infants have been reported. One is an open-label dose-escalating trial in neonates receiving intravenous sildenafil in the first ten days of life. A correlation between postnatal age and clearance was shown, as clearance increased threefold to adult values within the first seven days. Volume of distribution was fourfold higher compared to adults, resulting in a longer tissue half-time of approximately 6.8 hours[57]. The second study showed that a loading dose was desirable to establish plasma concentrations in a shorter period; however, in both studies this loading infusion needed to be given in at least three hours to avoid hypotension. Patients receiving the loading dose in 5 or 30 minutes often developed hypotension. Although patient numbers are small, an effect on oxygenation index was seen with a sildenafil plasma concentration of approximately 58ng/ mL and higher, with the use of a loading dose of at least 0.06mg/kg in three hours and infusion of 0.36 mg/kg/day[58]. The third study investigated the administration of oral sildenafil in post-ECMO infants. At dosages 1.3 - 10 mg/kg/day, plasma concentrations were 300 and 100 ng/mL at one and six hours, respectively; but both the interpatient and intrapatient variability were very high, possibly due to variable gut absorption. Dosing of 0.5 - 2 mg/kg six-hourly would provide an exposure comparable to the adult dose of 20 mg six-hourly[56,59]. A pharmacokinetic study evaluating the safety and dosing of sildenafil in preterm infants is currently underway (NCT01670136).

2.2.3 Clinical efficacy and role of sildenafil in current PH management

A Cochrane analysis from 2011 investigated the efficacy and safety of sildenafil treatment in neonates with PPHN[60]. Three randomized single-center trials were included, in which NO and ECMO were initially not available. In total, 51 patients received sildenafil and 37 patients received placebo[61-63]. Baquero *et al*. treated a small group of patients with a high risk of mortality (OI ≥ 40 and PAP ≥ 40mmHg) with 1 - 2 mg/kg sildenafil or placebo six-hourly and showed significant improvement of SpO_2 and OI after 24 hours in the sildenafil-treated group. Also, mortality was significantly lower, i.e. 15% versus 85%. For this reason the study was stopped early. The study of Herrera Torres *et al.* was

included in the Cochrane review and evaluated the effect of sildenafil 2mg/kg six-hourly in 13 patients versus 11 patients treated with placebo[60]. All patients were moderately sick with an OI > 25 and oxygenation and mean airway pressure (MAP) improved from the first dose. Vargas-Origel *et al.* showed a significant increase of PaO_2 after 7 hours of treatment with sildenafil 3mg/kg six-hourly and a decrease in MAP after 13 hours in a larger group with OI ≥ 20. Again, mortality was significantly lower in the sildenafil-treated group. iNO became available for the last 11 patients in this study but this did not influence the results. Although the patient numbers are small and not all outcome measures are adequately reported, the meta-analysis showed a significant reduction in mortality in the treated group (20% versus 54% in the placebo group). No adverse events were reported[63]. Another RCT in PPHN, not included in this Cochrane review, compared sildenafil with $MgSO_4$ in patients with OI ≥ 30 and PAP ≥ 40mmHg. Thirty-one patients were treated with 0.5 – 2 mg/kg sildenafil six-hourly and 34 with IV $MgSO_4$ (loading dose 200 mg/kg, maintenance 20 mg/kg/hr, max 100mg/kg/hr). Clinical response in the sildenafil group was significantly sooner, 36 versus 60 hours. This was defined as OI < 15 and PAP < 20mmHg. The mean blood pressure in the $MgSO_4$ group was significantly lower and these children needed more inotropic support[64]. A RCT comparing sildenafil and placebo in PPHN patients already on iNO is currently recruiting patients (NCT01720524). Another RCT in which iNO-naive PPHN patients were treated with sildenafil was terminated early due to insufficient recruitment (NCT01409031).

Sildenafil is also being used in cardiac surgery and this setting has been studied in four RCTs. Vassalos *et al.* administered oral sildenafil (0.5 mg/kg six-hourly) or placebo the day before cardiac surgery in 24 infants with either ventricular (VSD) or atrioventricular septal defects (AVSD) but without PH. All received invasive pressure monitoring in the main pulmonary artery and left atrium. Post-operative PAP, pulmonary vascular resistance index (PVRI), and MAP did not differ between the groups. However, pre- and postoperative left and right ventricular and septal systolic functions were significantly lower in the sildenafil group compared to placebo, persisting through day one. Also, the mean Doppler-derived cardiac index was significantly lower in the sildenafil group. Neither ventilation time nor length of ICU stay differed between groups. No adverse events were reported[65]. El Midany *et al.* enrolled 101 infants with large VSDs with moderate-to-severe PH (>40 mmHg and systolic PAP >50% of systemic pressure, PVR >3WU but <8WU) in a randomized open-label study. The sildenafil group started treatment 2 weeks before the surgery; a control group received sildenafil only postoperatively. There was no control group receiving only placebo[66]. The starting dose of 0.5 mg/kg six-hourly was increased to 2 mg/kg in both groups. In both groups, mean PAP decreased significantly at all time points of the study (which were all postoperatively) although starting sildenafil before surgery did not further decrease mPAP. Pretreatment of infants is not accepted in daily practice worldwide and these two studies confirm this. In a postoperative setting, a small

group of patients who underwent surgery for a VSD or AVSD were randomly assigned to intravenous sildenafil with the addition of iNO 20 ppm after 20 minutes or to iNO with the addition of sildenafil after 20 minutes. iNO reduced the PVRI and sildenafil further reduced the PVRI, and this double effect was also seen in patients receiving sildenafil first. However, sildenafil significantly lowered systemic blood pressure and systemic vascular resistance and increased the OI and alveolar-arterial gradient. The latter two findings were reasons to stop the study early[41]. A second RCT for postoperative PH in infants over 60 days was done in a multicenter setting[67]. Patients were assigned to one of three continuous doses of IV sildenafil or placebo. Unfortunately, the study was heavily underpowered with a total of 17 patients due to slow patient recruitment. Patients in the treatment groups required less additional therapy for PH, but this was not significant. Median time to extubation was significantly shorter for patients on sildenafil. In the first four hours, the reduction in systolic PAP was significantly greater in the sildenafil group, and only a minor reduction in systemic blood pressure was seen[67].

Besides the results from STARTS-2 mentioned above, several other safety issues deserve attention. Concerns have been raised about the effect of sildenafil on the systemic blood pressure. However, in the trials performed in PPHN, this was not seen[60,64]. In the pharmacokinetic studies on intravenous sildenafil, hypotension without severe hemodynamic instability was seen when using a short loading dose[57,58]. One study found a negative impact on cardiac function, but this was not reported in other studies[65]. The use of sildenafil in extreme premature infants has been associated with retinopathy of prematurity (ROP) in one case report[68]. Sildenafil is also an inhibitor of PDE6 receptors, and PDE6 is the regulator of cGMP in rod and cone photoreceptors. This association was not found in several series with small sample sizes[69-72]. In fact, in a mouse model sildenafil significantly decreased retinal vaso-obliteration and neovascularization[73]. Only one study evaluated neurodevelopmental outcome in a small group and found normal MRI, EEG, and neurological examination after the use of sildenafil[61]. In some case reports, intracerebral hemorrhagic events in premature infants have been linked to sildenafil. Such association has not been confirmed in trials[74].

For other patient groups, only retrospective data are available. A decrease in PVRI and an increase in cardiac output were found in a small group of oral sildenafil-treated infants with CDH refractory to iNO[75]. Intravenous sildenafil in CDH patients was associated with improved OI and the right-to-left shunt ratio over the PDA was reversed[76]. Oral sildenafil treatment in infants with chronic lung disease improved systolic PAP and interventricular septal flattening[77,78]. Despite the lack of randomized controlled trials in these groups, sildenafil is increasingly used in the acute and more chronic phases. A trial investigating the chronic use of sildenafil in CDH patients was terminated early due to change in clinical practice (NCT00133679).

Sildenafil is increasingly being used, also in infants. Even more so since the intravenous formulation became available. A few RCTs have shown a positive effect in that OI and mortality in infants with PPHN decreased in resource-limited settings. More studies with good pharmacodynamics measurement tools are needed to confirm this and to enable comparison of sildenafil with more commonly used vasodilators, such as iNO. All referred trials and studies have been summarized in Table 3.

2.3 Nitroglycerin

Nitroglycerin is metabolized to iNO to produce vasodilatation through smooth muscle cell relaxation in the vascular endothelium. Intravenous nitroglycerin has been used in patients with pulmonary hypertension but due to lack of selectivity for pulmonary vasculature its role in PH is limited[79]. However, inhalation of the drug has demonstrated significant decreases in PA pressure and PVRI without these systemic side effects in adults after cardiac surgery and in children during cardiac catheterization. But because of its short duration of action and risk of tachyphylaxis it is less suitable for long-term therapy in PH. Still there might be a role for inhaled nitroglycerin in the acute perioperative phase[80-82].

2.4 Riociguat

Riociguat is the first drug of a novel class of soluble guanylate cyclase (sGC) stimulators or agonists. sGC is an enzyme that catalyzes the synthesis of cGMP. The binding of NO to sGC increases the conversion of GTP to cGMP approximately 200-fold. Riociguat stabilizes the binding site on sGC for NO and also enhances the functioning of sGC in a NO-independent fashion. This new drug has not yet been studied in children. The few RCTs conducted in adults showed improved exercise capacity and PVR in patients with pulmonary arterial hypertension and chronic thromboembolic pulmonary hypertension[83,84].

3 MEDICATION INFLUENCING THE ENDOTHELIN PATHWAY

3.1 Bosentan

3.1.1 Introduction to bosentan

Bosentan is a potent oral endothelin-1 receptor antagonist for both endothelin A and B. Binding of bosentan to ETA is much more patent than its binding to ETB. Endothelin-1 (ET-1) is a very potent pulmonary vasoconstrictor produced in endothelial cells as a response to hypoxia, and plays a major role in pulmonary vascular remodeling and right heart hypertrophy. ET-1 binds to two receptor subtypes, ETA and ETB receptors, and the

Table 3 – Trials concerning the nitric oxide-cGMP pathway: sildenafil

Study	Number of patients	Indication	Methods	Age of patients	Outcome parameter	Pharmaco-dynamics	Outcome
El Midany *et al*, 2013[66]	101	VSD and PH, perioperative for surgical closure	Sildenafil perioperative versus postoperative	7-17 months	mPAP	mPAP	Significantly lower mPAP after sildenafil in both groups, no difference between groups
Fraisse *et al*, 2011[85]	17	Post cardiac surgery and PH	One of 3 doses of IV sildenafil or placebo	3 months – 14 years (median 5 months)	Additional therapy of PH, time on ventilator,LOS in hospital	sPAP, SBP, LAP, RAP, CVP	No difference in additional therapy. Time on ventilator and LOS shorter in sildenafil group, sPAP also lower. No decrease in SBP
Uslu *et al*, 2011[64]	65	PPHN	Sildenafil versus MgSO4	Term en near term newborns	PAP, OI, time on ventilator	OI, PAP, MBP, MAP	Earlier decrease in OI and PAP, shorter on ventilator, less hypotension in sildenafil group
Vassalos *et al*, 2011[65]	24	Pre-operative cardiac surgery	Sildenafil versus placebo	3.1-27.1 months, median 4.8 months	PVRI, PAP	PVRI, OI, left, right and septal systolic function, cardiac index	No effect on post-op PVRI, negative effect on oxygen delivery, ventricular function and oxygenation
Vargas-Origel, 2010[62]	51	PPHN	Sildenafil versus placebo	<48 hours	OI, time on ventilator	OI, MAP, PaO_2	Decrease in PaO_2, MAP and mortality
Baquero *et al*, 2006[61]	13	PPHN	Sildenafil versus placebo	3-72 hours	OI, feasibility of administration, gastric tolerance	SpO_2, PaO_2, OI	Significantly lower OI, SpO_2 and mortality in sildenafil group
Herrera Torres *et al*, 2006[86]	24	PPHN	Sildenafil versus placebo	Term newborns	OI, MBP, PaO2, PaCO2, mortality time on ventilator	OI, MBP, PaO_2, $PaCO_2$, MAP, A-a gradient	Improved OI within first hour in sildenafil group
Stocker *et al*, 2003[41]	16	VSD-, AVSD-closure postoperatively	Sildenafil with iNO after 20 min or iNO with sildenafil after 20 min	<1 year	PVRI, OI, A-a gradient	PRVI, SBP, CI, LAP, CVP, SVRI, OI, A-a gradient	In both groups synergistic effect of iNO and sildenafil on PRVI. With sildenafil SBP and SVRI lowered, worsened OI and A-a gradient

Notes: VSD: ventricular septal defect. PH: pulmonary hypertension. mPAP: mean pulmonary arterial pressure. IV: intravenous. SPAP: systemic pulmonary arterial pressure. SBP: systemic blood pressure. LAP: left arterial pressure. RAP: right arterial pressure. CVP: central venous pressure. PPHN: persistent pulmonary hypertension of the newborn. PAP: pulmonary artery pressure. OI: oxygenation index . MBP: mean blood pressure. MAP: mean arterial pressure. PVRI: pulmonary vascular resistance index. AVSD: atrioventricular septal defect. SVRI: systemic vascular resistance index.

binding of ET-1 to the ETA receptors on pulmonary smooth muscles causes vasoconstriction. On the other hand, activating ETB receptors causes vasorelaxation via prostacyclin and NO, but ETB receptors can also mediate vasoconstriction[87-90]. Bosentan is approved in adult patients with PAH; a pediatric formulation is approved only in Europe but it is available only as an oral drug[91].

3.1.2 Pharmacokinetics and metabolism of bosentan

Bosentan is administered orally, usually twice daily 1 mg/kg. The metabolism of bosentan is dependent on the activity of CYP2C9 and 3A4 isoenzymes, and elimination occurs mostly via the bile[92]. In children, the pharmacokinetic profile is characterized by a median peak concentration after 3 hours, followed by rapid disposition. An exposure plateau of its plasma levels is reached at 2 mg/kg/day, in accordance with the non-dose-proportional pharmacokinetics of bosentan in adults, but children's plasma concentrations are lower than those of adults. Age and body weight do not seem to have an effect on pharmacokinetics over the age of 2 years[93]. In younger children, however, pharmacokinetics are influenced by age, showing more inter individual differences in clearance, possibly due to difference in hepatic development[92]. There are no data available on children under the age of 25 days[94].

3.1.3 Clinical efficacy and role of bosentan in current PH management

Currently, only two RCTs with bosentan in infants have been performed, both in PPHN. Mohamed *et al*. treated 47 patients with bosentan twice daily or placebo in a single center without availability of iNO or ECMO[95]. Bosentan significantly improved oxygenation index and SpO_2 compared to placebo from six hours onwards after initiation of therapy. There was no evidence of rebound hypoxemia after discontinuation of bosentan. No adverse events were reported[95]. Steinhorn *et al*. treated 21 patients in 9 centers who were already on iNO with bosentan or placebo[88]. Bosentan did not improve oxygenation compared to placebo. However, steady state concentrations comparable to those in adult patients were achieved by day 5, and most patients received treatment for only 5 days[88]. A striking difference between these two studies besides the use of iNO is the difference in OI at the beginning of the study. Mean OI in the first trial was 43.6 and 45.1, respectively, in the second study mean OI was 21.1 and 17.3, respectively. Although these trials did not report any adverse events, cardiac failure, syncope, blood bilirubin increase, thrombocytopenia, and liver function test abnormalities have been reported by the FDA. However, the incidence of elevated aminotransaminase levels seems to be lower in children[96,97].

There is only observational experience with bosentan in infants with elevated vascular resistance pre- and post-operatively, before and after repair of cardiac defects with excess lung flow. Postoperative treatment of a small group of infants decreased PAP and

improved right and left ventricle cardiac index in most of them, even when refractory to sildenafil[98,99]. Preoperative treatment of an infant with PH due to complete atrioventricular septal defect also decreased PH[100]. Bosentan has also been used to decrease PH in patients in need for right-sided heart bypass surgery. Treatment with bosentan for 5 - 17 months showed a decrease of mPAP and PVR and also the blood marker BNP decreased significantly[101].

In a few case reports a positive clinical effect from bosentan was seen in children with refractory PH in CLD and in patients with transposition of the great arteries and PH[102-104].

A few trials are underway to evaluate the clinical effect and pharmacokinetics of bosentan. A new FUTURE 3 trial is ongoing, investigating twice daily dosing versus three times daily in PH patients (NCT01338415). One trial aims to evaluate the postoperative residual PH and mortality rate in patients with cardiac defects treated for weeks before surgery, and is recruiting (NCT01548950). Another trial in patients with PH and functional single ventricle has been completed but data are not yet available (NCT01662037).

3.2 Other endothelin-1 receptor antagonists

For adult use only, selective endothelin-1 receptor antagonists are available. Ambrisentan is selective for the type A endothelin-1 receptor; macitentan is non-selective but has a 50-fold higher selectivity for type A receptors compared to type B. No data are available on the use of these drugs in infants. Ambrisentan given to newborn rats with hyperoxia-induced lung injury prolonged survival by reversing PAH and RVH and reducing fibrin deposition in the lung. However, no benefits were seen on inflammation, alveolar enlargement, and vascularization[105]. In children with PH, retrospective data of ambrisentan showed significant improvement of PAP and WHO functional class. The

Table 4 – Trials concerning the endothelin pathway

Study	Number of patients	Indication	Methods	Age of patients	Outcome parameter	Pharmaco-dynamics	Outcome
Steinhorn *et al*, 2014[88]	21	PPHN	Bosentan versus placebo	newborn	OI, time on ventilator, time on iNO, treatment failure	unknown	No difference between groups
Mohamed *et al*, 2012[95]	47	PPHN	Bosentan versus placebo	newborn	OI, PAP	OI, PAP, SpO_2	Significant decrease in OI and SpO_2 in bosentan group, fewer sequelae after 6 months

Notes: PPHN: persistent pulmonary hypertension of the newborn. OI: oxygenation index. iNO: inhaled nitric oxide. PAP: pulmonary arterial pressure.

WHO functional classification system reflects the special physiology of PH in view of the importance of syncope and near-syncope. However, 26% of these children had side effects, mainly nasal congestion and headache[106].

So far, bosentan is the only endothelin receptor antagonist used in children and it cannot be recommended for the routine treatment of PH in infants due to a lack of valid data. Studies are on the way to evaluate the role of bosentan in PH in children and infants. All referred RCTs are summarized in Table 4.

4 MEDICATION INFLUENCING THE PROSTACYCLIN-CAMP PATHWAY

4.1 Prostanoids

4.1.1 Introduction to prostanoids

Prostanoids are arachidonic acid metabolites and include prostaglandins (PGI) and prostacyclins as well thromboxanes[107]. They play an important role in inflammation, platelet aggregation, and vasoconstriction and vasorelaxation[107]. The first prostanoid approved for the therapy of PH was epoprostenol in 1995, followed by treprostinil (subcutaneous 2002, intravenous 2004, inhaled 2009), iloprost (2004), and in 2010 room temperature stable epoprostenol[91]. Inhaled PGI is proven to be effective as a pulmonary vasodilator in adults with PH and acute respiratory distress syndrome (ARDS) and is recommended as standard therapy in adults[108-110].

4.1.2 Pharmacokinetics and metabolism

Epoprostenol is the intravenous formulation of prostaglandin I_2. It is rapidly degraded in blood into two primary metabolites and is excreted in urine. The half-life of epoprostenol is around 6 minutes[111]. The plasma steady-state concentration is reached within 15 minutes. The usual dose in children is 1-10ng/kg/min.

The synthetic prostacyclin analogue iloprost can be given via inhalation. The hemodynamic effects occur rapidly but will also decrease within 30 - 90 minutes. Therefore, it must be inhaled at least six to nine times a day.

4.1.3 Clinical efficacy and role of prostanoids in current PAH management

We identified four RCTs of prostanoids in infants and children[37,38,40,52,112]. Three focused on patients after cardiac surgery[37,38,52] and one on neonates with hypoxic respiratory failure[112].

Sood *et al*. initiated two pilot multicenter Phase II RCTs on the effect of inhaled prostaglandin E1 (IPGE1) on neonatal hypoxemic respiratory failure (NHRF). The first pilot study failed to recruit enough patients because prior administration of iNO was an exclusion

criterion. The next re-designed pilot study allowed for possible co-medication of iNO and IPGE1 but was also stopped prematurely for lack of enough suitable patients[112].

Goldman *et al*. studied the effects on vasodilation of iNO versus intravenous prostacyclin in infants with PH after cardiac surgery[52]. They included 13 patients between three days and 12 months of age who either received 20 ppm iNO or 20 ng/kg/min prostacyclin. Both groups showed a reduction of mPAP, but iNO lowered the mPAP more effectively than did prostacyclin (28.5 ± 2.9 mmHg versus 35.4 ± 2.1 mmHg). Furthermore, the PAP/SAP ratio was also significantly lower during iNO inhalation. It was concluded that iNO was preferable[52].

Loukanov *et al*. and Gorenflo *et al*. investigated the effect of iNO versus iloprost[37,38]. Gorenflo *et al*. initiated two different trials, one in children after cardiac surgery and one testing the acute pulmonary vasoreactivity in children with severe PH due to congenital heart disease[38]. The former showed no difference in the primary endpoint (occurrence of major or minor PHTC) between groups. In the latter, the effects on cardiac index and PVR did not differ between groups[38].

Loukanov *et al*. included 15 infants with left-to right shunt, of whom 11 children with Down syndrome. They received either 10 ppm iNO or 0.5 microgram/kg iloprost every 2 hours. Mean PAP and Pp/Ps ratio did not differ between the two groups, and neither did the primary endpoint (major or minor PHTC)[37].

In some centers prostanoids are routinely used in infants but so far RCTs are lacking. All referred trials and studies have been summarized in Table 5.

In 2014 Kahveci *et al*. published a clinical study which addressed the problem of treatment of PPHN in countries where expensive treatment methods such as iNO and ECMO are not wide available. The effect of sildenafil versus inhaled iloprost was evaluated in 47 neonates with PPHN. The iloprost group did not show any side effects and regarding the clinical parameters iloprost was more effective than sildenafil[113]. The use of iloprost might therefore be of interest for resource-poor countries.

4.2 Others

4.2.1 Milrinone

Milrinone is a phosphodiesterase 3 (PDE3) inhibitor which induces pulmonary vasodilatation by its action on cAMP in cardiac and vascular muscle. It has positive inotropic and vasodilator effects[114]. Milrinone is administered intravenously. It has been evaluated for inhalation in the setting of PH in older children with successful decrease of PAP and PVRI without significant effects on systemic hemodynamics[80].

In infants milrinone has only been used for PPHN in case series. Patients unresponsive to iNO had a substantial improvement of OI, PAP, left and right ventricular output, right ventricular strain, and strain rate after start of milrinone[115-117]. However, in a very small

Table 5 – Trials concerning the prostacyclin-cAMP pathway

Study	Number of patients	Indication	Methods	Age of patients	Outcome parameter	Pharmaco-dynamics	Outcome
Sood *et al.* 2014[112]	7	Neonatal hypoxemic respiratory failure	Low dose inhaled PGE1 versus high dose PGE1	Neonates	Feasibility trial	OI SAP	No serious side effects. Failing of adequate recruitment
Loukanov *et al.* 2011[37]	15	Left-to-right shunt and pulmonary hypertension after weaning from cardiopulmonary bypass	iNO versus Iloprost	77-257 days	Occurrence of PHTC, duration of mechanical ventilation, safety of administration, in-hospital mortality	mPAP PAP/SAP PaO2:FiO2	No difference, Iloprost with a favorable safety profile
Gorenflo *et al.* 2010[38]	15	Postoperative management of PH in infants and children with CHD	iNO versus inhaled iloprost (post cardiac surgery)	Median 3 months	Occurrence of major or minor PHTC, duration of mechanical ventilation	mPAP Cardiac index PVR	No difference
Sadiq *et al.* 2003[40]	87	Moderate PH	iNO versus control	Infants >35 weeks	Improvement of arterial paO2, Prevention of progression of PPHN, improvement of clinical outcome	Increase of arterial paO2, decrease of OI and alveolar-arterial OI	Improvement pf paO2 and ventilatory support, prevents progression to severe PPHN
Goldman *et al.* 1995[52]	13	Severe PH	iNO versus prostacyclin iv	3 days - 12 months	Decrease of mPAP	mPAP	No difference

Notes: PGE1: prostaglandin E1. OI: oxygenation index. SAP: systemic arterial blood pressure. iNO: inhaled nitric oxide. PHTC: pulmonary hypertensive crisis. mPAP: mean pulmonary arterial pressure. PAP: pulmonary arterial pressure. SAP: CHD: congenital heart disease. PVR: pulmonary vascular resistance. PH: pulmonary hypertension. PPHN: persistent pulmonary hypertension of the newborn.

case series there seemed to be an association with intraventricular hemorrhage[118,119]. To clarify the role of milrinone in PPHN and the risk of IVH due to milrinone treatment larger trials are needed. The upcoming MINT-1 trial will randomize PPHN patients on iNO to milrinone or placebo (ISRCTN12949496) and will hopefully provide some answers.

One retrospective study reports the use of milrinone in infants with CDH and PH combined with cardiac dysfunction. RV systolic and diastolic myocardial velocities were significantly increased and OI was significantly reduced without change in blood pressure[120].

Adverse events are not uncommon in milrinone-treated infants; hypotension requiring pressors was seen in 20% and PDA requiring treatment in 13% of patients. Thrombocytopenia is the commonest laboratory adverse event. Renal clearance of milrinone is age-dependent and in cases of renal impairment milrinone accumulates and leads to adverse events[121].

5 COMBINATION THERAPY

The guidelines for the treatment of PH in adults also prescribe combination therapy[2]. Combinations of sildenafil with bosentan or prostanoids have been found reasonable because they influence different pathways. The literature on this issue concerning infants is scarce. A few case reports have described positive effects of the use of sildenafil and iloprost or PGE1[122,123]. Combinations with iNO, sildenafil, and bosentan have also been described[124]. In a piglet model of PH due to meconium aspiration, combination treatment with iNO and sildenafil synergistically decreased PVR and PAP. Still, while sildenafil also increased cardiac output, it also significantly decreased arterial pO_2[125]. For extremely compromised infants, a combination therapy is regularly used on the basis of trial and error but there are no large case studies or RCTs available to support this use.

6 CONCLUSION

Treatment of PH in infants includes different medications influencing the three pathways involved in PH.

iNO has been investigated in many large RCTs in patients with PPHN and is widely used in general practice even though improved mortality rates have not been shown. However, iNO seems to exert a positive effect on PaO_2 and on the duration of ventilation. The few RCTs concerning sildenafil show a positive effect on OI and mortality in infants with PPHN. Only two studies, with few patients, looked at the pharmacodynamic

effects of bosentan in infants with PPHN, with contradictory results. A trial designed to investigate the effects of iloprost failed to recruit enough patients.

In cardiac infants iNO did not significantly reduce the incidence of PHTC or the duration of ventilation, except for one well conducted trial. The pre-operative use of sildenafil did not decrease PH, the trials evaluating the role of sildenafil in the postoperative phase are underpowered. Furthermore, iloprost has not been proven to be superior to iNO in post-cardiac surgery patients in two studies, both of which did not include a placebo group. RCTs on bosentan are still lacking.

iNO did not improve the outcome of patients with CDH. There are no reliable data on the use of sildenafil, bosentan, or prostacyclin in patients with CDH.

In premature infants iNO used as a rescue therapy did not improve survival and did not prevent the development of BPD. Its early routine use did not seriously affect the brain however. The use of iNO later in the course of the disease showed some beneficial effect to prevent BPD. Overall, PH in infancy is treated mainly on the basis of findings from case studies and personal experience and not on the basis of sufficiently powered RCTs. Furthermore, systematic survey's with a focus on the best pharmacodynamics outcome parameter in these patients are lacking.

7 EXPERT OPINION

The three major pathways influencing PH are the nitric oxide-cGMP pathway, the endothelin pathway, and the prostacyclin cAMP pathway. Medications affecting the nitric oxide-cGMP pathway are iNO and sildenafil. The endothelin pathway can be influenced using bosentan and other endothelin antagonists, and the prostacyclin pathway is affected with prostanoids. All of these medications are used in infants with PH mainly due to PPHN, premature infants, and post-cardiac surgery patients. Especially when considering the overall better survival of preterm infants and patients with CDH, it is expected that the number of affected infants with PH will increase. But so far RCTs in these patients are sparse. Even this broad literature review identified only 44 RCTs on PH in infants, mainly on iNO. For sildenafil, bosentan, and prostanoids further research is needed. The biggest challenge for the coming years is to set up large international RCTs for each group of patients separately as pathophysiology is likely to be different in every group and numbers per center are small.

The current pharmacokinetic knowledge of drugs like sildenafil and bosentan is inadequate, especially in newborns. To identify the therapeutic range and prevent both undertreatment and toxicity we need to know the plasma concentrations needed. Furthermore, bosentan is available only as an oral formulation and absorption is probably impaired in very ill infants. An intravenous formulation would solve this problem

and might increase its role in the treatment of PH in infants. The newer drug riocuguat influencing a new cGMP pathway seems very promising, but has not been adequately tested in infants so far. Though combination therapy is common practice in adults and is also used in infants with severe PH on a trial and error basis, more research is needed to elucidate the synergetic effects of these different drugs.

In adults, the pharmacodynamic effects of the different medications are evaluated with invasive measurements of PH, or through clinical parameters such as the NYHA classifications or the 6-minutes walking test. Heart catheterization or the use of PA catheters is seldom applied in infants because these procedures bear an increased risk of complications. Pharmacodynamic effects are commonly measured indirectly, for example through changes in OI or saturation, mortality and need for ECMO and not through actual measurement of improvement in mPAP. The lack of solid outcome parameters reflecting the actual changes of PH makes assessment of the different drugs more difficult and a comparison almost impossible. International consensus on the optimal cardiac ultrasound parameter could also help to solve the problem as well as consensus about the level of expertise needed to raise conclusions.

Plasma biomarkers could help with this dilemma. The biomarker BNP for example is used in PH, but has not been proven to be reliable enough so far for infants. Normal values of BNP and NT-proBNP in neonates both widely vary just after birth reflecting the physiological changes in this period[126]. On the other hand, it seems that BNP and NT-proBNP levels may serve as relative values as they correlated well with mortality[127]. There is a need for further research for other plasma biomarkers which are more reliable to detect and monitor PH, especially in infancy. Troponine T might be such a plasma biomarker reflecting myocardial damage which might be evident in PH with right ventricular failure. So far, there is no study investigating troponine T in infants with PH. Echocardiography is a well-established tool to estimate PH; it is available in most centers worldwide and can be performed easily in severely diseased infants. Echocardiography cannot replace invasive measurements. Even when using a broad echocardiogram-based definition of PH, most studies correlate these findings with clinical parameters such as the duration of oxygen supplementation or the occurrence of bronchopulmonary dysplasia[128]. Over the last years more advanced echocardiographic techniques have been introduced, such as TAPSE (Tricuspid annular plane systolic excursion), tissue Doppler imaging, and strain and strain rate imaging, which seem to have an additional benefit in the context of general echocardiographic evaluation[1,129,130]. These techniques might pave the way for trials in infants with a focus on more standardized pharmacodynamic effects of the different medications.

In conclusion, there is enough evidence for a positive effect of iNO on outcome in infancy – without a positive effect on mortality. Large international RCTs concerning the use of sildenafil, bosentan, and prostanoids in this age group are needed, using reli-

able and preferably noninvasive tools to measure the actual changes on PH itself. In the future, the use of newer medications and an upfront combination therapy might further improve the outcome in infants suffering from this life-threatening disease.

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Chapter 10

Standardized postnatal management of infants with congenital diaphragmatic hernia in Europe: The CDH EURO Consortium Consensus: 2015 update

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ABSTRACT

In 2010, the congenital diaphragmatic hernia (CDH) EURO Consortium published a standardized neonatal treatment protocol. Five years later, the number of participating centers has been raised from 13 to 22. In this article the relevant literature is updated, and consensus has been reached between the members of the CDH EURO Consortium. Key updated recommendations are: 1) planned delivery after a gestational age of 39 weeks in a high-volume tertiary center; 2) neuromuscular blocking agents to be avoided during initial treatment in the delivery room; 3) adapt treatment to reach a preductal saturation between 80 and 95% and postductal saturation >70%; 4) target $PaCO_2$ to be between 50 and 70 mmHg; 5) conventional mechanical ventilation to be the optimal *initial* ventilation strategy; and 6) intravenous sildenafil should be considered in CDH patients with severe pulmonary hypertension. This article represents the current opinion of all consortium members in Europe for the optimal neonatal treatment of CDH.

INTRODUCTION

In 2008, the congenital diaphragmatic hernia (CDH) EURO Consortium was set up and during a consensus meeting drafted a standardized neonatal treatment protocol to improve outcome and permit comparison of outcome data[1]. Since then the number of participating centers has increased from 13 to 22 specialized CDH centers from all over Europe, and the guidelines from 2010 have been widely cited. Moreover, after the implementation of the protocol, the survival rate has increased from 67% to 88% in 2 centers. This indicates the impact of the original standardized protocol. After 5 years of additional research including a multicenter randomized clinical trial on initial ventilation strategy (VICI-trial, Netherlands Trial Register NTR 1310), we aimed to update the standardized neonatal treatment protocol for CDH. All recommendations were summarized and compared to the protocol in 2010 (supplementary table 1).

METHODS

The studies were graded according to the Scottish Intercollegiate Guidelines Network (SIGN) criteria[2]. Five experts individually primarily determined the levels of evidence on the guidance of the SIGN checklist. Differences in opinion were primarily discussed between the five experts until full consensus was reached, and thereafter consensus was reached between all participating centers. The final consensus statement, therefore, represents the opinion of all participating centers based on the interpretation of the recent literature from 2010- 2015 and includes the main findings of the so-called VICI-trial[3]. A consensus meeting, in which neonatologists, pediatric intensivists, gynecologists, prenatal physicians, pediatric surgeons, pediatric cardiologists, and general pediatricians from 22 centers participated, was organized to discuss the most controversial recommendations. If it was very hard to reach consensus on a specific issue, the consortium concurred to investigate those issues in future randomized trials. The levels of evidence and grades of recommendation according to the SIGN criteria are presented in supplementary tables 2 and 3, respectively.

RESULTS

Prenatal management

With the increased use of second trimester 2D ultrasound and/ or MRI, CDH has become a prenatal diagnosis. Subsequently, a more detailed expert evaluation should be performed to determine the location of the defect, the observed/expected lung-to-head

ratio (O/E LHR) and the position of the liver (intra-abdominal or intrathoracic), in addition to ruling out additional congenital anomalies or syndromes[4,5]. Associated congenital anomalies, such as chromosomal or genitourinary anomalies, are present in about 25%[6] and cardiac anomalies in about 20% of cases[7]. Comprehensive assessment will also include invasive sampling for high-resolution genetic testing. Only once a comprehensive assessment has been made, multidisciplinary prenatal counseling by clinicians in tertiary centers can be offered to inform parents about the estimated prognosis after birth. Several other additional imaging methods, such as lung volumetry, 3D ultrasound and Doppler studies of the pulmonary vascularization, have been shown in individual series to be prognostic for pulmonary hypertension, the need for extracorporeal membrane oxygenation (ECMO), and survival[8]. All of these remain research tools, however, but may ultimately improve the predictive value of prenatal testing.

An experienced tertiary center with a high case volume (≥ 6 CDH patients per year) is the optimal environment for the delivery and neonatal treatment of prenatally diagnosed CDH fetuses[9,10]. Prenatal intervention by fetal endoscopic tracheal occlusion (FETO) has been proposed to promote lung growth[11]. Therefore, FETO is being evaluated in two randomized clinical trials both in moderate (first interim analysis stage reached; >100 patients randomized) and severe cases (>25 patients randomized) in centers in Europe, Australia and Canada ((TOTAL trial)[12] ; (NCT01240057)). Current reported survival rates are on average around 50%, yet there is a significant impact of gestational age at delivery. In the largest cohort study of which 17.1% of all patients were born under 32 weeks, the survival rate was 49.4% for isolated left CDH and 37.9% for isolated right CDH[13]. This suggests that FETO introduces a significant risk for prematurity and all its consequences. It is recommended therefore that – while waiting for the results – FETO should not be performed outside the trial[11]. According to the consensus statement of the National Institutes of Health (NIH), CDH fetuses at risk for delivery before 34 weeks of gestation should be given prenatal steroid therapy.

Delivery

The timing and preferred mode of delivery in CDH pregnancies are still controversial. Hutcheon et al. showed that neonatal and infant mortality significantly decreased with advancing gestation, from 25 and 36% at 37 weeks of gestation, respectively, to 17 and 20% at 40 weeks of gestation, respectively[14]. Moreover, a study from Odibo et al. among 107 CDH cases found that gestational age at delivery was inversely correlated to the need for ECMO[15]. However, Safavi et al. found no difference in mortality when dividing gestational age at delivery categorically as under 37 weeks; 37- 38 weeks and 39 weeks or beyond[16]. Neither did they find a difference in mortality between vaginal and cesarean delivery[16]. In the absence of true convincing data it seems intuitive to schedule delivery (induced delivery or cesarean section) carefully in the best possible conditions

also dependent of maternal indications, i.e. at 39 weeks or beyond and in the presence of the relevant clinicians.

Recommendations (prenatal management and delivery)

- Following prenatal diagnosis, disease severity should be assessed at an experienced center. This will involve measurement of the O/E LHR and position of the liver (grade of recommendation = D).
- In case of an anticipated birth prior to 34 weeks of gestation, antenatal steroids should be given (grade of recommendation = D).
- Delivery after a gestational age of 39 weeks in a high-volume tertiary center should be planned (grade of recommendation = D).

Delivery room management and treatment in the initial postnatal phase

Initial treatment and procedures in the delivery room are based on the updated Guidelines of the International Consensus on Cardiopulmonary Resuscitation and Emergency Cardiovascular Care Science with Treatment Recommendations[17].

Monitoring and goal of treatment

Measurements of heart rate, pre- and postductal saturations, and intra-arterial blood pressure are recommended. The key principles are the avoidance of high airway pressures and the establishment of adequate perfusion and oxygenation (based on preductal arterial saturation, SpO_2 measurements). In a study from Dawson et al. in term and preterm healthy neonates, the overall SpO_2 values at 10 min after birth were median 94% (interquartile range 91- 97%) in preterm infants and median 97% in term infants (interquartile range 92- 98%)[18]. Based on expert opinion, the consortium agreed on preductal SpO_2 boundaries in the delivery room of 80-95%. In the first 2 hours after birth, preductal SpO_2 levels as low as 70% are acceptable if they are improving without ventilator changes, if organ perfusion is satisfactory, as indicated by a pH > 7.2, and if ventilation is adequate ($PaCO_2$ <65 mmHg (8.6 kPa)). Since there is growing evidence that room air is less harmful than 1.0 fractional inspired oxygen (FiO_2) in the resuscitation of term infants[19,20], it may be better for CDH infants to start with fractional inspired oxygen lower than 1.0. The aim for preductal saturation is 80- 95% after the first hour of life. Thus, to avoid hyperoxia, supplemental oxygen should be diminished by reducing the oxygen fraction when preductal saturation exceeds 95%.

Intubation and ventilation

The consortium recommends intubating infants with prenatally diagnosed CDH immediately after birth as a standard of care. The position of the endotracheal tube should be confirmed by end-tidal CO_2 monitoring. However, based on expert opinion, in those

infants who are predicted to have good lung development based on their prenatal assessment (e.g. left sided defect, O/E LHR > 50%, and liver down), spontaneous breathing could be considered instead to prevent ventilator-induced lung injury. Low peak pressures, preferably <25cm H_2O, are recommended to avoid lung damage to the ipsilateral and contralateral lung.

Sedation and analgesia/paralysis for intubation

Carbajal et al. have studied physiological responses of neonates to awake intubation, and they reported significant rises in systemic arterial blood pressure and intracranial pressure, as well as significant decreases in heart rate and transcutaneous oxygen saturations[21]. In 166 infants Caldwell and Watterberg found that premedication for intubation significantly attenuated both the clinical pain score and the increase in blood glucose as marker of acute stress[22]. Moreover, it seems that intubation success rates progressively improve with premedication, although in some cases this is not possible due to a lack of vascular access[23]. Murthy et al. have shown no beneficial effects of administration of neuromuscular blocking agents immediately after intubation; in fact lung compliance deteriorated upon administration[24].

Naso- or orogastric tube

The consortium recommends immediate placing an oro- or nasogastric tube with continuous or intermittent suctioning in order to prevent bowel distension and any additional ipsilateral lung compression.

Vascular access

As preductal PaO_2 measurements reflect the delivered oxygen to the brain, the arterial line should preferably be inserted into the right radial artery, also for blood sampling and monitoring of the arterial blood pressure. Alternatively, an umbilical arterial line may be placed. This is less desirable, however, than a right radial artery line because it reflects the postductal situation, but on the other hand, it may give more secure longer -term arterial access. Each procedure should be performed as soon as possible. It is important, however, to prevent further agitation from recurrent insertion attempts as this may impair postnatal adaptation[25].

Blood pressure control

Measures to increase the systemic blood pressure may minimize the right-to-left shunting. However, there is no need to increase blood pressure levels to supranormal values if the preductal saturation remains above 80%. Therefore, the consortium recommends maintaining arterial blood pressure at normal levels for gestational age if preductal saturations remain between 80 and 95%. In the case of hypotension and/or poor tissue

perfusion, a fluid bolus of 10-20 ml/kg NaCl 0.9% should be administered, although no more than 2 times. If tissue perfusion and blood pressure do not improve, inotropic and/ or vasopressor medication should be considered according to local practice. Hydrocortisone may be used in the early phase for the treatment of hypotension after other treatment has failed[26].

Surfactant

There is no rationale for surfactant therapy because in CDH patients surfactant amounts are likely to be appropriate to lung size[27].

Recommendations

- After delivery, the infant should be intubated routinely without bag and mask ventilation (grade of recommendation = D).
- The goal of treatment in the delivery room is achieving acceptable preductal saturation targets, i.e. between 80 and 95% (grade of recommendation = D).
- Ventilation in the delivery room should be done with a peak pressure as low as possible, preferably with 25 cm H_2O, or below that (grade of recommendation = D).
- An oro- or nasogastric tube with continuous or intermittent suction should be placed (grade of recommendation = D).
- Arterial blood pressure has to be maintained at a normal level for gestation. In the case of hypotension and/ or poor tissue perfusion, 10- 20 ml/kg NaCl 0.9% should be administered 2 times (grade of recommendation = D).
- In cases of persistent hypotension after the administration of NaCl 0.9%, inotropic and vasopressor agents should be considered (grade of recommendation = D).
- In CDH infants who are predicted to have good lung development based on their prenatal assessment (e.g. left-sided defect, O/E LHR > 50%, and liver down), spontaneous breathing could be considered (grade of recommendation = D).
- Premedication should be given before intubation if possible (grade of recommendation = D).
- Neuromuscular blocking agents should be avoided during initial treatment in the delivery room (grade of recommendation = D).
- No routine use of surfactant in either term or preterm infants with CDH (grade of recommendation = D).

Ventilation management in the Intensive Care Unit

Permissive hypercapnia and 'gentle ventilation' have been reported to increase survival in neonates with CDH[28,29]. A ventilation strategy aiming for preductal saturation between 80 and 95%, postductal saturation above 70% and arterial CO_2 levels between 50 and 70 mmHg (6.9- 9.3 kPa) (permissive hypercapnia) is well accepted. In the first 2

hours after birth, preductal SpO_2 levels as low as 70% are acceptable provided they are slowly improving and organ perfusion is satisfactory (indicated by a pH >7.2, and if ventilation is adequate ($PaCO_2$ <65 mmHg, 8.6 kPa). Thereafter, preductal saturation levels are preferably kept between 85 and 95%. In individual cases, however, levels down to 80% may be accepted, providing organs are well perfused, as indicated by a pH >7.2, lactate levels < 5 mmol/l and urinary output >1 ml/kg/h. Postductal saturations should remain above 70%. Oxygen toxicity can be avoided by decreasing FiO_2 on the guidance of the saturation levels described above. The optimal *initial* ventilation strategy was investigated in a collaborative initiative from the CDH EURO Consortium (VICI-trial, NTR 1310)[30]. Although the primary outcome (death/ bronchopulmonary dysplasia at day 28) was not significantly different between the two groups, it was found that infants initially ventilated by conventional mechanical ventilation required a significantly shorter duration of ventilation, had less need for inhaled nitric oxide (iNO) or sildenafil, had a shorter duration of vasoactive medication and were less likely to require ECMO[3]. Therefore, the CDH EURO Consortium recommends conventional mechanical ventilation as the initial ventilation strategy. Recommendations for initial ventilation settings for pressure-controlled ventilation are summarized below. In the case of weaning, the peak pressure should primarily be reduced. Thereafter, frequency or PIP/ PEEP may be reduced as long as pCO_2 <50 mmHg (6.7 kPa). In general, the consortium recommends aiming for a limitation of peak pressures to 25 cm H_2O or less, a PEEP of 3-5 cm H_2O and adjustment of the ventilator rate to obtain $PaCO_2$ between 50 and 70 mmHg (6.9- 9.3 kPa). If a PIP of >28 cm H_2O is necessary to achieve pCO_2 and saturation levels within the target range, other treatment modalities (such as high-frequency oscillatory ventilation or ECMO) should be considered.

Chest radiograph

To assess the patient's initial condition, a chest radiograph should be obtained as soon as possible.

Recommendations

- Conventional mechanical ventilation is the optimal *initial* ventilation strategy (grade of recommendation = C).
- High-frequency oscillatory ventilation can be used as rescue therapy if conventional mechanical ventilation fails (grade of recommendation = D).
- Adapt ventilation settings to reach a preductal saturation between 80 and 95% and a postductal saturation above 70% (grade of recommendation = D).
- The target $PaCO_2$ should be between 50 and 70 mm Hg (6.9- 9.3 kPa) (grade of recommendation = D).

- Pressure-controlled ventilation: initial settings are a PIP <25 cm H_2O and a PEEP of 3- 5 cm H_2O; ventilator rate of 40- 60/ min (grade of recommendation = D).
- After stabilization, reduce FiO_2 if the preductal saturation is above 95% (grade of recommendation = D).

Further management in the Intensive Care Unit

Sedation and analgesia

A wide range of sedative and analgesic practices has been described[31,32]. Most centers use opioids such as morphine sulfate or fentanyl. Although there is no specific evidence in infants with CDH, neuromuscular blockade is associated with side-effects such as hypoxemia – and thus should be avoided. Infants should remain sedated during mechanical ventilation until weaning form mechanical ventilation is commenced.

Monitoring

Heart rate, invasive blood pressure, pO_2 and pCO_2, and pre- and postductal saturation should be monitored routinely. A head ultrasound scan should be performed at a time when there is little danger of arousing the newborn. Monitoring the regional cerebral oxygenation saturation with near infrared spectroscopy and transcutaneous saturation measurements may be indicated,[33] although its additional value in CDH infants is not yet clear. Sedation and analgesia should be started as soon as venous access is established. Careful monitoring of the blood pressure is then warranted because more fluid volumes or vasoactive drugs may be needed in view of the potential adverse hemodynamic effect of sedatives, in particular midazolam. Supportive care such as cocooning and swaddling is recommended to prevent stress from too much noise, light and nociceptive stimulation. The infant's condition should be regularly assessed using validated analgesia and sedation scoring systems, such as the COMFORT behavior score[34].

Hemodynamic management

Hemodynamic management should be aimed at achieving appropriate end-organ perfusion determined by heart rate, urine output, and lactate levels. If the heart rate is within the normal range[35], urine output is over 1.0 ml/kg/hour, lactate concentration is <3 mmol/l and there are no other symptoms of poor tissue perfusion, inotropic or vasopressor support is not required. Echocardiography is indicated if there are signs of poor perfusion or if the blood pressure is below the normal level for gestation with a preductal saturation below 80%. This may show whether the poor perfusion is due to hypovolemia or myocardial dysfunction. If there is hypovolemia, saline fluid therapy should be given (10-20 ml/kg NaCl 0.9% or Ringer lactate) up to 2 times during the first 2 hours)[36]. If necessary, this should be followed by inotropic and/ or vasopressor therapy.

Hydrocortisone may be used for the treatment of hypotension after other treatment has failed.

Recommendations

- Infants should be sedated and be monitored using validated analgesia and sedation scoring systems (grade of recommendation = D).
- Neuromuscular blocking agents should be avoided if possible (grade of recommendation = D).
- If symptoms of poor perfusion and/or blood pressure below the normal level for gestation occur and are associated with preductal saturation below 80%, echocardiographic assessment should be performed (grade of recommendation = D).
- In case of hypovolemia, fluid therapy (10-20 ml/kg NaCl 0.9% or Ringer lactate) up to 2 times during the first two hours may be given and followed if necessary by administration of inotropic and/ or vasopressor agents (grade of recommendation = D).

Pulmonary hypertension

A 2D echocardiography performed within the first 24 hours after birth remains the best modality to 1) rule out the presence of cardiac anomalies; 2) assess the right heart function; and 3) determine the amount of pulmonary hypertension classified accordingly (less or more than 2/3 systemic blood pressure)[37,38]. Especially in severe cases of pulmonary hypertension, a cardiac ultrasound may help to evaluate right ventricular dysfunction and/or right ventricular overload, which condition can also lead to left ventricular dysfunction[39].

There is no evidence for the usefulness of increasing systemic vascular resistance to treat right-to-left shunting, but a number of centers from the consortium suggest using inotropic or vasopressor agents such as dopamine, dobutamine and (nor)epinephrine to maintain blood pressure at normal levels for gestation[40]. If preductal saturation falls below 85% and/ or if there are signs of poor organ perfusion, treatment of pulmonary hypertension should be initiated. The first choice would be iNO, which is a pulmonary vasodilator. In neonates with pulmonary hypertension of the newborn (PPHN) or severe hypoxic respiratory failure, iNO improves oxygenation and decreases the need for ECMO[41,42]. At an oxygenation index of 20 or higher and/or a pre- and postductal saturation difference of 10% or more, iNO may be given for at least 1 hour. A consistent dose-dependent effect of iNO has not yet been shown[43]. As in one study[43] more infants treated with NO needed ECMO, we recommend to stop iNO therapy if no effect is seen after its initiation.

If there is no or an insufficient response to iNO, intravenous prostacyclin, intravenous phosphodiesterase type 5 inhibitor (sildenafil) or medication involving the endothelin pathway should be considered. These agents have been used successfully in treating

PPHN in neonates with and without CDH[44,45]. The effects of treatment may be best addressed by repeated cardiac evaluation[46]. This can lead to insufficient filling of the left ventricle and thereby to poor systemic perfusion. Re-opening of the ductus arteriosus with prostaglandin E1 may protect the right ventricle from excessive overload due to increased afterload[47]. Phosphodiesterase-3 inhibitor (Milrinone) was investigated in only 6 CDH patients by Patel et al. Right ventricular function and oxygenation index significantly improved[48]. Sildenafil® has been used in the treatment of pulmonary hypertension in infants with CDH. Intravenous sildenafil has recently become available, but its use has not yet been FDA approved.

Recommendations

- Perform echocardiography within the first 24 hours after birth to rule out structural cardiac anomalies (grade of recommendation = D).
- Blood pressure support should be given to maintain arterial blood pressure levels at normal levels for gestation (grade of recommendation = D).
- iNO administration for at least 1 hour in a dose of 10-20 ppm should be considered if there is evidence of extrapulmonary right-to-left shunting and the oxygenation index is above 20 and/or the saturation difference is more than 10% (grade of recommendation = D).
- In nonresponders iNO should be stopped. iNO responders are defined as follows: decline of 10- 20% in the pre-postductal saturation difference, or an increase of 10-20% of PaO_2, or improvement in hemodynamic parameters meaning a 10% increase in mean blood pressure, or a decrease in lactate levels (grade of recommendation = D).
- Intravenous sildenafil should be considered in CDH patients with severe pulmonary hypertension (grade of recommendation = D).
- In case of suprasystemic pulmonary artery pressure and right-to-left shunting through the foramen ovale, intravenous prostaglandin E1 should be considered (grade of recommendation = D).

Extracorporeal membrane oxygenation

The benefit of ECMO in the treatment of infants with CDH remains unclear. The ELSO registry showed a survival rate of 51% of patients with CDH who required ECMO[49]. The use of ECMO has decreased in recent years[50]; it is more used for preoperative stabilization, and the preferred method (venoarterial vs. venovenous) is still being debated. The VICI-trial showed no difference in survival between patients born in ECMO centers and patients born in non-ECMO centers[3].

Recommendations

– Criteria for ECMO (grade of recommendation = D):
  - Inability to maintain preductal saturations >85% or postductal saturations >70%
  - Increased $PaCO_2$ and respiratory acidosis with pH <7.15 despite optimization of ventilator management.
  - Peak inspiratory pressure >28 cm H_2O or mean airway pressure >17 cm H_2O is required to achieve saturation >85%.
  - Inadequate oxygen delivery with metabolic acidosis as measured by elevated lactate ≥ 5 mmol/L and pH <7.15.
  - Systemic hypotension, resistant to fluid and inotropic therapy, resulting in urine output <0.5 mL/kg/h for at least 12- 24 hours
  - Oxygenation index ≥ 40 present for at least 3 hours.

Surgical repair

Surgery should be performed electively. The effect of hospital volume on mortality is unclear. While a large study (2203 infants) concluded that hospitals with a high-volume of CDH repair have lower in-hospital mortality[51], a more recent study in 3738 infants showed no difference in mortality between lower and higher surgical volume centers[52]. Controversies about the exact timing of the surgical repair in patients on ECMO remain[53]. A recent study from Partridge et al. showed improved outcomes with surgical repair after ECMO, i.e. a higher likelihood of survival, less surgical bleeding and shorter duration of ECMO[54]. A relative small study (n=46) from Fallon et al. found that repair within the first 72 hours of ECMO correlated with a shorter duration of ECMO, less circuit complications and a trend towards improved survival[55].

The routine use of a chest tube postoperatively to drain the effusion filling the pleural cavity has been abandoned. This does not preclude its use in individual cases to drain an effusion that is symptomatic, for example due to chylothorax existing before surgery.

The optimal surgical technique also remains under debate. Minimal access surgery is gaining ground on the open approach (thoracotomy or laparotomy)[56]. Minimal access surgery has esthetic advantages and may be performed in patients with a left-sided defect and liver down, but carries a significantly higher risk of recurrence[56,57]. There is also concern about absorption of CO_2 used for insufflation in minimal access surgery[58], and CO_2 insufflation pressures should therefore be minimized. A meta-analysis from Lansdale et al. showed that thoracoscopic repair had greater recurrence rates and operative times but similar survival and patch usage compared with open surgery[59]. Recently, Costerus et al. concluded that thoracoscopic primary closure seems a safe and effective procedure, but efficacy of thoracoscopic patch repair has not been established[60]. To allow for better comparison of patient groups between studies it is recommended to record the diaphragmatic defect size[37] in all surgeries.

Recommendations

- Surgical repair of the diaphragmatic defect should be performed after clinical stabilization, defined as follows (grade of recommendation = D):
  - Mean arterial blood pressure normal for gestation.
  - Preductal saturation levels of 85- 95% on fractional inspired oxygen below 50%.
  - Lactate below 3 mmol/L.
  - Urine output more than 1 mL/kg/h.
- No routine chest tube placement postoperatively (grade of recommendation = D)
- Repair can be performed while the patient is on ECMO (grade of recommendation = D)

Fluid management, parenteral feeding, entering enteral feeding and gastroesophageal reflux

Restrictive fluid management in the first 24 hours after birth consists of 40 mL/kg/day of fluids including medication, with additional saline volume top-up for intravascular filling in the case of inadequate tissue perfusion or hypotension. Parenteral nutrition only is allowed until surgical repair and until postoperative enteral feeding has been achieved. Gastroesophageal reflux may be treated both by antireflux medication and by surgical intervention[61]. Maier et al. did not show evidence for profit beyond the first year of life after prophylactic Thal-procedure at primary CDH repair[62]. Diuretics should be given in the case of persisting positive fluid balance without hypovolemia, aiming for diuresis of >1 mL/kg/hour[63].

Recommendations

- 40 mL/kg/day saline including medication for the first 24 hours after birth; increase intake thereafter (grade of recommendation = D).
- Diuretics should be considered in case of persisting positive fluid balance; aim for a diuresis >1 mL/kg/hour (grade of recommendation = D).
- Preventive antireflux therapy should be started in combination with enteral feeding (grade of recommendation = D).
- Pre-operatively, patients should only receive parenteral nutrition (grade of recommendation = D).

CONCLUSION

The European task force for CDH (CDH EURO Consortium) has agreed on an updated protocol for standardized postnatal treatment guidelines. Although it is eminence-based medicine and many recommendations are level D, we think that a consensus of

many specialized centers on the use of a standardized treatment protocol will contribute to making more valid comparisons of patient data in ongoing and future multicenter prospective clinical studies.

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Supplementary table 1 – Recommendations

2010	2015
Prenatal management and delivery	
Following prenatal diagnosis, the absolute and O/E LHR and the position of the liver should be evaluated*	Following prenatal diagnosis, disease severity should be assessed at an experienced center. This will involve high resolution genetic testing, determination of the O/E LHR and position of the liver *
Planned vaginal delivery or caesarean section after a gestational age of 37 weeks in a high-volume tertiary center should be pursued*	Delivery after a gestational age of 39 weeks in a high-volume tertiary center, should be planned*
In case of preterm labor prior to 34 weeks of gestation, antenatal steroids should be given*	Unchanged
Delivery room management and treatment in a very early phase	
After delivery, the infant should be intubated immediately without bag and mask ventilation*	Unchanged
The goal of treatment in the delivery room is achieving acceptable preductal saturations levels between 80 and 95%*	Unchanged
Ventilation in the delivery room may be done by conventional ventilator or ventilation bag with a peak pressure as low as possible, preferably below 25 cm H_2O*	Unchanged
An oro- or nasogastric tube with continuous or intermittent suction should be placed*	Unchanged
Arterial blood pressure has to be maintained at a normal level for gestational age. In case of hypotension and/ or poor perfusion, 10- 20 ml/kg NaCl 0.9% should be administered 2 times*	Unchanged
Sedatives and analgesics should be given*	Neuromuscular blocking agents should be avoided during initial treatment in the labour ward*
	In CDH infants who are predicted to have good lung development based on their prenatal assessment (e.g. left sided, O/E LHR > 50%, and liver down), spontaneous breathing could be considered*
No routine use of surfactant in either term or preterm infants with CDH*	Unchanged
	In cases of persistent hypotension after administration of NaCl 0.9%, inotropic and vasopressor agents should be considered*
	Premedication should be given before intubation if possible*
Ventilation management in the Intensive Care Unit	
Adapt treatment to reach a preductal saturation between 85 and 95% and a postductal saturation above 70%*	Adapt treatment to reach a preductal saturation between 80 and 95% and a postductal saturation above 70%*
In individual cases, preductal saturation above 80% might be acceptable, as long as organs are well perfused*	Unchanged

Supplementary table 1 – (continued)

2010	2015
The target $PaCO_2$ range should be between 45 and 60 mmHg*	The target $PaCO_2$ should be between 50 and 70 mm Hg (6.9- 9.3 kPa)*
Pressure-controlled ventilation initial settings are a PIP of 20-25 cm H_2O and a PEEP of 2-5 cm H_2O; ventilator rate of 40- 60/min*	Pressure controlled ventilation: initial settings are a PIP of <25 cm H_2O and a PEEP of 3- 5 cm H_2O; ventilator rate of 40- 60/ min
HFOV: initial setting mean airway pressure 13-17 cm H_2O, frequency 10 Hz, ΔP 30-50 cm H_2O depending on chest wall vibration*	High-frequency oscillatory ventilation can be used as rescue therapy if conventional mechanical ventilation fails*
After stabilization, the FiO_2 should be decreased if preductal saturation is above 95%*	Unchanged
	Conventional mechanical ventilation is the optimal *initial* ventilation strategy**
Further management in the Intensive Care Unit	
Infants should be sedated and be monitored using validated analgesia and sedation scoring systems*	Unchanged (however in 2010, this recommendation was presented in a separate section).
Neuromuscular blocking agents should be avoided if possible*	Unchanged (however in 2010, this recommendation was presented in a separate section).
If symptoms of poor perfusion and/or blood pressure below the normal level for gestational age occur and are associated with preductal saturation below 80%, echocardiographic assessment should be performed*	Unchanged
In case of hypovolemia, isotonic fluid therapy 10-20 ml/kg NaCl 0.9% up to 3 times during the first 2 hours may be given and inotropics should be considered*	In case of hypovolemia, fluid therapy (10-20 ml/ kg NaCl 0.9% or lactated Ringers) up to two times during the first two hours may be given and followed if necessary, by administration of inotropic and/ or vasopressor agents should be considered*
Pulmonary hypertension	
Perform echocardiography within the first 24 h after birth*	Perform echocardiography within the first 24 hours after birth to rule out structural cardiac anomalies
Blood pressure support should be given to maintain arterial blood pressure levels at normal levels for gestational age*	Unchanged
iNO should be considered if there is evidence of extrapulmonary right-to-left shunting and the oxygenation index is above 20 and/or the saturation difference is more than 10%*	iNO administration for at least one hour in a dose of 10- 20 ppm should be considered if there is evidence of extrapulmonary right-to-left shunting and the oxygenation index is above 20 and/or the saturation difference is more than 10%.
	In non-responders iNO should be stopped. iNO responders are defined as follows: decline of 10- 20% in the pre-postductal saturation difference, or increase of 10- 20% of PaO_2, or improvement in hemodynamic parameters meaning a 10% increase in mean blood pressure, or decrease of lactate levels*
	Intravenous sildenafil should be considered in CDH patients with severe pulmonary hypertension*

Supplementary table 1 – (continued)

2010	2015
In case of suprasystemic pulmonary artery pressure and right-to-left shunting through the foramen ovale, i.v. prostaglandin E1 has to be considered*	Unchanged
Extracorporeal membrane oxygenation (ECMO)	
Criteria for ECMO*:	Criteria for ECMO*:
– Inability to maintain preductal saturations >85% or postductal saturations >70%.	Unchanged
– Increased $PaCO_2$ and respiratory acidosis with pH <7.15 despite optimization of ventilatory management.	Unchanged
– Peak inspiratory pressure >28 cm H_2O or mean airway pressure >17 cm H_2O is required to achieve saturation >85%.	Unchanged
– Inadequate oxygen delivery with metabolic acidosis as measured by elevated lactate ≥5 mmol/l and pH <7.15.	Unchanged
– Systemic hypotension, resistant to fluid and inotropic therapy, resulting in urine output <0.5 ml/kg/h for at least 12- 24 hours	Unchanged
– Oxygenation index (mean airway pressure x FiO_2 x 100/PaO_2) ≥40 consistently present	Oxygenation index (mean airway pressure x FiO_2 x 100/$PaCO_2$) ≥40 at least three hours present*
Surgical repair	
Surgical repair of the diaphragmatic defect should be performed after physiological stabilization, defined as follows*	Surgical repair of the diaphragmatic defect should be performed after physiological stabilization, defined as follows*
• Mean arterial blood pressure normal for gestation;	• Unchanged
• Preductal saturation levels of 85- 95% on fractional inspired oxygen below 50%;	• Unchanged
• Lactate below 3 mmol/ l;	• Unchanged
• Urine output more than 2 ml/kg/h	• Urine output more than 1 ml/kg/h
No routine chest tube placement*	Unchanged
Repair can be performed while the patient is on ECMO*	Unchanged
Fluid management, parenteral feeding, entering enteral feeding and gastroesophageal reflux	
40 ml/kg/day including medication for the first 24 hours after birth, increase intake thereafter*	Unchanged
Diuretics should be considered in case of persisting positive fluid balance, aim for diuresis 1- 2 ml/kg/hour*	Diuretics should be considered in case of persisting positive fluid balance, aim for a diuresis >1 ml/kg/hour*
Preventive antireflux therapy should be started in combination with enteral feeding*	Unchanged
	Pre-operatively, patients should only receive parenteral nutrition*

*Grade of recommendation = D. ** Grade of recommendation = C.

Supplementary table 2 – Levels of evidence

Level	Description of evidence
1++	High quality meta-analyses, systematic reviews of randomized controlled trials, or randomized controlled trials with a very low risk of bias
1+	Well conducted meta-analyses, systematic reviews of randomized controlled trials, or randomized controlled trials with a low risk of bias
1-	Meta-analyses, systematic reviews or randomized controlled trials, or randomized controlled trials with a high risk of bias
2++	High-quality systematics reviews of case-control or cohort studies, or high-quality case-control or cohort studies with a very low risk of confounding, bias or chance, and a high probability that the relationship is causal
2+	Well-conducted case-control or cohort studies with a low risk of confounding, bias or chance, and a moderate probability that the relationship is causal
2-	Case-control or cohort studies with a high risk of confounding, bias or chance, and a significant risk that the relationship is not causal
3	Nonanalytic studies, e.g. case reports, case series
4	Expert opinion

Supplementary table 3 – Grades of recommendation

Grade	Description of grade
A	At least 1 meta-analysis, systematic review, or randomized controlled trial rated as 1++ and directly applicable to the target population, or a systematic review of randomized controlled trials or a body of evidence consisting principally of studies rated as 1+ directly applicable to the target population and demonstrating overall consistency of results
B	A body of evidence including studies rated as 2++ directly applicable to the target population and demonstrating overall consistency of results, or extrapolated evidence from studies rated as 1++ or 1-
C	A body of evidence including studies rated as 2+ directly applicable to the target population and demonstrating overall consistency of results, or extrapolated evidence from studies rated as 2++.
D	Evidence level 3 or 4, or extrapolated evidence from studies rated as 2+

Part IV

Outcome

Chapter 11

Neurodevelopmental outcome in high-risk congenital diaphragmatic hernia patients: An appeal for international standardization

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ABSTRACT

Background: Since mortality in congenital diaphragmatic hernia (CDH) is decreasing, morbidity such as neurodevelopmental outcome is becoming increasingly important.
Objectives: We evaluated neurodevelopmental outcome in high-risk CDH patients treated according to the CDH EURO Consortium standardized treatment protocol.
Methods: This observational, prospective cohort study was conducted in two European centers. Neurodevelopment of 88 patients (Rotterdam n=49; Rome n=39) was assessed at 12 and 24 months with the Bayley Scales of Infant Development (BSID)-II-NL (Rotterdam) or BSID-III (Rome). Data of the centers were analyzed separately.
Results: Cognition was normal in 77.8% of children from Rotterdam and in 94.8% from Rome at 12 months, and in 70.7 and 97.4%, respectively, at 24 months. Motor function was normal in 64.3% from Rotterdam and in 81.6% from Rome at 12 months and in 45.7 and 89.8%, respectively, at 24 months. Longer length of hospital stay (LoS) was associated with worse cognitive outcome and motor function; LoS, low socioeconomic status, and ethnicity were associated with lower cognition.
Conclusions: At 2 years, most CDH patients have normal cognition, but are at risk for motor function delay. Due to differences in outcomes between centers, careful interpretation is needed before conclusions can be drawn for other centers. Future multicenter collaboration should not only focus on standardization of postnatal care, but also on international standardization of follow-up to identify risk factors and thereby reduce morbidity.

INTRODUCTION

Congenital diaphragmatic hernia (CDH) affects approximately 1 in 3000 newborns[1]. It is a life-threatening disease caused mainly by persistent pulmonary hypertension and pulmonary hypoplasia[1]. Severely ill CDH patients may receive extracorporeal membrane oxygenation (ECMO)[1]. In 2008, a standardized neonatal treatment protocol was developed at a consensus meeting of the CDH EURO Consortium[1]. The mortality rate has decreased from 33 to 12% since this protocol was implemented[2]. Consequently, worldwide the focus of interest has shifted to morbidity[3-5].

Previous studies found associated morbidity in about 87% of CDH survivors, mainly related to the lungs or the gastrointestinal tract[6,7]. While most follow-up studies have focused on morbidity, few have examined neurodevelopment. Most of those studies were cross-sectionally performed in small series in single centers[8-10]. Recently, longitudinal studies have shown neurodevelopmental delays in CDH patients[3,4,11]. To date, no published studies have examined neurodevelopmental outcome after implementation of a standardized treatment protocol in a multicenter setting.

We longitudinally investigated neurodevelopmental outcome in high-risk patients managed according to the CDH EURO Consortium standardized treatment protocol[1] in two high-volume CDH centers. Secondly, we identified determinants of neurodevelopmental outcome.

MATERIALS AND METHODS

This is an observational, prospective cohort study in patients born between January 2009 and May 2012 with high-risk CDH, i.e. antenatal diagnosis/ respiratory insufficiency within 6 hours postnatally. All patients were treated according to a standardized treatment protocol[1]; however, ECMO was only offered in Rotterdam. Rotterdam and Rome are two of the largest European CDH centers. As standard of care, survivors were offered a structured, longitudinal follow-up program, initiated in Rotterdam in 1999[11] and in Rome in 2004[12]. We evaluated prospectively collected data of repeated measurements at corrected ages of 12 and 24 months. Since subjects were not submitted to any handling and no rules of human behavior were imposed, institutional review board approval was waived. Parents were informed that data were used for research purposes.

Patient characteristics

Patient characteristics were retrieved from medical records. Prenatal, perinatal, perioperative, postnatal, demographic data and data at discharge were retrieved from medical records:

Prenatal: lung-to-head ratio (LHR), observed/ expected LHR (O/E LHR)[13] determined if ultrasound was performed before 32 weeks gestation, application of fetoscopic endoluminal tracheal occlusion (FETO).

Perinatal and postnatal: sex, birth weight, gestational age, Score for Neonatal Acute Physiology-II (SNAP-II score)[14], ECMO duration if applicable, initial ventilation type, length of ventilation, severity of chronic lung disease (CLD)[15], duration of morphinomimetics/ sedatives, use of anticonvulsants, length of initial hospital stay (LoS) in tertiary hospital, and episodes of general anesthesia within first 24 months.

Perioperative: age at repair, side of hernia, liver position, surgical approach (thoracoscopy or laparotomy), type of repair (patch or primary closure).

At discharge: tube feeding, physical therapy and involvement of a speech language pathologist for feeding problems or oral aversion.

Demographic: Ethnicity was classified as native or non-native. Socioeconomic status (SES) was classified by maternal highest education level[16].

Neurodevelopmental outcome was assessed with the Bayley Scales of Infant Development (BSID). In Rotterdam, the Dutch-language version of the BSID-II (BSID-II-NL) was administered with Dutch normative data[17]. In both centers cognitive development was assessed at both time points (by two developmental psychologists per center). Psychomotor development was assessed by developmental psychologists at both time points in Rome and at 12 months in Rotterdam. In Rotterdam, psychomotor development was assessed by one physiotherapist at 24 months. Nonverbal and verbal performance are combined in the Mental Developmental Index (MDI), and gross motor and fine motor developmental aspects are combined in the Psychomotor Developmental Index (PDI). In Rome, the Italian translation of the BSID-III[18] with American normative data was administered. The BSID-III provides three norm-reference index scores: cognition, motor (subscale: gross and fine motor) and language (subscale: receptive and expressive language) domains. The normalized population mean (SD) of each composite score is 100 (15) and that of the subscale domains is 10 (3). Scores were grouped as normal (>-1SD), mildly delayed ($-2<$SD<-1) and severely delayed (<-2SD).

Data analysis

Descriptive statistics are presented as number (%), mean (SD) or median (range). Independent samples t test, χ^2 tests and Mann-Whitney U tests, where appropriate, were used to compare characteristics between centers. One-sample t tests served to compare mean scores to the normalized population mean. Linear mixed models were used to estimate neurodevelopmental outcome over time. These models included only the time point (12 or 24 months) as the independent variable, and a random intercept to account for the within-subject correlations. Perinatal factors associated with adverse neurodevelopment were determined using multivariate linear regression. Cognition and motor

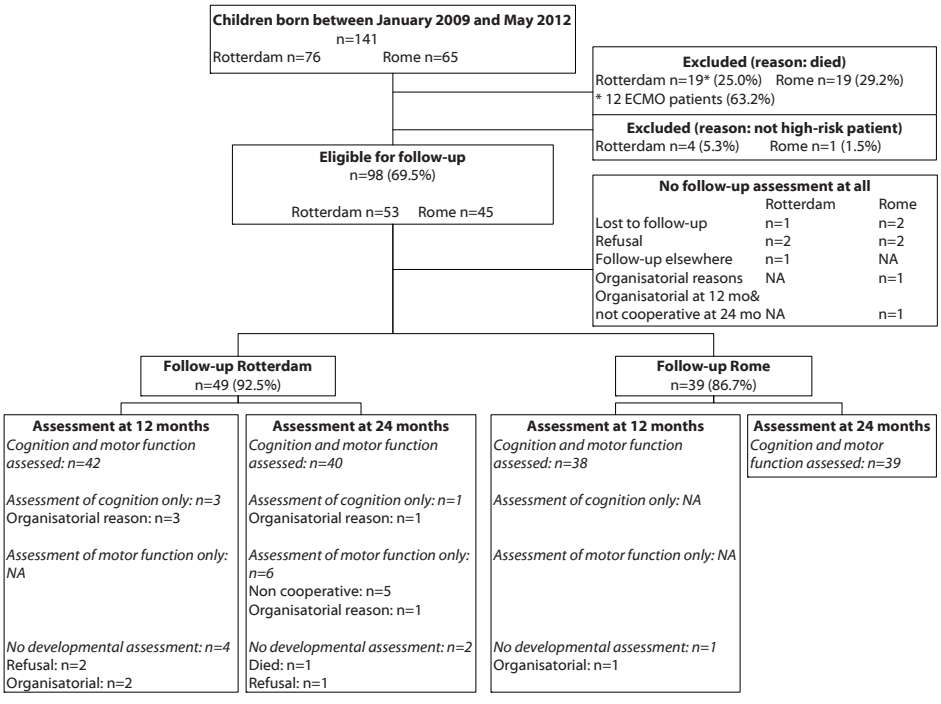
outcome were chosen as dependent variables in two different models; the data were analyzed separately for each assessment, age and each center. Based on clinical experience and the literature, the following independent variables were included in the linear regression analysis: tube feeding at discharge, ECMO support, ethnicity and SES as categorical variables, and SNAP-II score and length of hospital stay (LoS) (log-transformed due to skewed distribution) as continuous variables. Analyses were performed using SPSS version 20.

RESULTS

In the study period, 141 CDH patients were admitted (Rotterdam n=76; Rome n=65). Thirty-eight children died (Rotterdam n=19; Rome n=19) and 5 were not high-risk CDH patients; thus, 98 children were eligible for follow-up. Ultimately, 88 patients were assessed (Figure 1).

Baseline characteristics of participants (Table 1) were not significantly different from nonparticipants. Four patients (Rotterdam n=1, Rome n=3) underwent fetoscopic en-

Figure 1 – Flowchart of inclusion of all patients



Abbreviations: NA: not applicable

Table 1 – Background characteristics

	Rotterdam (n=49)	Rome (n=39)	p-value*
Perinatal and postnatal characteristics			
LHR	1.9 (0.8)	2.2 (0.8)	0.32
Missing	15	13	
O/E LHR	51.6 (17.4)	53.3 (15.8)	0.71
Missing	21	13	
Male sex	28 (57.1%)	25 (64.1%)	0.51
Birth weight, kg	3.07 (0.56)	2.99 (0.41)	0.45
Born before 37 weeks of gestation	8 (16.3%)	4 (10.3%)	0.41
SNAP-II score	18 (12)	13 (9)	0.07
Age at repair, days	3.8 (2.7)	3.6 (2.1)	0.77
Left-sided CDH	42 (85.7%)	38 (97.4%)	0.06
Liver: intrathoracic	19 (38.8%)	12 (30.8%)	0.44
Defect size			0.24
A	5 (10.2%)	4 (10.3%)	
B	18 (26.7%)	22 (56.4%)	
C	24 (49.0%)	11 (28.2%)	
D	2 (4.1%)	2 (5.1%)	
Patch repair	35 (71.4%)	11 (28.2%)	<0.001
Defect size A	n=1	n=0	
Defect size B	n=8	n=1	
Defect size C	n=24	n=8	
Defect size D	n=2	n=2	
Initial ventilation			0.83
CMV	24 (49.0%)	20 (51.3%)	
HFO	25 (51.0%)	19 (48.7%)	
Length of ventilation, days	8 (1- 271)	8 (2- 70)	0.55
Length of initial hospital stay, days	28 (6- 387)	30 (15-161)	0.54
CLD			0.12
No CLD	29 (59.2%)	32 (82.1%)	
Mild CLD	8 (16.3%)	2 (5.1%)	
Moderate CLD	1 (2.0%)	0 (0%)	
Severe CLD	11 (22.4%)	5 (12.8%)	
Length of morphinomimetics/ sedatives			0.28
<1 week	25 (51.0%)	14 (35.9%)	
1 week to 1 month	17 (34.7%)	20 (51.3%)	
>1 month	7 (14.3%)	5 (12.8%)	
Episodes of general anesthesia 0-24 months	2 (1- 13)	1 (1- 3)	0.01

Table 1 – (continued)

	Rotterdam (n=49)	Rome (n=39)	p-value*
At discharge			
Tube feeding	29 (59.2%)	20 (51.3%)	0.46
Physical therapy at home	20 (40.8%)	7 (17.9%)	0.02
Speech language pathologist involved	19 (38.8%)	3 (7.7%)	0.001
Demographic variables			
Ethnicity			0.13
Native	34 (69.4%)	33 (84.6%)	
Nonnative	14 (28.6%)	6 (15.4%)	
Unknown	1 (2.0%)	0 (0%)	
SES mother			0.12
Low	13 (26.5%)	9 (23.1%)	
Medium	15 (30.6%)	21 (53.8%)	
High	18 (36.7%)	9 (23.1%)	
Unknown	3 (6.1%)	0 (0%)	

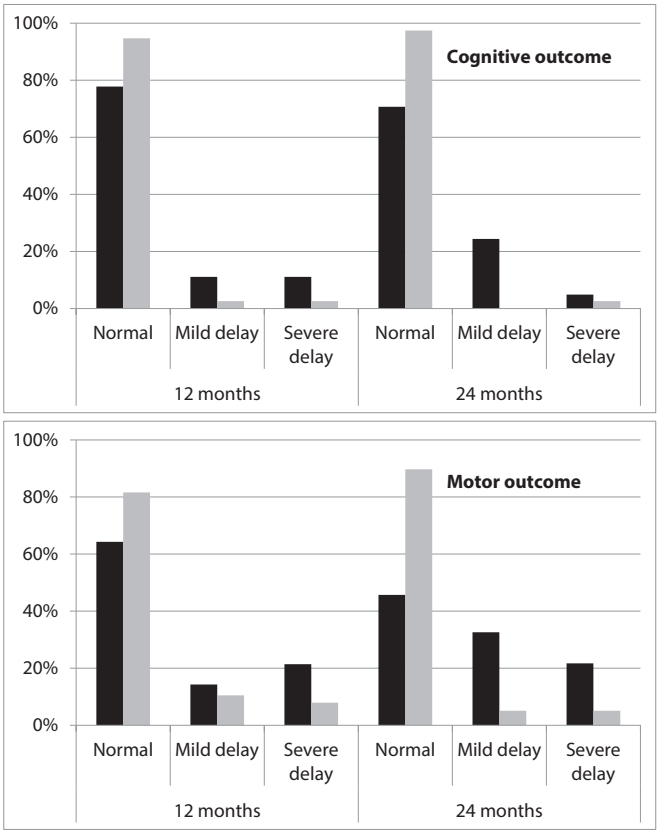
Categorical variables are shown as n (%) and compared between centers between centers using χ^2 tests, normally distributed variables are shown as means (SD) and compared between groups using independent samples t tests, and continuous variables that are not normally distributed are shown as medians (range) and compared between centers using Mann Whitney U tests.

Abbreviations: LHR = lung-head ratio; O/E LHR = observed to expected lung to head ratio; SNAP-II score: = Score for Neonatal Acute Physiology-II; CMV = conventional mechanical ventilation; HFO = high-frequency oscillation; CLD = chronic lung disease; SES= socioeconomic status.Comparison between Rotterdam and Rome.

dotracheal occlusion. In Rotterdam, 28 patients underwent thoracoscopic repair and 7 were converted to laparotomy. In Rome, all children underwent laparotomy. None of the children received anticonvulsants. Six patients from Rotterdam had a genetic or chromosomal abnormality that was not associated with neurodevelopment. Patch repair, episodes of general anesthesia 0-24 months, and involvement of physical therapist and speech-language pathologist were significantly different between Rotterdam and Rome (Table 1). After exclusion of ECMO-treated patients (Supplementary Table 1a), episodes of general anesthesia 0-24 months and involvement of physical therapy were not significantly different between the two centers (Supplementary Table 1b).

Based on the cutoff scores from the BSID manuals, cognition was normal in 71 children (85.5%) and motor function was normal in 58 children (72.5%) at 12 months; this held true for 67 patients (83.8%) and 56 patients (65.9%), respectively, at 24 months. Proportions of mild and severe neurodevelopmental delay are shown in Figure 2. Table 2 shows means (SD) of neurodevelopmental outcome. In supplementary Figure 2a and supplementary Table 2a, data were presented after exclusion of ECMO-treated patients.

Figure 2 – Neurodevelopmental outcome in CDH survivors at 12 and 24 months



Black: Rotterdam; Grey: Rome.

Table 2 – Neurodevelopmental outcome scores at 12 and 24 months (corrected for prematurity)

	12 months				24 months			
	Rotterdam[1]	**p-value[2]**	**Rome (n=38)**	**p-value[2]**	**Rotterdam[3]**	**p-value[2]**	**Rome (n=39)**	**p-value[2]**
Cognitive outcome[4]	97.8 (19.8)	0.46	97.9 (11.8)	0.28	96.0 (18.4)	0.17	102.1 (13.9)	0.36
Motor outcome[4]	87.7 (18.8)	<0.001	93.2 (12.2)	0.002	82.9 (16.7)	<0.001	98.2 (14.8)	0.45
Language outcome[4]	–	–	97.7 (8.6)	0.10	–	–	97.7 (12.6)	0.26
Receptive language[5]	–	–	9.4 (1.7)	0.04	–	–	10.2 (2.4)	0.60
Expressive language[5]	–	–	9.8 (1.9)	0.51	–	–	8.9 (2.5)	0.01
Fine motor skills[5]	–	–	10.1 (2.1)	0.76	–	–	10.5 (2.6)	0.23
Gross motor skills[5]	–	–	7.7 (2.5)	<0.001	–	–	8.9 (2.7)	0.01

[1]Cognitive outcome: n= 45; motor outcome: n= 42. [2]Outcome scores were compared to the expected normal score of reference population; Rotterdam: BSID-II-NL; Rome: BSID-III. [3]Cognitive outcome: n=41; motor outcome: n =46. [4]Mean (SD) of expected normal score of reference population is 100 (15). [5]Mean (SD) of expected normal score of reference population is 10 (3).

Table 3 – Determinants of impaired neurodevelopmental outcome; results of linear regression models

		Rotterdam		Rome	
	Independent variables	**Parameter estimate**	***95% CI***	**Parameter estimate**	***95% CI***
Cognition at 12 months	SNAP-II score	0.5	−0.2 to 1.2	0.1	−0.0 to 0.33
	ECMO	2.3	−25.8 to 30.3	–	–
	Length of hospital stay, days[1]	−9.6	−20.0 to 0.7	−13.7	−21.2 to −6.2
	Tube feeding at discharge	−0.2	−19.2 to 19.0	−1.9	−10.6 to 6.8
	Ethnicity	13.3	−7.7 to 34.3	1.5	−8.1 to 11.1
	SES mother				
	Low	−11.5	−34.9 to 11.9	1.9	−8.4 to 12.1
	Medium	4.6	−16.1 to 25.2	1.2	−7.7 to 10.1
	High[2]	–	–	–	–
Cognition at 24 months	SNAP-II score	0.1	−0.4 to 0.5	0.0	−0.2 to 0.3
	ECMO	20.8	−9.0 to 50.6	–	–
	Length of hospital stay, days[1]	−5.8	−14.1 to 2.6	−10.2	−20.0 to −0.4
	Tube feeding at discharge	−3.5	−18.4 to 11.3	−4.1	−16.2 to 8.0
	Ethnicity	25.1	5.9 to 44.2	−2.1	−15.5 to 11.4
	SES mother				
	Low	−36.2	−58.1 to −14.3	−7.0	−21.3 to 7.4
	Medium	−11.5	−27.9 to 4.8	−3.6	−16.0 to 8.7
	High[2]	–	–	–	–
Motor function at 12 months	SNAP-II score	0.6	−0.0 to 1.2	0.0	−0.2 to 0.2
	ECMO	5.6	−22.3 to 33.5	–	–
	Length of hospital stay, days[1]	−10.5	−21.6 to 0.5	−9.5	−17.7 to −1.4
	Tube feeding at discharge	−0.2	−18.4 to 17.9	−6.6	−16.1 to 2.8
	Ethnicity	15.3	−5.6 to 36.3	3.6	−6.8 to 14.1
	SES mother				
	Low	−0.8	−23.2 to 21.6	−3.1	−14.2 to 8.1
	Medium	1.2	−18.8 to 21.2	−2.4	−12.1 to 7.3
	High[2]	–	–	–	–
Motor function at 24 months	SNAP-II score	0.3	−0.2 to 0.8	0.1	−0.2 to 0.3
	ECMO	14.4	−7.8 to 36.6	–	–
	Length of hospital stay, days[1]	−7.8	−15.3 to −0.3	−16.7	−25.1 to −8.2
	Tube feeding at discharge	3.0	−11.0 to 17.0	0.8	−9.7 to 11.3
	Ethnicity	6.2	−7.4 to 19.9	2.1	−9.5 to 13.7
	SES mother				
	Low	−15.4	−32.1 to 1.4	−0.2	−12.7 to 12.2
	Medium	−2.7	−17.2 to 11.8	5.5	−5.2 to 16.2
	High[2]	–	–	–	–

SNAP-II score = Score for Neonatal Acute Physiology II. [1]Because of skewed distribution, these variables were log-transformed before including them into the linear regression model. [2]Reference category.

In Rotterdam, cognition and motor function remained stable over time. In Rome, cognition and motor function improved over time (mean differences 4.1 (p=0.02) and 5.8 (p=0.003), respectively).

In Rotterdam, cognitive outcome at 24 months was negatively associated with low socioeconomic status (SES) (B −36.2; 95% CI: [−58.1 to −14.3]) and non-Dutch ethnicity (B 25.1; 95% CI: [5.9 to 44.2]) (Table 3). Motor function was negatively associated with longer LoS at 24 months (B −7.8; 95% CI: [−15.3 to −0.3]). For example, taking into account the logarithmic transformation of LoS, children who were hospitalized twice as long scored 5.4 points lower (calculation: B x ln2= −7.8 x ln2) on motor function at 24 months. In non-ECMO- treated patients, the SNAP-II score was significantly associated with cognition and motor function at 12 months. At 24 months, low SES and non-Dutch ethnicity were associated with poor cognition, and low SES and LoS were associated with poor motor function (Supplementary Table 3a). In Rome, longer LoS was significantly associated with cognition at 12 months (B −13.7; 95% CI: [−21.2 to −6.2]) and 24 months (B −10.2; 95% CI: [−20.0 to −0.4]), and longer LoS and motor function at 12 months (B −9.5; 95% CI: [−17.7 to −1.4]) and 24 months (B −16.7, 95% CI: [−25.1 to −8.2]).

DISCUSSION

This longitudinal study was performed in two European high-volume centers that use the same neonatal treatment protocol[1] in high-risk CDH patients. We found normal cognition in 78% of children aged 12 months in Rotterdam and 95% in Rome. At 24 months this was 71% in Rotterdam and 97% in Rome. Normal development of motor function occurred in 64% in Rotterdam and 82% in Rome at 12 months. At 24 months, this was 46% in Rotterdam and 90% in Rome.

A few cross-sectional studies have reported neurodevelopmental outcome in CDH patients[8-10,19]. Danzer et al. reported delayed cognition in 32% of 41 children, assessed with BSID-II or BSID-III at a median age of 24 months[9], and 24% of 42 prospectively enrolled patients at 2-4 years assessed with BSID-III[8]. The prevalence of cognitive problems using similar outcome categories was comparable for Rotterdam, whilst fewer patients in Rome had cognitive delays. It is possible that patients in the study by Danzer et al. were more severely ill since they were ventilated longer and more infants received ECMO[8]. In a multicenter study by Wynn et al., mean cognitive scores –obtained with BSID-III in 48 CDH patients at 24 months – were significantly below normal (mean 93; SD 15)[19]. We found the same trend; although different BSID versions were applied, results from Rotterdam are comparable and results from Rome are better. Many factors may contribute to cognitive delay in CDH. Consistent with previous studies, low SES was a significant determinant of cognitive delay[19]. Previously reported predictive factors, such as tube

feeding at discharge and ECMO need[19], were not confirmed in our study. However, failure to reach statistical significance for ECMO need may have been due to the fact that 6 survivors needed ECMO treatment.

In general, impaired motor function is more frequent than cognitive problems in CDH. Our results on motor function were more favorable than those of Danzer et al., who reported mild (23%) and severe (31%) delay on motor function at 24 months[9]. Friedman et al.[3] reported motor problems in 60% at 1 year and 73% at 3 years. They retrospectively evaluated medical records documenting motor function. It is conceivable that standardized developmental assessment would have given other outcomes. On the other hand, Wynn et al.[19] found that motor function at 24 months was significantly lower than the population norm (mean 95; SD 11), which is comparable to Rome. Scores in Rotterdam, however, were on average 12 points lower. We could not confirm their results of adverse motor function in patients with low SES, but could confirm the result of another study[4] that LoS was associated with poorer motor function.

The strengths of our study were that children were treated according to a standardized protocol[1] and followed in a prospective, longitudinal, standardized follow-up program. We collected data on ethnicity and SES, which are well-known modifiers of neurodevelopmental outcome[19].

Neurodevelopmental outcomes were different between the centers, and changes between the two measurement moments were only seen in Rome. We suggest three possible reasons. The first is the use of different assessment instruments. Since recent studies reported that BSID-II and BSID-III are not comparable[20], we refrained from pooling the data. In the transition from BSID-II to BSID-III, 23 items from the BSID-II mental scale were moved to the BSID-III fine motor scale. Thus, with a higher proportion of items in the fine motor function domain in BSID-III compared to BSID-II (48 vs 28%, respectively) the fine motor score contributes relatively much to the total motor composite score in BSID-III. This is reflected by the fact that in Rome gross motor scores are lower than fine motor scores at both ages. We assume that the outcome for patients who have a developmental delay in both cognition and motor function will not be very different for the two BSID versions. However, results may differ for patients with CDH, who will typically show impaired motor function. Moreover, recent studies showed differences in reference scores of BSID-III between populations[21,22]. These considerations possibly explain the underestimation of neurodevelopmental delay in Rome. We have few standardized neurodevelopmental tests with population-matched reference data and the best option seems to compare neurodevelopmental outcome with healthy reference populations in each country. The use of American reference data in Rome might also have caused bias. In another study, neurodevelopmental outcomes were not different from those of

healthy matched controls aged 12 and 36 months[10]. Disease-determining parameters were not reported, which precludes comparison with our study.

Second, professional background may have played a role. In Rotterdam, motor function at 24 months was assessed by a pediatric physical therapist, whereas in Rome assessments were performed by developmental psychologists. Since results of our study did not significantly differ from the PDI assessed by developmental psychologists in a previous study of our group[11], we assume that this may not fully explain differences in motor function. Moreover, this contradicts our assumption that neurodevelopmental outcome has worsened due to survival of severely ill patients[2].

Third, center-specific differences may have played a role. Although both tertiary intensive care centers have a comparable referral area, patients from Rotterdam may have been more critically ill than those from Rome (higher proportion of large diaphragmatic defects and higher patch use). However, SNAP-II scores and observed to expected lung-to-head ratio were not significantly different. The surgical approach differed, too. Primarily, a thoracoscopic approach was performed in more than 50% in Rotterdam versus open surgery in all patients in Rome. A randomized controlled trial of open versus thoracoscopic repair in CDH concluded that thoracoscopic repair was associated with more prolonged and severe intraoperative hypercapnia and acidosis than open surgery[23]. Moreover, only in Rotterdam was ECMO available. Since only 6 survivors received ECMO and patient characteristics did not significantly change after exclusion of these children, we assume that this hardly contributes to the outcome differences. The influence of center-specific differences such as surgical care on neurodevelopmental outcome remains speculative, but these observations emphasize the need for multicenter studies with standardized protocols. Since scores in Rotterdam slowly deteriorate at 24 months, and it is known that early adverse development in children with major congenital anomalies is predictive of development at 5 years[5], follow-up was planned for patients from Rotterdam.

Next to the differences in study design, the fact that not every child was tested at both time points could be considered a limitation. However, the numbers of drop-outs were relatively low and the use of linear mixed models in the analysis over time accounted for missing data, provided that these outcomes were missing at random[24].

Interpreting outcomes in the context of international multicenter studies is difficult if the follow-up program is not standardized. International guidelines on standardization of follow-up programs in CDH, however, are still lacking. So far, the international CDH registry[25] collects only prenatal, perinatal and early postnatal data. We recommend setting up standardized follow-up programs using population-appropriate reference data and similar assessment instruments as well as assessments performed by profes-

sionals of the same background. In multicenter collaboration, video assessments should be included in the training sessions. We like to make an appeal for not only international standardization of postnatal care, but also for follow-up care beyond the neonatal period, as this is essential to improve outcome for CDH patients. This would require standardization of treatment protocols and decision flowcharts of referring criteria to pediatric physical therapists/ speech-language pathologists should be involved. Moreover, children should be followed up to adolescence since deficits may worsen.

In conclusion, although most CDH patients have normal neurodevelopment within the first 2 years of life, they are at risk for impaired motor function. Standardization of multicenter long-term follow-up programs using standardized assessment instruments and stratification to illness severity is necessary to compare neurodevelopmental outcomes between centers and to evaluate long-term effects of interventions.

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Supplementary Table 1a – Background characteristics (selection of no ECMO treated patients)

	Rotterdam (n=43)	Rome (n=39)	*p-value*
Perinatal and postnatal			
LHR	2.0 (0.9)	2.2 (0.8)	0.52
Missing	13	13	
O/E LHR	52.5 (17.9)	53.3 (15.8)	0.88
Missing	18	13	
Male sex	24 (55.8%)	25 (64.1%)	0.45
Birth weight (kg)	3.03 (0.58)	2.99 (0.41)	0.69
Born before 37 weeks of gestation	7 (16.3%)	4 (10.3%)	0.42
SNAP-II score	18 (12)	13 (9)	0.09
Age at repair (days)	3.5 (2.5)	3.6 (2.1)	0.77
Left sided CDH	39 (90.7%)	38 (97.4%)	0.20
Liver: intrathoracic	14 (32.6%)	12 (30.8%)	0.86
Defect size			0.57
A	5 (11.6%)	4 (10.3%)	
B	18 (41.9%)	22 (56.4%)	
C	18 (41.9%)	11 (28.2%)	
D	2 (4.7%)	2 (5.1%)	
Patch repair	29 (67.4%)	11 (28.2%)	<0.001
Defect size A	n=1	n=0	
Defect size B	n=8	n=1	
Defect size C	n=18	n=8	
Defect size D	n=2	n=2	
Initial ventilation			0.68
CMV	24 (55.8%)	20 (51.3%)	
HFO	19 (44.2%)	19 (48.7%)	
Length of ventilation (days)	7 (1- 177)	8 (2- 70)	0.10
Length of initial hospital stay (days)	21 (6- 387)	30 (15-161)	0.11
CLD			0.21
No CLD	29 (67.4%)	32 (82.1%)	
Mild CLD	8 (18.6%)	2 (5.1%)	
Moderate CLD	1 (2.3%)	0 (0%)	
Severe CLD	5 (11.6%)	5 (12.8%)	
Length of morphinomimetics/ sedatives			0.10
< 1week	25 (58.1%)	14 (35.9%)	
1 week- 1 month	16 (37.2%)	20 (51.3%)	
> 1 month	2 (4.7%)	5 (12.8%)	
Episodes of general anesthesia 0-24 months	1 (1- 12)	1 (1- 3)	0.09

Supplementary Table 1a – (continued)

	Rotterdam (n=43)	Rome (n=39)	*p-value*
At discharge			
Tube feeding	23 (53.5%)	20 (51.3%)	0.84
Physical therapy at home	14 (32.6%)	7 (17.9%)	0.13
Speech language pathologist involved	14 (32.6%)	3 (7.7%)	0.006
Demographic variables			
Ethnicity			0.15
Native	30 (69.8%)	33 (84.6%)	
Non-native	12 (27.9%)	6 (15.4%)	
Unknown	1 (2.3%)	0 (0%)	
SES mother			0.17
Low	8 (18.6%)	9 (23.1%)	
Medium	15 (34.9%)	21 (53.8%)	
High	17 (39.5%)	9 (23.1%)	
Unknown	3 (7.0%)	0 (0%)	

* Comparison between Rotterdam and Rome. Categorical variables are shown as n (%) and compared between centers between centers using chi-square tests, normally distributed variables are shown as mean (SD) and compared between groups using independent samples t-tests, and continuous variables that are not normally distributed are shown as median (range) and compared between centers using Mann-Whitney U tests. **Abbreviations:** LHR: lung to head ratio. O/E LHR: observed to expected lung to head ratio. SNAP-II score: Score for Neonatal Acute Physiology-II[14]. CDH: congenital diaphragmatic hernia. CMV: conventional mechanical ventilation. HFO: high frequency oscillation. CLD: chronic lung disease[15].

Supplementary Table 1b – Episodes of general anesthesia 0-24 months and associated anomalies

	Rotterdam n=49	**Rome n=39**
EPISODES OF GENERAL ANESTHESIA 0-24 MONTHS		
CDH repair		
Primary	49	49
Recurrence	16	3
ECMO related		
ECMO cannulation*	7	0
ECMO cannulation removal*	7	0
Other ECMO related procedures	1	0
Ventilation related		
Tracheostomy	5	0
Laryngeal tracheal cleft operation	0	1
Gastro-intestinal related		
Laparotomy (including 2nd and 3rd looks)	9	5
Nissen fundoplication	4	2

Supplementary Table 1b – (continued)

	Rotterdam n=49	Rome n=39
Gastrostomy	2	1 (during a Nissen)
Ileostomy or colostomy closure	2	0
Esophageal dilatation after Nissen fundoplication	1	0
Laparoscopy because of ileus	1	0
Posterior sagittal anorectoplasty	1	0
Anal dilatation	1	0
Diagnostic procedures		
Laryngotracheoscopy	5	0
Heart catheterization	4	0
Cystoscopy	3	0
Urodynamic investigation	2	0
Change of tracheal cannula	1	0
Bronchoscopy	1	0
Other		
Central venous line/ Broviac catheter placement	9	0
Orchidopexy/ orchidectomy	4	3
Hernia inguinal repair	3	0
Hypospadia	1	0
Bladder exstrophy procedure	0	1
Incisional hernia repair	1	0
Adenotonsillectomy	1	0
Surgical treatment of abscess	1	0
*1 patient had a second ECMO run.		
ASSOCIATED ANOMALIES		
Cardiopulmonary related		
Partial Anomalous Pulmonary Venous Return and ASD	1	0
Very mild aortic bow hypoplasia	0	1
Very mild hypoplasia left pulmonary artery	0	1
Pulmonary sequester left basal	0	2
Atrial septal defect	0	2
Mild hydronephrosis (also partial ASD)	1	0
Urogenital related		
Double system left kidney	1	0
Bladder hypotrophy, hypospadias, laryngeal cleft	0	1
Other		
Very mild epidermolysis bullosa	0	1
Anorectal malformation, bifid scrotum	1	0

Supplementary Table 1b – (continued)

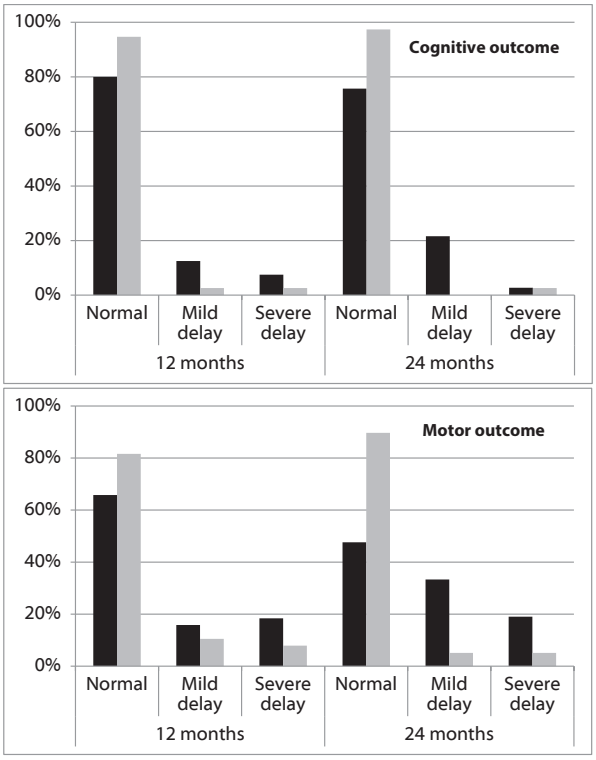
	Rotterdam n=49	Rome n=39
Generalized brain atrophy	1	0
Stroke left hemisphere	1	0
GENETIC/ CHROMOSOMAL ANOMALIES		
Duplications/ deletions		
Duplication in 1p21.2, duplication in 8q11.23 and deletion 2q37.3	1	0
Duplication in 15q11.2q13.1	1	0
Duplication in 10q26.13	1	0
Duplication in 2p13.1 and 8p11.21p11	1	0
Deletion in 16p13.3 and duplication 12p12.1	1	0
Deletion in 13q12.11	1	0

Supplementary Table 2a – Neurodevelopmental outcome scores at 12 and 24 months (corrected for prematurity) in no-ECMO treated patients

	12 months				24 months			
	Rotterdam (n=***)	p-value[a]	Rome (n=38)	p-value[a]	Rotterdam (n=****)	p-value[a]	Rome (n=39)	p-value[a]
Cognitive outcome*	99.7 (19.0)	0.91	97.9 (11.8)	0.28	98.4 (17.0)	0.57	102.1 (13.9)	0.36
Motor outcome*	89.0 (17.7)	<0.001	93.2 (12.2)	0.002	83.7 (16.3)	<0.001	98.2 (14.8)	0.45
Language outcome*	–	–	97.7 (8.6)	0.10	–	–	97.7 (12.6)	0.26
Receptive language**	–	–	9.4 (1.7)	0.04	–	–	10.2 (2.4)	0.60
Expressive language**	–	–	9.8 (1.9)	0.51	–	–	8.9 (2.5)	0.01
Fine motor skills**	–	–	10.1 (2.1)	0.76	–	–	10.5 (2.6)	0.23
Gross motor skills**	–	–	7.7 (2.5)	<0.001	–	–	8.9 (2.7)	0.01

[a] Outcome scores were compared to the expected normal score of reference population.
Rotterdam: BSID-II-NL. Rome: BSID-III.
* Mean (SD) of expected normal score of reference population is 100 (15).
** Mean (SD) of expected normal score of reference population is 10 (3).
*** Cognitive outcome: n=40. Motor outcome: n=38.
**** Cognitive outcome: n=37. Motor outcome: n=42.

Supplementary Figure 2a – Selection of no-ECMO treated patients
Neurodevelopmental outcome in CDH survivors at 12 and 24 months



Black: Rotterdam; Grey: Rome.

Supplementary Table 3a – Selection of no-ECMO treated patients
Determinants of impaired neurodevelopmental outcome; results of linear regression models

		Rotterdam		Rome	
	Independent variables	**Parameter estimate**	***95% CI***	**Parameter estimate**	***95% CI***
Cognition at 12 months	SNAP-II score	0.7	**0.1 to 1.3**	0.1	−0.0 to 0.33
	Length of hospital stay (d)*	−8.3	−18.4 to 1.9	−13.7	**−21.2 to −6.2**
	Tube feeding at discharge	−2.8	−20.5 to 14.9	−1.9	−10.6 to 6.8
	Ethnicity	20.2	−1.3 to 41.7	1.5	−8.1 to 11.1
	SES mother				
	Low	−23.7	−47.5 to 0.2	1.9	−8.4 to 12.1
	Medium	−1.3	−20.7 to 18.2	1.2	−7.7 to 10.1
	High**	–	–	–	–
Cognition at 24 months	SNAP-II score	0.2	−0.3 to 0.6	0.0	−0.2 to 0.3
	Length of hospital stay (d)*	−4.7	−12.1 to 2.7	−10.2	**−20.0 to −0.4**
	Tube feeding at discharge	−4.8	−17.9 to 8.4	−4.1	−16.2 to 8.0
	Ethnicity	−24.9	**8.0 to 41.9**	−2.1	−15.5 to 11.4
	SES mother				
	Low	−37.5	**−56.9 to −18.1**	−7.0	−21.3 to 7.4
	Medium	−12.4	−26.9 to 2.0	−3.6	−16.0 to 8.7
	High**	–	–	–	–
Motor function at 12 months	SNAP-II score	0.8	**0.2 to 1.4**	0.0	−0.2 to 0.2
	Length of hospital stay (d)*	−8.2	−19.6 to 3.1	−9.5	**−17.7 to −1.4**
	Tube feeding at discharge	−3.2	−20.1 to 13.7	−6.6	−16.1 to 2.8
	Ethnicity	17.5	−4.7 to 39.6	3.6	−6.8 to 14.1
	SES mother				
	Low	−6.7	−29.7 to 16.2	−3.1	−14.2 to 8.1
	Medium	−1.7	−20.5 to 17.0	−2.4	−12.1 to 7.3
	High**	–	–	–	–
Motor function at 24 months	SNAP-II score	0.5	0.0 to 0.9	0.1	−0.2 to 0.3
	Length of hospital stay (d)*	−7.3	**−14.4 to −0.2**	−16.7	**−25.1 to −8.2**
	Tube feeding at discharge	1.8	−10.9 to 14.5	0.8	−9.7 to 11.3
	Ethnicity	7.7	−5.9 to 21.3	2.1	−9.5 to 13.7
	SES mother				
	Low	−17.5	**−32.9 to −2.2**	−0.2	−12.7 to 12.2
	Medium	−3.5	−16.6 to 9.6	5.5	−5.2 to 16.2
	High**	–	–	–	–

Abbreviations: ECMO: extracorporeal membrane oxygenation. CI: confidence interval. SNAP-II score: score for neonatal acute physiology II. SES: socio-economic status. *Because of skewed distribution, these variables were log-transformed before including them into the linear regression model. **Reference category.

Part V

Consideration

Chapter 12

Congenital diaphragmatic hernia; trends in survival, use of ECMO and FETO and international comparison of four high-volume centers

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ABSTRACT

Objective: We aimed to determine trends in survival over the last decade. Secondly, we compared patient populations and survival rates between four high-volume centres and investigated which factors were associated with survival.

Summary Background Data: Congenital diaphragmatic hernia (CDH) is a severe congenital anomaly with significant mortality.

Methods: In four high-volume CDH centres from the CDH EURO Consortium, data from all CDH patients born between 2004- 2013 were analysed. The predictive value of variables known at birth, and influence of centre specific treatments (ECMO and FETO) on survival were evaluated in multivariable logistic regression analyses.

Results: Nine hundred and seventy-five patients were included in the analysis; 274 patients (28.1%) died. ECMO was performed in 259 patients of whom 81 (31.3%) died. 145 patients (14.9%) underwent FETO and from those 76 patients (52.4%) survived. Survival differed significantly between years ($p=0.006$) and between the four centres ($p<0.001$). In the multivariable logistic regression analysis, lung-to-head ratio, gestational age, ECMO, centre of birth, and year of birth were significantly associated with survival, whereas FETO was not.

Conclusions: There is a significant variability in survival over years and between centres which should be taken into consideration in the planning of future trials. Patient populations are highly different between centres which influences outcome. ECMO was significantly predictive for death, whereas FETO was not.

INTRODUCTION

Congenital diaphragmatic hernia (CDH) is a severe congenital anomaly with a high variability of outcome[1]. High-volume CDH centres achieve better survival rates as compared to low-volume CDH centres[2]. Moreover patient characteristics such as fetal liver position (intra-abdominal or intrathoracic)[3], stomach position[4], and lung-to-head ratio (LHR)[5]/ observed-to expected LHR[6] (O/E LHR) and the diaphragmatic defect size[7] are associated with outcome.

There are differences in opinion about whether extracorporeal membrane oxygenation (ECMO) improves outcome as no specific trials have ever been conducted with the primary aim to evaluate the role of ECMO in the treatment specifically for high-risk CDH patients[8,9]. The UK ECMO trial has investigated the role of ECMO for neonates of whom only 19% of the included patients had CDH, and no significant difference in this subgroup on survival was found[10]. In a multicentre randomized clinical trial (RCT) of initial ventilation strategy, both centres with and without ECMO availability were included, and no difference in survival between those centres was observed[11]. In the trial, nine CDH centres were included with different CDH populations. Many CDH centres chose not to use ECMO because of poor outcome of infants requiring ECMO[12]. Therefore, an important question is how CDH populations vary between centres and how that influences outcome. Moreover in the last decade individual centres have reported their very high survival rates up to 95% for cases with late presentation but no long-term reports on the consistency of these numbers are available in the literature[13].

In the most severe prenatally detected CDH cases, fetoscopic endotracheal occlusion (FETO) may improve outcome[14,15]. Ruano et al. recently performed a RCT of FETO versus postnatal management and found in 20 severe CDH patients that survival was significantly better in the FETO group[16]. In the TOTAL trial (NCT01240057), whether FETO improves outcomes in severe and moderate CDH infants is being assessed[17].

In four high-volume CDH centres with different treatment options and patient populations, we aimed to identify survival rates over the last decade. Secondly, we compared patient populations between four high-volume centres and investigated whether ECMO or FETO are associated with survival.

METHODS

This is an observational cohort study in patients with CDH who were born between January 2004 and December 2013 and treated in four high-volume centres of the CDH EURO Consortium. The four centres were Rotterdam, London, Mannheim, and Rome. Since 2008, all patients have been treated according to a standardized treatment protocol[18],

although ECMO was only used routinely in some centres. In Rotterdam and Mannheim, ECMO therapy was available during the whole inclusion period, in Rome ECMO was available in 2013 only, and in London infants could be transferred to an ECMO centre.

FETO therapy was only offered within the context of research trials (NCT01240057) from 2010 onwards and before 2010 as compassionate use. Inclusion criteria for FETO are: isolated left-sided CDH and severe pulmonary hypoplasia defined as observed-to-expected LHR <25% irrespective of the liver position as measured prior to 29 weeks+ 6 days at the FETO centre. ECMO criteria are: inability to maintain preductal saturations >85% or postductal saturations >70%; increased $PaCO_2$ and respiratory acidosis with pH <7.15 despite optimization of ventilatory management; peak inspiratory pressure >28 cm H_2O or mean airway pressure >17 cm H_2O is required to achieve saturation >85%; inadequate oxygen delivery with metabolic acidosis as measured by elevated lactate >5 mmol/l and pH <7.15; systemic hypotension, resistant to fluid and inotropic therapy, resulting in urine output <0.5 ml/kg/h for at least 12–24 h; oxygenation index (mean airway pressure x FiO_2 x 100/PaO_2) ≥40 consistently present. Before 2008, in Mannheim, above mentioned ECMO criteria were different on oxygenation index (>35 for 0.5- 6 hours) and pH (<7.25) or worse oxygenation or worse ventilation without strict limits.

Patient characteristics were retrieved from the medical records. Patient demographics, including prenatal diagnosis, LHR, FETO, gestational age, birth weight, gender, side of the defect, liver position (intrathoracic or intra-abdominal determined during surgical repair), type of repair (primary closure or patch repair), age at surgical repair, ECMO, ventilation days in survivors, inhaled nitric oxide (iNO) and survival were collected. Survival during the first year of life was determined.

Statistical analysis

Patient characteristics are described as number (%) or median (interquartile range; IQR). To determine whether differences in the demographics of the four centres were statistically significant, chi-square tests for categorical data, or Kruskal-Wallis tests for continuous data were used. Mann-Whitney U tests for continuous data and chi-square tests for categorical data were applied to compare centre of birth and patient characteristics that were known at birth between survivors and non-survivors. In these univariate comparisons, year of birth was treated as a categorical variable. Independent associations between prenatal diagnosis, LHR, FETO, gestational age, gender, side of the defect, ECMO, centre, and year of birth (coded as a continuous variable) as independent variables and survival were determined using multivariable logistic regression analysis. The goodness-of-fit of the logistic regression model was assessed using the Hosmer-Lemeshow test. Analyses were performed using SPSS 22.0 for Windows (SPSS, Inc., Chicago, IL). A two-sided p-value less than 0.05 was considered statistically significant.

RESULTS

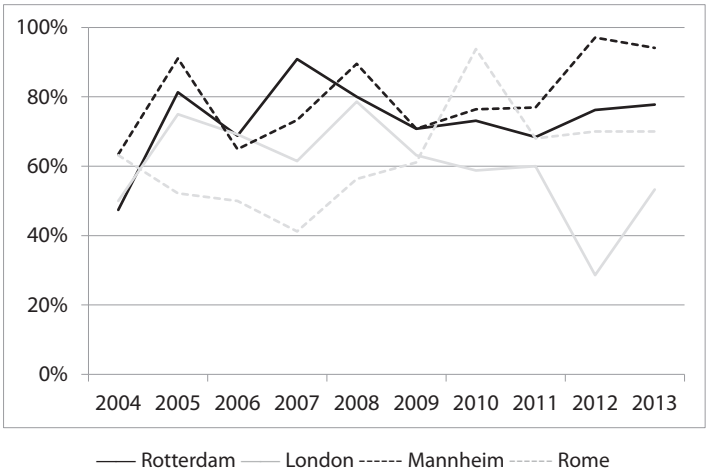
In the study period, 975 CDH patients were included. Overall, 274 patients (28.1%) patients died. A prenatal diagnosis was made in 820 (84.1%) patients. Prenatal diagnosis, LHR, FETO, gestational age, birth weight, gender, liver position at surgical repair, type of repair, age at surgical repair, ECMO, ventilation days in survivors, iNO and survival were significantly different between the four centres (Table 1). FETO was performed in 145 patients.

Table 1 – Background characteristics 2004-2013

	Rotterdam n= 195	London n= 127	Mannheim n= 469	Rome n= 184	*p*-value
Prenatal diagnosis	139 (71.3%)	125 (98.4%)	407 (86.8%)	149 (81.0%)	<0.001
LHR	1.47 (1.00- 1.99)	1.65 (1.33- 2.20)	1.60 (1.25- 1.99)	1.82 (1.44- 2.55)	<0.001
FETO	9 (4.6%)	84 (66.1%)	48 (10.2%)	4 (2.2%)	<0.001
Missing	0 (0%)	0 (0%)	108 (23.0%)	0 (0%)	
Gestational age (weeks)	38.3 (37.1- 39.1)	35.7 (33.7- 38.6)	37.6 (36.1- 38.4)	38.0 (37.0- 39.0)	<0.001
Birth weight (grams)	3000 (2598- 3239)	3200 (2345- 3600)	2870 (2480- 3210)	2930 (2600- 3230)	0.004
Male gender	113 (57.9%)	58 (45.7%)	274 (58.4%)	112 (60.9%)	0.04
Side of the defect					0.11
Left	164 (84.1%)	115 (90.6%)	381 (81.2%)	151 (82.1%)	
Right	31 (15.9%)	12 (9.4%)	83 (17.7%)	30 (16.3%)	
Bilateral	0 (0%)	0 (0%)	3 (0.6%)	3 (1.6%)	
Missing	0 (0%)	0 (0%)	2 (0.4%)	0 (0%)	
Liver position: intrathoracic	70 (40.2%)	48 (54.5%)	237 (58.5%)	48 (36.9%)	<0.001
Type of repair					<0.001
Primary closure	42 (21.5%)	38 (43.2%)	88 (18.8%)	89 (48.4%)	
Patch repair	132 (67.7%)	50 (56.8%)	306 (65.2%)	41 (22.3%)	
No repair	21 (10.8%)	39 (30.7%)	64 (13.6%)	54 (29.3%)	
Missing	0 (0%)	0 (0%)	11 (2.3%)	0 (0%)	
Age at surgical repair (days)	4.0 (3.0- 6.0)	5.0 (3.0- 7.0)	6.0 (3.0- 12.0)	3.0 (2.0 - 4.0)	<0.001
ECMO	62 (31.8%)	0 (0%)	196 (41.8%)	1 (0.5%)	0.01
Ventilation days in survivors	9.8 (6.0- 20.1)	13.0 (9.0- 18.0)	21.3 (11.0- 30.1)	9.0 (6.0- 15.0)	<0.001
iNO	105 (53.8%)	68 (53.5%)	266 (56.7%)	79 (43.2%)	<0.001
Survival	142 (72.8%)	75 (59.1%)	370 (78.9%)	114 (62.0%)	<0.001

Abbreviations: LHR: lung-to-head ratio; FETO: fetoscopic tracheal occlusion; ECMO: extracorporeal membrane oxygenation; iNO: inhaled nitric oxide. Liver position was determined at surgical repair.
Data were presented as n (%), median (interquartile range).

Figure 1 – Survival of CDH by centre over the years



Overall, we found a significant difference in survival over the years (p=0.006) (Figure 1). In Mannheim, 196 patients (41.8%) received ECMO and 153 (78.1%) of the ECMO-treated patients survived. In Rotterdam 62 patients (31.8%) received ECMO and 25 (40.3%) of the ECMO-treated patients survived. ECMO treated patients in Rotterdam had lower LHRs and more often a patch repair was performed compared to the ECMO treated patients in Mannheim. In Rome, ECMO was available from 2013 onwards, and in 2013 one patient received ECMO who died. None of the patients from London received ECMO. ECMO use between survivors and non-survivors was not statistically significant. FETO was significantly more often used in non-survivors (25%) than in patients who survived (13%). Furthermore, survivors had significantly less often a prenatal diagnosis, survivors had significantly higher LHRs and gestational ages, and more often left-sided defects (Table 2).

In the multivariable logistic regression analysis, we found that lower LHR, lower gestational age, ECMO, centre of birth and year of birth were significantly associated with death (Table 3). FETO was not significantly associated with death. The p-value of the Hosmer-Lemeshow test was larger than 0.05, indicating an adequate model calibration.

Table 2 – Background characteristics for survivors and non-survivors

	Survivors n=701	Non-survivors n=274	*p*-value
Prenatal diagnosis	570 (81.4%)	250 (85.0%)	<0.001
LHR	1.73 (1.40- 2.20)	1.35 (1.00- 1.73)	<0.001
FETO	76 (12.5%)	69 (25.2%)	<0.001
Missing	92 (13.1%)	16 (5.8%)	
Gestational age (weeks)	38.0 (37.0- 38.9)	37.0 (35.0- 38.0)	<0.001
Gender			0.50
Male	405 (57.8%)	152 (55.5%)	
Female	295 (42.1%)	122 (44.5%)	
Side of the defect			0.02
Left	595 (84.9%)	216 (78.8%)	
Right	103 (14.7%)	53 (19.3%)	
Bilateral	2 (0.3%)	4 (1.5%)	
Missing	1 (0.1%)	1 (0.4%)	
ECMO	178 (25.4%)	81 (29.6%)	0.20
Centre			<0.001
Rotterdam	142 (20.3%)	53 (19.3%)	
London	75 (10.7%)	52 (19.0%)	
Mannheim	370 (52.9%)	99 (36.1%)	
Rome	114 (16.3%)	70 (25.5%)	
Year of birth			0.01
2004	51 (58.6%)	36 (41.4%)	
2005	69 (78.4%)	19 (21.6%)	
2006	67 (63.2%)	39 (36.8%)	
2007	58 (67.4%)	28 (32.6%)	
2008	91 (81.2%)	21 (18.8%)	
2009	74 (67.9%)	35 (32.1%)	
2010	86 (75.4%)	28 (24.6%)	
2011	76 (71.7%)	30 (28.3%)	
2012	68 (75.6%)	22 (24.4%)	
2013	61 (79.2%)	16 (20.8%)	

Data are presented as n (%), or median (interquartile range).

Abbreviations: LHR: lung-to-head ratio; FETO: fetoscopic tracheal occlusion; ECMO: extracorporeal membrane oxygenation.

Numbers do not always add up to the total number of the group because of missing data.

Table 3 – Multivariable logistic regression analysis

Variable	OR	95% CI	*p*-value
LHR	4.30	2.98- 6.21	<0.001
FETO	0.67	0.36- 1.26	0.21
Gestational age	1.22	1.10- 1.35	<0.001
Gender	0.87	0.59- 1.29	0.87
Side			0.39
Left	Ref		
Bilateral	0.32	0.04-2.68	0.29
Right	1.27	0.73-2.20	0.40
ECMO	0.49	0.30- 0.81	0.005
Centre			<0.001
London	Ref		
Rotterdam	1.70	0.73- 3.95	0.22
Rome	0.35	0.15- 0.79	0.01
Mannheim	3.39	1.61- 7.14	0.001
Year of birth	1.09	1.01- 1.17	0.03

Abbreviations: LHR: lung-to-head ratio; FETO; fetoscopic tracheal occlusion; ECMO: extracorporeal membrane oxygenation; OR: odds ratio; 95% CI: 95% confidence interval; Ref: reference category.

DISCUSSION

In this study of prospectively collected data, we showed that there is a variability in survival between the centres and years. Secondly, patient populations were highly different between high-volume centres and this influenced outcome. ECMO was significantly related to reduced survival, whereas FETO did not reach statistical significance in the multivariable analysis.

The survival rate of CDH has significantly increased in recent decades[19]. Where 50 years ago fewer than 50% of the patients survived[20], nowadays about 75% of the patients survive[21]. Whereas in the 1980s significant improvements in survival were obtained by the introduction of delayed surgical repair[22] and the 'gentle ventilation strategy' with permissive hypercapnia[23], nowadays smaller improvement of survival rates may be reached by standardization of therapy and improvements such as medication for pulmonary hypertension[24]. The survival rate was very different each year (Figure 1), though these differences can be partly attributed to sampling variance. The variation in survival rate demonstrated over the years emphasizes the importance of a sufficient inclusion period in future studies to prevent that the natural variability is considered a positive trial effect.

In the univariable analysis, we did not find a significant difference in ECMO use between survivors and non-survivors. In the multivariable analysis with correction for

patient characteristics, however, we found that ECMO was significantly related to death. This may be explained by the fact that only the most severe CDH cases receive ECMO who subsequently die in approximately 50%. Therefore, the frequency of ECMO use is almost equally divided between survivors and non-survivors, thus no significant differences were expected in the univariable analysis. In the multivariable analysis, however, with correction for other patient characteristics, ECMO had a relatively large impact on survival.

The frequency of ECMO was very different between centres. In Mannheim 42% of the patients received ECMO and 78% of them survived, whereas in Rotterdam 32% of the patients received ECMO and only 41% of them survived. It may be that in Rotterdam in the most severe cases only, an ECMO procedure was initiated, whereas in Mannheim less severe CDH patients received ECMO, who would also have survived without ECMO. This is also supported by the fact that ECMO treated patients in Rotterdam had lower LHRs and more often a patch repair was performed compared to the ECMO treated patients in Mannheim. Moreover, despite standardized ECMO criteria including an oxygenation index of ≥ 40[18] from 2008 onwards in both centres, differences in ECMO criteria before 2008 are that in Rotterdam a slightly higher oxygenation index and lower pH were used as compared to Mannheim. In this retrospective study, most often the reason for initiation of ECMO in Mannheim was based on worse oxygenation or worse ventilation without documenting exact numbers. We can therefore doubt whether decisions to start ECMO therapy were strictly based on firm ECMO criteria[18] in both centres. To identify for which subgroup of CDH patients would be most beneficial, predictive postnatal clinical models such as the SNAP-II score[25] or the clinical prediction score by Brindle et al.[26] may have additional value. Therefore future studies should focus on this subject.

The percentage of FETO use in the different centres was very different, and by far the highest percentage of FETO treated patients was observed in London, which is a FETO referral centre. FETO was significantly more often used in non-survivors than in patients who survived. However, in the multivariable analysis FETO was not significantly related to survival demonstrating FETO improves survival in high-risk fetuses. A recent randomized trial performed by Ruano et al. found improved survival in the 20 patients treated with FETO[16], however the survival rate of 5% in the control group of 21 patients was extremely low. The TOTAL trial[17] may give a definitive answer to the benefit of FETO for patients with severe CDH.

Patient characteristics were very different between the four centres. Despite correction for patient characteristics in the multivariable analysis, we still found that centre significantly influenced survival. This emphasizes the need for correction for centre in all analyses in future multicentre studies on CDH. High-volume CDH centres have more experience in treating CDH infants than low-volume centres thus outcomes between high-volume CDH centres may be more comparable. Strengths of the current study

were the relatively large sample size (n=975) over a long period of time in four different high-volume centres. Our study is limited by the fact that although treatment strategies in general remained constant during the study period after 2008, we cannot rule out the possibility that differences of treating physicians, nursing staff and training may also have influenced our results. Second, although it is well known that diaphragmatic defect size and O/E LHR are associated with outcome[6,7], because of the start of the inclusion period in 2004, defect size was not documented for all patients. We think that analyses in future studies should also be corrected for this parameter[7,21]. However, since patch repair is more often performed in CDH patients with larger defect sizes, and patch repair was one of the collected parameters, an indication of defect size is provided.

In conclusion, variations in survival with differences in patient populations and over time between centres suggest that in future multicentre studies outcomes should be evaluated over a sufficient period of time and corrected for centre. Moreover, ECMO was significantly related to a decreased survival rate, whereas FETO was not.

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Part VI

Discussion and summary

Chapter 13

General discussion

The research described in this thesis concerned a wide spectrum of clinical care issues after the introduction of standardized postnatal management of infants with congenital diaphragmatic hernia (CDH) in Europe[1]. Most studies were performed in a multicentre context: the CDH EURO Consortium, which is a collaboration between tertiary European centres with an expertise in CDH and the willingness to adhere to guidelines and conduct clinical studies to enhance the level of care in patients with CDH. In fact, for years the therapy is at best trial and error due to the lack of properly conducted studies. The number of participating centres has increased from 13 to 22 specialized CDH centres from all over Europe. Additional insight in ventilation strategies for CDH was obtained in the VICI-trial, a multicentre randomized clinical trial to identify the best initial ventilation strategy.

PREDICTION

Clinical prediction models

The high variability in severity of illness in CDH means that outcomes can differ widely among patients. Both parents and clinicians would like to have optimal insight in the prognosis of the individual patient as soon as possible. Ideally, the anomaly is detected early during gestation so that a more detailed expert evaluation can be performed to determine the location of the defect, the observed/expected lung-to-head ratio (O/E LHR), the position of the liver (intra-abdominal or intrathoracic), in addition to ruling out additional congenital anomalies and genetic syndromes[2-5]. Multidisciplinary prenatal counselling on the basis of the prognosis can then be offered to the parents. It contributes to guide them on decisions such as termination of pregnancy or foetal therapy. The role of fetal therapy for severe and moderate pulmonary hypoplasia in CDH is being investigated in the international randomized Tracheal Occlusion To Accelerate Lung growth (TOTAL) trial (NCT0240057)[6].

We found that prenatal ultrasound lung measurements (observed-to-expected lung-to-head ratio (O/E LHR) can reliably predict survival and development of chronic lung disease in CDH survivors with a left-sided diaphragmatic defect. This is in line with studies from Jani and colleagues[3,7,8]. One of the strengths of our study is the two-centre, nationwide design in which postnatal neonatal treatment was standardized. Second, one single investigator performed all prenatal measurements. To study inter-observer variability of the O/E LHR measurements, a set of prenatal ultrasound measurements should be evaluated by several experienced investigators and their measurements should be compared.

The lung area in our study was measured by manual tracing of the limits of the lung (mm^2). This measurement together with the head circumference were used to calculate

the O/E LHR. Other techniques to calculate the O/E LHR are the method by multiplying the two longest perpendicular diameters, or by multiplying the anteroposterior diameter by the perpendicular diameter located at the midpoint. Britto et al. concluded that standardization of each of the measurements improved prediction of neonatal outcomes[9]. Therefore, a lack of standardization of prenatal lung measurements may lead to variations in the measurements and hence in accuracy of prediction.

Postnatally, the Score for Neonatal Acute Physiology-II (SNAP-II score) can be calculated after the first 12 hours of life. This clinical prediction score was initially developed for prematurely born neonates[10], but previous retrospective and single-centre studies[11-13] found that it had predictive value in neonates with CDH as well. We confirmed this in a large multicentre cohort of prenatally diagnosed CDH patients with regard to survival and the need for ECMO support (chapter 3). A new finding is that the SNAP-II score after adjustment for several parameters including ECMO did not predict the development of BPD in surviving CDH infants. Since the SNAP-II score is based on physiological parameters during the first 12 hours of life and the development of BPD is also dependent on ventilator-induced lung injury, the time point of 12 hours after birth might be too early to predict BPD in survivors.

Apart from having a reliable predictive value, a prediction score should be quick and easy to calculate. Since it takes only two to four minutes to collect data of the six scoring items, the SNAP-II score meets this criterion. A prediction model like that of Brindle et al[14] includes the presence of major cardiac anomaly, severe pulmonary hypertension and chromosomal anomaly, parameters for which several investigations such as genetic testing and echocardiography are needed. The results of these investigations are probably not yet known at 12 hours of life. Still, the combined results of both prediction models may well lead to a better prediction for CDH patients, which is an issue for new prospective studies. However, a perfect prediction of prognosis for the individual patient cannot be obtained. Clinical prediction scores such as SNAP-II cannot be used as a sole criterion for clinical decision making, such as initiation of ECMO. They nevertheless could be used for risk stratification.

Biomarkers

A biomarker is defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic response to therapeutic intervention"[15]. An ideal biomarker should be easy to use, consistent in repetitive measurements with a high sensitivity and specificity for early detecting, and have cut-offs to allow for risk stratification in the individual patient. In the design of the VICI-trial, blood, urine and tracheal aspirate samples were collected at predetermined time points (days 1, 3, 7, 14 and 28). We were confronted with an organizational issue during this study in that the day 1 sample could be taken within the

time span of immediately after delivery until 24 hours after birth. The "baseline" level of a biomarker could therefore have been influenced by the timing of the measurement in relation to therapeutic interventions. Collection of the first sample as soon as possible after birth, for example from arterial umbilical cord blood may overcome this problem. In clinical practice, however, it may be difficult to achieve this when it is not clear who is responsible for sample collection since many clinicians are involved in clinical care. The result may be a large number of missing values. Dedicated researchers are crucial in such studies.

For the two biomarkers investigated (NT-proBNP and hs-Troponin T), reference ranges are available for infants, which vary widely, however, also depending on the assay used[16-18]. Therefore, data of CDH infants often fall in the normal range. In the individual patient it may be more useful to measure biomarkers over a period of time and relate the course to severity of illness in this period. For example, an increase or decrease of biomarker level after start of an intervention might provide information for the predictive effect on the outcome of that intervention. A combination of biomarkers, such as endothelin-1[19], vascular endothelial growth factor A (VEGF-A)[20] and circulating microparticles from damaged endothelial cells ($CD62e^{+}$)[21,22] might have better predictive value than a single biomarker in patients with pulmonary arterial hypertension and CDH. Non-invasive biomarkers, such as biomarkers that can be measured in exhaled breath or urine, would be preferable. Since urinary F2-isoprostance was independently associated with mortality in adult patients with PAH[23] and fractional exhaled nitric oxide is shown to rise in concentration in response to therapy[24], these biomarkers might also be useful in CDH patients.

TREATMENT

The most important milestones in the treatment of CDH are the introduction of 'delayed surgical repair'[25] and 'gentle ventilation with permissive hypercapnia'[26]. Cartlidge et al. performed a study in 33 CDH patients who were treated by either early surgery or delayed surgery after preoperative stabilisation. The preoperative stabilisation was aimed at correcting acidosis and hypoxia, thereby reducing the severity of foetal circulation. Survival improved from 13% after early surgery to 53% after delayed surgery[25]. Wung et al found that permissive hypercapnia was successful in 15 infants suffering from persistence of foetal pulmonary circulation and presenting with severe respiratory failure. All infants survived and only one of them developed chronic lung disease[26]. Ventilation was focussed on minimizing barotrauma and $PaCO_2$ was not used as a controlling parameter. The most challenging aspects in the treatment of the individual patient with CDH nowadays are pulmonary hypertension and lung-related problems. Severe lung hypoplasia,

pulmonary hypertension and ventilator-induced lung injury are the most important risk factors of poor outcome. Ventilator-induced lung injury may lead to long-term pulmonary dysfunction including BPD and chronic pulmonary hypertension[27].

The primary outcome measure in the VICI-trial was 'mortality or development of BPD by day 28'. BPD was defined according to the definition of Jobe and Bancalari (oxygen dependency at day 28[28]). It can be debated, however, that the term chronic lung disease is more appropriate in CDH infants. During early lung development, terminal airspaces are formed, which are divided by the process of secondary septation[29]. This progressively generates an increasing number of alveoli with smaller size, and thereby substantially increases the surface area over which gas exchange takes place. Arrest of alveolarization may lead to BPD. In preterm born neonates, BPD is characterized by a heterogeneous pattern of persistent airway inflammation, parenchymal fibrosis, oedema and abnormal pulmonary vascular development[30]. In CDH patients, not arrested alveolarization, but rather a structural defect in early foetal development causes pulmonary hypoplasia and maldevelopment of the lungs. Pulmonary hypoplasia in CDH is characterized by thickened alveolar walls, increased interstitial tissue, reduced alveolar air space and reduced gas-exchange surface area[31]. In contrast to the lungs of prematurely born neonates, lungs of foetuses with CDH are not surfactant deficient[32], and surfactant replacement therapy has no beneficial effect in term neonates with CDH[33]. Even in prematurely born neonates with CDH, surfactant replacement therapy did not improve survival rates[34]. Therefore, although in the VICI-trial BPD was used, the term chronic lung disease may be more appropriate to use in future studies of CDH patients.

Ventilation

Because of the abnormal lung development, ventilation remains a challenge in CDH infants. Ventilator-induced lung injury must be prevented so as to reduce the risk of pulmonary morbidity including BPD[35]. A prospective evaluation of lung function in CDH survivors found decreased expiratory flows and higher functional residual capacity within the first year of life, the latter of which was associated with longer duration of ventilation and higher mean airway pressure[36]. Moreover, in two studies in 98 CDH survivors in total, longer duration of ventilation was associated with worse lung function[37,38]. None of these studies, however, addressed a possible impact of type of ventilation on lung function.

Two ventilation modalities were investigated in a multicentre international randomized clinical trial (chapter 6). The combined outcome of mortality or BPD in prenatally diagnosed CDH neonates born after a gestation of >34 weeks did not significantly differ between those who were initially ventilated by conventional mechanical ventilation (CMV) and those initially ventilated by high-frequency oscillation (HFO). In the CMV group, however, the duration of ventilation and inotropic support was significantly

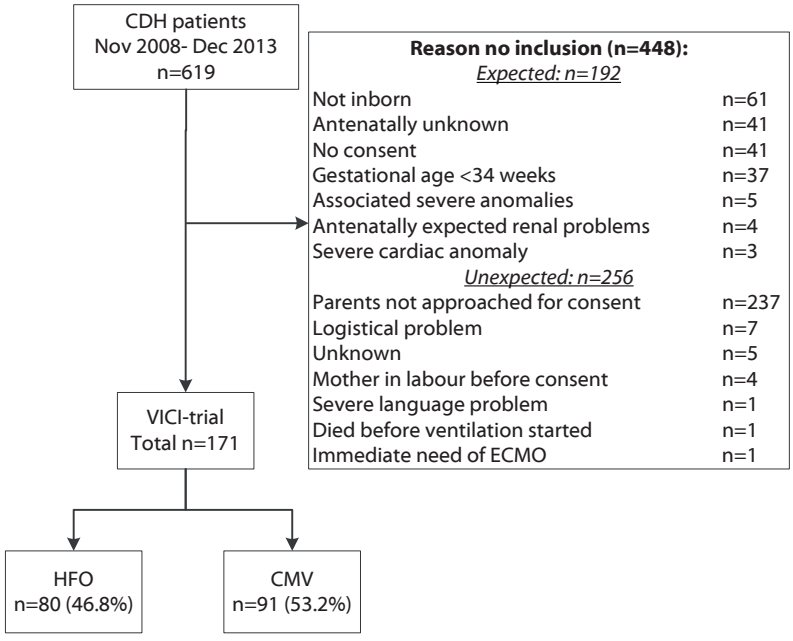
shorter, and these neonates were less likely to receive vasoactive medication or phosphodiesterase type 5 inhibitors or be placed on ECMO.

Other ventilation modalities than CMV and HFO, such as (partial) liquid ventilation, might be better suited for patients with the most severe lung hypoplasia (chapter 7). Lung compliance can be improved by eliminating the air-liquid interface. Perfluorooctylbromide, which has dense characteristics, can then gravitate to the dependent part of the lungs, so that collapsed regions are re-opened and the ventilation/perfusion ratio will improve[39]. On the other hand, spontaneous breathing, instead of routine intubation immediately after delivery, may be preferable in the less severely ill patients, so as to prevent ventilator-induced lung injury.

Although it was planned to include 400 infants with prenatally diagnosed CDH in the VICI-trial in three years, only 171 infants could be included in more than five years. In this period 619 CDH patients were born in one of the participating centres. Reasons for non-inclusion are summarized in Figure 1, distinguished in expected and unexpected non-inclusions.

The planned number of inclusions seemed reasonable since more than 600 patients were born in the study period. On the basis of the number of expected non-inclusions (192) still more than 400 patients could have been included. Expected non-inclusions are inevitable and should be taken into account, also in future trials. Only efforts to raise the consent rate may have any effect.

Figure 1



Reasons for the unexpected non-inclusions can be divided into research infrastructure/ organisational issues and human factors. Logistical problems and failure to ask parents for consent are examples of the first category. In one high-volume centre, seeking consent was stopped two years after the start of inclusion because a dedicated researcher was no longer available. This has resulted in a loss of 237 possible inclusions, which highlights that a good research infrastructure is a crucial element in trials.

Second, next to enrollment problems, some practical issues were present. With regard to human factors, one limiting factor was the large amount of time needed to complete the case record form. In many centres, clinicians were responsible for this and other aspects of the trial, which took much time in addition to their clinical shifts. This has probably contributed to lower inclusion rates and "trial fatigue" after several years in some centres. Girling recommended to collect only data necessary for answering the trial questions, and to check the reliability of data[40]. Therefore, in future studies the variables in the case record form should be strictly selected. A second potentially relevant human factor is discontinuity in management, and consequently communication between the centres, as three different study coordinators were involved over the five years' enrollment period.

One of the measures taken to increase the inclusion rate was to include more CDH centres in the trial. In the end, 13 centres officially participated, but patient inclusion took place in only nine centres. What is more, two centres each included only one patient, and two other centres fewer than ten patients in more than five years. Thus, in only five centres, more than 10 patients per centre were included. Although much effort was invested in adding more CDH centres to participate in the trial, the increased inclusion rate was less than expected. Therefore, in future studies, the research infrastructure should be optimized before any centre could join such a trial.

Nevertheless, the inclusion rate of the VICI-trial was relatively high for a RCT on a severe congenital anomaly. It might have helped that participation was discussed with parents early during prenatal counselling and that parents had enough time to overthink consequences and could ask questions in a later consultation. In future studies, this approach should be continued.

One of the strengths of the trial is the block randomization stratified per centre using a computer-generated randomization schedule for each centre by a 24-hour interactive web response system. Second, all patients were treated according to a standardized neonatal treatment protocol implemented prior to the start of inclusion. Zwitter et al recommended that the recruitment period should be short, and pointed out that unbiased randomisation, attention to the treatment protocol and to the rules of good clinical practice and honest evaluation of experience are essential[41]. Our trial largely met these essential criteria. Although much effort should be undertaken to achieve standardized

treatment for all centres, it is very important in multicentre studies especially when they concern a rare disease or congenital anomaly such as CDH.

Other research groups also had problems in setting up and performing multicentre trials. For example, Brown et al. mentioned some of the above-mentioned issues and noted that their study would have been better facilitated by a working administrative relationship between centres established at an early stage[42]. The National Institutes of Health, also recognizing the huge effort needed to conduct multicentre trials, has established clinical trial planning grants to support researchers[43]. The European Commission encourages to set up European reference networks (ERNs) and aims to join best specialists from across Europe to tackle complex or rare medical conditions that require highly specialised healthcare and a concentration of knowledge and resources[44]. If trials can be organized within such ERNs, and if support would be given to organize and maintain the research infrastructure, a part of research grants might be invested in research infrastructures.

Surgery

CDH used to be a surgical emergency, with surgical repair performed soon after birth when the infant was still unstable, based on a very mechanical concept that reduction of the intrathoracic contents would save the baby. From the 1980s onwards it was recognized that delayed surgical repair, after a period of clinical stabilization, improved survival rates[25,45]. Several triggers such as hypoxemia, hyperoxia (oxygen radicals and inflammation), and pulmonary vascular damage caused by mechanical ventilation sustain pulmonary hypertension through reactive vasoconstriction and vascular remodelling. Therefore, it would seem best to achieve optimal cardiopulmonary management and prevent further damage to the lung before surgical repair of the diaphragmatic defect is performed.

The CDH EURO Consortium members have reached consensus on criteria for surgical repair[1]. It would be interesting to evaluate whether this protocol is indeed followed for all patients in all participating centres. Different forms of surgical repair are possible: open repair via subcostal laparotomy/thoracotomy and minimal access surgery[46]. Advantages of the latter technique are better cosmetic results, less or at least no more postoperative pain and possibly improved respiratory compliance[47-50]. In a pilot randomized clinical trial of thoracoscopic versus open repair, however, thoracoscopic repair was found associated with prolonged and severe intraoperative hypercapnia and acidosis[51]. Besides, thoracoscopic repair is associated with significantly more recurrences[52-54]. Therefore, in many CDH centres thoracoscopic CDH repair is not performed and until research can prove that minimal access surgery is a safe procedure in CDH, it should not be performed outside a randomized trial. A major challenge for such a trial, next to the organisational problems of multicentre studies as described for the VICI-trial, would be the multidis-

ciplinary collaboration with anesthesiologists, pediatric surgeons, neonatologists and paediatric intensivists.

Pulmonary hypertension

In infants with CDH, pulmonary vascular resistance often remains elevated after birth resulting in pulmonary hypertension. Elevated pulmonary vascular resistance can lead to right-to-left shunting, resulting in hypoxia and a difference in preductal and postductal saturations[55]. It may be caused by either structural of functional abnormalities of pulmonary vasculature[56]. Consequences of pulmonary hypertension and pulmonary hypoplasia are the main determinants of survival, and therefore research has increasingly focused on this topic[57]. The altered vasoreactivity in combination with pulmonary vascular remodelling and varying degrees of pulmonary vascular bed hypoplasia may account for the extremely challenging management of this form of pulmonary hypertension[57].

Several drugs that act upon the three different pathways involved in the development of pulmonary hypertension have been used for pharmacotherapy. Pharmacodynamic considerations in the treatment of pulmonary hypertension in infants were described in chapter 9. Nitric oxide (NO) is a selective vasodilator affecting the NO-cGMP pathway. Inhaled nitric oxide (iNO) improved oxygenation in infants with persistent pulmonary hypertension or severe hypoxic respiratory failure[58,59], but so far has not shown a clear benefit in CDH infants[60]. Still many CDH centres use it as the first treatment choice in CDH patients with pulmonary hypertension. Moreover, since in one study more infants treated with iNO needed ECMO[61], this therapy should be stopped if no effect is seen after its initiation. The non-response to iNO-therapy is relatively high, and medications targeting other pathways of pulmonary hypertension might therefore be more suitable. Sildenafil, for example, is a selective phosphodiesterase type 5 (PDE5) inhibitor and PDE5 enzyme specifically degrades cGMP. A Cochrane analysis found that sildenafil significantly decreased mortality in neonates with pulmonary hypertension[62].

Increased endothelin-1 plasma concentrations have been found in neonates with persistent pulmonary hypertension and in CDH infants[19]. Bosentan is an endothelin antagonist, thus involved in the endothelin pathway, and can improve pulmonary hypertension in neonates[63]. Bosentan is available only as an oral formulation, however, which limits its application in very ill infants with impaired absorption. An intravenous formulation might increase its potential in treatment of pulmonary hypertension. Prostacyclin and treprostinil, which target the prostacyclin pathway, improved pulmonary hypertension in infants[64,65]. Lastly, milrinone is a phosphodiesterase 3 (PDE3) inhibitor which induces pulmonary vasodilation by its action on cAMP in cardiac and vascular muscle cells. In six CDH infants with severe pulmonary hypertension, milrinone significantly improved systolic and diastolic function in the right ventricle, and decreased oxygenation index

without changes of blood pressure[66]. Randomized clinical trials evaluating the effects of pharmacological therapies specifically for infants with CDH who have severe pulmonary hypertension are still lacking.

Monitoring of cardiopulmonary status

A two-dimensional echocardiography performed within the first 24 hours after birth remains the best modality to rule out the presence of cardiac anomalies, next to assessing the right heart function and the degree of pulmonary hypertension[67,68]. Pulmonary hypertension is most classified by the definition of Keller et al[19]. This definition was also used in our studies based on data of the VICI-trial[69]. In the VICI-trial the degree of pulmonary hypertension was measured within the first 24 hours of life. It appeared that about two-thirds of the patients had already pulmonary hypertension at the time, possibly because they were still in the transitional phase from intra-uterine to extra-uterine environment. Thus, to evaluate the persistence of pulmonary hypertension, echocardiographic assessment should also be performed at standardized time points later in life, for example at days 3, 7 and 14. A protocol describing measurements during echocardiographic evaluations should be developed to standardize the evaluation of severity of pulmonary hypertension, and assessment of ventricular function should be included in this protocol. An electronic system should be set up for training of individual centres to guarantee the level of evaluation.

Standardized treatment

Standardized treatment is the key to effective multicentre research. Standardized treatment[1] also significantly improved clinical outcome by lowering the mortality rate from 33% to 12% in two high-volume centres[70], and reducing the use of ECMO. In our studies, however, we found a higher mortality rate, which suggests that in the first years after implementation of the standardized protocol CDH patient populations with a relatively good prognosis were born.

Survival rates over the last decade in one high-volume CDH centre (Erasmus MC, Rotterdam, the Netherlands) are plotted in Figure 2. It is clear that there is a wide variation over the years. In the period after the inclusion period of Van den Hout et al[70], survival decreased again for some years. Thus this reflects a variation of survival over the years and the importance of a sufficient inclusion period in future studies. Another important consequence is the power calculation of future studies on interventions to prevent measuring the normal variation in survival which might be misinterpreted as a real effect of an alternative therapeutic approach.

Frequency of ECMO use and survival rates after ECMO over the last decade in the same centre are shown in Figures 3a and 3b. Figure 3a suggests that use of ECMO has decreased after the implementation of the standardized protocol. Since survival rates of

Figure 2 – Survival over the years in Erasmus MC, Rotterdam, the Netherlands

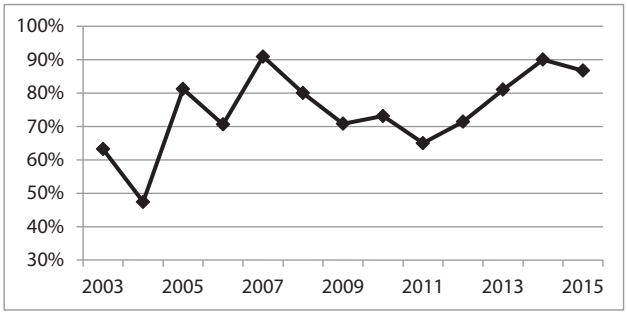


Figure 3a and 3b

Figure 3a: all patients

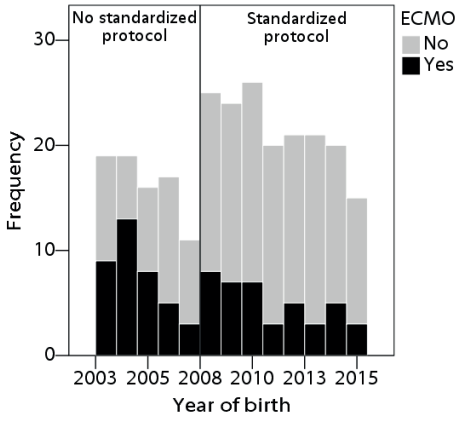
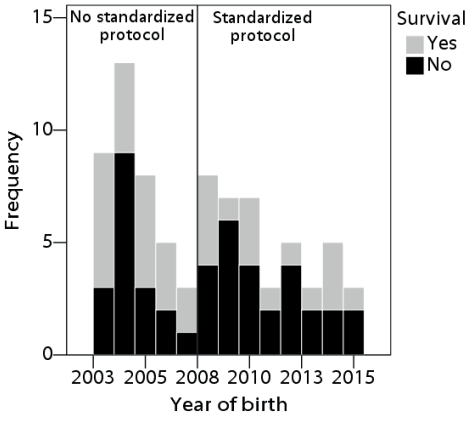


Figure 3b: ECMO treated patients



ECMO-treated CDH patients are about 50%, it is recommended to initiate ECMO treatment only in the most severe cases, based on the ECMO criteria from the protocol.

Chapter 10 of this thesis presents an update of this standardized treatment protocol. The effect on outcome of this updated protocol should be evaluated in the coming years. To guarantee that treatment in CDH is based on the most recent available evidence, the standardized neonatal treatment protocol should be renewed every 5 to 10 years during consensus meetings of the CDH EURO Consortium. Most of the recommendations are currently still based on expert opinions, and consensus meetings offer the opportunity to discuss conceptions and designs of future trials.

OUTCOME

The study described in chapter 11 found that neurodevelopmental outcome was significantly different between two high-volume CDH centres (Rotterdam[71] and Rome[72])[73]. Those two centres used standardized prospective long-term follow-up programs and the same standardized neonatal treatment protocol, but differed in assessment instruments used (Bayley Scales of Infant Development (BSID) version 2 and 3), reference data, and experience of staff in follow-up care. Since those differences probably have contributed to the differences in outcome, this study highlights the importance of standardized follow-up programs next to standardized care. Recently, several studies have suggested that there are differences in outcome score between the BSID-II and BSID-III[74-78]. For example, a healthy Australian cohort scored higher than the standardised norms on four of the subscales of BSID-III tests with American reference scores[79], which may lead to underestimation of neurodevelopmental delays since minor delays may not be identified. Therefore, ideally nationwide reference scores should be developed from a healthy population for each of the countries in which assessment instruments are used. In future studies, internationally validated tests making use of country-specific reference values should be used so that neurodevelopment can be reliably compared between populations.

As resources are limited and standardized long-term follow-up programs are costly, ideally children at high risk for neurodevelopmental problems should be identified. Screening tools such as the Parent-report Perceived Cognitive Function (PedsPCF) or Ages and Stages Questionnaire (ASQ), which both can be filled in by parents, may contribute to patient selection[80]. In paediatric oncology clinics, the PedsPCF significantly differentiated between patients with various clinical characteristics[81]. In a Dutch population, the ASQ-3 identified most of the children without a developmental delay according to the BSID-III-NL[82]. It should be tested whether the PedsPCF and ASQ can reliably differentiate between CDH patients with high and low risk of neurodevelopmental problems. If this should be the case, only patients with a high-risk on the PedsPCF test should then be assessed by BSID tests.

Next to neurodevelopmental problems, CDH survivors may suffer from long-term problems on other domains such as the gastrointestinal tract and the respiratory tract[36,83]. As mentioned earlier, a prospective evaluation of lung function in CDH survivors found decreased expiratory flows and higher functional residual capacity within the first year of life, the latter of which was associated with longer duration of ventilation and higher mean airway pressure[36]. Moreover, in two studies in 98 CDH survivors in total, longer duration of ventilation was associated with worse lung function[37,38]. None of these studies, however, addressed a possible impact of type of ventilation on lung function. Spoel et al found in a small cohort of CDH survivors that functional and microstructural changes of

the lung persist into adulthood[84]. None of these studies, however, has addressed a possible impact of type of ventilation on lung function. Although we have not focused on long-term pulmonary morbidity in this thesis, it would be interesting to further explore this aspect.

In conclusion, risk stratification is essential to identify infants with subtle impairments who are at risk for long-term problems such as neurodevelopmental delays and pulmonary morbidity. Possible determinants of long-term problems need to be established, therefore, such as long duration of hospital stay/ventilation time, low socioeconomic status and other ethnicity.

CONSIDERATION

Multicentre research has advantages, but these can be undermined by centre-specific differences in for example the patient population and availability of treatment modalities, even though the same standardized neonatal treatment protocol is in place. In chapter 12 we described that not in all high-volume CDH centres ECMO was available. Although ECMO criteria were included in the standardized neonatal treatment protocol, in one centre ECMO was used much more often used than in the other centre. Moreover, one centre often made use of fetoscopic tracheal occlusion (FETO), which is only reserved for the prenatally most severely ill patients. We have also shown that patient mix can have an impact on outcome, as even after correction for patient characteristics, centre of birth was still significantly associated with survival. Therefore, in future multicentre studies all analyses should be corrected for centre, next to patient characteristics that influence outcome. Differences in outcome between centres can partly be explained by differences in available treatment options such as ECMO and FETO. Moreover, experience in treatment options such as ECMO may account for differences in outcome between CDH populations.

The additional contribution of ECMO to outcome is still being debated. In the VICI-trial, mortality rates were not significantly different between centres with ECMO availability and centres without ECMO availability[69]. In 2006 a meta-analysis of randomized controlled trials with small sample sizes indicated a reduction in early mortality with ECMO, yet did not demonstrate a long-term benefit[85]. The same study included a meta-analysis of the retrospective studies and concluded that the introduction of ECMO has improved survival in infants with CDH[85]. The use of ECMO has decreased in recent years[86], with a shift to preoperative stabilization. The best suited mode (veno-arterial vs. venovenous) remains unclear[87] and this aspect should be studied prospectively in a randomized trial. The still high morbidity in ECMO survivors, for example reflected by the fact that more than 90% of the patients developed BPD (chapter 3), suggests that only severely ill in-

fants are kept alive by ECMO. Therefore, future studies should focus on the true benefit of ECMO in CDH; and if a benefit can be proven, criteria should be developed for the selection of patients who would benefit most from ECMO.

Another point of consideration is testing of the rate of compliance with the standardized treatment protocol in each of the centres participating in a multicentre trial. For example, compliance with initiating an ECMO procedure when the ECMO criteria are met could be tested. If compliance would be found to be low in some of the centres, and outcomes of ECMO survivors would differ between centres, the ECMO criteria should perhaps be adjusted.

FUTURE RESEARCH PERSPECTIVES

Since many aspects of the current treatment are based on expert opinion only and are not evidence-based, there is plenty of room for further research. Also taking into account the findings from the studies presented in this thesis, we propose the following recommendations, in line with the issues addressed in the four parts of this thesis:

Prediction

- Combining several prenatal and postnatal clinical prediction scores, such as the O/E LHR, prenatal liver position (intrathoracic/ intra-abdominal), SNAP-II score[10] and the prediction model of Brindle et al[14], may further improve risk stratification of CDH infants.
- Instead of the current "trial-and-error application" of biomarkers with possible predictive value in CDH, a pathophysiological based approach should be used. For example, since most CDH infants die from consequences of severe pulmonary hypertension, biomarkers in one of the three pathways involving development of pulmonary hypertension, such as VEGF or endothelin, should be considered. It should be studied whether biomarkers could predict cardiopulmonary status at later age (such as chronic pulmonary hypertension).
- Since routine measurements of biomarkers do not seem useful, future research should include a baseline level, such as umbilical cord blood, and study whether biomarker levels determined before and after the start of clinical interventions (such as iNO or ECMO) could better predict outcome.

Treatment

- Regular communication between all participating centres in a multicentre trial is essential to evaluate patient inclusion rates and discuss practical problems that limit patient inclusion. For example in the form of three-monthly teleconferences on

the initiative of the coordinating centre. Then, each centre should report inclusion numbers so that interventions can be initiated if these are lagging behind.

- It might be useful to ask the advice of patient organisations on trial designs and research objectives from the patient's perspective. Moreover, involving patient support groups in the information provision on clinical trials to future parents may help improve patient inclusion rates.
- Very low volume centres or centres with a lack of a research infrastructure should not participate in multicentre trials. Despite much effort to organize the participation of those centres (such as ethical committee consent), it is doubtful whether those centres indeed can enrol patients.
- A web-based portal should be set up for the international collection of data which all members of the CDH EURO Consortium can use to answer specific research questions. Electronical case record forms should be incorporated so that all changes can automatically be saved and no patient data are lost. The portal should also offer a central randomization procedure for future multicentre RCTs.
- A part of the budget for research should be invested in the research infrastructure and data management, for example to cover the salary of a dedicated researcher or research nurse. This will ensure the continuity of patient inclusion and continuity of (blood) sampling, independent of working hours or presence of the responsible clinician.

Next to the above-mentioned recommendations for future multicentre trial designs, several randomized clinical trials on important aspects of CDH can be proposed to further improve outcome;

- *Pulmonary hypertension:* Model-based dosing with population pharmacokinetic models of commonly used drugs such as sildenafil should primarily be defined. Then, a multicentre randomized clinical trial should be initiated to identify the optimal treatment strategy of severe pulmonary hypertension in CDH infants; for example early intravenous sildenafil versus inhaled nitric oxide.
- *Extracorporeal membrane oxygenation:* RCT on ECMO versus no ECMO treatment in CDH patients who fulfil the ECMO criteria[1].
- *Surgical repair:* Minimal access surgery versus open repair in selected CDH patients (left sided, liver down). Importantly, standard operative procedures should be developed, as well as follow-up programs to evaluate intra- and postoperative morbidity including recurrences, musculoskeletal abnormalities and neurodevelopment. The cost-effectiveness of both strategies, including direct and indirect costs, should also be addressed.

Outcome

- Most importantly, in the coming years centres should focus on international standardization of follow-up programs on cardiopulmonary status and neurodevelopment, which should include standardized cardiac evaluation and standardized neurodevelopmental assessment tests. It would be interesting to find if and how ventilation strategy influences long-term pulmonary morbidity. Therefore, CDH survivors from the VICI-trial should be examined at later age by lung imaging, cardiopulmonary exercise tests and lung function tests, next to standardized neurodevelopmental assessment.
- Because of limited resources, a minimal generic dataset on risk stratification for the different domains of morbidity should be developed to identify patients who are at highest risk for long-term problems.
- Centre-specific "specialties" as research approaches may be helpful to answer many research questions in a relatively short period of time.
- A system for international data collection should provide for electronic data transfer to expert centres.

CONCLUSIONS

In conclusion, there is a huge need for multicentre research. Since CDH is a rare congenital anomaly, multicentre collaboration is necessary to evaluate significant numbers of patients over time. Preferably, future randomized clinical trials should be performed in multicentre collaboration. CDH centres participating in the CDH EURO Consortium are listed in the appendix and their logos appear in Figure 4. Standardized treatment between centres is essential to evaluate patient populations and to ensure that infants are treated according to the latest evidence. Therefore, the treatment protocol should regularly be updated during consensus meetings. In addition, monitoring including cardiac assessment and follow-up programs should be standardized between centres. A good research infrastructure providing for sufficient financial resources and data management is essential for multicentre collaboration. In this thesis we have shown that despite of practical problems, multicentre collaboration is possible and can indeed lead to improved insights and thereby improved clinical outcome.

APPENDIX

Members of the CDH EURO Consortium Group:
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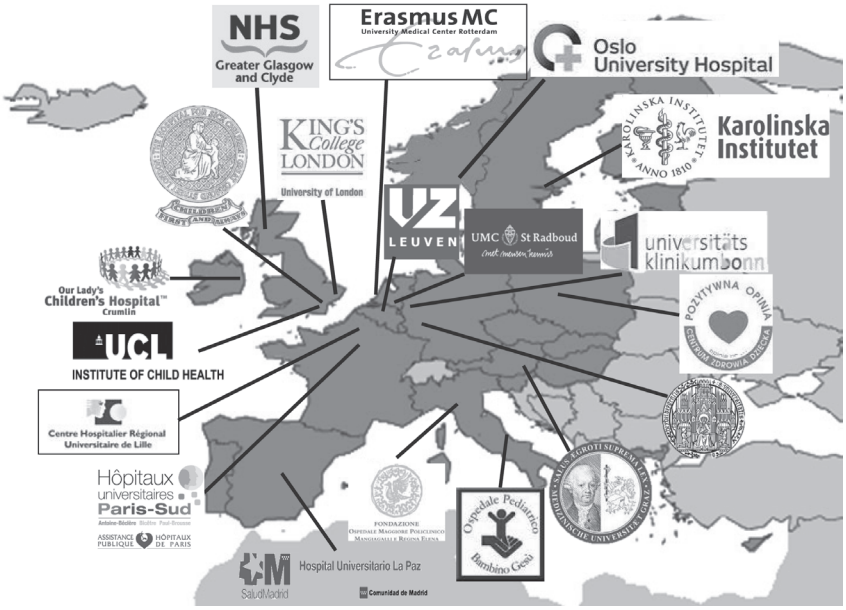
Figure 4



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Chapter 14

Summary
Nederlandse samenvatting

The research described in this thesis addressed several aspects of the management of congenital diaphragmatic hernia (CDH) after the introduction of a standardized neonatal treatment protocol. Most studies involved multicentre collaborative efforts of members of the CDH EURO Consortium. This is a collaboration between tertiary European centres with an expertise in CDH.

Part II focusses on clinical parameters and biomarkers with a potential predictive role. In **part III** different aspects of the treatment of CDH patients are described, including findings of a randomized clinical trial on initial ventilation strategy. **Part IV** studies the neurodevelopmental outcome in high-risk CDH infants at the ages of one and two years. In **Part V** the influences of patient characteristics and center specific differences are evaluated with respect to outcome.

PART I – INTRODUCTION

In **Chapter 1** the history of CDH is described, and attention is paid to the antenatal period in which the diaphragmatic defect should be discovered by prenatal ultrasonography. Antenatal predictive markers for outcome such as the lung-to-head ratio (LHR) and observed-to-expected LHR (O/E LHR) are explained. In the postnatal period the focus is on clinical prediction models including their limitations, next to biomarkers related to pulmonary hypertension. The way in which the CDH EURO Consortium was founded is described, as well as achievements such as consensus on the standardized neonatal treatment protocol and initiation of a randomized clinical trial on initial ventilation strategy. Then, a short overview of morbidity and neurodevelopment related to CDH. Lastly, differences between CDH centres such as availability or unavailability of extracorporeal membrane oxygenation (ECMO) are discussed.

PART II – PREDICTION

Chapter 2 describes a nationwide study in the Netherlands on the predictive role of the O/E LHR on survival of CDH infants in an era of standardized postnatal treatment. O/E LHR was measured using 2D ultrasonography at ≤24 weeks gestational age (GA), between 24-30 weeks GA, and ≥30 weeks GA by one single observer who performed all prenatal measurements. The first measured O/E LHR per patient significantly predicts survival and development of chronic lung disease in survivors (defined as oxygen dependency at day 28). Longitudinal analyses of the O/E LHR measurements over time during gestation showed no significant association with survival.

Chapter 3 concerns a prospective study of 171 prenatally diagnosed CDH patients in which we found that the Score for Neonatal Acute Physiology– II (SNAP-II) predicts mortality and need for ECMO, after adjustment for side of the defect, liver position, ventilation mode, gestational age, centre, and O/E LHR. This simple and rapid scoring system provides further insight into the prognosis within one day after birth.

Sphingolipid profiles were investigated in **chapter 4**. Tracheal aspirates were collected at days 1, 3, 7, and 14 in 72 patients from four centres. In multivariable logistic regression analysis with correction for side of the defect, liver position and O/E LHR, none of the changes in sphingolipid levels was significantly associated with mortality/development of bronchopulmonary dysplasia. At day 14, long-chain ceramides 18:1 and 24:0 were significantly elevated in patients initially ventilated by conventional mechanical ventilation compared to high-frequency oscillation, which could be explained by high peak inspiratory pressures and remodelling of the alveolar membrane.

In **chapter 5**, the value of high-sensitivity Troponin T (hsTnT) and N-terminal pro-brain natriuretic peptide (NT-proBNP) as predictive biomarker was investigated at days 1, 3, 7, and 14. In the multivariable analysis with correction for multiple testing, none predicted severe pulmonary hypertension, death, need for ECMO or bronchopulmonary dysplasia. Future research should study whether biomarker levels determined before and after the start of clinical interventions (such as start of iNO or initiation of an ECMO procedure) could better predict outcome.

PART III – TREATMENT

In **chapter 6**, a multicenter international randomized clinical trial on initial ventilation strategy is described. The trial included 171 prenatally diagnosed CDH infants born after a gestational age of more than 34 weeks. They were randomized to either high-frequency oscillation (HFO) or conventional mechanical ventilation (CMV). The primary outcome measure was bronchopulmonary dysplasia (BPD)/ death at day 28. Forty-one (45.1%) of the 91 patients randomized to CMV died/ had BPD compared with 43 (53.8%) of the 80 patients in the HFO group. The odds ratio for death/ BPD for CMV vs. HFO was 0.62, after adjustment for centre, head-lung ratio, side of the defect, and liver position. Patients initially ventilated by CMV were ventilated for fewer days, less often needed ECMO support, inhaled nitric oxide, sildenafil, had a shorter duration of vasoactive drugs and less often failed treatment as compared with infants initially ventilated by HFO. A major limitation was that the calculated sample size was not achieved due to limited financial resources and a lack of research infrastructure in one high-volume centre.

An editorial on liquid ventilation is presented in **chapter 7**. This editorial was based on a laboratory study of fetal instillation of perfluorocarbon in rabbits with induced CDH.

We reviewed studies on liquid ventilation in CDH and concluded that once adverse long-term effects of liquid ventilation have been excluded, a randomized clinical trial for the most severe CDH cases could be the next step.

Chapter 8 describes the prenatal and postnatal clinical course of five newborns with a prenatal diagnosis of CDH who were not routinely intubated immediately after birth. We concluded that this strategy is feasible in a selected subgroup of CDH infants (LHR >2.5 or O/E LHR >50%, liver down) depending on the nature of the child's transitional phase.

Pulmonary hypertension is the major cause of death in infants with CDH. In **chapter 9** we reviewed pharmacodynamics of drugs used in the treatment of pulmonary hypertension in infants. We concluded that reliable data of pharmacodynamics tested in adequate series or in randomized controlled trials in children are lacking for most of these drugs.

Chapter 10 provides an update of the original standardized neonatal treatment protocol published in 2010. Consensus was reached between the 22 centres of the CDH EURO Consortium. Five experts individually determined the levels of evidence. Differences in opinion were discussed until full consensus was reached. Key updated recommendations are: 1) planned delivery after a gestational age of 39 weeks in a high-volume tertiary centre; 2) neuromuscular blocking agents should be avoided during initial treatment in the delivery room; 3) adapt treatment to reach a preductal saturation between 80- 95% and postductal saturation >70%; 4) target $PaCO_2$ to be between 50- 70 mmHg; 5) conventional mechanical ventilation is the optimal *initial* ventilation strategy; and 6) intravenous sildenafil should be considered in CDH patients with severe pulmonary hypertension.

PART IV – OUTCOME

In an observational, prospective cohort study, presented in **chapter 11**, neurodevelopmental outcome was evaluated of 88 high-risk CDH patients treated according to the standardized neonatal treatment protocol. Patients from two centres in the CDH EURO Consortium (Rotterdam or Rome), were included. Cognition and motor function were assessed with the Bayley Scales of Infant Development (BSID). In Rotterdam the BSID-II version with Dutch normative data was used and in Rome the BSID-III version with American normative data was used. At 12 months, cognition was normal in 77.8% of children from Rotterdam and in 94.8% from Rome; at 24 months, in 70.7% and 97.4%, respectively. Motor function was normal in 64.3% of children from Rotterdam and in 81.6% from Rome at 12 months, and in 45.7% and 89.8%, respectively, at 24 months. These striking differences between the two centres warrant careful interpretation of results for other centres. Future multicentre collaboration should not only focus on

standardization of postnatal care but also on international standardization of follow-up to identify risk factors and thereby reduce morbidity.

PART V – CONSIDERATION

In **chapter 12,** differences between patient populations from four high-volume centres (two of which with ECMO availability) were retrospectively evaluated. 975 CDH patients, born between 2004 and 2013, were included. We found a significant variability in survival over the years and between centres. Characteristics of the patient populations were highly different between centres, which influences outcome. Therefore we suggested that in future multicentre studies outcomes should be evaluated over a sufficient period of time and corrected for centre.

PART VI – GENERAL DISCUSSION

The general discussion in **chapter 13** addresses the research described in this thesis in connection with the literature, as well as the implications for future multicentre research. Both the strengths and limitations of the presented studies are discussed. The major findings and recommendations are the following:

- Multicentre collaboration is essential in a relatively rare congenital anomaly such as CDH.
- Standardized treatment between centres is essential to evaluate patient populations.
- Crucial for multicentre research collaborations is a good research infrastructure including financial resources and data management.

NEDERLANDSE SAMENVATTING

Het onderzoek van dit proefschrift beschrijft verschillende aspecten van congenitale hernia diaphragmatica (CDH) na de introductie van een gestandaardiseerd neonataal behandelingsprotocol. De meeste studies betreffen multicenter samenwerkingsverbanden van het CDH EURO Consortium. Dit is een samenwerking tussen tertiaire Europese centra met een expertise in CDH.

Deel II richt zich op klinische parameters en biomarkers met een mogelijk voorspellende waarde. In **deel III** worden verschillende aspecten van de behandeling van CDH patiënten beschreven, waaronder de uitkomsten van een gerandomiseerde klinische studie over initiële beademingsstrategie. In **deel IV** wordt op de leeftijd van één en twee jaar de neuropsychologische ontwikkeling onderzocht in hoog-risico kinderen met CDH. In **deel V** wordt het effect van patiëntkarakteristieken en centra specifieke verschillen op uitkomst onderzocht.

DEEL I – INTRODUCTIE

In **hoofdstuk 1** wordt de geschiedenis van CDH beschreven. Daarnaast wordt de antenatale periode waarin het diafragma defect ontdekt zou moeten worden door prenatale echoscopie toegelicht. Antenataal voorspellende markers voor uitkomst zoals de "long-hoofd ratio" (LHR) en de "geobserveerde ten opzichte van de verwachte long-hoofd ratio" (O/E LHR) worden uitgelegd. Vervolgens wordt de postnatale periode besproken waarin de focus ligt op predictiemodellen en daarnaast biomarkers die betrokken zijn bij pulmonale hypertensie. Hiervan worden ook de beperkingen besproken. De manier waarop het CDH EURO Consortium was opgericht, wordt beschreven. Vervolgens wordt uitgelegd wat er momenteel al door het consortium bereikt is, zoals de consensus van het gestandaardiseerde behandelingsprotocol en het opzetten van de klinische studie naar initiële behandelingsstrategie. Vervolgens wordt kort de morbiditeit besproken en de neuropsychologische ontwikkeling van CDH patiënten. Tot slot worden verschillen tussen CDH centra bediscussieerd zoals de beschikbaarheid van extracorporele membraan oxygenatie (ECMO).

DEEL II – PREDICTIE

In **hoofdstuk 2** wordt een landelijke studie in Nederland beschreven over de voorspellende waarde van de O/E LHR voor overleving van CDH patiënten in een tijdperk van gestandaardiseerde postnatale behandeling. De O/E LHR werd middels 2D echografie

gemeten op drie tijdsmomenten; <24 weken zwangerschapsduur, tussen 24- 30 weken zwangerschap en na 30 weken zwangerschapsduur. Alle metingen werden verricht door één persoon. De eerst gemeten O/E LHR per patiënt voorspelde significant overleving en ontwikkeling van chronische long ziekte in de overlevenden. Chronische long ziekte was gedefinieerd als zuurstofafhankelijkheid op dag 28. Longitudinale analyse van de O/E LHR metingen tijdens de zwangerschap liet geen significante associatie met overleving zien.

Hoofdstuk 3 betreft een prospectieve studie van 171 prenataal gediagnosticeerde CDH patiënten waarin we vonden dat de Score voor Neonatale Acute Fysiologie – II (SNAP-II score) overleving en ECMO behoefte kon voorspellen. Deze analyses werden gecorrigeerd voor kant van het defect, de lever positie, initiële beademingsstrategie, zwangerschapsduur, centrum van geboorte en O/E LHR. Dit simpele en snelle scoringssysteem geeft inzicht in de prognose binnen één dag na de geboorte.

Sphingolipiden profielen werden onderzocht in **hoofdstuk 4**. Trachea aspiraten werden verzameld op de dagen 1, 3, 7 en 14 na de geboorte in 72 CDH patiënten, geboren in totaal vier centra. In de multivariable logistische regressie analyse gecorrigeerd voor kant van het defect, lever positie en O/E LHR, bleek dat geen van de veranderingen van de sphingolipiden waardes significant geassocieerd was met mortaliteit/ ontwikkeling van bronchopulmonale dysplasie. Op dag 14 bleek dat enkel de lange keten ceramides 18:1 en 24:0 significant verhoogd waren in patiënten die initieël beademd werden middels conventionele mechanische beademing ten opzichte van patiënten die initieël met hoog-frequente oscillatie beademd werden. Dit zou verklaard kunnen worden door hoge piek inspiratoire drukken en remodelling van de alveolair membraan.

In **hoofdstuk 5** werd de waarde van hoog-sensitieve Troponine T (hsTnT) en N-terminal pro-brain natriuretic peptide (NT-proBNP) als predictieve biomarker onderzocht op de dagen 1, 3, 7 en 14. In de multivariable analyse met correctie voor multiple testen bleek dat geen van de waarden kon voorspellen of er sprake was van ernstige pulmonale hypertensie, overlijden, ECMO behoefte of ontwikkeling van bronchopulmonale dysplasie. Toekomstig onderzoek zou zich daarom moeten richten of biomarker waarden bepaald voor en na de start van klinische interventies, zoals de start van iNO of start van ECMO, beter uitkomst zou kunnen voorspellen.

DEEL III – BEHANDELING

In **hoofdstuk 6** wordt een multicenter internationaal gerandomiseerd klinisch onderzoek beschreven over initiële beademingsstrategie. In dit onderzoek werden 171 prenataal gediagnosticeerde CDH kinderen, die geboren werden na een zwangerschapsduur van meer dan 34 weken, geïncludeerd. Zij werden gerandomiseerd voor hoog-frequente

oscillatie (HFO) of conventionele mechanische beademing (CMV). De primaire uitkomstmaat was bronchopulmonale dysplasia (BPD) of overlijden op dag 28. Eénenveertig (45.1%) van de 91 patiënten gerandomiseerd voor CMV overleden/ ontwikkelden BPD vergeleken met 42 (53.8%) van de 80 patiënten in de HFO groep. De odds ratio voor overlijden/ BPD voor CMV versus HFO was 0.62 na correctie voor centrum van geboorte, LHR, kant van het defect en lever positie. Wij vonden dat patiënten die initieël beademd werden met CMV korter werden beademd, minder vaak ECMO nodig hadden, minder vaak iNO en sildenafil nodig hadden, korter vasoactieve medicatie nodig hadden en minder vaak falen van behandeling ondervonden in vergelijking met patiënten die initieel met HFO waren beademd. De belangrijkste beperking van het onderzoek was dat de van tevoren berekende steekproefgrootte niet gehaald werd vanwege beperkte financiële middelen en een gebrek aan een onderzoeksinfrastructuur in één groot centrum.

Een editorial over vloeibare beademing wordt beschreven in **hoofdstuk 7**. Dit editorial was gebaseerd op een laboratoriumonderzoek waarbij foetale instillatie van perfluorocarbon bij konijnen met geïnduceerde CDH onderzocht werd. Wij hebben verschillende studies over vloeibare beademing bij CDH bestudeerd. We concludeerden dat zodra er bewijs is dat er geen negatieve lange termijn effecten zijn van deze vorm van beademing, een gerandomiseerd onderzoek specifiek voor de meest ernstige CDH patiënten mogelijk een volgende stap zou kunnen zijn.

In **hoofdstuk 8** wordt het prenatale en postnatale beloop van vijf neonaten met een prenataal gediagnosticeerde CDH beschreven. Deze patiënten werden niet routinematig onmiddellijk na de geboorte geïntubeerd. Wij concludeerden dat deze methode toe te passen is in een selectie van CDH kinderen (LHR >2.5 of O/E LHR >50% en lever gepositioneerd in het abdomen), afhankelijk van het beloop van de transitionele fase.

Pulmonale hypertensie is de belangrijkste oorzaak van overlijden in kinderen met CDH. In **hoofdstuk 9** hebben we de farmacodynmiek van medicamenten beschreven welke gebruikt worden in de behandeling van pulmonale hypertensie bij kinderen. Wij concludeerden dat betrouwbare data van de farmacodynamiek onderzocht in onderzoeken met grote patiëntenaantallen of gerandomiseerd gecontroleerd onderzoek in kinderen nog steeds ontbreekt voor de meeste medicamenten.

Hoofdstuk 10 beschrijft een update van het originele gestandaardiseerde neonatale behandelingsprotocol welke in 2010 gepubliceerd werd. Consensus werd bereikt in 22 centra van het CDH EURO Consortium. Vijf experts hebben individueel het niveau van het beschikbare bewijs beoordeeld. Verschillen in mening werden bediscussieerd totdat volledige consensus bereikt werd. De belangrijkste nieuwe aanbevelingen zijn: 1) geplande geboorte na een zwangerschapsduur van 39 weken in een groot tertiair centrum; 2) neuromusculaire blokkade zou vermeden moeten worden tijdens de initiële behandeling in de opvangkamers; 3) preductale saturatie tussen 80-95% en postductale

saturatie >70% zou moeten worden nagestreefd middels het aanpassen van de behandeling; 4) streef naar $PaCO_2$ waarden tussen 50- 70 mmHg; 5) conventionele mechanische beademing is de optimale initiële beademingsstrategie; en 6) intraveneus sildenafil moet overwogen bij CDH patiënten met ernstige pulmonale hypertensie.

DEEL IV – UITKOMST

In een observationale, prospectieve cohort studie, beschreven in **hoofdstuk 11,** is de neuropsychologische uitkomst bestudeerd in 88 hoog-risico CDH patiënten. Deze patiënten werden behandeld volgens het gestandaardiseerde neonatale behandelingsprotocol. Patiënten geboren in twee centra van het CDH EURO Consortium (Rotterdam of Rome) konden geïncludeerd worden. Cognitie en motoriek werden onderzocht met behulp van de Bayley Scales of Infant Development (BSID) test. In Rotterdam werd de BSID-II versie met Nederlandse normaalwaarden gebruikt en in Rome werd de BSID-III versie met Amerikaanse normaalwaarden gebruikt. Op de leeftijd van 12 maanden was de cognitie normaal in 77.8% van de kinderen uit Rotterdam en in 94.8% van de kinderen uit Rome. Op de leeftijd van 24 maanden was dat in respectievelijk 70.7% en 97.4% het geval. Motoriek was normaal gescoord in 64.3% van de kinderen uit Rotterdam en in 81.6% van de kinderen uit Rome op de leeftijd van 12 maanden. Op de leeftijd van 24 maanden was dat in respectievelijk 45.7% en 89.8% het geval. Deze opvallende verschillen in uitkomsten tussen de twee centra laten zien dat de resultaten voorzichtig geïnterpreteerd moeten worden voor andere centra. Toekomstige multicenter samenwerking zou zich daarom ook niet alleen moeten richten op standaardisatie van postnatale behandeling maar ook op internationale standaardisatie van de follow-up met als doel om risicofactoren te identificeren en daarmee de morbiditeit te verminderen.

DEEL V – BESCHOUWING

In **hoofdstuk 12** worden verschillen tussen patiëntpopulaties van vier grote centra, waarvan twee met beschikbaarheid van ECMO, retrospectief geëvalueerd. In deze studie werden 975 CDH patiënten geboren tussen 2004 en 2013 geïncludeerd. Wij vonden dat de overleving significant varieerde over de jaren en tussen centra. De karakteristieken van de patiëntenpopulaties waren zeer verschillend tussen de centra en dit was van invloed op de toekomst. Daarom zijn we van mening dat in toekomstige multicenter onderzoeken uitkomsten geëvalueerd zouden moeten worden over een lange tijd en dat daarnaast de analyses gecorrigeerd moeten worden voor het centrum van geboorte.

DEEL VI – ALGEMENE DISCUSSIE

In de algemene discussie in **hoofdstuk 13** worden verbanden tussen het beschreven onderzoek uit dit proefschrift en de literatuur gelegd. Daarnaast worden implicaties voor toekomstig multicenter onderzoek gegeven. Zowel de sterke punten als de limitaties van de beschreven studies worden bediscussieerd. De belangrijkste bevindingen en aanbevelingen zijn de volgende:

- Multicenter samenwerking is essentieel in een relatief zeldzaam aangeboren afwijking zoals CDH.
- Gestandaardiseerde behandeling tussen centra is essentieel om patiëntenpopulaties te evalueren.
- Cruciaal voor multicenter onderzoek is een goede onderzoek infrastructuur waar financiële ondersteuning en databeheer ook bij hoort.

Part VII

Appendices

ABOUT THE AUTHOR

Kitty Geertruide Snoek was born on the 29th of January 1989 in Rotterdam, The Netherlands. She grew up in Rotterdam, where she completed secondary school at the Marnix Gymnasium in 2006. That same year, she started her medical training at the Erasmus MC, University Medical Center in Rotterdam. In the fourth year of her medical training, she went to Cape Town for a six-month research project at the pediatric surgery department under supervision of Dr. M. van Dijk, Dr. R. Albertyn and Prof. Dr. D. Tibboel. These research projects resulted in publication of a case report and three articles, for which she received the "Bataafsch Genootschap der Proefondervindelijke Wijsbegeerte" award. As a medical student she worked at the departments of Pediatric Psychiatry, Hematology, Ophthalmology, and Intensive Care and Department of Pediatric Surgery.

Kitty obtained her medical degree in 2012 (cum laude). As of January 2013 she commenced her PhD project on congenital diaphragmatic hernia at the Intensive Care and Department of Pediatric Surgery (Prof. Dr. D. Tibboel, Prof. Dr. R.M.H. Wijnen (promotors) and Dr. H. IJsselstijn (copromotor)). During this PhD project, she also worked as a resident at the Department of Pediatric Surgery (head Prof. Dr. R.M.H. Wijnen) at the Erasmus MC-Sophia Children's Hospital, University Medical Center in Rotterdam.

In July 2016 she will start her residency training in Anesthesiology at the University Medical Center Utrecht.

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1. **Snoek KG**, Jacobsohn M, van As AB. Bifocal Spinal Cord Injury without Radiographic Abnormalities in a 5-Year Old Boy: A Case Report. Case Rep Pediatr. 2012;2012:351319.
2. van Dijk M, Timmers M, **Snoek K**, Scholten WK, Albertyn R. How health professionals rate painfulness of childhood injuries and illnesses: a survey study. J Pain Palliat Care Pharmacother. 2012 Jun;26(2):105-10.
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7. **Snoek KG**, Capolupo I, Braguglia A, Aite L, van Rosmalen J, Valfrè L, Wijnen RM, Bagolan P, Tibboel D, IJsselstijn H. Neurodevelopmental outcome in high-risk congenital diaphragmatic hernia patients: An appeal for international standardization. Neonatology. 2016;109(1):14-21.
8. Kraemer U, Cochius-den Otter S, **Snoek KG**, Tibboel D. Pharmacodynamic considerations in the treatment of pulmonary hypertension in infants: challenges and future perspectives. Expert Opin Drug Metab Toxicol. 2016 Jan;12(1):1-19.
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10. **Snoek KG**, Capolupo I, Morini F, van Rosmalen J, Greenough A, van Heijst A, Reiss IK, IJsselstijn H, Tibboel D, CDH EURO Consortium. Score for Neonatal Acute Physiology-II predicts outcome in congenital diaphragmatic hernia patients. **Accepted for publication** – Pediatr Crit Care Med 2016, February.
11. **Snoek KG**, Kraemer US, ten Kate CA, Greenough A, van Heijst A, Capolupo I, Schaible T, van Rosmalen J, Wijnen RM, Reiss IK, Tibboel D. High-sensitivity troponin T and N-terminal pro-brain natriuretic peptide in prediction of outcome in congenital dia-

phragmatic hernia: results from a multicenter, randomized controlled trial. **Accepted for publication** – J Pediatr 2016, March.

12. **Snoek KG**, Reiss IK, Tibboel J, van Rosmalen J, Capolupo I, van Heijst A, Schaible T, Post M, Tibboel D. Sphingolipids in congenital diaphragmatic hernia; results from an international multicenter study. **Provisionally accepted for publication** – PLOS ONE 2016, March.
13. **Snoek KG**, Cochius- den Otter SC, Eggink AJ, Cohen-Overbeek TE, Wijnen RM, Reiss IK, Tibboel D. Routine intubation in the newborn with congenital diaphragmatic hernia; resetting our minds. **Submitted for publication.**
14. **Snoek KG**, Greenough A, van Rosmalen J, Capolupo I, Schaible T, Ali K, Wijnen RM, Tibboel D. Congenital diaphragmatic hernia; trends in survival, use of ECMO and FETO and international comparison of four high-volume centers. **Submitted for publication.**
15. **Snoek KG**, Peters NC, van Rosmalen J, van Heijst A, Eggink AJ, Wijnen RM, IJsselstijn H, Cohen-Overbeek TE, Tibboel D. The validity of the observed-to-expected lung-to-head ratio in congenital diaphragmatic hernia in an era of standardized neonatal treatment; a multicenter study. **Submitted for publication.**

Book chapter:

16. E. Brosens, **K.G. Snoek**, D. Veenma, H. Eussen, D. Tibboel, A. de Klein. Copy Number Variation in monozygous twins. In Genome-Wide Association Studies: From Polymorphism to Personalized Medicine. Edited by Appasani K, Cambridge University Press 2014.

PhD PORTFOLIO

Name PhD student:	Kitty G. Snoek
Erasmus MC Department:	Intensive Care and Department of Pediatric Surgery
PhD period:	2013-2016
Promotors:	Prof. Dr. D. Tibboel
	Prof. Dr. R.M.H. Wijnen
Copromotor:	Dr. H. IJsselstijn

	Year	Workload (ECTS)
General academic skills		
BROK (Basiscursus Regelgeving Klinisch Onderzoek) Erasmus MC	2013	1.0
MolMed - Research Integrity, dept. medical ethics and philosophy	2013	2.0
Systematical Literature Retrieval (medical library)	2013	0.5
Biomedical English Writing and Communication	2014	3.0
CPO short course	2014	0.3
MolMed - Biomedical English writing course	2014	2.0
Research skills		
NIHES - Biostatistical methods 1: basic principles	2013	5.7
MolMed - Basic introduction Course on SPSS	2013	1.0
MolMed - Research management	2014	1.0
NIHES - Advanced analysis of prognostic studies	2015	0.9
NIHES - Applied regression analysis	2015	1.4
Symposia and workshops		
Symposium: non-scrotal testis	2013	0.1
Symposium: schisis and craniofacial disorders	2013	0.1
Capita selecta medical genetics, dept. Clinical genetics, Erasmus MC	2013	0.5
Symposium agressie en claimend gedrag op de werkvloer; hoe ga je ermee om?	2013	0.1
Medical ethical meetings pediatric intensive care unit	2013	0.3
NICHD's 2012-2013: principles of Pediatric Clinical Pharmacology	2013	0.2
Multidisciplinary research meeting: will imaging help us to improve care of CDH patients?	2013	0.1
Capita selecta pain medicine, dept. Anesthesiology, Erasmus MC	2014	0.5
Publishing Connect Webinars Series	2014	0.2
Massive blood loss from incident to ICU	2015	0.1
International conferences		
2013 International CDH workshop Rotterdam, The Netherlands	2013	0.8
Esophageal atresia: "Bridging the gap", Rotterdam, The Netherlands	2014	0.3
5th Congress of the European Academy of Paediatric Societies, Barcelona, Spain: *short oral presentation (1x), oral presentation (2x)*	2014	4.0

	Year	Workload (ECTS)
Invitational Conference CDH, Rotterdam, The Netherlands: *oral presentation*	2015	1.5
16th Congress of European Pediatric Surgeon's Association, Ljubljana, Slovenia: *oral presentation*	2015	2.0
2015 International CDH workshop, Toronto, Canada: *oral presentation*	2015	2.0
2015 Annual Meeting of the Canadian Association of Paediatric Surgeons, Niagara Falls, Canada: *oral presentation*	2015	2.0
Teaching activities		
Tutor first year medical students	2013	1.5
Supervising 'acquaintance with the profession of medical doctor' ('Kennismaking Beroeps Praktijk'); first year medical students, Erasmus MC	2014	0.5
Lectures on primary school, Calvijn College, Rotterdam	2014	1.0
Educational lecture for ICU nurses	2015	0.1
Other		
Research meetings pediatric surgery	2013-2014	0.5
PhD day Erasmus, several workshops	2013	0.2
Research meetings follow-up	2013-2015	0.7
Research days Erasmus MC- Sophia Children's Hospital	2013-2016	1.0
PhD day Erasmus MC	2014	0.2
CDH patient day: *oral presentation*	2014	0.2
Advanced Pediatric Life Support, SSHK, Riel	2015	1.5

ECTS= European Credit Transfer and Accumulation System
1 ECTS represents 28 hours

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Mijn twee lieve paranimfen, Dorian en Joreintje.

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