From the first breath of life:
congenital diaphragmatic hernia, the child at risk

Lisette Leeuwen

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het kind met een congenitale hernia diafragmatica

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Contents

Chapter 1

General introduction

Adapted from:
Congenital diaphragmatic hernia
Lisette Leeuwen and Dominic A. Fitzgerald

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Congenital diaphragmatic hernia

Congenital diaphragmatic hernia (CDH) is a congenital anomaly of the diaphragm and both lungs, which occurs in approximately 1 per 2,500 births.[1] Normally in humans, the diaphragm starts to develop around four weeks of gestation and is fully formed by the twelfth week of gestation.[2] In infants with CDH, the diaphragm does not form properly creating a defect that allows abdominal viscera to enter into the thoracic cavity. Multiple factors including genetic and environmental factors as well as compression by the abdominal organs impede the normal development of the lungs. Maldevelopment of the terminal bronchioles, alveoli and pulmonary vessels is the result,[3, 4] and most patients experience severe respiratory failure soon after birth because of pulmonary hypoplasia and pulmonary hypertension.

Postnatal management

As long ago as 1701, Holt reported the first case of CDH in a child.[5] The first successful repair of the diaphragmatic defect was performed in 1902 by Heidenhain.[5] As it was hypothesized that early repair could improve survival of the child, the aim was to perform surgery at an early age. In 1946, Gross et al.[5] reported the first successful surgical repair of a diaphragmatic defect in a neonate within the first 24 hours after birth. From then on, CDH was considered a surgical emergency, and immediate surgical repair after birth was the cornerstone of treatment. Over the years, however, the idea developed that this may not be the best treatment option. Indeed, several studies found that delayed surgery, allowing physiologic stabilization of the patients, improved their outcome.[6, 7] Other studies, in contrast, did not find a difference in outcome between acute and delayed surgical repair.[8, 9] Despite the lack of clear evidence, surgeons have embraced the concept of delayed surgical repair increasingly since the 1980s, and this is now considered as standard of care.[10]

Other advances in surgical treatment include the use of minimally invasive techniques and closure of the defect with a patch. The method of closure depends on the size of the diaphragmatic defect. Standardized classification of the defect size is advocated by using the letters A to D, with 'A' reflecting the smallest and 'D' the largest defect size.[11] Small defects (A and B) are repaired primarily, whereas large defects require closure with a patch. Nowadays, surgical repair can be accomplished by open and minimally invasive techniques. Large defects are repaired using the open technique whereas smaller defects can be repaired by laparoscopy or thoracoscopy. Benefits of the latter techniques are a lesser need of surgery for early adhesive small bowel disease and a shorter hospital stay.[12] On the other side, a higher rate of diaphragmatic hernia recurrence with minimally invasive techniques can be considered a disadvantage.[12]

The ventilation strategy for CDH is based on the principle of gentle ventilation, whereby the peak inflation pressure is controlled by limiting the pressure of ventilation while tolerating an oxygen saturation of 85% and a rise of the arterial pressure of carbon dioxide (permissive hypercapnia).[13] Apart from stimulating spontaneous ventilation, this approach achieves adequate oxygenation while it avoids injury to the lungs by volutrauma, shear forces, and oxygen free radicals. Another ventilation strategy that is increasingly used for CDH patients is high frequency oscillation ventilation (HFOV). HFOV allows adequate oxygenation and carbon dioxide elimination at low ventilation pressures thereby decreasing iatrogenic barotrauma.[14] A trial in 171 CDH patients treated with conventional (gentle) ventilation or HFOV as the initial ventilation mode found no difference in mortality or chronic lung disease between the treatment groups.[15] Still, the group initially treated with conventional ventilation were ventilated for fewer days, were treated with vasoactive medication for a shorter time, and a smaller proportion required extracorporeal membrane oxygenation (ECMO) and medication to treat pulmonary hypertension.[15] Therefore, the CDH EURO Consortium now recommends conventional mechanical ventilation as the initial ventilation strategy, and HFOV should be used as rescue therapy when conventional ventilation fails.[10]

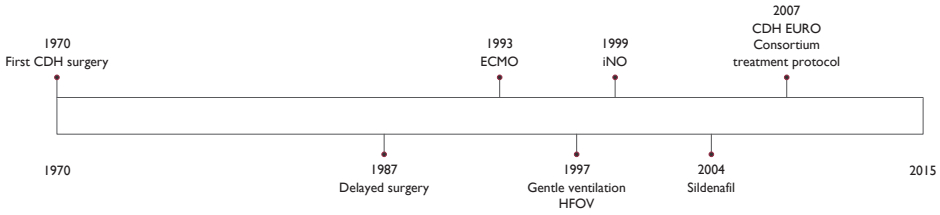
Pulmonary hypertension associated with CDH is a major cause of mortality.[16,17] A reduced number of pulmonary arteries in combination with altered vasoreactivity and abnormal pulmonary vascular remodeling in CDH contribute to the development of pulmonary hypertension.[18] The optimal treatment is unknown; currently, inhaled nitric oxide (iNO) is the most used acute treatment for pulmonary hypertension in CDH infants,[19] although its beneficial effects on oxygenation and survival have been questioned.[20] Another treatment option for persistent pulmonary hypertension in CDH patients is sildenafil, which appears to be more effective than iNO.[21] However, both the efficacy and safety of iNO and sildenafil have not been studied in a large trial. Other treatment options include other vasodilators such as prostacyclins, milrinone and bosentan, which have been even less studied.[22,23] Therefore, further studies are necessary to clarify the efficacy and safety of medication for pulmonary hypertension in CDH patients.

ECMO is reserved as a life-saving treatment option for CDH patients with severe reversible respiratory failure when other treatment options fail. Still the use of ECMO for CDH patients is controversial, and a Cochrane systematic review in 2008 concluded: 'the benefit of ECMO for babies with CDH is unclear'.[24] While some studies have shown increased survival rates with ECMO treatment,[25,26] other studies have demonstrated no improved survival.[27] Recently, the Extracorporeal Life Support Organization Registry reported a survival rate of 51% in CDH patients who require ECMO treatment.[28] In addition to this low survival rate, adverse long-term outcomes such as impaired neurodevelopmental outcome, increased respiratory morbidity and growth failure have been found in these patients.[29-31] We should not forget, however, that they represent a subgroup of CDH patients with more severe disease and, in all likelihood a greater probability of an adverse outcome. To date, we still

do not know whether ECMO is beneficial in CDH patients and how candidates for ECMO should be selected.[10]

In summary, current postnatal management of CDH is based on the standardized and revised guidelines of the CDH EURO Consortium protocol,[10] and this includes gentle ventilation with permissive hypercapnia, delayed surgical repair after stabilization, with the use of iNO, HFOV and ECMO as rescue therapies. A timeline showing when these therapy options were introduced in the Erasmus MC-Sophia Children's Hospital is presented in figure 1.

Figure 1. Timeline showing the introduction of new postnatal treatment strategies for congenital diaphragmatic hernia patients in the Erasmus MC-Sophia Children's Hospital, Rotterdam, the Netherlands



Abbreviations: CDH=congenital diaphragmatic hernia; ECMO=extracorporeal membrane oxygenation; HFOV=high frequency oscillation ventilation; iNO=inhaled nitric oxide.

Mortality

Reported mortality rates for CDH patients vary considerably in the literature. One of the reasons for this difference in reported mortality rates is the presence of 'hidden mortality'.[32] The term 'hidden mortality' accounts for intrauterine deaths because of CDH and CDH patients who die before arriving at the reporting institution and who, therefore, are not reported by institution-based studies. Nevertheless, it appears that advances in treatment, such as delayed surgical repair, 'gentle ventilation', and the use of HFOV and ECMO have decreased mortality rates occurring during initial hospital stay in CDH patients. Especially, the beneficial effects of standardized evidence-based management on survival have been acknowledged in recent years. With standardized treatment, institutions report a survival between 82% and 88%.[33-35] With this in mind, it is important that CDH research also focuses on long-term outcomes of CDH patients.

Morbidities

As mentioned above, the improved survival has made the long-term outcomes and quality of life of CDH survivors increasingly important issues. During initial hospital stay, patients are at risk for morbidities related to their critical illness, their underlying diagnosis as well as their medical treatment. Furthermore, CDH survivors are at risk for long-term sequelae including pulmonary disease, gastro-intestinal morbidity, poor growth, and neurological impairment.[36] The high prevalence of long-term morbidities among CDH survivors emphasizes the importance of close follow-up and long-term care.

Endocrine morbidity

Neonates with CDH are considered as critically ill: they are treated with mechanical ventilation, and most of them require vasoactive medication and treatment for pulmonary hypertension. As pointed out above, ECMO is offered to those with severe reversible respiratory failure.[10, 37] Critical illness may be associated with endocrine dysfunction such as non-thyroidal illness syndrome (NTIS), which is characterized by low thyroxine and low triiodothyronine, increased reverse triiodothyronine, and normal or low thyroid-stimulating hormone.[38] Low thyroid hormone levels have been reported in critically ill neonates with various diseases such as congenital heart disease, sepsis, persistent pulmonary hypertension, hyaline membrane disease, pneumonia, and CDH.[39-41] These low thyroid hormone levels can be detected during neonatal screening for congenital hypothyroidism. As thyroid hormones are essential for normal development of the central nervous system,[42] NTIS could have important consequences for neurodevelopmental outcome. Information on the incidence and clinical consequences of NTIS in CDH patients is not yet available. It is also unknown whether disturbed thyroid hormone concentrations persist.

Pulmonary morbidity

CDH patients are immediately intubated after birth and ventilated for a varying period of time. Approximately 30% of CDH survivors develop chronic lung disease.[10] Lung function is also affected in the first year of life, with decreased expiratory flows and increased functional residual capacity.[30] Lung function appears to gradually improve to normal or near-normal during childhood.[43] Although in a follow-up study by Spoel et al.[44] CDH survivors who had received ECMO treatment had compromised lung function at the ages of 5, 8, and 12 years, with deterioration over time. A recent study likely showed that airflow obstruction and diffusion capacity of CDH survivors deteriorated mildly from childhood into adulthood.[45] Spoel et al.[46] additionally found ventilation abnormalities in six out of nine patients on hyperpolarized helium-3 MRI imaging at adult age. They also found structural changes indicative of enlarged alveolar dimensions in the ipsilateral lung. These findings demonstrate the importance of long-term follow-up of pulmonary morbidities.

As described in a previous section, pulmonary hypertension is common among CDH infants and is a major cause of early death. Its persistence after the first days of life is associated with worse outcome.[17,47] Studies have found that pulmonary hypertension persisted in half of CDH patients at 3 weeks of age,[47] and in 18% at 3 months of age.[17,36] No study has as yet routinely assessed pulmonary hypertension in the follow-up of CDH patients after the age of 3 months.[17] It is therefore hard to tell which CDH patients qualify for long-term follow-up of pulmonary hypertension.

Growth failure and nutritional morbidity

Many CDH survivors show poor growth during early life. Reported prevalence rates of wasting, which reflects acute malnutrition, at 1 year of age range from 8% to 69%.[48,49] The cause for poor growth in CDH patients is probably multifactorial, including increased catabolic stress in the neonatal period, gastroesophageal reflux disease, and persistent pulmonary impairment.[50] A contributing factor is oral aversion, which has been found in about one quarter of CDH infants.[50] While one study has reported catch-up growth after the first 6 months,[49] a recent longitudinal study showed that growth failure still persisted in 13.5% of patients at 6 years.[51] Adequate nutritional intake is important as malnutrition in infancy has been associated with adverse long-term outcomes such as impaired neurodevelopment in otherwise healthy children.[52,53] Currently it is unknown whether poor growth in CDH patients persists during childhood. Additionally, it is unknown whether physical growth affects neurodevelopmental outcomes of CDH patients at all.

Neurodevelopmental and neuropsychological morbidity

Neurodevelopmental dysfunction is considered one of the most significant morbidities among CDH survivors. Neurodevelopmental problems seem to vary with age and widely varying outcomes have been reported. Danzer et al.[54] reported mildly and severely delayed neurocognitive and language scores in 17% and 15%, respectively, of 41 CDH patients at the median age of 24 months. Psychomotor development was mildly and severely delayed in 23% and 31%, respectively.[54] However, Gischler et al.[55] found normal mental development scores in 12 CDH patients at 24 months of age, but psychomotor development scores were also delayed in this cohort. Neurocognitive and motor problems have also been found at school age. While Danzer et al.[56] reported normal intelligence quotient scores and normal academic achievement sores in 35 CDH patients at 5 years of age, visual-motor-integration scores of these children were lower than those of the norm population. Further, more of them had reactive (23%) or pervasive developmental problems (27%) compared with the norm population.[56] Madderom et al.[57] likewise found a normal intelligence in a cohort of 35 CDH patients at 8 years of age, whereas the proportions of patients with concentration and behavioral attention problems were higher than the norm population. Currently, we do not know which specific neuropsychological domains are most affected in CDH patients

at school age. Worse still, we do not know which patients are most at risk of long-term impairments and neither do we know whether severity of illness scores or early growth parameters are predictive of neuropsychological outcome at this age.

Conclusion

Advanced postnatal treatment options have led to improved survival of CDH patients, even of those who have severe pulmonary hypoplasia and pulmonary hypertension. These CDH survivors seem to carry a high but varying risk of developing postnatal morbidities. An increasing number of institutions in different countries now acknowledge long-term longitudinal follow-up of CDH patients is needed. Currently, little is known about the development and persistence of postnatal morbidities including thyroid hormone disturbances, growth failure, and adverse neurodevelopmental outcomes in this group of CDH patients who received modern treatment strategies including HFOV, ECMO and medication for pulmonary hypertension. Further, the evidence in the current literature on risk factors for postnatal morbidities as well as its consequences is not conclusive.

Outline of this thesis

The aim of this thesis is to evaluate the postnatal risks and outcomes of CDH patients both at the short-term and long-term, and to identify possible risk factors associated with outcomes. The reported studies included CDH patients treated and followed in the Erasmus MC-Sophia Children's Hospital in Rotterdam, the Netherlands (chapters 2, 3, 4, 7, and 8) and the Westmead Children's Hospital in Sydney, Australia (chapters 5 and 6). The outline of this thesis is schematically shown in figure 2.

Chapter 2 describes the incidence and consequences of aberrant screening results for congenital hypothyroidism in ECMO-treated CDH patients and other ECMO-treated neonates. Additionally, we determine associations between clinical variables and an aberrant congenital hypothyroidism screening result, as well as the association between congenital hypothyroidism screening results and mortality.

Chapter 3 describes the disturbances in thyroid hormone levels during neonatal ECMO for CDH and other life-threatening conditions requiring ECMO. Further, the neuropsychological outcome at 2 years of age is assessed.

Chapter 4 reports the prevalence of persistent pulmonary hypertension in CDH patients during the first year of life. Further, we compare characteristics of patients with persistent pulmonary hypertension with patients without persistent pulmonary hypertension, and give advice about the follow-up of pulmonary hypertension.

Chapter 5 describes the physical growth during the first year of life of CDH patients. Risk factors for failure to thrive are also determined.

Chapter 6 assesses the neurodevelopmental outcome at 1 year and 3 years of age of CDH patients. Neurodevelopmental outcomes of CDH patients are compared with a healthy age-matched control group.

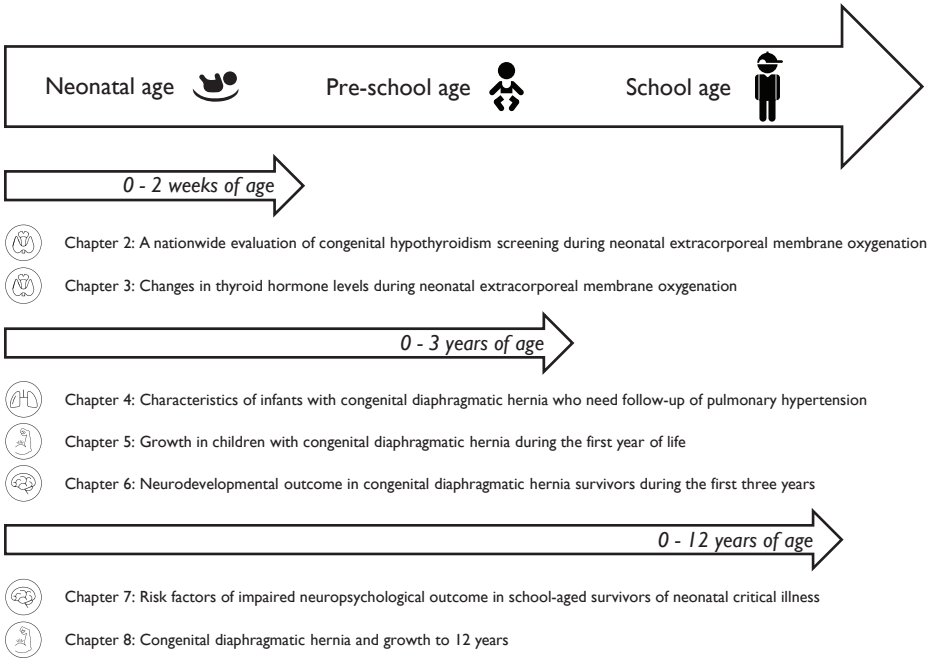
Chapter 7 describes the neuropsychological outcomes at 8 years of age of patients with CDH and patients treated with neonatal ECMO following other diagnoses. Further, associations between neuropsychological outcomes and early physical growth as well as severity of illness are determined.

Chapter 8 describes physical growth of CDH patients until 12 years of age. Further, associations between physical growth and clinical variables are investigated. Potential causes of poor growth are identified through dietary consultations with indirect calorimetry measurements in a subset of patients with growth failure.

In **chapter 9** the study results are discussed and put into perspective.

The results of all studies are summarized in English (**chapter 10**) and Dutch (**chapter 11**).

Figure 2. Schematic overview of the chapters in this thesis showing which studies were performed at the different follow-up ages



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Chapter 2

Nationwide evaluation of congenital hypothyroidism screening during neonatal extracorporeal membrane oxygenation

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Neonatology 2017; 111: 93-99

Abstract

Background: Thyroid hormone concentrations may deviate from normal values during critical illness. This condition is known as non-thyroidal illness syndrome (NTIS), and it can influence the results of screening for congenital hypothyroidism (CH) during neonatal extracorporeal membrane oxygenation (ECMO).

Objectives: To determine the incidence of aberrant CH screening results in ECMO-treated neonates, to identify possible determinants, and to follow up patients with abnormal thyroid hormone concentrations.

Methods: In this retrospective cohort study, we included 168 ECMO-treated neonates admitted from 2004 to 2014 and screened by protocol and divided them into the following 3 groups: group 1 (screened during ECMO, n=107), group 2 (screened shortly before ECMO, n=26), and group 3 (screened shortly after ECMO, n=35).

Results: CH screening results were aberrant in 67.3% (72/107) of the neonates screened during ECMO, in 73.1% (19/26) of the neonates screened before ECMO, and in 31.4% (11/35) of the neonates screened after ECMO ($p<0.001$). Of the neonates with an aberrant screening result, all but 2 (i.e. 98%) had a low thyroxine concentration with a normal thyrotropin concentration at screening, as is seen in NTIS. None was diagnosed with CH. Mortality did not significantly differ between neonates with an aberrant screening result (32.4%) and neonates with a normal screening result (22.7%; $p=0.18$). Screening before ECMO (odds ratio: 5.92; 95% confidence interval: 1.93-18.20), screening during ECMO (odds ratio: 4.49; 95% confidence interval: 1.98-10.19), and a higher Pediatric Logistic Organ Dysfunction-2 score (odds ratio: 1.31; 95% confidence interval: 1.04-1.66) were associated with an aberrant screening result.

Conclusions: Aberrant CH screening results were found in most ECMO-treated neonates screened before or during ECMO, which is likely due to NTIS. Follow-up of thyroid hormone concentrations is best started after recovery from critical illness. Our results suggest that thyroxine therapy is not required during ECMO.

Introduction

As thyroid hormones are essential for normal brain development, children with congenital hypothyroidism (CH) are at risk for intellectual disability.[1] CH screening and early treatment have almost eliminated intellectual disabilities caused by untreated CH. In the Netherlands, CH screening is primarily based on thyroxine (T4) screening with supplementary thyrotropin (TSH) and thyroxine-binding globulin measurements aimed at identifying not only children with primary hypothyroidism but also children with central hypothyroidism.[2] However, interpretation of the screening results can be complicated by factors that can transiently reduce thyroid hormone concentrations, such as prematurity,[3] drug use,[4] cardiac surgery,[5] and critical illness.[6] This condition of disturbed thyroid hormone concentrations (low T4 and low triiodothyronine, increased reverse triiodothyronine, and normal or low TSH) during critical illness is referred to as non-thyroidal illness syndrome (NTIS) or euthyroid sick syndrome. It is important to distinguish between CH and NTIS because CH therapy should be initiated as soon as possible, whereas treatment for NTIS is not recommended.[6] In previous studies critically ill neonates have had lower thyroid hormone concentrations than healthy neonates,[7-9] and this has been associated with a prolonged hospital stay and a higher mortality.[7, 9] Low thyroid hormone concentrations consistent with NTIS have been found in neonates on extracorporeal membrane oxygenation (ECMO).[10] However, it is unknown whether ECMO exerts an influence on CH screening results and whether there are any implications of a positive CH screening result during ECMO.

We therefore wanted to determine the incidence of aberrant CH screening results in ECMO-treated neonates, identify possible determinants of aberrant screening results, and follow up neonates with abnormal thyroid hormone concentrations.

Methods

Patients

In this retrospective cohort study, we included ECMO-treated neonates admitted between January 1, 2004, and December 31, 2014, who underwent CH screening in either of the two pediatric ECMO centers in the Netherlands (i.e. the Erasmus MC-Sophia Children's Hospital, Rotterdam and the Radboud UMC-Amalia Children's Hospital, Nijmegen). These centers use the same entry criteria and treatment protocols, but the prime fluid is different: erythrocyte concentrate is used in the Erasmus MC-Sophia Children's Hospital, and erythrocyte concentrate with fresh frozen plasma (FFP) is used in the Radboud UMC-Amalia Children's Hospital. ECMO was initiated in case of reversible severe respiratory failure with an estimated mortality risk >80% as described by Stolar et al.[11] The exclusion criteria were: screening not in conformity with the Dutch neonatal screening protocol, incomplete or no screening results available, no registration in the Dutch civil registry (not invited for screening), and ECMO initiated after 8 days of life (not critically ill at the scheduled time of screening).

We created the following 3 groups: group 1 (screened during ECMO), group 2 (screened shortly before ECMO), and group 3 (screened shortly after ECMO). Neonates in groups 2 and 3 were considered critically ill at screening, but they did not receive ECMO at that time. CH screening results were obtained from the National Institute for Public Health and Environment (RIVM). In the Netherlands, CH screening is primarily based on T4 measurements by immunochemical blood spots sampled 72-168 hours after birth. The method is described in detail in the supplementary material. We classified the initial screening results as either normal or aberrant (abnormal or borderline result on the first heel puncture). In neonates with an aberrant result, the pattern of changes in thyroid hormone concentrations was classified into 3 categories: abnormally low T4 (≤-3.0 standard deviation score (SDS)) with normal TSH (≤7.0 mU/l), mildly lowered T4 (-3.0< T4≤-1.6 SDS) with normal TSH (≤7.0 mU/l), and mildly lowered T4 (-3.0<T4≤-1.6 SDS) with mildly elevated TSH (7.0<TSH≤21.0 mU/l). Relevant clinical data were collected. The primary diagnosis was defined as the underlying diagnosis requiring ECMO support. Illness severity was estimated using the Pediatric Risk of Mortality III (PRISM III) score,[12] calculated for the first 24 hours of pediatric intensive care unit (PICU) stay, and the Pediatric Logistic Organ Dysfunction-2 (PELOD-2) score,[13] computed for the 24 hours prior to the first heel puncture. Povidoneiodine and radiocontrast materials were not used.

Ethical approval was obtained from the local ethics committees (CMO 2005/253).

Follow-up of neonates with abnormal thyroid hormone concentrations

Follow-up serum free T4 (FT4) and TSH concentrations were obtained from the medical charts of neonates with an abnormal CH screening result. FT4 and TSH were compared to the reference values established by Lem et al.[14] An abnormal value was defined as <-2 SDS or >+2 SDS compared to the reference values.

Data analysis

Data are expressed as means±standard deviation, medians (interquartile range), or numbers (%), as appropriate. Categorical variables were compared using the chi-square test or Fisher's exact test. Student's t-test or a one-way analysis of variance was used for normally distributed variables, and the Mann-Whitney U test or the Kruskal-Wallis test was applied for non-normally distributed variables. To correct for multiple testing, the Bonferroni correction was used, and adjusted p-values are given. Univariate logistic regression analyses served to identify associations between clinical variables (primary diagnosis, time of screening (before/during/after ECMO), PRISM III score, PELOD-2 score, and drug usage) and aberrant screening results. Use of the following intravenous drugs ≤8 hours prior to screening was recorded: dopamine, dobutamine, furosemide, morphine, fentanyl, and hydrocortisone. From a few hours after administration, these drugs can temporarily affect thyroid hormone concentrations; pretreatment levels are reached within hours after discontinuation of the drug.[4, 15-19] Variables with $p \leq 0.20$ were included in the multiple logistic regression analysis. Multicollinearity was not found.

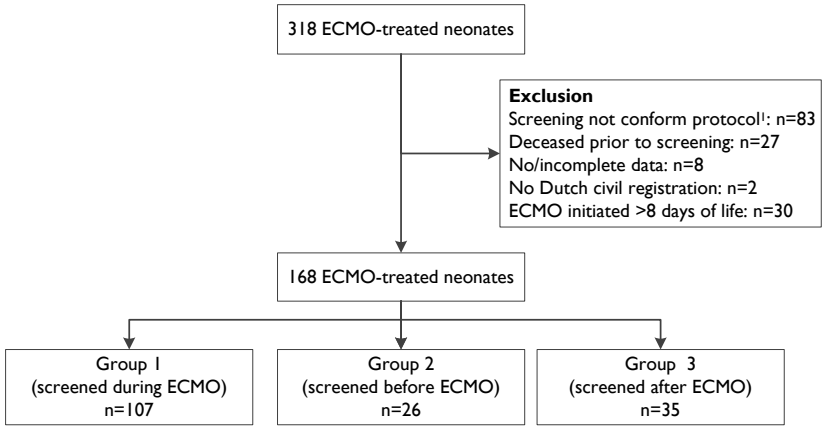
Data were analyzed using SPSS 21.0 for Windows (IBM Corporation, Armonk, New York, USA). Statistical significance was accepted at the 5% level.

Results

Patients

ECMO was initiated in 318 neonates (figure 1). We included 168 neonates divided into 3 groups. Table 1 shows the patient characteristics. In group 3, ECMO was started later, and CH screening was performed later. Further, the duration of ECMO therapy was shorter in group 3. The median PELOD-2 score was higher in group 1 than in group 3 (adjusted p=0.045). Eight patients received levothyroxine therapy (supplementary table 2). Three of them had a normal initial screening result and developed hypothyroxinemia later, which was identified by routine laboratory tests. One patient was diagnosed with thyroid dyshormonogenesis and required lifelong treatment.

Figure 1. Flowchart of the study population



[1] Screening performed too early: n=1; Screening performed too late: n=82.
Abbreviation: ECMO=extracorporeal membrane oxygenation.

Congenital hypothyroidism screening results

Table 2 shows the screening results. The incidence of aberrant results was higher in group 1 (67.3%) and group 2 (73.1%) than in group 3 (31.4%; both adjusted p<0.001). At the initial screening, the combination of low T4 and normal TSH was found in 100 of 102 neonates (table 2). Neonates with an aberrant screening result were treated longer with ECMO and had higher PELOD-2 scores than those with a normal screening result (supplementary table 3). The incidence of aberrant screening results in group 1 in both centers was similar and thus irrespective of the use of FFP in the prime fluid (67.2% with FFP versus 67.3% without FFP; p=1.00). The proportion of aberrant screening results in group 1 was not dependent on the time elapsed between the start of ECMO and blood sampling for CH screening (data not shown).

Table 1. Patient characteristics

	Group 1 n=107	Group 2 n=26	Group 3 n=35	p-value
Gestational age (weeks)	39.9 (38.1-41.0)	40.0 (37.0-41.0)	40.4 (39.0-41.1)	0.21
Birth weight (grams)	3247±631	3256±704	3541±595	0.06
Male	62 (57.9%)	17 (65.4%)	20 (57.1%)	0.77
Primary diagnosis				0.08
Meconium aspiration syndrome	41 (38.3%)	9 (34.6%)	17 (48.6%)	
Congenital diaphragmatic hernia	35 (32.7%)	6 (23.1%)	5 (14.3%)	
Sepsis	11 (10.3%)	3 (11.5%)	5 (14.3%)	
Persistent pulmonary hypertension	10 (9.3%)	6 (23.1%)	8 (22.9%)	
Other[1]	10 (9.3%)	2 (7.7%)	0 (0%)	
Age at start ECMO therapy (day of life)	1 (0-2)	6 (5-7)	1 (0-1)	<0.001
Duration of ECMO therapy (days)	7 (5-9)	6 (4-7)	3 (2-4)	<0.001
PRISM III score (first 24 hours of PICU admission)	17 (15-23)	17 (9-21)	17 (12-21)	0.12
Age at CH screening (day of life)	4 (4-5)	4 (4-4)	6 (5-7)	<0.001
PELOD-2 score (24 hours prior to CH screening)	6 (6-7)	5 (5-7)	6 (5-7)	0.03

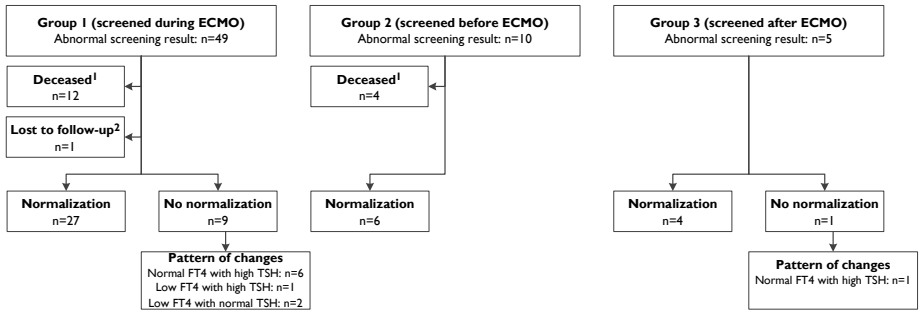
Data are expressed as mean±standard deviation, median (interquartile range) or number (percentage), as appropriate.
[1] Other: congenital heart disease; congenital cystic adenomatoid malformation; recurrent pneumothoraces; infant respiratory distress syndrome in combination with pneumothorax; meconium peritonitis; respiratory failure of unknown cause.
Abbreviations: CH=congenital hypothyroidism; ECMO=extracorporeal membrane oxygenation; PELOD-2=Pediatric Logistic Organ Dysfunction-2; PICU=pediatric intensive care unit; PRISM III=Pediatric Risk of Mortality III.

Table 2. Congenital hypothyroidism screening results

	Group 1 (n=107)	Group 2 (n=26)	Group 3 (n=35)
Initial screening: normal screening result → no further action	35 (32.7%)	7 (26.9%)	24 (68.6%)
Initial screening: abnormal screening result → referral[1]	42 (39.3%)	10 (38.5%)	5 (14.3%)
Pattern of changes in thyroid hormone concentrations			
Abnormally low T4; normal TSH[2]	42	10	5
Initial screening: dubious screening result → second screening	30 (28.0%)	9 (34.6%)	6 (17.1%)
Pattern of changes in thyroid hormone concentrations			
Mildly lowered T4; normal TSH[3]	28	9	6
Mildly lowered T4; mildly elevated TSH[4]	2	-	-
Second screening: died before second screening	1	0	0
Second screening: normal screening result → no further action	22	9	6
Second screening: abnormal screening result → referral[1]	7	0	0
Pattern of changes in thyroid hormone concentrations			
Abnormally low T4; normal TSH[2]	1	-	-
Mildly lowered T4; normal TSH[3]	2	-	-
Mildly lowered T4; mildly elevated TSH[4]	4	-	-

[1] Referral=referral to pediatrician for further analysis.
[2] T4≤-3.0 SDS and TSH≤7.0 mU/l.
[3] -3.0<T4≤ -1.6 SDS and TSH≤7.0 mU/l.
[4] -3.0<T4≤ -1.6 SDS and 7.0<TSH≤21.0 mU/l.
Abbreviations: ECMO=extracorporeal membrane oxygenation; SDS=standard deviation score; T4=thyroxine; TSH=thyrotropin.

Figure 2. Flowchart: follow-up of neonates with an abnormal congenital hypothyroidism screening result



[1] Deceased before follow-up of thyroid hormone concentrations was performed.
[2] Transferred to another hospital.
Abbreviations: ECMO=extracorporeal membrane oxygenation; FT4=free thyroxine; TSH=thyrotropin.

Follow-up of neonates with abnormal thyroid hormone concentrations

The follow-up results are shown in figure 2. Sixteen neonates died before the follow-up was initiated, and 1 neonate was transferred to another hospital. Thyroid hormone concentrations normalized in 37 neonates at the median age of 10 days (interquartile range: 8-17.5). In 29 of those neonates, this happened before 28 days of life; thyroid hormone concentrations normalized in the other 8 neonates between 30 and 108 days of life. Normalization of thyroid hormone concentrations could not be confirmed in 10 children (figure 2). At the last follow-up, low FT4 values ranged from 12.0 to 12.7 pmol/l and high TSH values ranged from 5.7 to 14.9 mU/l in these children.

Aberrant congenital hypothyroidism screening results and mortality

The mortality rate before hospital discharge was 28.6% (48/168). The mortality rate was not significantly higher in children with an aberrant CH screening result (32.4%) than in children with a normal screening result (22.7%; p=0.18).

Associations between clinical variables and an aberrant screening result

In the univariate logistic regression analyses, screening before ECMO, screening during ECMO, and a higher PELOD-2 score were significantly associated with an aberrant screening result (table 3). In the multivariate model, screening before and during ECMO remained associated with an aberrant screening result.

Table 3. Results of logistic regression analyses: clinical variables associated with an aberrant congenital hypothyroidism screening result

	Univariate model OR (95% CI)	p-value	Multivariate model[1] aOR (95% CI)	p-value
Time of screening				
After ECMO (ref)	1.00	-	1.00	-
Before ECMO	5.92 (1.93-18.20)	0.002	4.59 (1.11-18.93)	0.04
During ECMO	4.49 (1.98-10.19)	<0.001	3.19 (1.22-8.32)	0.02
PELOD-2 score	1.31 (1.04-1.66)	0.02	1.27 (0.99-1.63)	0.06
Primary diagnosis				
Meconium aspiration syndrome (ref)	1.00	-	1.00	-
Congenital diaphragmatic hernia	1.78 (0.82-3.89)	0.15	1.41 (0.60-3.34)	0.44
Others	1.51 (0.73-3.13)	0.27	1.34 (0.56-3.16)	0.51
Dopamine				
No (ref)	1.00	-	1.00	-
Yes	1.63 (0.84-3.18)	0.15	1.01 (0.46-2.21)	0.98
Morphine				
No (ref)	1.00	-	1.00	-
Yes	1.98 (0.75-5.19)	0.17	1.60 (0.54-4.73)	0.40

[1] adjusted for all other variables shown in the table.

Abbreviations: aOR=adjusted odds ratio; CI=confidence interval; ECMO=extracorporeal membrane oxygenation; OR=odds ratio; PELOD-2=Pediatric Logistic Organ Dysfunction-2; ref=reference category.

Discussion

More than two thirds of the initial CH screening results were aberrant in ECMO-treated neonates screened before and during ECMO. Low T4 in combination with a normal TSH concentration was found in all but two neonates with an aberrant screening result, which normalized rapidly without treatment in most cases. None of these children was diagnosed with CH. This suggests that aberrant screening results are due to NTIS, and that T4 therapy during ECMO is not required. The incidence of aberrant screening results in the group of neonates screened after ECMO was significantly lower than that in the two other groups, possibly reflecting that clinical recovery occurs during ECMO.

Reduced thyroid hormone concentrations have been found in critically ill term neonates treated with or without ECMO.[7-10] The effect of neonatal ECMO on thyroid hormone concentrations has been studied only by Stewart et al.[10] They found a decline in all thyroid hormone concentrations directly after the start of ECMO in a cohort of 14 neonates, which suggests a dilutional effect. Additionally, Agus and Jaksic[20] reported that thyroid hormone concentrations in ECMO prime fluid were significantly below normal. They suggested that these concentrations would be even lower in prime fluid without FFP.[20] However, in the present study the incidence of aberrant screening results was not higher in the center without FFP in the prime fluid. Although we acknowledge that the occurrence of hemodilution after ECMO cannulation plays a role in the lowering of thyroid hormone concentrations, we think that NTIS contributes more to this, and that hemodilution may further lower thyroid hormone concentrations in critically ill neonates. This assumption is supported by our finding that the incidences of aberrant screening results did not significantly differ between screening before (73.1%) and during (67.3%) ECMO.

Screening before and during ECMO and a higher PELOD-2 score were associated with an aberrant screening result. Further, neonates with an aberrant screening result had higher PELOD-2 scores than neonates with a normal screening result, suggesting an influence of illness severity. Nevertheless, the use of the PELOD-2 score has some limitations. Though validated for a broad group of PICU patients,[13] its performance in ECMO-treated neonates is unknown. Further, the PELOD-2 score is affected by treatment. Therefore, we cannot firmly conclude that illness severity plays a role in causing aberrant screening results.

Dopamine has been found to suppress TSH in previous studies,[17,19] but we did not find an association between dopamine and an aberrant screening result. Therefore, dopamine does not seem to be the main cause of aberrant CH screening results. However, our study does not allow pinpointing of the exact effect of dopamine on thyroid hormone concentrations.

Now, clinicians will be most concerned with the implications of aberrant CH screening results. As we found a lower risk of aberrant screening results in neonates screened after ECMO, follow-up of thyroid hormone concentrations should be started after ECMO and ideally after recovery from critical illness. However, because T4 treatment should not be

started later than the first two weeks of life to prevent intellectual disabilities,[21] we recommend that thyroid function tests should be repeated before this age.

The consequences of low thyroid hormone concentrations during critical illness remain controversial. Correlations between low thyroid hormone concentrations and adverse outcomes have been found, including increased mortality.[8, 9, 22] We did not find a significant difference in mortality rates between neonates with an aberrant screening result and neonates with a normal screening result. However, it should be noted that our sample size was small, and the results could have been biased by the lack of screening results for the 27 neonates who died before screening was performed.

As thyroid hormones are essential for early brain development, transiently reduced thyroid hormone concentrations during early life could affect neurodevelopment, which has been shown in premature infants.[23, 24] In that case, supplementation of thyroid hormones during NTIS could be beneficial. On the other hand, a recent study in young adults did not confirm this association between transient hypothyroxinemia of prematurity and adverse neurodevelopmental outcomes.[25] Furthermore, neonatal T4 supplementation was not beneficial in premature infants.[26] As T4 therapy has not been proven to be beneficial, and thyroid hormone concentrations normalized rapidly in most cases, we do not advise starting T4 therapy in ECMO-treated neonates based on an aberrant screening result in the first two weeks of life.

To our knowledge, this is the first study reporting on CH screening results in ECMO-treated neonates with follow-up of thyroid function. A strength is the use of an efficient CH screening method. Further, this is the largest cohort study investigating CH screening results in ECMO-treated neonates. Limitations are its retrospective design, its small sample size, and the lack of a strict schedule to follow-up abnormal thyroid hormone concentrations. The small sample size does not allow drawing of a strong conclusion about the influence of the time of sampling during ECMO on screening results. Further, the exact date of normalization in some patients with abnormal thyroid hormone levels remains uncertain.

In conclusion, we found a higher incidence of aberrant CH screening results in neonates screened before (73.1%) or during (67.3%) ECMO than in neonates screened after (31.4%) ECMO. All but two neonates (i.e. 98%) with an aberrant screening result had low T4 concentrations with normal TSH, as is seen in NTIS. Thyroid hormone concentrations normalized rapidly in most, and none of these children was diagnosed with CH. Our results suggest that T4 therapy is not required during ECMO. Follow-up of abnormal thyroid hormone concentrations is best performed after recovery from critical illness.

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Supplements

Supplementary methods. Dutch neonatal screening method

In the Netherlands, congenital hypothyroidism screening is primarily based on thyroxine (T4) measurement by immunochemical assay in blood spots sampled 72 to 168 hours after birth. The concentration of T4 is compared with the day mean, and expressed as a standard deviation score (SDS). If T4 is >-0.8 SDS, the result is normal. If T4 is ≤-0.8 SDS, the thyrotropin (TSH) concentration (expressed in mU/l blood) is additionally measured. If T4 is ≤-1.6 SDS, the thyroxine-binding globulin (TBG) concentration (expressed in nmol/l blood) is additionally measured. A T4/TBG ratio is calculated as follows: ((T4 SDS+5.1)x1,000)/TBG. If T4 is ≤-3.0 SDS and TBG is >40.0 nmol/l or TSH is >21.0 mU/l, the screening result is abnormal, and referral to a pediatrician is indicated (supplementary table 1). Before July 2012, a T4≤-3.0 SDS was considered as abnormal and in that case TBG was not determined. Since July 2012, TBG is always determined if T4≤-1.6 SDS. A result indicating TBG deficiency (T4≤-3.0 SDS and TBG≤40 nmol/l) is considered as normal. In case of a borderline result (-3.0<T4≤-0.8 SDS in combination with a T4/TBG ratio≤17 or 7<TSH≤21 mU/l), a second heel puncture is performed. If the second screening result is abnormal or borderline again, the result is considered as abnormal, and referral is indicated.

The referral criterion for premature neonates born ≤36.0 weeks gestation or with a birth weight of ≤2500 grams is based on TSH.

Supplementary table 1. Interpretation of the Dutch screening procedure for congenital hypothyroidism

	TSH >21.0 mU/l	**7.0<TSH≤21.0 mU/l**	**TSH≤7.0 mU/l**
T4 ≤-3.0 SDS and TBG >40.0 nmol/l	Abnormal: referral[2]	Abnormal: referral[2]	Abnormal: referral[2]
-3.0<T4≤-1.6 SDS and T4/TBG ratio≤ 17.0[1]	Abnormal: referral[2]	Borderline: repeat[3]	Borderline: repeat[3]
-3.0<T4≤-1.6 SDS and T4/TBG ratio>17.0[1] OR T4≤-3.0 SDS and TBG≤40.0 nmol/l OR T4>-1.6 SDS	Abnormal: referral[2]	Borderline: repeat[3]	Normal: no action

[1] T4/TBG ratio = ((T4 SDS+5.1)x1,000)/TBG.

[2] Referral: referral to pediatrician for further analysis.

[3] Repeat: repeat screening.

Abbreviations: SDS=standard deviation score; T4=thyroxine; TBG=thyroxine-binding globulin; TSH=thyrotropin.

Supplementary table 2. Patient characteristics of neonates treated with levothyroxine

	Primary diagnosis	Initial screening result	Cause of low thyroxine	L-T4 treatment (days)	Outcome
Patient 1	Congenital diaphragmatic hernia	Normal	Non-thyroidal illness syndrome	3	Death
Patient 2	Congenital diaphragmatic hernia	Normal	Non-thyroidal illness syndrome	61	Survived
Patient 3	Congenital diaphragmatic hernia	Abnormal	Non-thyroidal illness syndrome	4	Death
Patient 4	Congenital diaphragmatic hernia	Abnormal	Non-thyroidal illness syndrome	46	Survived
Patient 5	Congenital diaphragmatic hernia	Borderline	Non-thyroidal illness syndrome	1	Death
Patient 6	Congenital diaphragmatic hernia	Abnormal	Octreotide treatment for chylothorax	40	Survived
Patient 7	Meconium aspiration syndrome	Abnormal	Octreotide treatment for chylothorax	24	Survived
Patient 8	IRDS with pneumothorax	Normal	Thyroid dyshormonogenesis	Lifelong	Death

Abbreviation: IRDS=infant respiratory distress syndrome; L-T4=levothyroxine; NTIS=non-thyroidal illness syndrome.

Supplementary table 3. Patient characteristics of neonates with aberrant versus normal screening results

	Aberrant screening result n=102	Normal screening result n=66	p-value
Gestational age (weeks)	39.9 (38.1-41.0)	40.3 (38.3-41.2)	0.37
Birth weight (grams)	3311±611	3310±694	1.00
Male	66 (64.7%)	33 (50.0%)	0.06
Primary diagnosis			0.30
Meconium aspiration syndrome	36 (35.3%)	31 (47.0%)	
Congenital diaphragmatic hernia	31 (30.4%)	15 (22.7%)	
Sepsis	11 (10.8%)	8 (12.1%)	
Persistent pulmonary hypertension	18 (17.6%)	6 (9.1%)	
Other[1]	6 (5.9%)	6 (9.1%)	
Age at start ECMO therapy (day of life)	3 (2-4)	2 (1-3)	0.06
Duration of ECMO therapy (days)	6 (4-8)	5 (3-6)	0.001
PRISM III score (first 24 hours of PICU admission)	18±6.4	17±7.2	0.32
Age at CH screening (day of life)	4 (4-5)	4 (4-5)	0.32
PELOD-2 score (24 hours prior to CH screening)	6 (6-8)	6 (5-7)	0.02

Data are expressed as mean±standard deviation, median (interquartile range) or number (percentage), as appropriate.

[1] Other: congenital heart disease; congenital cystic adenomatoid malformation; recurrent pneumothoraces; infant respiratory distress syndrome in combination with pneumothorax; meconium peritonitis; respiratory failure of unknown cause.

Abbreviations: CH=congenital hypothyroidism; ECMO=extracorporeal membrane oxygenation; PELOD-2=Pediatric Logistic Organ Dysfunction-2; PICU=pediatric intensive care unit; PRISM III=Pediatric Risk of Mortality III.

Chapter 3

Changes in thyroid hormone concentrations during neonatal extracorporeal membrane oxygenation

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Abstract

Objective: Thyroid hormone concentrations can be disturbed during critical illness. Our aim was to determine changes in thyroid hormone concentrations during neonatal extracorporeal membrane oxygenation (ECMO).

Methods: We included 21 ECMO-treated neonates. Age-specific standard deviation scores (SDS) of free and total thyroxine (FT4; TT4), reverse and total triiodothyronine (rT3; TT3), thyroid-stimulating hormone (TSH) and thyroxine-binding globulin (TBG) were determined at six fixed time-points. Data were analyzed using general linear models.

Results: At baseline, mean SDS FT4 (-0.78, 95% confidence interval: -1.37 to -0.19), TT4 (-1.97, 95% confidence interval: -2.76 to -1.18), TT3 (-0.88, 95% confidence interval: -1.13 to -0.63), TSH (-2.14, 95% confidence interval: -2.93 to -1.35) and TBG (-3.52, 95% confidence interval: -4.55 to -2.50) were low with high mean SDS rT3 (0.53, 95% confidence interval: 0.28 to 0.78). One hour after start ECMO, TT4, TSH and TBG had further declined; 12 hours after start ECMO TT3 had declined (all $p<0.05$). After this decline, mean SDS TSH increased to the baseline level 12 hours after start ECMO (-2.50, 95% confidence interval: -3.22 to -1.79), and was higher than baseline 48 hours after start ECMO (-0.56, 95% confidence interval: -1.29 to 0.17). This TSH increase was followed by increases in TT4 and TT3. FT4 remained constant within the normal range during ECMO.

Conclusions: Thyroid hormone concentrations before ECMO were suggestive of non-thyroidal illness syndrome. During ECMO, increases in TSH, TT4 and TT3 after an initial decline possibly reflect spontaneous restoration of the hypothalamic–pituitary–thyroid axis. FT4 remained constant within the normal range. This suggests that thyroxine therapy is not required during ECMO.

Introduction

Thyroid hormone concentrations can be affected by several factors including gestational age,[1] cardiac surgery,[2,3] use of drugs such as dopamine,[4] and critical illness.[5-7] Critically ill patients typically present with lower concentrations of total triiodothyronine (TT3) and total thyroxine (TT4), increased reverse triiodothyronine (rT3) and low to normal thyroid-stimulating hormone (TSH) concentrations, which is known as non-thyroidal illness syndrome (NTIS) or euthyroid sick syndrome.[5-7] The magnitude of changes in TT3 and TT4 concentrations has been related to the severity of illness. Both low TT3 and low TT4 have been associated with adverse patient outcomes such as increased mortality in neonatal, pediatric and adult patients.[8-14]

Recently we reported that 67.3% of neonates screened during extracorporeal membrane oxygenation (ECMO) had an aberrant screening result for congenital hypothyroidism.[15] The one study so far on thyroid hormone concentrations during neonatal ECMO found a decline in all thyroid hormone concentrations in 14 critically ill neonates directly after the start of ECMO.[16] It is not known, however, whether and how thyroid hormone concentrations change during and after neonatal ECMO, and whether there are any potential therapeutic consequences of changes. Therefore, we determined longitudinal changes in thyroid hormone concentrations before, during and after neonatal ECMO therapy.

Methods

Patients

We included 21 neonates with a diagnosis of meconium aspiration syndrome (MAS) or congenital diaphragmatic hernia (CDH), which are the two most common indications for neonatal ECMO treatment.[17] Neonates were admitted to either of the two ECMO centers in the Netherlands (the Erasmus MC-Sophia Children's Hospital, Rotterdam or the Radboud University Medical Center-Amalia Children's Hospital, Nijmegen), both level III university hospitals. ECMO therapy was initiated in case of reversible severe respiratory failure with an estimated mortality risk of >80% as described by Stolar et al. and on the standardized CDH EURO Consortium Consensus treatment protocol.[18, 19]

Relevant clinical data were collected including gestational age, birth weight, gender, age at start ECMO, duration of ECMO, neonatal screening results for congenital hypothyroidism and survival. Severity of illness was estimated with the Pediatric Risk of Mortality III (PRISM III) score and the Pediatric Logistic Organ Dysfunction-2 (PELOD-2) score.[20, 21] PRISM III is a physiology-based score for predicting the mortality risk in pediatric intensive care unit (PICU) patients. It is calculated from the most abnormal values of 17 physiologic variables obtained in the first 24 hours of PICU care (score range: 0-74).[20] The PELOD-2 is a descriptive score that allows daily assessment of the severity of multiple organ dysfunction syndrome in PICU patients. It includes 10 variables involving five organ dysfunctions with a score range of 0-33.[21] PELOD-2 scores were calculated for the 24 hours prior to blood sampling. Use of dopamine ≤8 hours prior to blood sampling was recorded because dopamine affects thyroid hormone concentrations within the first hours after initiation of treatment, and pre-treatment concentrations are reached within hours after discontinuation.[22] None of the patients received supplemental levothyroxine at any time-point. At 2 years of age, the Bailey Scales of Infant Development -Second Edition- Dutch version was used to assess cognitive outcomes of patients using the mental development index score. The mean score of the mental development index is 100 with a standard deviation of 15.[23] Informed consent was obtained from parents. Ethical approval for this study was obtained from both local Ethics Committees (CMO 2005/253).

ECMO procedure

In the Erasmus MC-Sophia Children's Hospital, the ECMO circuit was primed with erythrocyte concentrate, Ringer's solution, albumin, Tris buffer, sodium bicarbonate, calcium gluconate and heparin. In the Radboud University Medical Center-Amalia Children's Hospital, the ECMO circuit was primed with erythrocyte concentrate, fresh frozen plasma (FFP), sodium bicarbonate and heparin. The total prime volume was approximately 350 ml in both centers. Once the circuit was fully primed, ECMO cannulation was performed. The pediatric intensivist or neonatologist and pediatric surgeon decided on the choice of ECMO modality (veno-venous or veno-arterial).

Thyroid function measurements

Blood was taken from an arterial catheter at six fixed time-points: 1 hour prior to ECMO (baseline value); 1, 12, 48 and 72 hours after start of ECMO; >24 hours after cessation of ECMO. Plain tubes were centrifuged and serum was stored at -20 °C. Free thyroxine (FT4), TT4, TT3 and TSH concentrations were determined using chemiluminescence assays (Vitros ECI; Ortho Clinical-Diagnostics, Rochester, New York, USA). rT3 was measured by radioimmunoassay (ZenTech S.A., Liége, Belgium). Thyroxine-binding globulin (TBG) was measured on an Immulite XPi system (Siemens Healthcare Diagnostics B.V., Los Angeles, California, USA). The intra- and interassay coefficients of variation were <5.4% for FT4, <6.4% for TT4, <8.7% for rT3, <4.3% for TT3, <4.1% for TSH and <6.4% for TBG. Thyroid hormone concentrations and TBG were calculated into standard deviation scores (SDS) using age-specific reference values.[24] The normal range was considered between -2 SDS and +2 SDS compared to the reference values, which are shown in supplementary table 1.

Statistical analyses

Baseline characteristics of MAS and CDH patients were compared using the Fisher's exact test for categorical variables. The independent samples t-test was used for normally distributed variables, and the Mann-Whitney U-test for continuous variables that were not normally distributed. Differences in thyroid hormone concentrations at baseline between survivors and non-survivors were compared using the Mann-Whitney U-test. We used general linear models to describe the longitudinal changes in thyroid hormone concentrations and TBG. The dependent variables in these general linear models were the SDS of FT4, TT4, rT3, TT3, TSH and TBG. The independent variables were gestational age, diagnosis, use of dopamine ≤8 hours prior to the laboratory measurement, treatment center (use of FFP) and time-point (treated as a categorical variable). A random intercept was used in the general linear models to account for the within-subject correlations. Multicollinearity was assessed by calculating correlations between independent variables. The results of the general linear models are presented using the estimated marginal means, which are the predicted values of the dependent variable adjusted for covariates in the model, and the associated 95% confidence interval. To estimate the effect of critical illness, the PELOD-2 score and survival were added as independent variables in subsequent analyses. The statistical tests were two-sided and used a significance level of 0.05. All analyses were performed using SPSS 21.0 for Windows (IBM Corporation, Armonk, New York, USA).

Results

Patients

We included 21 ECMO-treated neonates diagnosed with either MAS (n=11) or CDH (n=10). The CDH patients had a significantly lower gestational age than the MAS patients, and were all treated with veno-arterial ECMO (table 1). The CDH patients had a significantly higher PRISM III score than the MAS patients. Mental development index scores of CDH and MAS patients were within the normal range of the Dutch norm population (within 1 SDS of the mean). One MAS patient could not be tested because of severe mental disabilities due to perinatal asphyxia. Eighteen patients were screened for congenital hypothyroidism in accordance with the Dutch neonatal screening protocol (others: screening too late (n=2); screening result missing (n=1)). Two of the three patients (66.7%) screened before ECMO had an aberrant initial screening result. Seven of the 12 patients (58.3%) screened during ECMO had an aberrant initial screening result. None were diagnosed with congenital hypothyroidism. The three patients screened after ECMO all had a normal screening result. Three (14.3%) patients died from pulmonary hypertension after ECMO decannulation: two CDH patients at the age of 9 and 11 days, respectively; one MAS patient at the age of 9 days.

Table 1. Patient characteristics

Characteristics	MAS patients n=11	CDH patients n=10	p-value
Gestational age (weeks)	40.7±0.6	37.6±1.6	<0.001
Birth weight (grams)	3.38±0.37	3.00±0.62	0.10
Male	6 (54.5%)	5 (50.0%)	1.00
PRISM III score (first 24 hours of PICU admission)	13±6	21±7	0.01
Veno-arterial ECMO	4 (36.4%)	10 (100%)	0.004
Age start ECMO (days)	3 (1-4)	1 (1-2)	0.31
Duration of ECMO therapy (hours)	146±80	165±65	0.56
MDI[1]	92±8[2]	93±16[3]	0.97

Data are expressed as mean±standard deviation, median (interquartile range) or number (percentage).

[1] The Bailey Scales of Infant Development -Second Edition- Dutch version was used to assess mental development index in survivors.

2 Nine MAS patients were tested: one patient died and one patient could not be tested because of severe mental disabilities due to perinatal asphyxia.

3 Seven CDH patients were tested: two patients died and one patient was lost to follow-up

Abbreviations: CDH=congenital diaphragmatic hernia; ECMO=extracorporeal membrane oxygenation; MAS=meconium aspiration syndrome; MDI=mental development index; PICU=pediatric intensive care unit; PRISM III=Pediatric Risk of Mortality III.

Thyroid hormone and thyroxine-binding globulin concentrations at baseline

Mean baseline values of FT4, TT4, TT3, TSH and TBG were significantly below the reference norm (SDS=0), and rT3 was significantly higher than the norm (table 2). Mean baseline values of TSH and TBG were abnormally low before ECMO. Baseline values did not differ between MAS patients and CDH patients or between patients in the two different treatment centers (data not shown). At baseline, the three non-survivors had lower median TT3 SDS (-1.32, interquartile range (IQR): -1.46 to -1.29 versus -0.85, IQR: -1.10 to -0.63) and lower median TBG SDS (-5.70, IQR: -6.17 to -4.90 versus -2.71, IQR: -3.89 to -1.69) than survivors (both p=0.03).

Table 2. Thyroid hormone and thyroxine-binding globulin concentrations at baseline compared with the reference norms

SDS of	ECMO patients n=21	p-value
FT4	-0.78 (-1.37 to -0.19)	0.01
TT4	-1.97 (-2.76 to -1.18)	<0.001
TT3	-0.88 (-1.13 to -0.63)	<0.001
rT3	0.53 (0.28 to 0.78)	<0.001
TSH	-2.14 (-2.93 to -1.35)	<0.001
TBG	-3.52 (-4.55 to -2.50)	<0.001

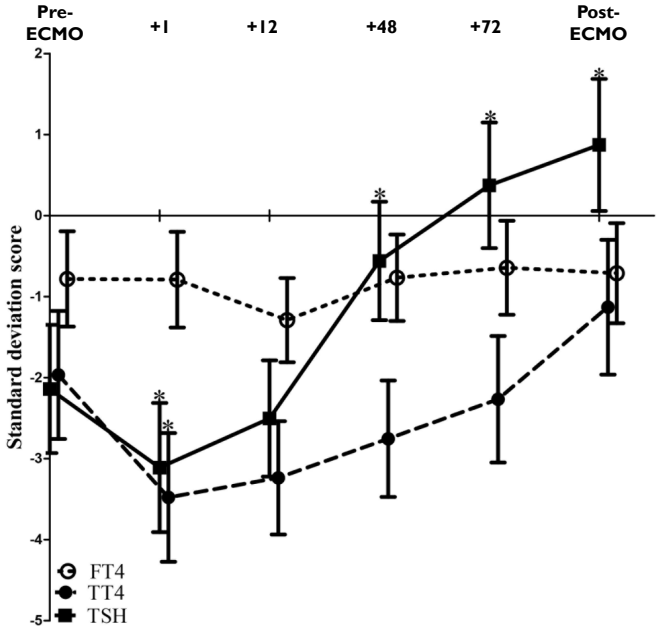
Data are presented using the estimated marginal means (associated 95% confidence interval).
Abbreviations: ECMO=extracorporeal membrane oxygenation; FT4=free thyroxine; rT3=reverse triiodothyronine; SDS=standard deviation score; TBG=thyroxine-binding globulin; TSH=thyroid-stimulating hormone; TT3=total triiodothyronine; TT4=total thyroxine.

Thyroid hormone and thyroxine-binding globulin concentrations during neonatal ECMO

Figure 1 shows the changes in thyroid hormone concentrations. One hour after start of ECMO, all thyroid hormone concentrations and TBG showed a downward slope. Mean TT4, rT3, TSH and TBG significantly declined 1 hour after start of ECMO (p=0.003, p=0.002, p=0.02, p<0.001, respectively). Mean TT3 was significantly lower than baseline 12 hours after start of ECMO (p=0.02). After this initial decline, first TSH increased to baseline 12 hours after start of ECMO. TT4, rT3, TT3 and TBG reached their baseline values 48 hours after start of ECMO. After the cessation of ECMO, TT3 and TSH concentrations were significantly higher than baseline (p=0.009, p<0.001, respectively). Mean TBG was still abnormally low after cessation of ECMO. FT4 concentrations did not significantly change during ECMO (p=0.53), and remained within the normal range.

Figure 1. Changes in thyroid hormone and thyroxine-binding globulin concentrations during neonatal extracorporeal membrane oxygenation

A. Changes in FT4, TT4 and TSH concentrations



B. Changes in rT3, TT3 and TSH concentrations

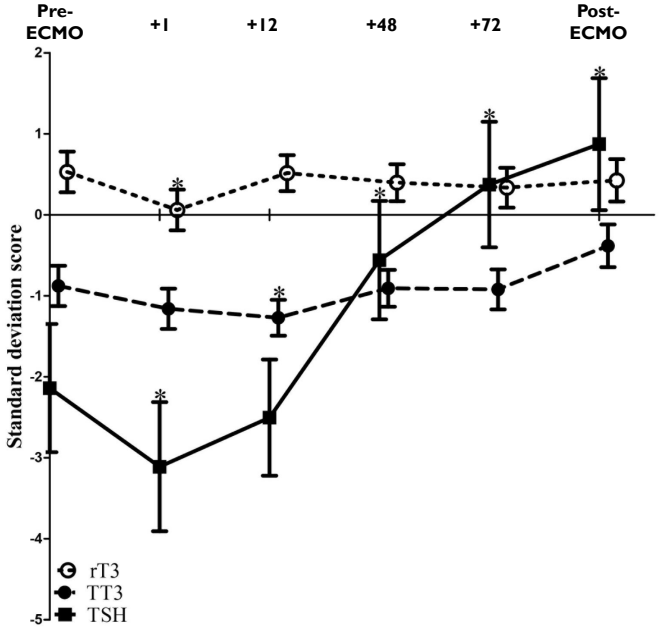
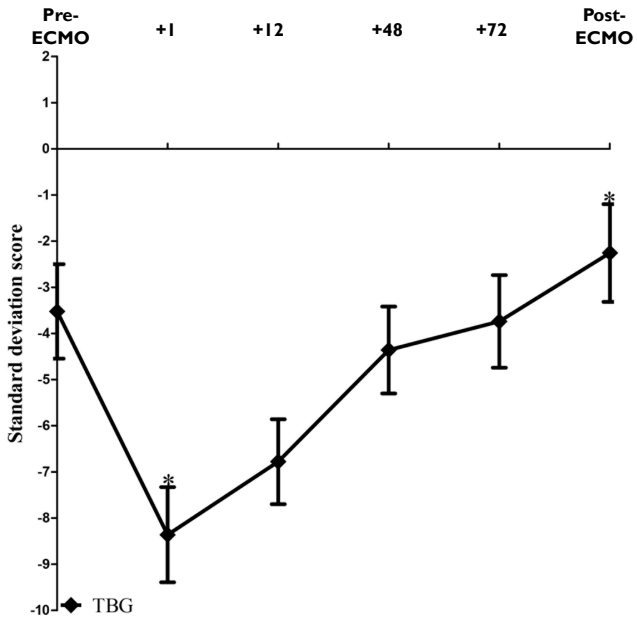


Figure 1. Continued

C. Changes in TBG concentration



Estimated marginal means with error bars representing the 95% confidence intervals were plotted. Pre-ECMO: 1 hour prior to ECMO (baseline value); +1: 1 hour after start of ECMO; +12: 12 hours after start of ECMO; +48: 48 hours after start of ECMO; +72: 72 hours after start of ECMO; Post-ECMO: >24 hours after cessation of ECMO.

* Indicates a significant change compared with the baseline value.

Abbreviations: ECMO=extracorporeal membrane oxygenation; FT4=free thyroxine; rT3=reverse triiodothyronine; TBG=thyroxine-binding globulin; TSH=thyroid-stimulating hormone; TT3=total triiodothyronine; TT4=total thyroxine.

Clinical variables associated with thyroid hormone and thyroxine-binding globulin

The estimated coefficients of time-point, gestational age, diagnosis of CDH, dopamine use and treatment center (use of FFP) on thyroid hormone concentrations are given in supplementary table 2. The numbers of children that used dopamine are shown in supplementary table 3. Time-point had a significant effect on all thyroid hormone and TBG concentrations, except FT4. Use of FFP was positively associated with TSH and TBG, but was negatively associated with FT4. A diagnosis of CDH was negatively associated with TSH. PELOD-2 score or survival had no significant associations with thyroid hormone concentrations or TBG (data not shown). Assessment of multicollinearity revealed a strong correlation between gestational age and diagnosis (r=0.85). Excluding diagnosis as a covariate in the general linear models did not considerably change the estimated associations (data not shown).

Discussion

We found that the concentrations of FT4, TT4, TT3, TSH and TBG were already low and the rT3 concentration already high in critically ill neonates prior to the start of ECMO, resembling the pattern described in NTIS.[5-7] The biologically active hormone FT4 was statistically significantly below the reference norm, but it remained within the normal range. After an initial decline directly after start ECMO, first TSH increased to a value above baseline 48 hours after ECMO. This TSH increase was followed by increases in TT4 and TT3, possibly reflecting clinical recovery with restoration of the hypothalamic–pituitary-thyroid axis.

Stewart et al.[16] studied thyroid hormone concentrations during ECMO in 14 neonates with severe respiratory failure from different causes, and also found a decline in all thyroid hormone concentrations directly after the start of ECMO. Further, a recent study in pediatric patients undergoing cardiac surgery with cardiopulmonary bypass specifically addressed the thyroid response in a subgroup of 57 neonates.[3] Similar to our results, TSH strongly declined after surgery. However, they also found a small drop in FT4. Agus et al.[25] found that low concentrations of thyroid hormones are present in ECMO prime fluid. In the current study, the prime volume was 350 ml. We think that the immediate decline of thyroid hormone concentrations can be ascribed to a dilutional effect. Based on the average birth weights in our study, we estimated that the dilutional factor in MAS patients was 55% (350/(350+287) x100%) and in CDH patients was 58% (350/(350+255)x100%). We think that this small difference in dilutional factor did not cause a significant difference in the decline of thyroid hormone levels between both groups. However, a larger difference in dilutional factor could lead to a different response. Agus et al.[25] suggested that concentrations of thyroid hormones would be higher when FFP is used in the prime fluid. Indeed we found a positive association between TSH and TBG and the use of FFP. However, this positive association was not found for TT4, rT3 and TT3, and contrary to this suggestion, we found that the use of FFPs was associated with lower FT4 levels. From this study, we cannot make a definite conclusion about the effect of the use of FFP in prime fluid on thyroid hormone levels.

Hemodilution may not be the only explanation for this decline in thyroid hormone concentrations. The severe decline in TBG concentration could also be explained by a global inflammatory response with consequent capillary leakage, which occurs during the initial phases of cardiopulmonary bypass and ECMO.[26-28] This capillary leak allows plasma proteins to move from the intravascular to the extravascular space, which lowers plasma protein concentrations.

Other factors contributing to the changes in thyroid hormone concentrations may be the severity of critical illness and use of dopamine. Previous studies in neonates have found a relation between low TT3 and low TT4 and adverse patient outcomes such as increased mortality, longer duration of mechanical ventilation and longer hospital stay.[12-14] Therefore, we expected thyroid hormone concentrations to be lower in neonates with a greater severity of

illness. Indeed, we found lower concentrations of TT3 and TSH at baseline in non-survivors. However, we did not find an association between the PELOD-2 score or survival and thyroid hormone concentrations in the general linear model. This could be due to the relatively small sample size. Secondly, although the PELOD-2 score has been validated for PICU patients,[21, 29] the score is affected by treatment such as ventilator settings and use of vasoactive drugs. Therefore, it does not solely reflect the severity of illness. Possibly, the PELOD-2 score does not distinguish enough between treatment effects and severity of illness in this specific group of ECMO-treated neonates. Furthermore, dopamine could have played a role, as it has been found to suppress TSH concentrations.[22] Dopamine could be discontinued in most patients during ECMO, and we think this indeed played a role in the TSH increase during ECMO. However, we did not find a significant association between dopamine and thyroid hormone concentrations, which may be due to the influence of other factors that potentially affect thyroid hormone concentrations during ECMO. In the present study, the combination of low TT3, TT4 and TSH concentrations prior to the start of ECMO indicates an aberrant feedback regulation of the hypothalamic–pituitary–thyroid axis, as has been previously described during NTIS.[5-7] During ECMO, however, first the TSH concentration significantly increased, which was later followed by an increase in TT4 and TT3. Other studies have found the same pattern of TSH, TT4 and TT3 increases at the start of restoration of the hypothalamic-pituitary–thyroid axis.[30, 31] Therefore, we think these changes in thyroid hormone concentrations during ECMO suggest clinical recovery with restoration of the hypothalamic–pituitary–thyroid axis. Therefore, we think these changes in thyroid hormone concentrations during ECMO suggest clinical recovery with restoration of the hypothalamic–pituitary–thyroid axis.

In a previous study we found a higher incidence of aberrant screening results for congenital hypothyroidism in neonates screened before (73.1%) or during ECMO (67.3%) than in neonates screened after (31.4%) ECMO.[15] In the present study, aberrant screening results were found only in neonates screened before or during ECMO. None of the children with an aberrant screening result had congenital hypothyroidism. Because thyroid hormone concentrations significantly changed during ECMO, we recommend to repeat thyroid function tests after ECMO. Thyroid function tests should be repeated before the age of 14 days, however, because thyroxine treatment started later than this age can lead to intellectual disabilities.[32]

Treatment of low thyroid hormone concentrations during NTIS remains a matter of debate. As thyroid hormone is important for neurodevelopment during early life, a low concentration during this period could possibly affect neurodevelopmental outcomes. Yet, studies on thyroid supplementation therapy in infants with hypothyroxinemia of prematurity found no beneficial effect.[33] Further, a recent study found that transient hypothyroxinemia of prematurity was not associated with adverse neurodevelopmental outcomes at the age of 19 years.[34] A randomized controlled trial in pediatric patients undergoing cardiac surgery found that T3 supplementation in patients <5 months of age was associated with reduced time of mechanical ventilation.[35] However, T3 supplementation in pediatric patients undergoing car-

diopulmonary bypass has not been found to improve their long-term neurodevelopmental outcomes.[36] We found a constant FT4 concentration during ECMO, which was only slightly lower than the reference norm. Further, cognitive outcomes of MAS and CDH patients were similar to cognitive outcomes of the norm population, except for one MAS patient with severe perinatal asphyxia. This, in combination with the lack of evidence for thyroid replacement therapy and the increases in thyroid hormone concentrations occurring during ECMO suggest that early treatment is not required during ECMO. However, larger studies with long-term follow-up of neurodevelopmental outcomes should be performed to draw definite conclusions.

A strength of this study is the determination of all thyroid hormone concentrations and TBG at fixed time-points before, during and after neonatal ECMO. Furthermore, it is the first study that calculated these concentrations into SDS using recently established age-specific reference values.[24] A limitation of this study is that we did not measure thyroid hormone concentrations in the prime fluid used during ECMO. Thyroid hormone concentrations in prime fluid could have varied, and measurements of thyroid hormone concentrations in the prime fluid would have given more information about the hemodilution effect during ECMO.

In conclusion, we found that thyroid hormone disturbances similar to NTIS were present in critically ill neonates prior to the start of ECMO. After an initial decline directly after the start of ECMO, first TSH increased, followed by TT4 and TT3. This may reflect restoration of the hypothalamic–pituitary–thyroid axis during ECMO. FT4 remained constant within the normal range during the ECMO course. These results suggest that thyroid hormone replacement therapy is not required during ECMO.

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Supplements

Supplementary table 1. Age-specific reference values for thyroid hormone and thyroxine-binding globulin levels in neonates published by Lem et al.[24]

	Age	-2 SDS	-1 SDS	0 SDS	+1 SDS	+2 SDS
FT4 (pmol/l)	Day of birth	12.12	15.81	21.78	32.65	56.54
	1 day-7 days	12.32	15.91	21.61	31.65	52.54
	8 days-1 month	12.81	16.12	21.12	29.28	44.33
TT4 (nmol/l)	Day of birth	75.26	99.44	132.38	177.64	240.37
	1 day-7 days	75.90	99.96	132.64	177.36	239.10
	8 days-1 month	77.93	101.62	133.44	176.47	235.16
TT3 (nmol/l)	Day of birth	0.30	1.23	2.18	3.13	4.09
	1 day-7 days	0.32	1.25	2.18	3.13	4.08
	8 days-1 month	0.39	1.29	2.21	3.13	4.06
rT3 (nmol/l)	Day of birth	0.30	0.48	0.87	1.88	5.51
	1 day-7 days	0.30	0.48	0.86	1.82	5.20
	8 days-1 month	0.30	0.47	0.83	1.67	4.36
TSH (mU/l)	Day of birth	2.43	3.84	6.44	11.75	24.03
	1 day	1.90	3.21	5.54	9.76	17.58
	2 days	1.40	2.61	4.64	7.94	13.10
	3 days	0.94	2.03	3.75	6.24	9.65
	4 days	0.60	1.48	2.85	4.63	6.82
	5 days-7 days	0.58	1.18	2.14	3.57	5.58
	8 days-1 month	0.58	1.18	2.14	3.57	5.57
TBG (mg/L)	Day of birth	19.17	23.34	28.68	35.61	44.70
	1 day-7 days	19.16	23.34	28.67	35.59	44.68
	8 days-1 month	19.12	23.28	28.61	35.51	44.59

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Abbreviations: FT4=free thyroxine; rT3=reverse triiodothyronine; SDS=standard deviation score; TBG=thyroxine-binding globulin; TSH=thyroid-stimulating hormone; TT4=total thyroxine; TT3=total triiodothyronine.

Supplementary table 2. Associations between thyroid hormone and thyroxine-binding globulin concentrations and clinical variables

SDS of	Variable	Estimated fixed effect coefficient	95% confidence interval	p-value
FT4	Time-point (compared to baseline):			0.53
	1 hour after start ECMO	-0.01	-0.72 to 0.70	
	12 hours after start ECMO	-0.51	-1.25 to 0.23	
	48 hours after start ECMO	0.01	-0.73 to 0.76	
	72 hours after start ECMO	0.14	-0.65 to 0.93	
	Post-ECMO	0.07	-0.76 to 0.90	
	Gestational age (weeks)	0.20	-0.07 to 0.47	0.15
	Diagnosis of CDH	0.44	-0.58 to 1.46	0.37
	Use of dopamine	-0.26	-0.84 to 0.31	0.36
	Center that used FFP	-0.67	-1.26 to -0.07	0.03
TT4	Time-point (compared to baseline):			<0.001
	1 hour after start ECMO	-1.51	-2.48 to -0.54	
	12 hours after start ECMO	-1.27	-2.27 to -0.27	
	48 hours after start ECMO	-0.79	-1.81 to 0.23	
	72 hours after start ECMO	-0.30	-1.37 to 0.77	
	Post-ECMO	0.84	-0.29 to 1.96	
	Gestational age (weeks)	0.26	-0.09 to 0.62	0.13
	Diagnosis of CDH	-0.38	-1.69 to 0.93	0.55
	Use of dopamine	-0.20	-0.96 to 0.57	0.61
	Center that used FFP	0.67	-0.10 to 1.43	0.08
rT3	Time-point (compared to baseline):			0.03
	1 hour after start ECMO	-0.47	-0.76 to -0.18	
	12 hours after start ECMO	-0.01	-0.32 to 0.29	
	48 hours after start ECMO	-0.13	-0.44 to 0.17	
	72 hours after start ECMO	-0.19	-0.52 to 0.13	
	Post-ECMO	-0.10	-0.44 to 0.23	
	Gestational age (weeks)	-0.02	-0.15 to 0.11	0.74
	Diagnosis of CDH	0.18	-0.30 to 0.66	0.44
	Use of dopamine	0.07	-0.18 to 0.31	0.58
	Center that used FFP	-0.21	-0.50 to 0.07	0.13
TT3	Time-point (compared to baseline):			<0.001
	1 hour after start ECMO	-0.28	-0.60 to 0.04	
	12 hours after start ECMO	-0.39	-0.72 to -0.07	
	48 hours after start ECMO	-0.03	-0.36 to 0.30	
	72 hours after start ECMO	-0.04	-0.39 to 0.31	
	Post-ECMO	0.49	0.13 to 0.86	
	Gestational age (weeks)	0.10	-0.002 to 0.20	0.05
	Diagnosis of CDH	-0.02	-0.39 to 0.34	0.90
	Use of dopamine	-0.19	-0.43 to 0.04	0.11
	Center that used FFP	0.11	-0.11 to 0.32	0.30

Supplementary table 2. Continued

SDS of	Variable	Estimated fixed effect coefficient	95% confidence interval	p-value
TSH	Time-point (compared to baseline):			<0.001
	1 hour after start ECMO	-0.97	-1.77 to -0.17	
	12 hours after start ECMO	-0.36	-1.20 to 0.48	
	48 hours after start ECMO	1.58	0.72 to 2.44	
	72 hours after start ECMO	2.51	1.61 to 3.42	
	Post-ECMO	3.01	2.06 to 3.97	
	Gestational age (weeks)	-0.38	-0.88 to 0.12	0.13
	Diagnosis of CDH	-2.25	-4.10 to -0.40	0.02
	Use of dopamine	-0.50	-1.23 to 0.24	0.18
	Center that used FFP	1.37	0.27 to 2.46	0.02
TBG	Time-point (compared to baseline):			<0.001
	1 hour after start ECMO	-4.84	-5.92 to -3.76	
	12 hours after start ECMO	-3.26	-4.39 to -2.13	
	48 hours after start ECMO	-0.84	-1.99 to 0.31	
	72 hours after start ECMO	-0.22	-1.44 to 1.00	
	Post-ECMO	1.27	-0.02 to 2.55	
	Gestational age (weeks)	0.12	-0.49 to 0.74	0.68
	Diagnosis of CDH	-1.33	-3.60 to 0.94	0.24
	Use of dopamine	-0.32	-1.29 to 0.65	0.52
	Center that used FFP	1.63	0.29 to 2.97	0.02

Abbreviations: CDH=congenital diaphragmatic hernia; ECMO=extracorporeal membrane oxygenation; FFP=fresh frozen plasma; FT4=free thyroxine; rT3=reverse triiodothyronine;TBG=thyroxine-binding globulin;TSH=thyroid-stimulating hormone;TT3=total triiodothyronine;.TT4=total thyroxine.

Supplementary table 3. Patients that used dopamine during extracorporeal membrane oxygenation

Time-point	Use of dopamine
1 hour before start ECMO (baseline)	94.7% (18/19)
1 hour after start ECMO	94.7% (18/19)
12 hours after start ECMO	55% (11/20)
48 hours after start ECMO	52.6% (10/19)
72 hours after start ECMO	50.0% (8/16)
Post-ECMO	42.9% (6/14)

Abbreviation: ECMO=extracorporeal membrane oxygenation.

Chapter 4

Characteristics of infants with congenital diaphragmatic hernia who need follow-up of pulmonary hypertension

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Abstract

Objective: Pulmonary hypertension (PH) is one of the main causes of mortality and morbidity in patients with congenital diaphragmatic hernia (CDH). Currently, it is unknown whether PH persists or recurs during the first year of life.

Design: Prospective longitudinal follow-up study.

Setting: Tertiary university hospital.

Patients: Fifty-two CDH patients admitted between 2010 and 2014.

Measurements: PH was measured using echocardiography and electrocardiography at 6 and 12 months of age. Characteristics of patients with persistent PH were compared with those of patients without persistent PH.

Main results: Persistent PH was found in four patients. At 6 months of age, in three patients (patients A-C), and at 12 months of age, in two patients (patients C and D). Patients with persistent PH had a longer duration of mechanical ventilation (median 77 days (interquartile range: 49-181) versus median 8 days (interquartile range: 5-15; $p=0.002$) and hospital stay (median 331 days (interquartile range: 198-407) versus median 33 days (interquartile range: 16-59; $p=0.003$) than patients without persistent PH. The proportion of patients with persistent PH (n=4) treated with inhaled nitric oxide (100% vs. 31%; $p=0.01$), sildenafil (100% vs. 15%; $p=0.001$) and bosentan (100% vs. 6%; $p<0.001$) during initial hospital stay was higher than that of patients without persistent PH (n=48). A higher proportion of patients with persistent PH required tube feeding and treatment with supplemental oxygen and sildenafil at follow-up. The diagnosis of PH would have been missed in two patients with PH on echocardiography, if only electrocardiography had been performed.

Conclusions: Less than 10% of CDH patients had persistent PH at ages 6 and 12 months. Follow-up for PH should be reserved for CDH patients with echocardiographic signs of persistent PH at hospital discharge and those treated with medication for PH at hospital discharge.

Introduction

Congenital diaphragmatic hernia (CDH) is a severe birth defect associated with significant mortality and morbidity due to pulmonary hypoplasia and pulmonary hypertension (PH). Although survival rates have improved, overall mortality remains high.[1] Increased survival rates have also led to increased comorbidity. A recent study found that 74% of CDH survivors had pulmonary, gastrointestinal, or neurological morbidities at hospital discharge.[2] One of the most important causes of mortality and morbidity in CDH patients is persistent PH. The development of persistent PH is complex. A reduced number of pulmonary arteries in combination with altered vasoreactivity and abnormal pulmonary vascular remodeling contribute to the development of PH in neonates with CDH.[3] In approximately half of CDH patients PH resolves at 3 weeks of age.[4,5] Nevertheless, a recent study showed that PH still persisted in 18% of 113 CDH patients at 3 months of age.[6]

Current guidelines advise to treat severe PH with pulmonary vasodilators such as inhaled nitric oxide (iNO), sildenafil and/or bosentan,[7] although there is no evidence from properly designed randomized controlled trials in this specific group of patients. Management of PH in CDH patients remains challenging, and persistence of PH is associated with a worse outcome including higher mortality and respiratory morbidity.[1, 4-6] In 2008, the American Academy of Pediatrics recommended screening for PH at follow-up when echocardiography was previously abnormal or when the child required supplemental oxygen therapy.[8] More recently, Hollinger et al.[9] advised to routinely follow up PH only in high risk CDH patients at 3 and 6 months of age. Still, little is known about the course of PH in CDH survivors after hospital discharge.

In this study, we determined the prevalence of persistent PH at 6 and 12 months of age in CDH patients who received standardized postnatal treatment.[10] Furthermore, we evaluated whether characteristics of patients with persistent PH differed from patients without persistent PH.

Methods

Patients

This prospective longitudinal study was conducted within the framework of our structured follow-up program for CDH patients monitoring growth, lung function and neurodevelopment until 18 years of age.[11] We included children admitted between January 2010 and October 2014. Exclusion criteria were: CDH diagnosed after 7 days of age, a paraesophageal diaphragmatic defect, or a congenital heart defect with known increased pulmonary vascular resistance. Postnatal management was performed according to the guidelines of the standardized CDH EURO Consortium Consensus treatment protocol.[10] Echocardiography was performed within the first 24 hours of pediatric intensive care unit admission. The treatment of PH was as follows: If preductal saturations fell below 85% with signs of poor organ perfusion, blood pressure support was given to maintain arterial blood pressure levels at normal levels for gestational age. Inhaled nitric oxide (iNO) was started in case of extra-pulmonary right-to-left shunting and if the oxygenation index was above 20 and/or the saturation difference was more than 10%. Sildenafil was started when severe PH persisted after the use of iNO. Bosentan was started in case of persistent PH despite the use of iNO and sildenafil. Sildenafil and/or bosentan were continued after discharge in patients with severe PH during hospital admission with the need for supplemental oxygen therapy at hospital discharge. CDH patients were discharged with supplemental home oxygen if they were unable to maintain an oxygen saturation above 92% on room air. At follow-up, sildenafil and/or bosentan were weaned if children had no signs of PH on echocardiography and no supplemental oxygen therapy. The Erasmus MC Medical Ethical Review Board stated that 'the Medical Research in Human Subjects Act does not apply to this study', and waived institutional review board approval (MEC-2017-256).

Data collection

The following baseline data were collected: gestational age, birth weight, gender, ethnicity, side of diaphragmatic defect, prenatal diagnosis, in case of prenatal diagnosis observed-to-expected lung-to-head ratio (LHR) and liver position at third prenatal ultrasound (30-32 weeks gestational age), age at repair of the diaphragmatic defect, surgical technique for repair (laparotomy or thoracoscopy), requirement of patch repair, need for extracorporeal membrane oxygenation (ECMO) treatment, age at start of ECMO treatment, duration of ECMO treatment, duration of initial mechanical ventilation, presence of chronic lung disease (oxygen dependency at 28 days of life),[12] treatment with iNO, sildenafil, and/or bosentan during hospital stay, duration of initial hospital stay, presence of cardiac anomalies, and need for supplemental oxygen, diuretics, sildenafil and/or bosentan at discharge and follow-up.

Pulmonary hypertension assessments

Echocardiograms and electrocardiographs (ECGs) were performed at 6 and 12 months of age (or >12 months of age when indicated). An experienced pediatric cardiologist obtained standard echocardiographic views using a GE Vivid S6 ultrasound system with a 6S or 10S MHz transducer or a Philips iE 33 ultrasound system with a S8 or S12 MHz transducer according to the ECS/ERS guidelines.[13] Echocardiographic images were stored electronically. The following echocardiographic criteria were assessed to determine the presence of PH: peak tricuspid regurgitation (TR) gradient estimated by the modified Bernoulli equation, peak pulmonary regurgitation gradient, shunt direction across a patent ductus arteriosus, interventricular septum position (normal, flattened, or D-shaped interventricular septum), global right and left ventricular function (normal, moderately decreased, severely decreased), dilation of the vena cava inferior, and collapse of the vena cava inferior during inspiration. The diagnosis of PH was made if one of the following criteria were present: elevated TR gradient, right-to-left or bidirectional shunt across the patent ductus arteriosus, flattened or D-shaped interventricular septum position, impaired right ventricular function and/or dilation.

ECGs were assessed for the presence of sinus rhythm, heart rate and the following signs of PH: right QRS axis deviation of ≥110° or right ventricular hypertrophy. Right ventricular hypertrophy was defined by the presence of either R-wave amplitude in lead V1≥98th percentile of age or S-wave amplitude in lead V6≥98th percentile of age.[14] Right atrial enlargement was defined as ≥2.5 mV. On available chest x-rays, the heart position was determined on chest x-rays (normal or dextroposition defined as heart located primarily in the right chest).

A second pediatric cardiologist who was blinded to the clinical outcomes independently assessed all echocardiograms and ECGs. There was 100% concordance in diagnosing PH on echocardiograms and ECGs between the two pediatric cardiologists. Lastly, we evaluated results of two patients with persistent PH who underwent cardiac catheterization during follow-up.

Statistical analyses

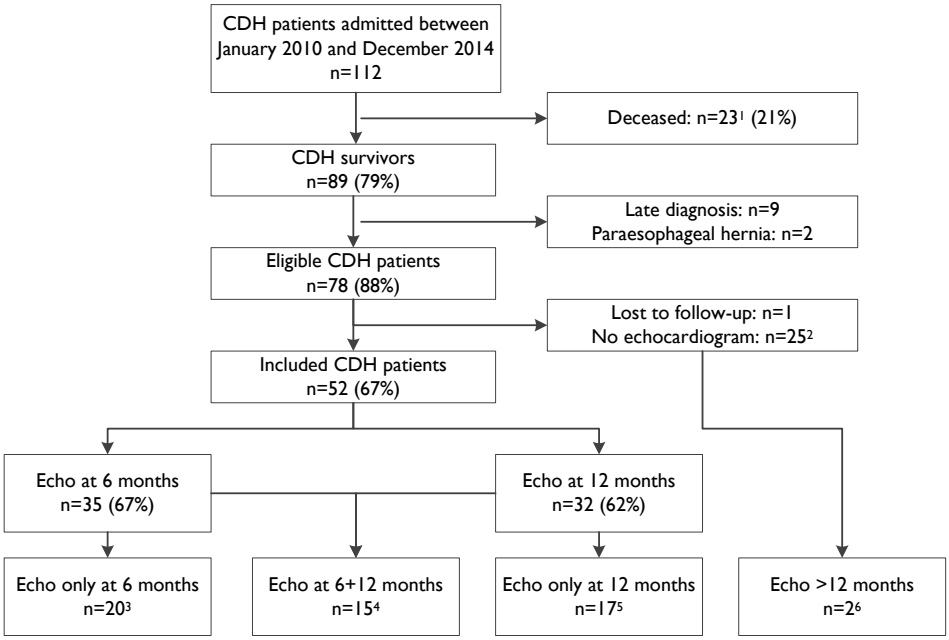
To evaluate differences between characteristics of included and non-included patients and between patients with and without persistent PH at 6 or 12 months of age, we performed chi-square or Fisher's exact tests for categorical variables, as appropriate. Independent samples t-tests were used for normally distributed variables, and Mann-Whitney U tests for continuous variables that were not normally distributed. Analyses were performed using SPSS 21.0 for Windows (IBM Corporation, Armonk, New York, USA). The statistical tests were two-sided and used a significance level of 0.05.

Results

Patients

During the study period, 112 CDH patients were admitted. Twenty-three (21%) died before hospital discharge (figure 1). Eleven patients met exclusion criteria (late diagnosis: n=9; paraesophageal hernia: n=2). Twenty-six patients were excluded because they did not have an echocardiogram at follow-up (n=25) and one patient was lost to follow-up. Comparison of data of the included 52 patients (67%) with those of the 26 eligible nonparticipants revealed no important differences in patient characteristics (supplementary table 1). Included patients were more frequently born in our institution than non-included patients, and a higher proportion of included patients required supplemental oxygen at discharge. Other patients' characteristics did not differ. Forty-nine (94%) of the 52 included patients had an echocardiogram at hospital admission at the median age of 1 day. This revealed PH in 37 cases (71%). Four infants (8%) had PH at 6 and/or 12 months of age. Patient characteristics are shown in table 1. Patients with persistent PH had a significantly longer duration of mechanical ventilation and hospital stay than patients without persistent PH. All patients with persistent PH underwent a laparotomy for repair of the diaphragmatic defect, and all were treated with iNO, sildenafil and bosentan during initial hospital stay.

Figure 1. Flowchart of the study population



[1] Died before hospital discharge.

[2] Reasons for no echocardiogram at 6 and 12 months of age (n=25): logistic (n=23); MRSA infection (n=2).

[3] Reasons for no echocardiogram at 12 months of age (n=17): logistics (n=14); poor image quality of echocardiogram (n=2); uncooperativeness (n=1).

[4] Two children died after hospital discharge. One patient, without pulmonary hypertension at 6 and 12 months follow-up, died at the age of 15 months due to sepsis. One patient, with pulmonary hypertension at ages 6 and 12 months, died at the age of 14 months due to a cyanotic spell.

[5] Reasons for no echocardiogram at 6 months of age (n=20): logistics (n=16); hospital admission (n=2); poor image quality of echocardiogram (n=1); echocardiography in other center (n=1).

[6] Reasons for echocardiogram >12 months of age (n=2): small atrial septal defect (n=1); refusal of follow-up at 6 and 12 months of age (n=1).

Abbreviation: CDH=congenital diaphragmatic hernia.

Table 1. Characteristics of patients with and without pulmonary hypertension at 6 and/or 12 months follow-up

	Patients with PH[1] n=4	Patients without PH n=48	p-value
Gestational age (weeks)	38.4 (38.1-38.8)	38.3 (37.5-38.9)	0.62
Birth weight (kilograms)	3.0 (2.8-3.1)	3.0 (2.6-3.3)	0.95
Male	3 (75%)	27 (56%)	0.63[2]
Ethnicity			1.00[2]
Dutch	4 (100%)	39 (81%)	
Other	0 (0%)	9 (19%)	
Inborn	4 (100%)	39 (81%)	1.00[2]
Left sided hernia	4 (100%)	42 (88%)	1.00[2]
Prenatally			
Prenatal diagnosis	4 (100%)	38 (79%)	0.58[2]
Prenatal liver position			0.18[2]
Up	3 (75%)	11 (29%)	
Down	1 (25%)	21 (55%)	
Missing		6 (16%)	
Observed-to-expected LHR	44.0% (34.0-44.6)	47.6% (41.6-56.7)	0.29
Surgery			
Age at surgery (days)	5 (4-7)	3 (2-4)	0.13
Thoracoscopic repair	0 (0%)	29 (60%)	**0.03[2]**
Patch repair	4 (100%)	35 (73%)	0.56[2]
ECMO			
ECMO use	2 (50%)	4 (8%)	0.06[2]
Age start ECMO (days)	10 (8-11)	3 (3-5)	0.06
Hours on ECMO	321 (315-327)	97 (91-237)	0.36
Hospital admission			
Duration of mechanical ventilation (days)	77 (49-181)	8 (5-15)	**0.002**
Presence of chronic lung disease	4 (100%)	23 (51%)[3]	0.11[2]
Duration of initial hospital stay (days)	331 (198-407)	33 (16-59)	**0.003**
Inhaled nitric oxide use	4 (100%)	15 (31%)	**0.01[2]**
Sildenafil use	4 (100%)	7 (15%)	**0.001[2]**
Bosentan use	4 (100%)	3 (6%)	**<0.001[2]**
Hospital discharge			
Supplemental oxygen at discharge	4 (100%)	4 (8%)	**<0.001[2]**
Diuretics at discharge	4 (100%)	8 (17%)	**0.002[2]**
Sildenafil at discharge	4 (100%)	5 (10%)	**<0.001[2]**
Bosentan at discharge	3 (75%)	1 (2%)	**0.001[2]**

Data are presented as median (interquartile range) or number (percentage), as appropriate.

P-value=significant difference between the groups.

[1] Pulmonary hypertension at 6 and/or 12 months of age.

[2] Fisher's exact test was used.

[3] Missing: n=3.

Abbreviations: ECMO=extracorporeal membrane oxygenation; LHR=lung-to-head ratio; PH=pulmonary hypertension at 6 and/or 12 months of age.

Echocardiography at 6 months

At 6 months follow-up, 35 patients underwent echocardiography (figure 1). A higher proportion of the 35 patients with echocardiography at 6 months were treated with sildenafil at hospital discharge compared with the 17 included patients who did not receive echocardiography at 6 months (26% versus 0%; p=0.02). Other patient characteristics did not differ significantly (data not shown). Three patients (patients A-C, see supplementary table 2) had PH at 6 months with flattening of the interventricular septum and/or right ventricular dilation. The ductus arteriosus had closed in all. TR was only found in patient A, with a gradient of 30 mm Hg. Patient B was diagnosed with a partial anomalous pulmonary venous return (PAPVR) with an atrial septal defect patients PH at 6 months follow-up required supplemental oxygen, tube feeding, diuretics, sildenafil and bosentan at 6 months of age, which differed from patients without PH (table 2).

Table 2. Follow-up details of patients with and without pulmonary hypertension at 6 months of age

	Patients with PH n=3	Patients without PH n=32	p-value
Supplemental oxygen use	3 (100%)	3 (9%)	**0.003**[1]
Tube feeding	3 (100%)	8 (25%)	**0.03**[1]
Diuretics	3 (100%)	5 (16%)	**0.009**[1]
Sildenafil	3 (100%)	3 (9%)	**0.003**[1]
Bosentan	3 (100%)	2 (6%)	**0.002**[1]

Data are presented as number (percentage).
P-value=significant difference between the groups.
[1] Fisher's exact test was used.
Abbreviation: PH=pulmonary hypertension.

Echocardiographic at 12 months and later

Echocardiographic data at 12 months of age were available for 32 patients (figure 1). A higher proportion of the 32 patients with echocardiography at 12 months underwent a laparotomy for CDH repair compared with the 20 patients without echocardiography (56% versus 25%; p=0.03), while other patient characteristics did not differ significantly (data not shown). PH was found in two patients (6%; patients C and D, see supplementary table 2). Both patients with persistent PH at 12 months received sildenafil at 12 months of age (table 3). Patient C had PH at the ages of 6 months and 12 months. At 12 months of age, the echocardiogram showed signs of severe right ventricular failure despite the use of sildenafil and bosentan. This patient died at 14 months of age due to a cyanotic spell in combination with severe PH. Parents did not agree to autopsy. Patient D had a normal echocardiogram at 6 months of age under sildenafil treatment, which was continued because this patient still required supplemental oxygen therapy at that time. At 12 months of age, this patient had slight right ventricular dilation without TR and a closed ductus arteriosus. Sildenafil was discontinued at

18 months of age. Two weeks later and 6 months after stopping sildenafil, echocardiographic evaluation confirmed that PH had not recurred in these patient. Under treatment with sildenafil, PH had disappeared at 12 months of age in patients A and B. In patient A, sildenafil and bosentan were stopped at 3 years of age, and an echocardiogram made 2 weeks later confirmed absence of PH. In patient B, sildenafil and bosentan were stopped at 2.9 years of age, and an echocardiogram made 2 months later confirmed absence of PH. Two patients received sildenafil until 14 months of age but they had no echocardiographic signs of PH at 6 and 12 months of age (Table 3).

Table 3. Follow-up details of patients with and without pulmonary hypertension at 12 months of age

	Patients with PH n=2	Patients without PH n=30	p-value
Supplemental oxygen use	1 (50%)	2 (7%)	0.18[1]
Tube feeding	2 (100%)	8 (26%)	0.10[1]
Diuretics	1 (50%)	3 (10%)	0.24[1]
Sildenafil	2 (100%)	3 (10%)	**0.02**[1]
Bosentan	1 (50%)	2 (7%)	0.18[1]

Data are presented as number (percentage).
P-value=significant difference between the groups.
[1] Fisher's exact test was used.
Abbreviation: PH=pulmonary hypertension.

Cardiac catheterization at follow-up

During follow-up, cardiac catheterization was performed in two patients who had PH after the neonatal period (patients A and B). In patient A, cardiac catheterization evaluating pulmonary vascular reactivity at 4.5 months of age found a right ventricular pressure of 55-60% of the left ventricular pressure, and PH did not change with the use oxygen or iNO. Patient B underwent cardiac catheterization three times (at 9, 10 and 36 months of age). The first confirmed the diagnosis of a PAPVR with a median sized atrial septal defect with a left-to-right shunt ratio of 1.7. During the second time, an atrial septal defect device could not be placed. The third cardiac catheterization showed a slight right atrium dilation due to PAPVR with an atrial septal defect without signs of PH.

ECG at follow-up

Twenty-nine of 35 patients had an ECG at 6 months of age (including patients A and C with PH). Patient C had right ventricular hypertrophy on the ECG. All other patients (including patient A and one patient with dextroposition on the chest x-ray) had no signs of PH on ECG. At 12 months of age, an ECG was performed in 29 of 32 patients (including patient D with PH). All these patients had no signs of PH on ECG (including patient D and two patients with cardiac dextroposition).

Discussion

Routine echocardiography showed persistence of PH in a minority (8%) of included CDH patients, while 71% of surviving CDH patients had PH at hospital admission. Patients with persistent PH had a longer duration of mechanical ventilation and hospital stay than patients without persistent PH, and they were all treated with iNO, sildenafil and bosentan. This indicates that persistent PH is associated with a greater severity of illness during early life.

As stated earlier, the development of PH in CDH patients is a complex matter, with involvement of reduction in the number of pulmonary arteries in combination with altered vasoreactivity and abnormal pulmonary vascular remodeling.[3] Patients with most severe lung hypoplasia requiring prolonged ventilatory support and hospitalization seem most at risk of persistent PH, as is seen in infants with bronchopulmonary dysplasia.[15] In addition to abnormal lung and pulmonary vessel development, the iatrogenic lung injury due to prolonged mechanical ventilation may be a contributing factor to persistence of PH. In our study, 94% and 96% of CDH patients had a normal echocardiogram at 6 and 12 months of age, respectively. The persistence of PH in CDH patients has been studied previously (supplementary table 3). Most of the previous studies on persistence of PH in CDH patients were retrospective cohort studies assessing PH with echocardiography. Interpretation and comparison of the study results is hindered by to the use of different definitions for PH resulting in varying prevalence rates of PH (supplementary table 3). Lusk et al.[5] found a prevalence of 57% at 4 weeks of age, and Wynn et al.[6] reported a prevalence of 49% at 1 month of age. In other studies, the reported prevalence of persistent PH at 6 to 7 months of age varied between 0% and 27%.[16, 17] Selection bias due to different patient populations and loss to follow-up seems also involved in these varying prevalence rates.

Although cardiac catheterization is the gold standard for assessing PH,[13, 18] echocardiography is more frequently used in children. Cardiac catheterization carries the risk of complications such as hypotension requiring intervention, hypertensive crisis, and cardiac arrest, which occur in 10% of patients.[19] Still, the reliability of echocardiography in CDH has been questioned because of the risk of false-negative results. Using cardiac catheterization, Zussman et al.[20] found an elevated mean pulmonary artery pressure and pulmonary vascular resistance in three (38%) of eight children with CDH, whereas they had no signs of PH on the echocardiogram. The authors argue that early consideration of cardiac catheterization may be warranted in CDH patients. All these patients had a clinical indication for cardiac catheterization, however, which makes it impossible to extrapolate these results. In our series, two patients with persistent PH underwent cardiac catheterization on clinical indication, which confirmed the diagnosis of PH in both. In addition to cardiac catheterization and echocardiography, ECG may provide supportive evidence of PH, although a normal ECG cannot exclude PH.[13, 21] Indeed, we think echocardiography should be the diagnostic tool of choice for follow-up of PH in CDH patients as two patients with persistent PH on echocardiography would have been missed if only an

ECG had been performed.

Echocardiography can be challenging in CDH patients as the heart is often shifted to an abnormal position, and no single reliable quantitative measure of pulmonary artery pressure and/or right ventricular function exists. In our study, one patient who received sildenafil treatment had echocardiographic signs of PH at 12 months of age, but not at 6 months of age. We assume that the echocardiogram at 6 months of age had underestimated the pulmonary artery pressure, which led to a false-negative diagnosis. To improve diagnostic accuracy, more recent echocardiographic studies in CDH patients have used more advanced techniques such as tissue Doppler.[5, 6, 16, 17, 22] Estimation of pulmonary artery pressures is mainly based on the existence of TR, and qualitative assessment including position of the interventricular septum and/or signs of right ventricular dilation. Yet, TR is often not present in CDH patients with PH. Lusk et al.[5], for example, found that TR was present in only 38% of patients with PH. We found TR in only two of the four patients with PH. So far, there is no standardized echocardiography protocol for CDH patients that minimizes the risk of missing PH. More advanced techniques such as tissue Doppler may improve diagnostic accuracy of PH in CDH patients, and as mentioned above, this technique is increasingly being used in more recent studies.[5, 6, 16, 17, 22] A standardized protocol with more advanced echocardiographic methods may improve diagnostic accuracy and follow-up of PH in the future.

As echocardiography is both time-consuming and costly, ideally the risk of missing PH should be minimal. Our results indicate that routine follow-up of PH with echocardiography is not useful during infancy because PH was only found in a small subset of patients who were treated with sildenafil. The American Academy of Pediatrics recommends follow-up of PH when echocardiography was previously abnormal or when the child is on supplemental oxygen.[8] More recently, Hollinger et al.[9] advised to routinely follow up PH only in high risk CDH patients (defect size type C or D, requirement of patch repair, ECMO and/or supplemental oxygen) until 6 months of age, and after this age on indication. Nowadays, sildenafil and bosentan are increasingly being prescribed after hospital discharge, and follow-up of PH is important in these patients. Therefore, we recommend using risk stratification on the basis of presence or absence of PH on echocardiography at hospital discharge and/or the need for PH treatment at hospital discharge to guide follow-up of PH.

Little is known about the efficiency and safety of sildenafil and bosentan in the treatment of PH in CDH patients, and valid data or international guidelines are lacking.[23, 24] Research addressing the reactivity of the pulmonary vasculature to medication is needed to assess the effectiveness of treatment. So far, only one study has addressed the weaning off sildenafil in 17 infants with CDH. Sildenafil could be ceased in ten patients after a long period (median 343 days) but no echocardiographic results were presented.[25] In our study, three patients without PH were still treated with sildenafil and/or bosentan at 6 months of age, and two patients with resolved PH at 12 months of age received sildenafil until 2.7 years and 3.0 years of age, respectively. These patients may have been treated with sildenafil and

bosentan longer than needed, especially considering that PH did not recur. Although we did not address this issue in our study, we propose that sildenafil and bosentan can be weaned much faster than described by Behrsin et al.[25] Based on our experience we recommend a control echocardiogram one month after discontinuation of sildenafil and/or bosentan to confirm that PH has not recurred.

Another important question is whether PH recurs when CDH patients get older, which may affect their exercise capacity. A previous study found only a mildly reduced exercise capacity without echocardiographic evidence of PH at rest in 23 CDH patients at the median age of 13.3 years.[26] These patients had been born in the period 1985-1991, in which survival and treatment were different than in our study period. More recently, Van der Cammen-Van Zijp et al.[27] showed that exercise capacity was below the norm in CDH patients at 5 years of age and had deteriorated over time in ECMO-treated CDH patients. However, echocardiography was not performed in this study. Based on our data and current literature we cannot exclude that altered vasoreactivity and abnormal growth of pulmonary vessels in CDH patients affects their exercise capacity. Future multicenter studies should focus on evaluating the course of PH in CDH patients later in life. Further in the future, exercise echocardiography can possibly be used to assess pulmonary vascular function during exercise.[28]

This is the first prospective study assessing routine follow-up for PH during infancy in CDH patients. All echocardiograms were performed by an experienced pediatric cardiologist and assessed by a second pediatric cardiologist, which showed 100% concordance in diagnosing PH. This is also the first mid-term follow-up study that focuses on the follow-up of PH in patients treated with sildenafil and/or bosentan. Some limitations need to be addressed as well. First, echocardiographs made within the first year of life were not available for 26 patients, and not all included patients had an echocardiogram at both time-points. Second, the limited number of patients with PH did not allow for regression analyses. Multicenter studies using the same standard of care should further investigate risk factors for persistence of PH as well as the consequences of persistent PH.

In conclusion, echocardiographic follow-up of PH during infancy should not be advocated for all CDH patients. It should be reserved for patients with persistent PH on echocardiography at hospital discharge and/or those who still require treatment with sildenafil and/or bosentan at hospital discharge. Outcomes of studies on the efficacy of medication such as sildenafil and/or bosentan in lowering the pulmonary artery pressure might be helpful for developing guidelines on PH treatment in CDH patients. Development of a standardized protocol prescribing validated echocardiography measures for the accurate echocardiographic diagnosis of PH in children with CDH is currently addressed by the CDH EURO Consortium.

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Supplements

Supplementary Table 1. Characteristics of included patients and non-included patients

	Included patients n=52	Non-included patients n=26	p-value
Gestational age (weeks)	38.3 (37.6-38.9)	38.6 (38.1-39.7)	0.11
Birth weight (kilograms)	3.0 (2.6-3.2)	3.2 (2.9-3.5)	0.48
Male	30 (58%)	15 (58%)	1.00
Ethnicity			0.54
Dutch	43 (83%)	20 (77%)	
Other	9 (17%)	6 (23%)	
Inborn	43 (83%)	16 (62%)	**0.04**
Left sided hernia	46 (89%)	22 (85%)	0.72[1]
Prenatally			
Prenatal diagnosis	42 (81%)	16 (62%)	0.07
Prenatal liver position			0.21
Up	14 (33%)	6 (38%)	
Down	22 (52%)	8 (50%)	
Missing	6 (14%)	2 (12%)	
Observed-to-expected LHR	44.9% (41.3-56.3)	43.0% (37.1-59.8)	0.88
Surgery			
Age at surgery (days)	3 (2-5)	4 (3-5)	0.23
Thoracoscopic repair	29 (56%)	17 (65%)	0.42
Patch repair	39 (75%)	15 (58%)	0.12
ECMO			
ECMO use	6 (12%)	3 (12%)	1.00[1]
Age start ECMO (days)	5 (3-8)	2 (2-5)	0.30
Hours on ECMO	207 (96-327)	264 (190-293)	0.80
Hospital admission			
Duration of mechanical ventilation (days)	8 (5-19)	8 (5-16)	0.53
Presence of chronic lung disease[2]	27 (52%)[3]	12 (46%)	0.48
Duration of initial hospital stay (days)	34 (16-93)	35 (22-57)	0.55
Inhaled nitric oxide use	19 (37%)	9 (35%)	0.87
Sildenafil use	11 (21%)	2 (8%)	0.20[1]
Bosentan use	7 (14%)	1 (4%)	0.26[1]
Hospital discharge			
Supplemental oxygen at discharge	8 (15%)	0 (0%)	**0.047[1]**
Diuretics at discharge	12 (23%)	5 (19%)	0.70
Sildenafil at discharge	9 (17%)	1 (4%)	0.15[1]
Bosentan at discharge	4 (8%)	0 (0%)	0.30[1]

Data are presented as median (interquartile range) or number (percentage), as appropriate.
P-value=significant difference between the groups.
[1] Fisher's exact test was used.
[2] Presence of chronic lung disease (oxygen dependency at 28 days of life)[12]
[3] Missing: n=3.
Abbreviations: ECMO=extracorporeal membrane oxygenation; LHR=lung-to-head ratio.

Supplementary table 2. Characteristics of the four patients with pulmonary hypertension at follow-up

	Patient A	Patient B	Patient C	Patient D
Hospital admission				
PH on echo	Yes	Yes	Yes	Yes
Duration of mechanical ventilation (days)	258[1]	48	104	49
Duration of initial hospital stay (days)	274	388	426	122
6 months follow-up				
PH on echo	Yes	Yes	Yes	No
PH on ECG	No	Missing	Yes	No
Supplemental oxygen	Yes	Yes	Yes	Yes
Tube feeding	Yes	Yes	Yes	Yes
Diuretics	Yes	Yes	Yes	Yes
Sildenafil	Yes	Yes	Yes	Yes
Bosentan	Yes	Yes	Yes	No
12 months follow-up				
PH on echo	No	No	Yes	Yes
PH on ECG	No	Missing	Missing	No
Supplemental oxygen	No	Yes	Yes	No
Tube feeding	Yes	Yes	Yes	Yes
Diuretics	Yes	Yes	Yes	No
Sildenafil	Yes	Yes	Yes	Yes
Bosentan	Yes	Yes	Yes	No

[1] Use of tracheostomy tube

Abbreviations: ECG=electrocardiography; PH=pulmonary hypertension.

Supplementary table 3. Overview of echocardiographic studies assessing pulmonary hypertension at follow-up in congenital diaphragmatic hernia patients published from 2000 onwards

Study	Study design	Number of patients	Year of birth	Time frame of follow-up
Lusk et al. 2015	Retrospective cohort study	140	2002-2012	-1 week -2 weeks -3 weeks -4 weeks -6 weeks
Healy et al. 2015	Retrospective cohort study	66	2004-2011	-6 months -12 months -24 months -36 months
Wynn et al. 2013	Prospective cohort study	220	2009-2010	-1 month -3 months
Datta et al. 2012	Retrospective cohort study	31	1992-2007	-90 days
Egan et al. 2012	Case control study	7 CDH patients versus 16 controls	Unknown	-Mean 6.2±1.7 years
Zussman et al. 2012	Case control study	8 CDH patients versus 10 controls with a patent ductus arteriosus	2006-2009	-Mean 16.9±9.3 months
Trachsel et al. 2006	Case control study	23 CDH patients versus 23 controls	1985-1991	-Mean 13.3±2.2 years
Kamata et al. 2005	Retrospective cohort study	33	1986-2000	-Mean 4.1±2.5 years
Dillon et al. 2004	Retrospective cohort study	47	1991-2002	-1 week -2 weeks -3 weeks
Stefanutti et al. 2004.	Cross-sectional cohort study	24	1985-1994	-Mean 8.15±2.80 years

Abbreviations: CDH=congenital diaphragmatic hernia; ECMO=extracorporeal membrane oxygenation; PH=pulmonary hypertension; TR=tricuspid regurgitation.

Definition PH	Most important results
PH: ≥2/3 of systemic systolic pressure.	12% of survivors had persistence of PH prior to discharge. PH was associated with ECMO and prolonged ventilatory support.
PH: TR jet velocity of ≥2.6 m/sec or evidence of ventricular-septal flattening with one or more of the following: right atrial enlargement, right ventricular hypertrophy, or right ventricular dilation.	27% had PH assessed at the median age of 7 (5.7-13.3) months of age.
PH: ≥1/2 of systemic systolic pressure.	49% had PH at 1 month of age. Worse PH at 1 month of age was associated with prenatal diagnosis, patch repair, post-operative complications, low birth weight and ECMO. 18% had PH at 3 months of age. Worse PH at 3 months of age was associated with patch repair, non-isolated CDH, a genetic diagnosis, and a lower birth weight.
Chronic PH: evidence of right ventricular systolic pressure above normal <90 days of life with requirement of oxygen or vasodilatory medication on follow-up at 90 days of life.	32% had chronic PH. Chronic PH was associated with higher ventilatory pressure after ECMO decannulation, need for high-frequency oscillation ventilation, prolonged duration of ventilation and hospital stay.
PH: flattened interventricular septum or a TR jet >2.5 m/sec.	None of the patients had persistence of PH.
PH: TR or patent ductus arteriosus gradient estimating right-sided pressure >40% of systemic pressure.	38% had persistence of PH on echocardiogram. At cardiac catheterization, mean pulmonary artery pressure and pulmonary vascular resistance was significantly higher in CDH patients than in controls.
PH: not defined.	None of the patients had persistence of PH.
PH: not defined.	None of the patients had persistence of PH.
PH: estimated pulmonary artery pressure to systemic arterial pressure ratio ≥0.5	33% of survivors had persistence of PH.
PH: not defined.	None of the patients had persistence of PH.

Chapter 5

Growth in children with congenital diaphragmatic hernia during the first year of life

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Abstract

Purpose: Infants with congenital diaphragmatic hernia (CDH) have high rates of mortality and long-term morbidity, including poor growth and failure to thrive. The aim of this study was to describe growth patterns during the first year of life in infants with CDH not treated with extracorporeal membrane oxygenation.

Methods: Medical records of infants with CDH admitted to our center between January 2005 and December 2011 were reviewed. Infants with anthropometric measurements at 3, 6 and 12 months were included. Anthropometric measurements were obtained for the first year of life. Logistic regression analyses were performed to find predictive associations with failure to thrive (weight-for-age and/or weight-for-length z-score <-2).

Results: Of the 45 survivors, 38 were seen twice (84%) and 24 (53%) were seen on three occasions to age 12 months. Poor growth was observed with weight being most affected. Failure to thrive was present in 63% during the first 6 months of life. Days of mechanical ventilation were the only predictor of failure to thrive. Besides poor weight gain, height and head circumference were also reduced. However, catch-up growth occurred during the second half of infancy and at age 12 months failure to thrive had reduced by two thirds to 21%.

Conclusions: Poor growth is a common early finding in CDH patients, which improves during infancy. This emphasizes the importance of close follow-up and aggressive nutritional management in CDH patients.

Introduction

Infants with congenital diaphragmatic hernia (CDH) have significant rates of mortality and long-term morbidity, which are primarily related to lung hypoplasia and associated pulmonary hypertension.[1,2] In recent years, the survival of CDH infants has progressively improved with survival rates now approaching 90% at some centers.[3,4] Advances in treatment, like extracorporeal membrane oxygenation (ECMO), have been thought to be partly responsible for the improved survival rates in CDH infants.[5,6] However, the beneficial effect of ECMO on survival remains controversial,[7,8] and the use of ECMO therapy has been found to be associated with adverse outcomes in CDH survivors.[9-11] With increased survival, however, the long-term prognosis and quality of life of CDH survivors have become an increasingly important issue.

Failure to thrive (FTT) is a significant comorbidity that has been reported in up to 69% of CDH survivors.[12-16] Catch-up growth in children with CDH has been reported in the second year of life,[15] although this finding is not universal. Contributing to poor growth are feeding difficulties and oral aversion, which are reported to occur in up to 27% of CDH infants.[12-14] However, growth over time has been poorly studied in CDH survivors, and as such growth patterns during the early years remain unclear.

The aim of this study was to describe the effect of CDH on growth in children during the first year of life.

Methods

In this study, medical records of all infants with CDH admitted to the Children's Hospital at Westmead between 1 January 2005 and 31 December 2011 were reviewed. Infants were treated according to a standardized protocol including gentle ventilation and delayed surgical repair following stabilization. Synchronized conventional ventilation with tidal volume monitoring was the preferred initial ventilator strategy. Inhaled nitric oxide, vasopressor support and high frequency ventilation were used if infants could not be stabilized with conventional ventilation (table 1). ECMO was not offered as a routine treatment modality in our center.

Table 1. Postnatal management protocol for congenital diaphragmatic infants

Treatment with synchronized conventional ventilation	PIP max <26 cm H_2O; PEEP: 2-5 cm H_2O[1]
	Adapt FiO_2 to obtain preductal saturation between 85% and 88% and postductal saturations above 70%
	Ventilator breath rate: 40-60/min to allow permissive hypercapnia (pCO_2 45-65 mm Hg) with a pH of 7.25-7.35[2]
	Aim for 3-4 ml/kg tidal volume
Indications for inhaled nitric oxide	Presence of pulmonary hypertension confirmed by echocardiography
Indications for vasopressor support	Mean blood pressure <10th percentile for gestational age
Indications for high-frequency ventilation	Preductal saturation <85% Respiratory acidosis: pH <7.25 and/or pCO_2 >65 mm Hg

[1] If oxygenation is a problem consider trialing increased PEEP if chest x-ray reveals underinflation. Also consider a longer inspiratory time and an inspiration:expiration ratio of 1:1 to maintain airway pressure.
[2] Maintain spontaneous respiration if possible.
Abbreviations: CO_2=carbon dioxide; FiO_2=inspiratory oxygen fraction; PEEP=positive end expiratory pressure; PIP=peak inspiratory pressure.

After hospital discharge, follow-up visits were scheduled in the infant lung clinic on a regular basis. All infants with anthropometric measurements at 3, 6 and 12 months of age were included. Anthropometric measurements were plotted on the World Health Organization 2006 growth charts (http://www.who.int/childgrowth/en/) and z-scores were calculated. A z-score of 0 represents the mean for the reference population. All anthropometric measurements were adjusted for gestation in children born <37 weeks.

FTT was defined as having a z-score <-2 for weight-for-age and/or weight-for-length. After identification of FTT, we searched for variables associated with FTT including prenatal diagnosis, prematurity, birth weight, gender, side of hernia, type of repair, presence of congenital anomalies, presence of moderate to severe pulmonary hypertension (arterial pressures greater than half systemic on echocardiography together with the need for inhaled nitric oxide and/or sildenafil postoperatively), days of mechanical ventilation, supplemental oxygen at discharge, duration of hospital stay and presence of gastroesophageal reflux disease (GERD).

Data were analyzed using SPSS version 20.0 for Windows (SPSS Inc., Chicago, Illinois, USA).

Categorical variables were compared using chi-square test or Fisher's Exact test. Student's t-tests were used for continuous normally distributed variables and nonparametric tests for non-normally distributed continuous variables. Growth parameters over time were compared with the Wilcoxon signed-ranks test. Logistic regression analyses were performed to find predictive associations with FTT. A p-value <0.05 was considered significant.

Results

Fifty-five infants with CDH were admitted to the Children's Hospital at Westmead during the 7-year period of data collection. The overall survival rate during the entire follow-up was 82%. Nine children died in hospital owing to a combination of respiratory failure, pulmonary hypoplasia and persistent pulmonary hypertension. Five children died before undergoing surgical repair and four patients died postoperatively before hospital discharge (age of death: 11, 21, 26, and 112 days). One child died from viral pneumonia after hospital discharge at 10 weeks of age in the emergency department of a regional hospital.

Of the 45 survivors, 38 (84%) were seen twice, between 3 and 12 months of age. A subset of 24 infants (53%) was seen on three occasions (3 months, 6 months and 12 months). Seven patients were lost to follow-up. Infant characteristics are shown in table 2.

Table 2. Characteristics of congenital diaphragmatic hernia infants

Characteristics	CDH patients (n=24)
Gestational age (weeks)	37.5±3.1
Birth weight (kilograms)	2.96±0.73
Gender	
Male	12 (50%)
Female	12 (50%)
Apgar Score	
1 min	6 (1-9)
5 min	8 (5-9)
Time of diagnosis	
Prenatal	18 (75%)
Postnatal	6 (25%)
Side of hernia	
Left	20 (83%)
Right	4 (17%)
Type of repair	
Primary repair	14 (58%)
Patch	10 (42%)
Age at surgery (days)	3 (1-14)
Associated congenital anomalies	10 (42%)
Congenital heart disease	7 (29%)
Moderate to severe pulmonary hypertension	9 (38%)

Data are presented as mean±standard deviation, median (range) or number of patients (percentage).

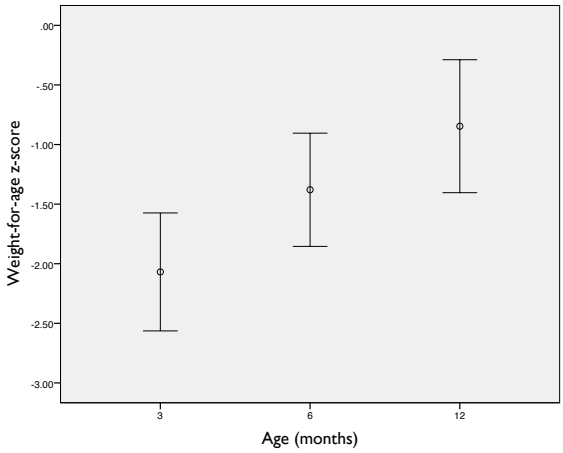
Structural heart disease was the most commonly associated congenital anomaly noted in CDH survivors. Minor structural heart disease (atrial septal defect, ventricular septal defect and atrioventricular septal defect) was present in six children and one child had tetralogy of Fallot. Other associated congenital anomalies were malformation of both upper limbs, imperforate anus and a balanced translocation of chromosome 1:16. Moderate to severe

pulmonary hypertension was defined by pulmonary arterial pressures greater than half systemic on echocardiography together with the need for inhaled nitric oxide and/or sildenafil postoperatively. Moderate to severe pulmonary hypertension was present in 9/24 (38%) of infants. Pulmonary hypertension, as assessed by echocardiography, had resolved in all by the age of 13 months.

After hospital discharge, 14/24 (58%) of children received supplemental calories with polyjoule, 10/24 (42%) of patients required gastric tube feeding, and 2/24 (8%) of children required gastrostomy placement. Poor growth was observed in many infants, especially in early infancy with weight being most affected (figure 1).

Figure 1. Growth of congenital diaphragmatic hernia infants during the first year of life

A. Weight-for-age z-scores during the first year of life



B. Height-for-age z-scores during the first year of life

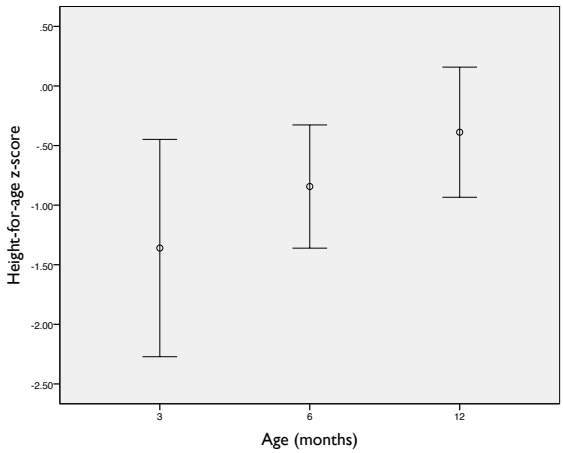
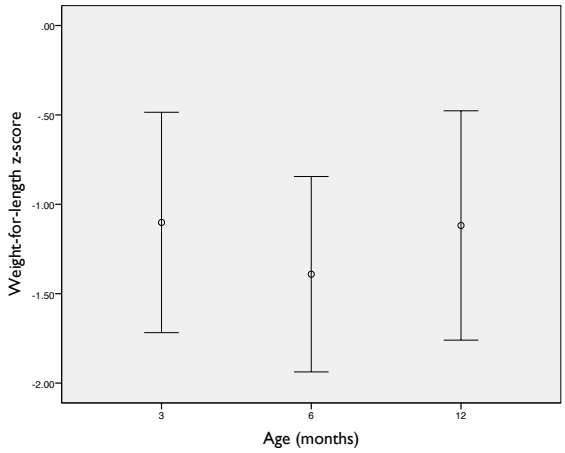
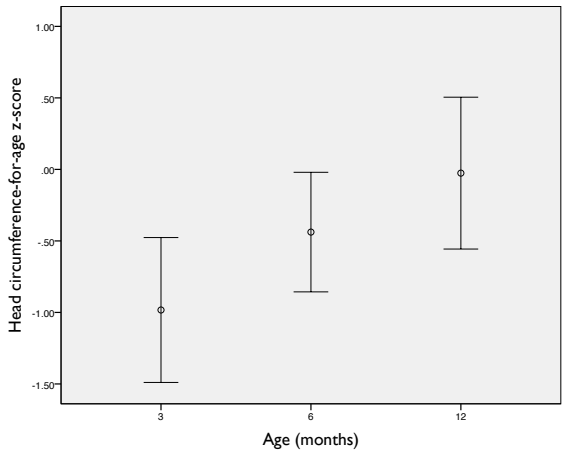


Figure 1. Continued

C. Weight-for-length z-scores during the first year of life



D. Head circumference-for-age z-score during the first year of life



The figure presents error bars, indicating mean values and 95% confidence intervals.

Catch-up growth was observed in a significant proportion of children during infancy. Weight improved from a mean±standard deviation (SD) z-score of -2.07±1.17 at 3 months of age to -0.85±1.32 at 12 months of age (p=0.001). FTT was present in 15/24 (63%) of the children during the first 6 months of life, however, considerable catch-up growth was observed over the latter part of infancy (figure 1). At the age of 12 months, FTT remained in only 5/24 (21%) of the children (p=0.01). When the 38 subjects seen on only two occasions were considered, FTT was present in a similar proportion of the cohort with 50% at 3 to 6 months of age and in 19% at 12 months of age. Days of mechanical ventilation were the only variable predictive of FTT at 6 months of age (p=0.03). We found no predictors

for FTT at 12 months of age. Linear growth was reduced but also improved over time with a mean±SD z-score of -1.36±1.43 at 3 months of age and -0.39±1.30 at 12 months of age (p=0.04). As a consequence of improved weight gain as well as linear growth, weight-for-length scores remained relatively stable during the first year of life. Weight-for-length mean±SD z-scores were -1.10±0.97 and -1.12±1.37, at respectively 3 and 12 months of age (p=0.86). Head circumference was the growth parameter best preserved with mean±SD z-scores of -0.98±1.08 and -0.03±1.13 at respectively 3 and 12 months of age (p=0.001).

Twenty-one percent of patients required additional visits to a multidisciplinary feeding clinic owing to persistent feeding difficulties. Oral aversion was observed in 1/24 (4%) of children. Other feeding difficulties reported were: poor suck-swallowing reflex, immature oral skills, feeding difficulties owing to increased work of breathing and GERD. The number of children treated for symptomatic GERD during infancy was 9/24 (38%). Almost all of these children were successfully managed with antireflux medication, however, only one (4%) child required fundoplication for persistent symptoms despite medical treatment.

Discussion

Our survival rate of 82% is consistent with recent studies showing improved survival in CDH patients.[3, 4, 17] Despite our encouraging survival outcomes, this study indicates that poor growth and FTT are frequent findings in CDH survivors during the first year of life. The cause for poor growth in CDH patients is multifactorial, including increased catabolic stress in the neonatal period, GERD, and persistent pulmonary impairment.[13] Poor growth is similarly seen in infants with bronchopulmonary dysplasia during the early months after initial hospitalization, which is caused by their increased work of breathing and higher metabolic rates.[18, 19] The number of children with poor growth and FTT in the present cohort is consistent with the findings of others.[13] However, some studies have reported even higher numbers of patients with growth failure.[12, 15]

Most children in our cohort demonstrated catch-up growth during the first year of life. FTT was present in 63% of children during the first 6 months of life but remained in only 21% at 12 months of age. Other studies have also reported improved growth with time, but mainly after the first year of life.[13, 14] The early follow-up visits in the infant lung clinic and regular medical review together with the elaborate use of calorie-enhanced feeds may have contributed to the faster catch-up growth observed in our patients. Besides poor weight gain, length was reduced in many children during the follow-up period. Linear growth in CDH patients is infrequently described in the literature, and the finding of reduced length in CDH patients has only been reported in one other study.[12]

In this study, days of mechanical ventilation were the only predictor of FTT in the first 6 months of life. This is interpreted to reflect a more severe pulmonary disease phenotype of CDH with poor growth as a consequence of increased respiratory effort or work of breathing, which is consistent with the current literature. One study found an association between FTT and duration of mechanical ventilation as well as hospital stay.[20] Muratore et al.[13] have also demonstrated ECMO and the need for oxygen at discharge as predictors of FTT. GERD has also been associated with FTT.[13, 21] However, we did not find an association between GERD and FTT as was consistent with another study.[22] These differences could be owing to the variable severity of GERD present in CDH infants and diagnostic methods of GERD. The importance of optimizing early nutrition is likely to improve respiratory status, reduce the metabolic cost of an elevated work of breathing, both in terms of respiratory effort and rate, and allow the utilization of calories for growth.

Oral aversion is another difficult problem that is reported in up to 27% of CDH infants,[12-14] however, only one (4%) child in our cohort was diagnosed with oral aversion. Our restrictive approach towards gastrostomy tube placements and early stimulation of oral feeding could be an explanation for the low rate of oral aversion seen.

The incidence of GERD found in this study was lower (38%) than reported in most other studies (45% to 89%).[12, 23] Further, only one (4%) child required fundoplication in contrast

with 19% in another study.[13] This variability in incidence is also likely related to the different diagnostic methods used in studies including pH monitoring, upper gastrointestinal contrast studies or clinical diagnosis of GERD, as well as thresholds for initiating treatment.

The strengths of this study are the early follow-up of all four growth parameters in CDH patients during review in the follow-up clinic. Limitations of our study are the retrospective study design and relatively small sample size. Multicenter studies should be encouraged to recruit a sufficient number of CDH patients into a randomized trial of more aggressive feeding initiatives overseen by a dietician to improve growth in early infancy.

Conclusion

Poor growth continues to be a common finding in infants with CDH during the first year of life. Close follow-up of growth and nutrition with early aggressive nutritional management is essential. Additional calories should be provided in the first year of life to optimize growth and ameliorate FTT in these infants.

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Chapter 6

Neurodevelopmental outcome in congenital diaphragmatic hernia survivors during the first three years

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Abstract

Objective: To determine neurodevelopmental outcome in congenital diaphragmatic hernia (CDH) survivors during the first 3 years of life.

Methods: Admitted CDH patients were assessed at the ages of 12 (n=18) and 36 months (n=15) using the Bayley scales of infant and toddler development-third edition. Neurodevelopmental results of CDH patients were compared with published norms and with a healthy matched control group.

Results: At 12 months of age, receptive language was mildly delayed in 6% of patients, and 6% of patients demonstrated mildly delay in expressive language and gross motor skills at this age. Eighteen percent of CDH patients had severely delayed scores for gross motor skills. At 36 months of age, expressive language scores were mildly delayed in 21% of patients. When compared to the control group, CDH patients had similar neurodevelopmental scores at 12 and 36 months of age.

Conclusion: CDH is not necessarily associated with impaired neurodevelopmental outcomes during the first 3 years of life.

Introduction

Neurodevelopmental outcomes of congenital diaphragmatic hernia (CDH) survivors are a major cause for concern, with studies reporting a significantly increased risk of impaired neurodevelopmental outcomes.[1-3] The incidence of impaired neurodevelopmental outcomes in CDH patients during the first years of life has varied considerably in the literature ranging from 12% to 77%.[4,5] Three recent longitudinal studies reported adverse neurodevelopmental and neuromotor outcomes in CDH patient during the first years of life.[6-8] It appears that infants requiring extracorporeal membrane oxygenation (ECMO) have the highest risk for adverse neurodevelopmental outcomes.[1-3] However, reports of neurodevelopmental outcomes in CDH patients are generally based upon small cohorts without control subjects for comparison.

The aim of this study was to determine the neurodevelopmental outcomes in CDH patients in relation to contemporarily recruited healthy controls during the first 3 years of life.

Methods

In this study, neurodevelopmental outcome was assessed in CDH patients admitted to the Children's Hospital at Westmead between 1 January 2006 and 31 December 2009. Neurodevelopmental assessments were performed at the corrected age of 12 months and at 36 months using the Bayley scales of infant and toddler development-third edition (Bayley-III). The Bayley-III is a standardized test that assesses neurodevelopment across five distinct scales: cognition, receptive language, expressive language, fine motor and gross motor. Neurodevelopmental results of CDH patients were compared with published norms and a locally recruited, contemporary control group. The Bayley-III scaled scores have a mean of 10 with a standard deviation (SD) of 3. A Bayley-III scaled score <1 SD below the mean was considered as normal, a score of 1-2 SD below the mean as mildly delayed and a score of >2 SD below the mean as severely delayed. Additionally, audiology assessments using tympanometry were performed annually as part of routine follow-up. All neurodevelopmental measurements were adjusted for preterm delivery. Each infant with CDH was matched with two healthy control infants of the same gestational age and gender. The control group comprised healthy infants born during the same time period, who did not have any surgery up to the time of their assessment. The healthy controls were recruited from the co-located tertiary maternity units. Controls were the children born in one of the three tertiary maternity units, whose date and time of birth was closest to that of the CDH patient. Informed consent was directly obtained from parents.

Data were analyzed using SPSS version 20.0 for Windows (SPSS Inc., Chicago, Illinois, USA). Student's t-test was used for continuous normally distributed variables and non-parametric tests for non-normally distributed continuous variables. A p-value <0.05 was considered statistically significant.

Ethical approval for this study was obtained from the Ethics Committee of The Children's Hospital at Westmead (Activity N4060).

Results

During the study period, 34 CDH patients were admitted to our center. The overall survival rate was 85%. Of 29 survivors, 11 patients could not be included due to refusal of parents to join the study or loss to follow-up. Eighteen CDH patients were matched with 36 controls and assessed with the Bayley-III at the corrected age of 12 months and 15 of these children were tested again at 36 months of age. One child was later diagnosed with a chromosomal abnormality and was excluded from the analyses. Therefore, results for 17 CDH patients at 12 months and 14 CDH patients at 36 months of age are shown. Patient characteristics of CDH patients and controls are shown in table 1. Structural heart disease was the most commonly associated congenital anomaly noted in CDH survivors, other associated congenital anomalies were Pentalogy of Cantrell, an imperforate anus and a balanced translocation of chromosome 1:16. Minor structural heart disease (atrial septal defect, ventricular septal defect, moderate pulmonary valve stenosis) was present in six children and the child with Pentalogy of Cantrell was diagnosed with Tetralogy of Fallot.

Table 1. Patient characteristics of congenital diaphragmatic hernia patients and controls

Characteristics	CDH patients (n=17)	Controls (n=36)
Gestational age (weeks)	38.8±1.6	38.8±1.5
Birth weight (grams)	3344±644	3348±555
Gender		
Male	8 (47.1%)	18 (50.0%)
Female	9 (52.9%)	18 (50.0%)
Time of diagnosis		
Prenatal	9 (52.9%)	
Postnatal	8 (47.1%)	
Side of hernia		
Left	14 (82.4%)	
Right	3 (17.6%)	
Type of repair		
Primary repair	13 (76.5%)	
Patch	4 (23.5%)	
Associated congenital anomalies	9 (52.9%)	
Congenital heart disease	7 (41.2%)	
Moderate to severe pulmonary hypertension[1]	5 (29.4%)	

Data are presented as mean±standard deviation or number of patients (percentage).

[1] Pulmonary arterial pressures greater than half systemic on echocardiography together with the need for inhaled nitric oxide and/or sildenafil post-operatively.

Abbreviation: CDH=congenital diaphragmatic hernia.

At 12 months of age, all CDH patients scored within the normal range on the cognitive and fine motor scale. Receptive language was mildly delayed in one patient (6%), and one patient (6%) demonstrated mild delay in expressive language and gross motor skills. Three patients (18%) had severely delayed scores for gross motor skills at the age of 12 months.

All children with mildly or severely delayed scores at 12 months of age were reassessed at 36 months of age. At 36 months of age, expressive language scores were mildly delayed in three children (21%), and one child (7%) had a mildly delayed score on the receptive language scale as well. All the other outcome scores were normal at 36 months of age.

We found no difference in neurodevelopmental outcomes between CDH patients and the control group at 12 months and 36 months of age (table 2). However, there was a trend toward a significant difference for expressive language at 36 months of age, with CDH patients having lower scores. There was no difference in maternal or paternal education between the CDH patients and the control group. No sensorineural hearing loss was diagnosed in our cohort during the follow-up period.

Our study did not find any predictors for delayed neurodevelopmental outcome on any of the subscales.

Table 2. Neurodevelopmental outcome scores at 12 months and 36 months of age

Scale	Outcome score at 12 months			Outcome score at 36 months		
	CDH (n=17)	Controls (n=36)	p-value	CDH (n=14)	Controls (n=30)	p-value
Cognitive	11.41 (10.13-12.70)	11.14 (10.34-11.94)	0.76	10.36 (9.43-11.28)	10.73 (9.79-11.68)	0.97
Receptive language	10.18 (9.11-11.24)	11.17 (10.28-12.05)	0.14	10.42 (8.95-11.89)	11.23 (10.56-11.91)	0.25
Expressive language	9.35 (8.25-10.46)	10.06 (9.33-10.78)	0.22	9.33 (8.21-10.46)	10.87 (9.88-11.85)	0.06
Fine motor	9.82 (8.91-10.74)	9.97 (9.23-10.71)	0.77	10.64 (9.61-11.67)	11.00 (10.22-11.78)	0.39
Gross motor	8.24 (6.57-9.90)	8.97 (8.13-9.81)	0.61	9.86 (9.08-10.64)	10.21 (9.30-11.12)	0.93

Data are presented as mean (95% confidence interval).
Abbreviation: CDH=congenital diaphragmatic hernia.

Discussion

We did not find any differences in neurodevelopmental outcomes in CDH patients when compared with healthy controls at 12 months and 36 months of age. Three other longitudinal studies evaluated neurodevelopmental and neuromotor outcomes in CDH patients.[6-8] In our study, grossmotor skills were severely delayed in 18% of CDH patients at 12 months of age with all of these children achieving normal scores for grossmotor skills at 36 months of age. Friedman et al.[6] showed considerably higher rates of motor problems in CDH patients compared with our cohort. Similarly to our study, Danzer et al.[8] and Gischler et al.[7] demonstrated poor psychomotor outcomes in CDH patients during infancy. CDH survivors may require prolonged recovery periods in hospital. As a consequence, they have little physical activity and therefore fewer opportunities to obtain motor skills early in the first year of life. Nonetheless, our results demonstrate that CDH patients with delays in grossmotor skills catch-up with their peers after the first year of life. The two other studies also observed an improvement of psychomotor scores after 12 months of age.[7, 8] At 12 months of age, our control group scored one point below the mean score of the general population on the gross motor scale, which was still in the normal range within one standard deviation from the mean score.

At 36 months of age, 21% of CDH patients demonstrated mild delay on the expressive language scale compared to 6% of children at 12 months of age. Furthermore, there was a trend toward a difference for expressive language at 36 months of age between CDH patients and controls that did not reach statistical significance. Friedman et al.[6] also found language problems to be increased at 3 years of age compared to age 1 year, whereas the other two longitudinal studies did not show a decline in language scores.[7, 8] A possible explanation for these differences at 12 and 36 months of age is that subtle neurocognitive problems will become more evident with age. Therefore, problems are more likely to be diagnosed at 36 months of age. This emphasizes the importance of long-term follow-up of CDH survivors.

ECMO is frequently found to be associated with adverse neurodevelopmental outcome in CDH patients.[1-3] None of the CDH survivors in our cohort were treated with ECMO, which could be a contributing factor toward favorable neurodevelopmental outcome in our cohort of CDH patients.

Sensorineural hearing loss is another neurological disability that has been associated with CDH.[9] No sensorineural hearing loss was diagnosed in our cohort during the follow-up period, which could have contributed to the encouraging results in our cohort.

The most important limitation of this study includes the small sample size of CDH patients. However, we did assess our subjects in relation to contemporary controls from the same geographic population. Larger numbers of patients should be recruited to further explore this issue into school age when subtler learning difficulties may become apparent.

Conclusion

In this study, neurodevelopmental outcomes of CDH patients born since 2006 were no different to healthy matched controls at 12 and 36 months of age. This suggests that CDH is not necessarily associated with impaired neurodevelopmental outcomes during the first 3 years of life.

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Chapter 7

Risk factors of impaired neuropsychological outcome in school-aged survivors of neonatal critical illness

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Abstract

Purpose: Until now, long-term outcome studies have focused on general cognitive functioning and its risk factors following neonatal extracorporeal membrane oxygenation (ECMO) and/or congenital diaphragmatic hernia (CDH). This study aimed to identify affected neuropsychological domains and its clinical determinants in survivors of neonatal ECMO and/or CDH.

Design: Prospective follow-up study.

Setting: Tertiary university hospital.

Patients: Sixty-five eight-year-old survivors of neonatal ECMO and/or CDH.

Interventions: None.

Measurements and Main Results: Intelligence, attention, memory, executive functioning and visuospatial processing were evaluated using validated tests and compared to Dutch reference data. Assessed risk factors of outcome were illness severity indicators (need for ECMO, type of ECMO, maximum vasoactive-inotropic score, sepsis, ventilator-free days during the neonatal period, and duration of initial hospital stay), number of anesthetic procedures in the first year of life and growth at 1 year of age.

Patients had an average intelligence (mean IQ±standard deviation (SD): 95±16), but a significantly poorer sustained attention (mean z-score±SD: -2.73±2.57), verbal (immediate: -1.09±1.27; delayed: -1.14±1.86) and visuospatial memory (immediate: -1.48±1.02; delayed: -1.57±1.01; recognition: -1.07±3.10) than the norm. ECMO-treated CDH patients had a significantly lower mean IQ (84±12) than other neonatal ECMO patients (94±10) and CDH patients not treated with neonatal ECMO (100±20). Maximum vasoactive-inotropic score was negatively associated with delayed verbal (*B*=-0.02; 95% confidence interval: -0.03 to -0.002, p=0.026) and visuospatial memory (*B*=-0.01; 95% confidence interval: -0.02 to -0.001, p=0.024).

Conclusions: We found memory and attention deficits in eight-year-old neonatal ECMO and CDH survivors. The maximum dose of vasoactive medication was negatively associated with verbal and visuospatial memory, which may suggest an effect of early cerebral hypoperfusion in determining these abnormalities.

Introduction

The majority of children growing up following neonatal extracorporeal membrane oxygenation (ECMO) and/or congenital diaphragmatic hernia (CDH) have a generally average intelligence quotient (IQ), yet show impaired neurodevelopmental outcome and school problems.[1-3] Until now, most long-term outcome studies of school-aged survivors have focused on IQ and attention, hampering our understanding of the specific neuropsychological problems after neonatal critical illness.[1-3] Furthermore, it remains largely unclear which patients are at risk of impaired outcome and why. For early identification and intervention of those patients at risk, it is crucial to increase our understanding of the neuropsychological domains most frequently affected and the risk factors determining impaired outcome.

Earlier studies have found that markers of illness severity, such as ECMO requirement and the presence of chronic lung disease, were predictive of neuropsychological deficits in CDH patients.[2] Still, clinically useful risk factors of such deficits following neonatal critical illness remain unknown. Our group has recently shown specific hippocampal volume alterations that were related to verbal memory impairments in school-aged neonatal ECMO survivors.[4, 5] The hippocampus appears specifically vulnerable to hypoxic-ischemic injuries.[6, 7] Using measures of hypoperfusion could possibly aid in predicting neuropsychological outcomes following neonatal critical illness, as hypoperfusion may result in hypoxic-ischemic and eventually reperfusion injury in the hippocampus. Additionally, growth during early life may affect neurodevelopment. Poor growth in the first year of life has been reported in CDH and ECMO-treated patients,[8, 9] and has been associated with worse neurodevelopmental outcome both in CDH patients and in premature infants.[10, 11] However, the effects of poor growth on specific neuropsychological functions in school-aged survivors of neonatal critical illness remain unknown.

In this study, neuropsychological outcome was evaluated in school-aged CDH survivors treated or not treated with neonatal ECMO and in neonatal ECMO-treated survivors following other diagnoses. We hypothesized that children would mainly have attention and memory deficits, despite a generally average IQ. We expected markers of increased severity of illness and hypoperfusion as well as poor growth in the first year of life to have negatively affected neuropsychological outcome at school age.

Methods

Population

We included CDH and neonatal ECMO patients born between January 2006 and March 2009. All participants were routinely seen at 8 years of age as part of a structured prospective longitudinal follow-up program that includes regular physical and neurodevelopmental assessments until 18 years of age.[12] ECMO treatment had been applied in case of severe respiratory failure using the entry criteria described by Stolar et al.[13] Since November 2007, CDH patients were treated according to the standardized CDH EURO Consortium treatment protocol.[14] In case of persistent poor tissue perfusion and/or hypotension (arterial blood pressure below normal levels for gestational age and not improving after fluid boluses), treatment with dobutamine and/or dopamine was initiated, followed by norepinephrine, epinephrine or milrinone in case of persistent hypotension. Exclusion criteria were: genetic syndromes known to affect neurodevelopment, severe neurologic or developmental impairments preventing standardized assessments, late CDH diagnosis (>7 days of life), or a paraesophageal hernia. We used a protocol with extended neuropsychological assessments, implemented in January 2014 (Supplemental File 1).[1,2] Included children were divided into three groups: neonatal ECMO patients following other diagnoses than CDH ('ECMO-other'), CDH patients treated with neonatal ECMO ('CDH-ECMO') and CDH patients not treated with neonatal ECMO ('CDH non-ECMO'). This post-ECMO/CDH follow-up program is standard of care, therefore Institutional Review Board approval was waived (MEC-2017-185).[2,15]

Data collection

Relevant clinical data were collected at the time of hospitalization (supplementary methods). The Pediatric Logistic Organ Dysfunction-2 (PELOD-2) score[16] was collected in the first 24 hours of pediatric intensive care unit (PICU) stay (or up to ECMO cannulation in ECMO-treated patients if ECMO was initiated within the first 24 hours of PICU stay), the maximum vasoactive-inotropic score (VIS)[17] was recorded up until ECMO cannulation for the ECMO-treated patients or up until hernia repair for the CDH non-ECMO patients.

Follow-up data included growth measurements (height, weight, head circumference) at 6 months and 1 year, which were converted into z-scores (individual score minus the norm score divided by the norm standard deviation).[19] Height-corrected-for-target height z-score was calculated as follows: height-for-age z-score minus target height z-score.[20]

Neuropsychological evaluation was performed by an experienced pediatric psychologist. Socioeconomic status was assessed from maternal education level (referring to highest level of completed education by the mother).[21]

Neurodevelopmental outcome tests

Validated neuropsychological tests were administered in their Dutch versions to assess skills in six domains (see brief descriptions of the tests in supplementary methods):

1 *IQ:* two-subtest short-form (block design and vocabulary) of the Wechsler intelligence scale for children (WISC-III-NL).[22]
2 *Attention*:
   a. *Processing speed:* trail making test section A (TMT A).[23,24]
   b. *Selective attention and cognitive flexibility:* Stroop color word test (STROOP)[23,24] and trail making test section B (TMT B).[23,24]
   c. *Sustained attention:* dot cancellation test (DCT).[25]
3 *Verbal memory:*
   a. *Working memory:* subtest digit span of the WISC-III-NL.[22]
   b. *Immediate and delayed recall:* Rey auditory verbal learning test (RAVLT).[26]
4 *Visuospatial memory:*
   a. *Working memory:* subtest spatial span of the Wechsler nonverbal scale of ability (WNV).[27]
   b. *Immediate and delayed recall:* Rey complex figure test (RCFT).[28]
5 *Executive functioning:* key search and modified six elements of the Behavioral assessment of the dysexecutive syndrome for children (BADS-C-NL).[29]
6 *Visuospatial processing:* copy of the Rey complex figure test (RCFT copy).[28]

Neuropsychological test scores were converted into z-scores. Scores were inverted where appropriate so that a higher score always indicated a better performance. Z-scores ≤-1 were regarded as likely to represent impaired functioning (general population (mean z-score=0; standard deviation=1)).[23]

Statistical analysis

Differences in patient characteristics between the three groups ('ECMO-other', 'CDH-ECMO' and 'CDH-non-ECMO') were evaluated with chi-square or Fisher's exact tests for categorical variables, and with independent samples t-tests and one-way analysis of variance (ANOVA) for normally distributed variables. Mann-Whitney U tests and Kruskal-Wallis tests were used for continuous variables that were not normally distributed. Differences in neuropsychological outcome between the three groups were assessed with one-way ANOVA.

Univariable analyses were performed to assess the influence of clinical characteristics on the following neuropsychological outcomes of interest: intelligence, sustained attention, verbal memory immediate recall, verbal memory delayed recall, visuospatial memory immediate recall and visuospatial memory delayed recall. The independent variables included: maximum VIS, ECMO, type of ECMO(veno-arterial or veno-venous), sepsis, ventilator-free days in the first 28 days of life, duration of initial hospital stay, growth z-scores at 1 year (paired t-test showed largest growth deflection from 6 months to 1 year) and number of anesthetic pro-

cedures during the first year of life. Next, multivariable linear regression analyses were used to identify which independent variables remained significant predictors in a multivariable model. The assumptions for linear regression analysis were checked with normal probability plots of the residuals. Multicollinearity was evaluated in the multivariable models using the criterion that variance inflation factors should not exceed 2.5.[30]

Analyses were performed with SPSS 21.0 (IBM, Chicago, Illinois, USA), a two-sided p-value of <0.05 was considered statistically significant.

Results

Patient characteristics

Sixty-five patients aged 8.0±0.6 years were included: 25 ECMO-other patients, 10 CDH-ECMO patients, and 30 CDH-non-ECMO patients (supplemental figure 1). Illness severity during hospital admission differed between the three groups (table 1). The CDH-ECMO patients had the highest PELOD-2 score, the highest maximum VIS, the highest rate of sepsis, and the longest duration of mechanical ventilation and hospital stay. Sepsis occurred in three ECMO-other patients (during ECMO: n=2; after ECMO: n=1), four CDH-ECMO patients (after ECMO: n=4), and one CDH-non-ECMO patient after hernia repair. Four (50%) patients required vasoactive medication during sepsis. The median maximum VIS during sepsis was lower than the median maximum VIS before the ECMO run and none of the patients had a higher maximum VIS during sepsis (table 1). Characteristics of eligible patients who were lost to follow-up or refused follow-up (n=14) did not differ from included patients (data not shown). None of the patients treated with ECMO (both ECMO-other and CDH-ECMO) had signs of cerebral hemorrhage or vessel occlusion on cranial ultrasounds performed before and after the ECMO run.

Neuropsychological outcome

IQ fell within the normal range for the group as a whole. Sustained attention, verbal memory (immediate and delayed recall) and visuospatial memory (immediate and delayed recall as well as recognition) were below average compared to the general population (table 2). The majority of patients had normal outcomes in working memory, executive functioning and visuospatial processing. However, over 50% had impaired outcomes (z-score≤-1) on one or more of the memory and attention tests (figure 1).

When analyzing the three groups separately, we found that CDH survivors treated with ECMO had a significantly lower IQ compared to both other groups. However, no significant differences were found between the three groups on any of the other neuropsychological outcomes (table 2).

Table 1. Patient characteristics

	ECMO-other[1] (n=25)	CDH-ECMO (n=10)	CDH non-ECMO (n=30)	p-value
Gestational age (weeks)	40.9 (40.0-41.1)	39.2 (36.7-40.7)	38.5 (38.0-39.3)	**<0.001**
Birth weight (kilograms)	3.5±0.5	3.1±0.8	2.9±0.4	**0.001**
Male	14 (56%)	5 (50%)	18 (60%)	0.84[2]
Ethnicity				0.60[2]
Dutch	19 (76%)	9 (90%)	26 (87%)	
Other	6 (24%)	1 (10%)	4 (13%)	
Maternal education level				0.59[2]
Low	6 (24%)	3 (33%)	8 (29%)	
Moderate	12 (48%)	3 (33%)	16 (57%)	
High	7 (28%)	3 (33%)	4 (14%)	
Unknown	0	1	2	
Inborn	4 (16%)	4 (40%)	18 (60%)	**0.003[2]**
ECMO-related				
Highest oxygenation index prior to ECMO	33 (28-40)	38 (26-54)		0.72
Age start ECMO (days)	2 (1-4)	1 (1-2)		0.30
Duration of ECMO (hours)	92 (54-100)	172 (131-212)		***<0.001***
ECMO mode				***<0.001[2]***
VA	7 (28%)	10 (100%)		
VV converted to VA	1 (4%)			
VV	17 (68%)			
CDH-related				
Left sided hernia		8 (80%)	25 (83%)	1.00[2]
Age at surgery (days)		4 (3-6)	3 (2-4)	0.32
Patch repair		9 (90%)	17 (57%)	0.07[2]
Surgical technique				
Laparotomy		10 (100%)	20 (67%)	**0.04[2]**
Thoracoscopy			10 (33%)	
Hospital admission-related				
PELOD-2 score[3]	7 (7-9)	9 (8-9)	6 (5-7)	**<0.001**
Maximum VIS[4]	40 (35-70)	107 (91-142)	40 (5-76)	**<0.001**
Dobutamine	24 (96%)	10 (100%)	19 (63%)	**0.04[2]**
Dopamine	22 (88%)	7 (70%)	13 (43%)	**0.01[2]**
Norepinephrine	13 (52%)	10 (100%)	15 (50%)	**0.02[2]**
Epinephrine	2 (8%)	-	2 (7%)	1.00[2]
Milrinone	-	2 (20%)	-	**0.03[2]**
Vasopressin	-	-	-	-
CPR during initial hospital stay	2 (8%)	1 (10%)	0 (0%)	0.18[2]
Sepsis during initial hospital stay[5]	3 (12%)	4 (40%)	1 (3%)	**0.01[2]**
Days of mechanical ventilation	10 (7-11)	40 (16-51)	10 (5-18)	**0.001**
Ventilator-free days in the first 28 days of life	18 (17-21)	0 (0-12)	19 (10-23)	**0.001**
Days of initial PICU stay	13 (10-16)	70 (24-101)	21 (12-35)	**<0.001**
Days of initial hospital stay	24 (21-29)	91 (48-156)	36 (20-53)	**0.004**

Table 1. Continued

	ECMO-other[1] (n=25)	CDH-ECMO (n=10)	CDH non-ECMO (n=30)	p-value
Pulmonary hypertension				
Yes	13 (57%)	8 (80%)	12 (48%)	0.25[2]
No	10 (43%)	2 (20%)	13 (52%)	
Missing	2	0	5	
Inhaled nitric oxide treatment	22 (88%)	10 (100%)	10 (33%)	**<0.001[2]**
Sildenafil treatment	2 (8%)	6 (60%)	1 (3%)	**<0.001[2]**
Chronic lung disease[6]				
Yes	2 (10%)	8 (80%)	8 (28%)	**<0.001[2]**
No	19 (90%)	2 (20%)	21 (72%)	
Missing	4		1	
Follow-up-related				
Number of anesthetic procedures first year of life	2 (2-3)	4 (3-4)	1 (1-2)	**<0.001**
Weight-for-height z-score at 1 year	-0.38±0.86	-1.80±0.76	-0.88±0.97	**0.001**
Height-corrected-for-target height z-score at 1 year	-0.27±0.96	-0.36±0.69	-0.43±0.78	**0.02**
Head circumference-for-age z-score at 1 year	-0.47±1.16	-0.50±1.31	-0.39±1.15	0.96

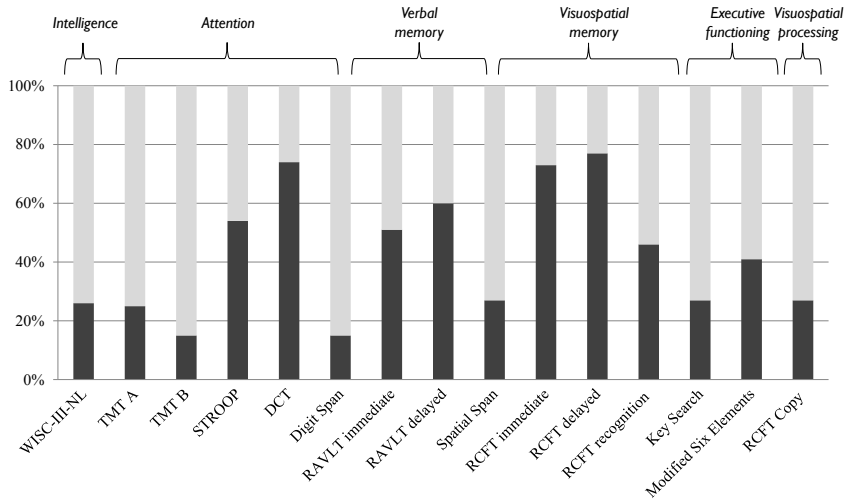
Data are expressed as mean±standard deviation, median (interquartile range) or number (percentage), as appropriate.
P-value=significant difference between the groups.
[1] ECMO treatment was given in case of meconium aspiration syndrome (n=18), persistent pulmonary hypertension of the newborn (n=2), congenital heart disease (monoventricular heart with transposition of the great vessels and total anomalous pulmonary venous return) (n=1), sepsis (n=1), respiratory insufficiency due to respiratory syncytial virus (n=1), infant respiratory distress syndrome with bilateral pneumothorax (n=1), pulmonary hypoplasia due to bilateral hydrothorax (n=1).
[2] Fisher's exact test was used.
[3] PELOD-2 score in the first 24 hours of PICU stay or up to ECMO cannulation if ECMO was initiated in the first 24 hours of PICU stay was calculated.[16]
[4]The maximum VIS recorded during PICU stay up until ECMO cannulation for the ECMO-treated patients or up until hernia repair for the CDH non-ECMO patients.[17] VIS was maximal at the median age of 1 (1-1) day in the ECMO patients, at the median age of 1 (0-1) day in the CDH-ECMO patients, and at the median age of 0 (0-1) days in the CDH non-ECMO patients.
[5] Sepsis during initial hospital stay (clinical suspicion of sepsis with positive blood culture). ECMO-other: two patients had sepsis during ECMO; one patients after ECMO; maximum VIS during sepsis: 10 (5-15). CDH-ECMO: 4 patients had sepsis after ECMO; maximum VIS during sepsis: 2.5 (0-32.5). CDH non-ECMO: one patient had sepsis after hernia repair; maximum VIS during sepsis: 0.
[6] Chronic lung disease defined as oxygen dependency at 28 days of life.[18]
Abbreviations: CDH=congenital diaphragmatic hernia; CPR=cardiopulmonary resuscitation; ECMO=extracorporeal membrane oxygenation; PELOD-2=Pediatric Logistic Organ Dysfunction-2; PICU=pediatric intensive care unit; VA=veno-arterial; VIS=va-soactive-inotropic score; VV=veno-venous.

Table 2. Overview of neuropsychological assessment outcome

Neuropsychological test	All (n=65)	ECMO-other (n=25)[1]	CDH-ECMO (n=10)	CDH-non-ECMO (n=30)	p-value
Intelligence					
WISC-III-NL	95±16	94±10	84±12	100±20	**0.029**
Attention					
TMT A	-0.33±0.86	-0.25±1.05	-0.45±0.53	-0.36±0.79	0.963
TMT B	-0.18±0.98	0.08±1.03	-0.28±1.08	-0.37±0.88	0.267
STROOP	-0.61±1.01	-1.00±0.76	-0.29±1.11	-0.39±1.10	0.081
DCT	-2.73±2.57	-2.88±2.09	-3.88±2.91	-2.25±2.76	0.173
Verbal memory					
WISC-III-NL digit span	0.06±1.09	-0.08±1.15	-0.34±0.94	0.31±1.07	0.706
RAVLT immediate	-1.09±1.27	-1.26±1.24	-1.55±1.03	-0.79±1.33	0.664
RAVLT delayed	-1.14±1.86	-1.38±1.46	-1.87±1.16	-0.70±2.24	0.117
Visuospatial memory					
WNV spatial span	-0.31±0.99	-0.39±0.79	-0.85±0.76	-0.06±1.14	0.613
RCFT immediate	-1.48±1.02	-1.52±1.02	-1.86±0.69	-1.31±1.09	0.417
RCFT delayed	-1.57±1.01	-1.56±1.01	-1.89±0.77	-1.47±1.09	0.689
RCFT recognition	-1.07±3.10	-1.09±1.51	-0.62±0.95	-0.47±1.22	0.117
Executive functioning					
Key search	-0.12±0.94	-0.26±0.98	-0.04±1.09	-0.05±0.89	0.694
Modified six elements	-0.60±0.87	-0.95±0.90	-0.46±0.47	-0.46±0.92	0.194
Visual spatial processing					
RCFT copy	-0.26±1.02	-0.36±1.00	-0.69±1.02	-0.02±1.01	0.107

Mean (standard deviation)=average IQ score or average z-score of the neuropsychological test.
One-way analysis of variance was used to identify differences between the groups on neuropsychological outcome.
P-value=significant difference between the groups.
[1] ECMO treatment was given in case of meconium aspiration syndrome (n=18), persistent pulmonary hypertension of the newborn (n=2), congenital heart disease (monoventricular heart with transposition of the great vessels and total anomalous pulmonary venous return) (n=1), sepsis (n=1), respiratory insufficiency due to respiratory syncytial virus (n=1), infant respiratory distress syndrome with bilateral pneumothorax (n=1), pulmonary hypoplasia due to bilateral hydrothorax (n=1).
Abbreviations: CDH=congenital diaphragmatic hernia; DCT=dot cancellation test; ECMO=extracorporeal membrane oxygenation; RAVLT=Rey auditory verbal learning test; RCFT=Rey complex figure test; STROOP=Stroop color word test; TMT=trail making test; WISC-III-NL=Wechsler Intelligence Scale for Children-Dutch version; WNV=Wechsler nonverbal scale of ability.
Please refer to supplementary file 1 for a description of the neuropsychological tests.

Figure 1. Presence of neuropsychological impairments in the study population



Percentage of patients with a z-score ≤-1 (impaired; as shown by the dark colored bars) and >-1 (normal; as shown by the grey colored bars) on each of the neuropsychological tests.
Abbreviations: WISC-III-NL=Wechsler intelligence scale for children-Dutch version; TMT=trail making test; STROOP=Stroop color word test; DCT=dot cancellation test; RAVLT=Rey auditory verbal learning test; RCFT=Rey complex figure test. Please refer to the supplementary file for a description of the neuropsychological tests; STROOP=Stroop color word test; TMT=trail making test; WISC-III-NL=Wechsler intelligence scale for children-Dutch version.

Predictors of neuropsychological outcome

Table 3 shows the results of the regression analyses. In the univariable analyses, severity of illness indicated by the need for ECMO, treatment with venoarterial-ECMO, maximum VIS, ventilator-free days during the neonatal period, and duration of initial hospital stay were associated with neuropsychological outcome, in particular with IQ. The number of anesthetic procedures during the first year of life was associated with IQ and sustained attention, and weight-for-height at 1 year was positively associated with IQ.

In the multivariable analyses, a higher maximum VIS remained associated with worse verbal and visuospatial memory delayed recall, such that an increase in maximum VIS of 20 points would result in a decrease of the verbal and visuospatial memory delayed recall z-scores of 0.4 and 0.2, respectively. Patients with impaired verbal memory had a significantly higher mean±standard deviation maximum VIS (72±44) than patients with normal verbal memory (40±32; p=0.003). Patients with impaired visuospatial memory had a higher mean maximum VIS (64±45) than patients with normal visuospatial memory (44±33), although this difference did not reach significance (p=0.118). The VIS remained a significant predictor of verbal and visuospatial memory delayed recall when group (ECMO-other, CDH-ECMO, CDH-non-ECMO) was added to the model (data not shown).

Growth or other indicators of severity of illness were no longer associated with neuropsychological outcome at 8 years of age in the multivariable analyses (supplementary table 1).

Table 3. Patient characteristics and neuropsychological outcome at 8 years of age

	Intelligence	Sustained attention	Verbal memory immediate
Univariable analyses with medical predictors			
CDH-non-ECMO[1]	**B=9.84, p=0.031 (CI: 0.96 to 18.72)**	**B=1.73, p=0.016 (CI: 0.34 to 3.12)**	B=0.70, p=0.068 (CI: -0.05 to 1.46)
CDH-ECMO[2]	**B=-14.11, p=0.016 (CI: -25.48 to -2.74)**	B=-1.84, p=0.060 (CI: -0.08 to 3.76)	B=-0.73, p=0.144 (CI: -1.73 to 0.26)
ECMO-other[3]	B=-1.59, p=0.745 (CI: -11.37 to 8.19)	B=-0.77, p=0.312 (CI: -2.30 to 0.75)	B=-0.29, p=0.475 (CI: -1.11 to 0.53)
VA-ECMO[4]	**B=-12.59, p=0.002 (CI: -19.97 to -5.20)**	B=-0.42, p=0.670 (CI: -2.46 to 1.61)	B=-0.38, p=0.457 (CI: -1.41 to 0.66)
Sepsis[5]	B=-12.36, p=0.064 (CI: -25.46 to 0.74)	B=-0.86, p=0.451 (CI: -1.42 to 3.15)	B=-0.52, p=0.363 (CI: -1.65 to 0.62)
VIS[6]	B=-0.08, p=0.105 (CI: -0.17 to 0.02)	B=-0.01, p=0.217 (CI: 0.01 to -0.03)	**B=-0.01, p=0.014 (CI: -0.02 to -0.002)**
Ventilator-free days[7]	**B=0.70, p=0.006 (CI: 0.22 to 1.19)**	B=0.07, p=0.098 (CI: 0.16 to -0.01)	**B=0.04, p=0.035 (CI: 0.00 to 0.09)**
Initial hospital stay (days)	**B=-0.16, p=0.004 (CI: -0.27 to -0.06)**	B=-0.01, p=0.133 (CI: 0.00 to -0.03)	**B=-0.01, p=0.045 (CI: -0.02 to 0.000)**
Anesthetics[8]	**B=-2.92, p=0.027 (CI: -5.49 to -0.35)**	**B=-0.44, p=0.036 (CI: -0.03 to -0.85)**	B=-0.15, p=0.179 (CI: -0.37 to 0.72)
Univariable analyses with growth predictors			
Weight-for-height z-score at 1 year	**B=6.41, p=0.002 (CI: 2.48 to 10.33)**	B=0.42, p=0.275 (CI: -0.35 to 1.19)	B=0.35, p=0.053 (CI: -0.01 to 0.69)
Height-corrected-for-target height z-score at 1 year	B=0.54, p=0.835 (CI: -4.70 to 5.79)	B=0.21, p=0.639 (CI: 0.68 to 1.10)	B=0.28, p=0.203 (CI: -0.16 to 0.71)
Head circumference-for-age z-score at 1 year	B=3.98, p=0.060 (CI: -0.18 to 8.13)	B=0.27, p=0.413 (CI: -0.39 to 0.94)	B=0.21, p=0.237 (CI: -0.14 to 0.55)
Multivariable analyses[9]			
VIS			

Maternal education level, ethnicity and gender were adjusted for in all models.
Variables found to be significant predictors in the univariable analyses were added into the multivariable model. Only those variables found to be significant in the multivariable analyses are reported.
Results indicate significant associations at p-value<0.05.
[1] CDH non-ECMO patients were compared to patients treated with ECMO.
[2] CDH-ECMO patients were compared to patients treated with ECMO following other diagnoses and to CDH patients not treated with ECMO.
[3] ECMO patients following other diagnosis were compared to CDH patients treated with and without ECMO.
[4] Patients treated with VA-ECMO were compared to patients treated with VV-ECMO.

Verbal memory delayed	Visuospatial memory immediate	Visuospatial memory delayed
B=0.86, p=0.145 (CI: -0.31 to 2.02)	B=0.31, p=0.257 (CI -0.23 to 0.86)	B=0.30, p=0.302 (CI: -0.28 to 0.88)
B=-0.97, p=0.204 (CI: -2.48 to 0.54)	B=-0.45, p=0.205 (CI: -1.16 to 0.26)	B=-0.37, p=0.328 (CI: -1.12 to 0.38)
B=-0.31, p=0.622 (CI: -1.55 to 0.94)	B=-0.48, p=0.868 (CI: -0.63 to 0.53)	B=-0.09, p=0.772 (CI: -0.70 to 0.53)
B=-0.25, p=0.677 (CI: -1.47 to 0.97)	B=-0.23, p=0.530 (CI: -0.97 to 0.51)	B=-0.26, p=0.499 (CI: -1.02 to 0.51)
B=-0.93, p=0.281 (CI: -2.63 to 0.78)	B=-0.23, p=0.565 (CI: -1.04 to 0.57)	B=-0.25, p=0.556 (CI: -1.10 to 0.60)
B=-0.02, p=0.004 (CI: -0.03 to -0.01)	**B=-0.01, p=0.028 (CI: -0.01 to -0.001)**	**B=-0.01, p=0.006 (CI: -0.01 to -0.002)**
B=0.08, p=0.025 (CI: 0.01 to 0.14)	**B=0.03, p=0.040 (CI 0.00 to 0.06)**	**B=0.03, p=0.043 (CI: 0.01 to 0.07)**
B=-0.01, p=0.133 (CI: -0.03 to 0.004)	B=-0.00, p=0.281 (CI: -0.01 to 0.003)	B=-0.00, p=0.347 (CI: -0.01 to 0.004)
B=-0.23, p=0.175 (CI: -0.57 to 0.11)	B=-0.04, p=0.615 (CI: -0.20 to 0.12)	B=-0.04, p=0.626 (CI: -0.21 to 0.13)
B=0.29, p=0.323 (CI: -0.30 to 0.89)	B=0.23, p=0.087 (CI: -0.03 to 0.49)	B=0.22, p=0.112 (CI: -0.05 to 0.50)
B=0.38, p=0.287 (CI: -0.33 to 1.09)	B=-0.01, p=0.936 (CI: -0.34 to 0.31)	B=-0.05, p=0.766 (CI: -0.39 to 0.29)
B=0.23, p=0.445 (CI: -0.37 to 0.83)	B=0.13, p=0.351 (CI: -0.14 to 0.40)	B=0.11, p=0.423 (CI: -0.17 to 0.39)
B=-0.02, p=0.026 (CI: -0.03 to -0.002)		**B=-0.01, p=0.024 (CI: -0.02 to -0.001)**

[5] Sepsis during initial hospital stay (clinical suspicion of sepsis with positive blood culture).

[6] The maximum VIS recorded during pediatric intensive care unit stay up until ECMO cannulation for the ECMO-treated patients or up until hernia repair for the CDH non-ECMO patients.

[7] Ventilator-free days in the first 28 days of life.

[8] Number of anesthetic procedures in the first year of life.

[9] Results of all variables added into the multivariable model are shown in supplementary table 1.

Abbreviations: CDH=congenital diaphragmatic hernia; CI=95% confidence interval; ECMO=extracorporeal membrane oxygenation; VA-ECMO=veno-arterial extracorporeal membrane oxygenation; VIS=vasoactive-inotropic score; VV-ECMO =veno-venous extracorporeal membrane oxygenation.

Discussion

This is the first study evaluating all major neuropsychological domains in school-aged survivors of neonatal ECMO and/or CDH. We found sustained attention and verbal and visuospatial memory deficits in over half of the patients, while other neuropsychological domains fell within the average range. CDH survivors treated with ECMO had a lower IQ than the other two groups, who had an average IQ. Nonetheless, the observed attention and memory problems were more severe than would be expected based on their IQ. This incongruity between attention and memory problems with IQ for all three groups indicates specific impairments in these domains. A higher dose of vasoactive medication (indicated by the maximum VIS recorded up until ECMO cannulation or hernia repair) was associated with lower scores on verbal and visuospatial memory delayed recall. Interestingly, impaired memory and attention were found in all diagnostic groups, except for better, although not significantly, verbal memory in the CDH non-ECMO group (within one standard deviation of the norm). Attention deficits have been reported in these patients previously, also despite generally average IQ.[1-3, 15] However, other neuropsychological domains were not assessed in these studies. In the present study we evaluated all major neuropsychological domains, and thereby identified a specific neuropsychological profile following neonatal ECMO and/or CDH. Our findings may serve as a starting point for intervention-based studies designed to improve cognitive functioning in these children.

As we found memory and attention deficits in the majority of patients, it is imperative to identify potential risk factors. Over the years, several severity of illness scoring systems have been developed including the VIS.[17] In the univariable analyses, maximum VIS as well as veno-arterial ECMO, ventilator-free days during the neonatal period and duration of initial hospital stay were independently associated with neuropsychological outcome, mainly with IQ. This indicates that severity of illness plays an important role in determining cognitive outcome in these survivors. Interestingly, in the multivariable analyses, only the maximum VIS recorded up until ECMO cannulation or hernia repair remained associated with delayed verbal and visuospatial memory. The hippocampus is highly involved in memory and has previously been shown to be altered following neonatal ECMO and/or CDH.[4, 5, 31, 32] The hippocampus has been found to be particularly susceptible to cerebral hypoperfusion resulting in hypoxia-ischemia.[6, 7, 33] Although this study does not show a causative effect of vasoactive medication regarding memory problems, we speculate that receiving high levels of vasoactive medication in the first period of life may be an indirect marker of temporarily (regional) inadequate brain perfusion. A high need for vasoactive medication could therefore be a useful component in estimating severity of illness and risk of memory impairments at school age in these survivors. Although we cannot make any recommendations based on our findings, the VIS may be valuable in determining the need for and timing of ECMO treatment in neonates with severe respiratory failure, which should be investigated in future studies. As the

VIS has been validated to predict clinical outcomes in infants who require cardiac surgery,[17, 34] representing another group of critically ill children requiring circulatory support by vaso-active drugs. Future studies are needed to further validate the usefulness of this score for long-term outcome in neonatal critical illness survivors, and to study the direct association between maximum VIS and brain areas susceptible to ischemic-reperfusion injury such as the hippocampus.

Attention and memory problems at school age have also been found in other groups of critically ill neonates such as premature infants and infants with congenital heart disease.[31, 35] Studies in premature infants have found that lower scores on executive functioning were associated with the severity of neonatal illness (indicated by presence of sepsis, meningitis, bronchopulmonary dysplasia or longer duration of hospital stay),[36] although others have not confirmed this.[37] Multicenter studies are needed to develop a multimodal prediction model which may be the key to earlier identification of critical illness survivors at risk of impaired neurodevelopmental outcome. Predictors of interest would be specific markers of illness severity, such as the maximum VIS, in combination with predetermined assessment of neurobiological correlates, such as imaging of the hippocampus.

We did not find any associations between growth at 1 year and long-term neuropsychological outcome. Although many CDH patients show poor growth during the first year of life,[9] only one study has found an association between growth (weight and head circumference) at 2 to 3 years of age and general cognitive functioning, although not at the age of 5 years.[11] In premature infants, several studies have demonstrated a positive association between weight gain and head growth and cognitive outcomes.[10] However, a recent study in children with extremely low birth weight showed no effect of catch-up-growth in the first 2 years of life on neurocognitive outcome at 11 years.[38] Most studies in premature infants did not take into account the difference in severity of illness. It is therefore uncertain whether poor growth itself or severity of critical illness leading to poor growth, is more important in determining adverse neuropsychological outcome in premature infants. Although we cannot draw definitive conclusions, our study indicates that in ECMO and CDH patients, the severity of illness has a greater impact on neuropsychological outcome than growth in the first year of life.

Our study has some limitations. First, the relatively small sample sizes of the three diagnostic groups, especially of the CDH-ECMO group, is a frequent problem in follow-up studies including patients with rare diagnoses, but limits the interpretability of our regression analyses. Multicenter collaborations with standardized management and standardized structured follow-up are important to increase sample sizes and get a better understanding of the pathophysiology underlying long-term outcome. Second, MRI data were not available in this study and we therefore could not examine whether maximum VIS was specifically associated with brain structures susceptible to cerebral hypoperfusion. Standardized neuroimaging studies both at neonatal age and school age will aid in understanding pathophysiologic concepts of early brain development as well as long-term outcome and are therefore important

in future studies. Third, there are likely multiple factors involved in the development of long-term neuropsychological impairments following neonatal ECMO and/or CDH, such as exposure to inflammation[7], anesthetics[39], and endogenous and exogenous stressors[40] in early life, or probably a complex interplay amongst these factors. As of now, techniques to reliably measure these mechanisms and their interactions are lacking. Future studies are needed to develop specific brain monitoring techniques that can be used during PICU stay for early identification of patients at risk of long-term impairments.

In conclusion, we found sustained attention, verbal and visuospatial memory deficits in eight-year-old survivors of neonatal ECMO and/or CDH. These findings emphasize the need for standardized neuropsychological follow-up including attention and memory assessments until school age and beyond in these survivors. Maximum VIS in the first day(s) of PICU admission was negatively associated with verbal and visuospatial memory at eight years of age. This suggests that this measure of severity of illness, possibly indicating (cerebral) hypoperfusion during early life, is related to specific neuropsychological functions in eight year-old neonatal ECMO and/or CDH survivors. Future studies using advanced neuroimaging techniques in combination with clinical characteristics and neuropsychological evaluation will aid in a better understanding of this finding and are needed for early identification and intervention of patients at risk. Our findings of specific attention and memory problems can serve as a starting point for developing and implementing early intervention strategies that focus on improving attention and memory in these patients.

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Supplements

Supplementary methods. Description of data collection

Relevant clinical data were collected at the time of hospitalization, including: gestational age, birth weight, gender, ethnicity (Dutch/≥1 non-native Dutch parent), inborn, the need for extracorporeal membrane oxygenation (ECMO), Pediatric Logistic Organ Dysfunction-2 (PELOD-2) score[16] in the first 24 hours of pediatric intensive care unit (PICU) stay (or up to ECMO cannulation in ECMO-treated patients if ECMO was initiated in the first 24 hours of PICU stay), the maximum vasoactive-inotropic score (VIS)[17] was recorded up until ECMO cannulation for the ECMO-treated patients or up until hernia repair for the CDH-non-ECMO patients, calculated as follows: dopamine dose (ug/kg/min)+dobutamine dose (ug/kg/min)+(100xepinephrine dose (ug/kg/min))+(10xmilrinone dose (ug/kg/min))+(10,000xvasopressin dose (U/kg/min))+(100xnorepinephrine dose (ug/kg/min)), cardiopulmonary resuscitation(CPR) during initial hospital stay, sepsis during initial hospital stay (clinical suspicion of sepsis with positive blood culture), duration of initial mechanical ventilation, ventilator-free days in the first 28 days of life, duration of PICU stay, duration of initial hospitalization, pulmonary hypertension on echocardiography during PICU admission, inhaled nitric oxide requirement, sildenafil requirement, the presence of chronic lung disease(oxygen dependency at 28 days of life),[18] and number of anesthetic procedures in the first year of life (including CDH repair and/or ECMO (de)cannulation). Additional characteristics for ECMO patients included: highest oxygenation index before ECMO, age at start ECMO, type of ECMO (veno-arterial or veno-venous), ECMO duration, and cranial ultrasound result before and after ECMO. Additional data for CDH patients were: diaphragmatic defect side, surgical repair technique (thoracoscopy or laparotomy), age at surgery, and patch repair requirement.

Supplementary methods. Descriptions of the neuropsychological tests

Intelligence

Wechsler Intelligence Scale for Children (WISC-III-NL)*:* the Wechsler intelligence scale for children was used. Both tests assessing verbal and non-verbal intelligence, have been shown to have good reliability and validity,[1,2] and have been used interchangeably by our group before.[3] For both tests, a normalized population mean of 100 with a standard deviation of 15 is used.[1,2]

Attention

Trail making test (TMT)*:* this paper and pencil test consists of two parts. In the first part (part A), the subject must draw lines to consecutively connect numbered circles on a sheet. In the second part (part B), the subject must consecutively but alternately connect numbered and lettered circles on another worksheet. The goal of the test is to finish each part as quickly as possible. The test can be administered to children and adults in the age range 6-89 years. This test measures visual conceptual and visuomotor tracking as well as divided attention.[4,5]

Stroop color word test (STROOP): the STROOP consists of three trials: in the first trial (Stroop 1) the subject must read color names, in the second trial (Stroop 2) name printed colors, and in the third trial (Stroop 3) name printed colors not denoted by the color name. The test can be administered to children and adults in the age range 8-65 years. Selective attention is measured with this test.[4, 5]

Dot cancellation test: this paper and pencil test measures sustained selective attention and concentration in terms of speed and accuracy. It consists of a paper on which figures made of 3, 4 or 5 dots are displayed in 33 rows. The child is instructed to cross off all figures with 4 dots, as precise and as fast as they can.[6]

Verbal memory

WISC-III-NL – subtest digit span: the digit span consists of random number sequences that increase in length and that the examiner reads aloud at the rate of 1 number per second. The child has to reproduce these numbers in the same order. Next, the sequences must be recalled backwards (3-5-7 becomes 7-5-3). The first part of the test measures short-term auditory memory and short-term retention capacity. The second part measures auditory working memory. A difference of ≥4 points between forward and backward digit span in favor of forward is indicative of a working memory problem.[7]

Rey auditory verbal learning test (RAVLT): the RAVLT consists of five presentations with recall of a 15-word list, a sixth recall trial after 30 minutes, and a seventh recognition trial. This test measures memory span, short- and long-term verbal memory, verbal recognition, learning curve, and retroactive or proactive interference. It can be administered to children and adults in the age range 6-89 years.[8, 9]

Visuospatial memory

Wechsler nonverbal scale of ability (WNV) – subtest spatial span: the spatial span requires the child to touch a group of blocks arranged on a board in a nonsystematic manner in the same and reverse order as demonstrated by the examiner. The first part of the test measures short-term visuospatial memory and short-term retention capacity. The second part measures visuospatial working memory.[10]

Rey complex figure test (RCFT): the RCFT consist of three trials. First the child has to copy a complex figure. Then after 3 and after 30 minutes the figure must be drawn from memory. Next, different figures are shown and the child has to indicate whether these figures were in the original figure. The last two trials measure short- and long-term visual-spatial memory, and visuospatial recognition. This test can be completed by children and adults in the age range 6-89 years.[11, 12]

Executive functioning

Key search: a test of strategy formation. The child is asked to demonstrate how they would search a field for a set of lost keys and their strategy is scored according to its functionality.[13]

Modified six elements: the child is asked to work on six different tasks for which they have 5 minutes. There are some rules the child has to obey during the task, while making sure that by the end of the 5 minutes, all six of the tasks have been done and the child has done as much as possible of each task. This is a test of planning, task scheduling and performance monitoring.[13]

Visuospatial processing

Rey complex figure test (RCFT): the RCFT consists of three trials. First the child has to copy a complex figure. Then after 3 and after 30 minutes the figure must be drawn from memory. Next, different figures are shown and the child has to indicate whether these figures were in the original figure. The first trial measures visual integration. This test can be completed by children and adults in the age range 6-89 years.[11, 12]

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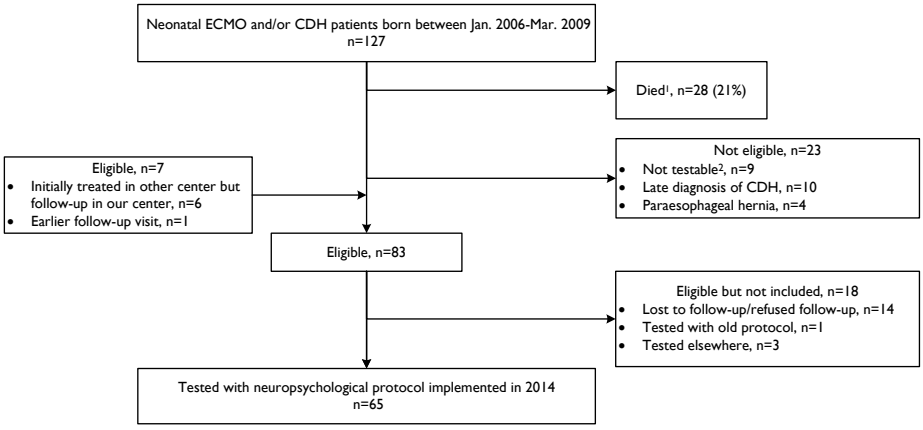
Supplements

Supplementary figure 1. Flowchart of the study population



[1] ECMO-other group: n=12; ECMO-CDH-group: n=10; CDH-non-ECMO group: n=6.

[2] Severe neurologic or developmental impairments (n=5; 3 patients had primary hemorrhage at cranial ultrasound performed after the ECMO run); Simpson-Golabi-Behmel Syndrome (n=2); Down Syndrome (n=1); mitochondriopathy (n=1).

Abbreviations: CDH=congenital diaphragmatic hernia; ECMO=extracorporeal membrane oxygenation.

Supplementary table 1. Patient characteristics and neuropsychological outcome at 8 years of age

	Intelligence	Sustained attention	Verbal memory immediate
Univariable analyses with medical predictors			
CDH-non-ECMO[1]	**B=9.84, p=0.031 (CI: 0.96 to 18.72)**	**B=1.73, p=0.016 (CI: 0.34 to 3.12)**	B=0.70, p=0.068 (CI: -0.05 to 1.46)
CDH-ECMO[2]	**B=-14.11, p=0.016 (CI: -25.48 to -2.74)**	B=-1.84, p=0.060 (CI: -0.08 to 3.76)	B=-0.73, p=0.144 (CI: -1.73 to 0.26)
ECMO-other[3]	B=-1.59, p=0.745 (CI: -11.37 to 8.19)	B=-0.77, p=0.312 (CI: -2.30 to 0.75)	B=-0.29, p=0.475 (CI: -1.11 to 0.53)
VA-ECMO[4]	**B=-12.59, p=0.002 (CI: -19.97 to -5.20)**	B=-0.42, p=0.670 (CI: -2.46 to 1.61)	B=-0.38, p=0.457 (CI: -1.41 to 0.66)
Sepsis[5]	B=-12.36, p=0.064 (CI: -25.46 to 0.74)	B=-0.86, p=0.451 (CI: -1.42 to 3.15)	B=-0.52, p=0.363 (CI: -1.65 to 0.62)
VIS[6]	B=-0.08, p=0.105 (CI: -0.17 to 0.02)	B=-0.01, p=0.217 (CI: 0.01 to -0.03)	**B=-0.01, p=0.014 (CI: -0.02 to -0.002)**
Ventilator-free days[7]	**B=0.70, p=0.006 (CI: 0.22 to 1.19)**	B=0.07, p=0.098 (CI: 0.16 to -0.01)	**B=0.04, p=0.035 (CI: 0.00 to 0.09)**
Initial hospital stay (days)	**B=-0.16, p=0.004 (CI: -0.27 to -0.06)**	B=-0.01, p=0.133 (CI: 0.00 to -0.03)	**B=-0.01, p=0.045 (CI: -0.02 to 0.000)**
Anesthetics[8]	**B=-2.92, p=0.027 (CI: -5.49 to -0.35)**	**B=-0.44, p=0.036 (CI: -0.03 to -0.85)**	B=-0.15, p=0.179 (CI: -0.37 to 0.72)
Univariable analyses with growth predictors			
Weight-for-height z-score at 1 year	**B=6.41, p=0.002 (CI: 2.48 to 10.33)**	B=0.42, p=0.275 (CI: -0.35 to 1.19)	B=0.35, p=0.053 (CI: -0.01 to 0.69)
Height-corrected-for-target height z-score at 1 year	B=0.54, p=0.835 (CI: -4.70 to 5.79)	B=0.21, p=0.639 (CI: 0.68 to 1.10)	B=0.28, p=0.203 (CI: -0.16 to 0.71)
Head circumference-for-age z-score at 1 year	B=3.98, p=0.060 (CI: -0.18 to 8.13)	B=0.27, p=0.413 (CI: -0.39 to 0.94)	B=0.21, p=0.237 (CI: -0.14 to 0.55)
Multivariable analyses			
CDH-non-ECMO[1]	B=7.33, p=0.213 (CI: -4.42 to 19.07)	B=1.29, p=0.110 (CI: -2.90 to 0.31)	
CDH-ECMO[2]	B=-1.66, p=0.851 (CI: -19.51 to 16.18)		
VA-ECMO[3]	B=-8.03, p=0.131 (CI: -18.83 to 2.76)		
VIS[6]			B=-0.01, p=0.055 (CI: -0.02 to -0.000)
Ventilator-free days[7]	B=-0.28, p=0.571 (CI: -1.29 to 0.72)		B = 0.02, p=0.580 (CI -0.06 to 0.10)
Initial hospital stay (days)	B = -0.14, p=0.225 (CI: -0.36 to 0.09)		B=0.00, p=0.784 (CI: -0.01 to 0.02)
Anesthetics[8]	B=0.40, p=0.831 (CI: -3.36 to 4.15)	B=0.25, p=0.285 (CI: -0.22 to 0.72)	
Weight-for-height at 1 year	B=3.85, p=0.180 (CI: -1.88 to 9.58)		

Verbal memory delayed	Visuospatial memory immediate	Visuospatial memory delayed
B=0.86, p=0.145 (CI: -0.31 to 2.02)	B=0.31, p=0.257 (CI -0.23 to 0.86)	B=0.30, p=0.302 (CI: -0.28 to 0.88)
B=-0.97, p=0.204 (CI: -2.48 to 0.54)	B=-0.45, p=0.205 (CI: -1.16 to 0.26)	B=-0.37, p=0.328 (CI: -1.12 to 0.38)
B=-0.31, p=0.622 (CI: -1.55 to 0.94)	B=-0.48, p=0.868 (CI: -0.63 to 0.53)	B=-0.09, p=0.772 (CI: -0.70 to 0.53)
B=-0.25, p=0.677 (CI: -1.47 to 0.97)	B=-0.23, p=0.530 (CI: -0.97 to 0.51)	B=-0.26, p=0.499 (CI: -1.02 to 0.51)
B=-0.93, p=0.281 (CI: -2.63 to 0.78)	B=-0.23, p=0.565 (CI: -1.04 to 0.57)	B=-0.25, p=0.556 (CI: -1.10 to 0.60)
B=-0.02, p=0.004 (CI: -0.03 to -0.01)	**B=-0.01, p=0.028 (CI: -0.01 to -0.001)**	**B=-0.01, p=0.006 (CI: -0.01 to -0.002)**
B=0.08, p=0.025 (CI: 0.01 to 0.14)	**B=0.03, p=0.040 (CI 0.00 to 0.06)**	**B=0.03, p=0.043 (CI: 0.01 to 0.07)**
B=-0.01, p=0.133 (CI: -0.03 to 0.004)	B=-0.00, p=0.281 (CI: -0.01 to 0.003)	B=-0.00, p=0.347 (CI: -0.01 to 0.004)
B=-0.23, p=0.175 (CI: -0.57 to 0.11)	B=-0.04, p=0.615 (CI: -0.20 to 0.12)	B=-0.04, p=0.626 (CI: -0.21 to 0.13)
B=0.29, p=0.323 (CI: -0.30 to 0.89)	B=0.23, p=0.087 (CI: -0.03 to 0.49)	B=0.22, p=0.112 (CI: -0.05 to 0.50)
B=0.38, p=0.287 (CI: -0.33 to 1.09)	B=-0.01, p=0.936 (CI: -0.34 to 0.31)	B=-0.05, p=0.766 (CI: -0.39 to 0.29)
B=0.23, p=0.445 (CI: -0.37 to 0.83)	B=0.13, p=0.351 (CI: -0.14 to 0.40)	B=0.11, p=0.423 (CI: -0.17 to 0.39)
B=-0.02, p=0.026 (CI: -0.03 to -0.002)	B=-0.01, p=0.084 (CI: -0.01 to -0.001)	**B=-0.01, p=0.024 (CI: -0.02 to -0.001)**
B=0.02, p=0.555 (CI: -0.06 to 0.10)	B= 0.01, p=0.587 (CI: -0.03 to 0.05)	B=0.00, p=0.813 (CI: -0.03 to 0.04)

Maternal education level, ethnicity and gender were adjusted for in all models.
Variables found to be significant predictors in the univariable analyses were added into the multivariable model. Only those variables found to be significant in the multivariable analyses are reported.
Results indicate significant associations at p-value<0.05.
[1] CDH non-ECMO patients were compared to patients treated with ECMO.
[2] CDH-ECMO patients were compared to patients treated with ECMO following other diagnoses and to CDH patients not treated with ECMO.
[3] ECMO patients following other diagnosis were compared to CDH patients treated with and without ECMO.
[4] Patients treated with VA-ECMO were compared to patients treated with VV-ECMO.
[5] Sepsis during initial hospital stay (clinical suspicion of sepsis with positive blood culture).
[6] The maximum VIS recorded during pediatric intensive care unit stay up until ECMO cannulation for the ECMO-treated patients or up until hernia repair for the CDH non-ECMO patients.
[7] Ventilator-free days in the first 28 days of life.
[8] Number of anesthetic procedures in the first year of life.
Abbreviations: CDH=congenital diaphragmatic hernia; CI=95% confidence interval; ECMO=extracorporeal membrane oxygenation; VA-ECMO=veno-arterial extracorporeal membrane oxygenation; VIS=vasoactive-inotropic score; VV-ECMO =veno-venous extracorporeal membrane oxygenation.

Chapter 8

Congenital diaphragmatic hernia and growth to 12 years

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Abstract

Objectives: Growth problems are reported in patients with congenital diaphragmatic hernia (CDH) during the first years of life. However, it is unknown if poor growth persists during childhood. We therefore evaluated growth of patients longitudinally until 12 years.

Methods: This prospective study included 172 patients (43 treated with extracorporeal membrane oxygenation (ECMO)) born from 1999 to 2014. Z-scores of height-for-age, weight-for-height, and distance-to-target height were calculated at 6 months of age and at 1, 2, 5, 8, and 12 years of age. Data were analyzed by using general linear models.

Results: At 1 year of age, the mean weight-for-height z-score had declined in ECMO (-1.30, 95% confidence interval: -1.62 to -0.97) and non-ECMO patients (-0.72, 95% confidence interval: -0.91 to -0.54; $p<0.05$). Thereafter in ECMO patients, the mean height-for-age z-score deteriorated between 1 year (-0.43, 95% confidence interval: -0.73 to -0.13) and 5 years of age (-1.08, 95% confidence interval: -1.38 to -0.78; $p<0.01$). In non-ECMO patients, the mean height-for-age z-score deteriorated between 2 years (-0.35, 95% confidence interval: -0.53 to -0.17) and 5 years of age (-0.56, 95% confidence interval: -0.75 to -0.37; $p=0.002$). At 12 years of age, the mean height-for-age z-score was still less than the norm in both groups: ECMO (-0.67, 95% confidence interval: -1.01 to -0.33) versus non-ECMO (-0.49, 95% confidence interval: -0.77 to -0.20; $p<0.01$). Adjusting for target height improved the mean height z-scores but did not bring them to normal.

Conclusions: Poor linear growth persisted at 12 years of age. The pattern of early deterioration of weight gain followed by a decline in linear growth is suggestive of inadequate nutrition during infancy. Therefore, nutritional assessment and intervention should be started early, and should be continued during childhood.

Introduction

Patients with congenital diaphragmatic hernia (CDH) may suffer from long-term morbidities that could affect development later in life, including obstructive lung function,[1] gastroesophageal reflux,[2] and impaired physical growth.[3] This indicates the need for long-term follow-up of patients with CDH. Therefore, in 2008 the American Academy of Pediatrics published guidelines for structured long-term follow-up.[4] Growth problems are an important issue in patients with CDH. Prevalence rates of wasting, indicating acute malnutrition, vary between 8% and 69% at 1 year of age.[5, 6] Malnutrition in infancy has been associated with adverse long-term outcomes such as impaired neurodevelopment in otherwise healthy children.[7, 8] Previous studies reported growth problems in CDH survivors during the first months of life with catch-up growth starting after the first 6 months.[6, 9] Still, a recent longitudinal study showed that growth failure persisted in 13.5% of patients at 6 years of age.[10]

Growth problems are worse among patients who were treated with neonatal extracorporeal membrane oxygenation (ECMO), possibly because they have more severe lung hypoplasia and, consequently, higher energy needs to compensate for the increased work of breathing.[3, 11] So far, growth of patients with CDH after the age of 6 years has not been studied longitudinally.

We aimed to evaluate growth longitudinally until 12 years of age in patients with CDH who were treated with and without ECMO. We hypothesized that patients were at risk for impaired growth in the long-term, especially those treated with neonatal ECMO. Furthermore, we aimed to find clinical variables associated with growth.

Patients and methods

Patients

In 1999, we started a prospective longitudinal follow-up program to monitor growth, lung function and neurodevelopment of patients with CDH until 18 years of age.[12] This prospective longitudinal study was conducted within the framework of our follow-up program and included surviving patients with CDH admitted to the Erasmus MC-Sophia Children's Hospital between January 1999 and December 2014. For the purposes of this evaluation, we excluded data from patients diagnosed after 7 days of age, those with paraesophageal diaphragmatic defects, those with a diaphragmatic eventration, and those with genetic syndromes that affect physical growth. ECMO treatment was applied in case of reversible severe respiratory failure by using the entry criteria reported by Stolar et al.[13] until November 2007. After November 2007, children were treated according to the standardized CDH EURO Consortium Consensus treatment protocol.[14]

The Erasmus MC Medical Ethical Review Board stated that the 'Medical Research in Human Subjects Act does not apply to this study,' and waived institutional review board approval (MEC-2016-111).

Data collection

The following data were collected: gestational age; birth weight; sex; ethnicity; side and type of repair of the diaphragmatic defect; age at repair; duration of initial mechanical ventilation; ECMO treatment; duration of pediatric intensive care unit (PICU) stay; presence of chronic lung disease (oxygen dependency at 28 days of life)[15]; need for supplemental oxygen at discharge; and presence of cardiac anomalies; gastroesophageal reflux disease (GERD) or Nissen fundoplication. All patients were treated with antireflux medication until evaluation of GERD by using 24-hour pH-metry. Until 2013, GERD was evaluated by 24-hour pH-metry only; afterwards, we used 24-hour pH-metry with impedance.

Growth was evaluated at each routine outpatient visit at 6 months of age and at 1, 2, 5, 8, and 12 years of age. At each follow-up visit, we recorded the use of tube feeding, calorie enriched feeds, and GERD medication.

Growth measurements

Growth parameters were expressed as z-scores in relation to Dutch norms by using the reference data from the Fifth Dutch Growth study.[16] Reference values for Dutch children of Moroccan or Turkish origin were used where applicable.[17] Height-for-age (HFA) and weight-for-height (WFH) z-scores were calculated.[18] Target height (TH) and TH z-scores were calculated using parental heights.[19] To correct HFA z-score for TH, we calculated distance-to-target height (DTH) z-score as follows: DTH z-score=HFA z-score minus TH z-score. Stunting was defined as HFA z-score <-2.[20] Wasting was defined as WFH z-score

<-2.[20] Stunting corrected for TH was defined as DTH z-score <-2.

Pilot study: indirect calorimetry

To assess the role of increased energy expenditure in 11 patients with growth problems, we measured resting energy expenditure (REE) by indirect calorimetry (Cosmed Quark RMR with Canopy, Tulipmed, Nieuwegein, the Netherlands). In addition, these patients had dietary consultations. For more details, see supplemental methods.

Statistical Analyses

To evaluate differences in patient characteristics between ECMO and non-ECMO patients, we performed chi-square or Fisher's exact tests for categorical variables. Independent samples t-tests were used for normally distributed variables, and Mann-Whitney U tests for variables not normally distributed.

To evaluate growth longitudinally and compare growth of patients with the norm population (z-score=0), we used general linear models. This method can account for within subject correlations and allows for missing values in the dependent variable. We compared ECMO and non-ECMO patients with the norm population by using general linear models that included z-scores for HFA, WFH and DTH as dependent variables, and time-point, ECMO, and the interaction between time-point and ECMO as independent variables. Paired sample t-tests were used to compare mean z-scores of HFA and DTH at each time-point. To find associations between growth and clinical variables, we estimated general linear models in which the following independent variables were considered, in addition to ECMO and time-point: gestational age, patch repair, presence of cardiac anomalies, duration of initial mechanical ventilation, duration of PICU stay, supplemental home oxygen at discharge, the presence of Nissen fundoplication, the use of tube feeding, the use of calorie-enriched feeds, and the presence of a new treatment protocol (after November 2007). We used a stepwise backward approach to select the independent variables from this list of variables, with a p-value cut-off of p=0.20 for removal of independent variables. Two-way interaction effects were then added to the resulting model if the interaction effect was statistically significant. Pearson correlation coefficients between independent variables were calculated to assess the level of multicollinearity. All correlations among the included independent variables were lower than 0.6. To account for within-subject correlations, all general linear models were estimated with an unstructured error covariance matrix for the repeated measurements of each patient. The results of general linear models were summarized by using the estimated marginal means, which are the predicted values of the dependent variable adjusted for the effects of the independent variables.

Analyses were performed using SPSS 21.0 (IBM Corporation, Armonk, New York, USA). Statistical tests were 2-sided and used a significance level of 0.05.

Results

Patients

Of 298 patients with CDH, 78 (26%) died before hospital discharge (figure 1). Forty-eight of the 220 patients who survived were excluded for various reasons. Therefore, the study group consisted of 172 patients, of whom 43 (25%) received ECMO treatment. None of the 172 included children had a Morgagni-Larrey hernia.

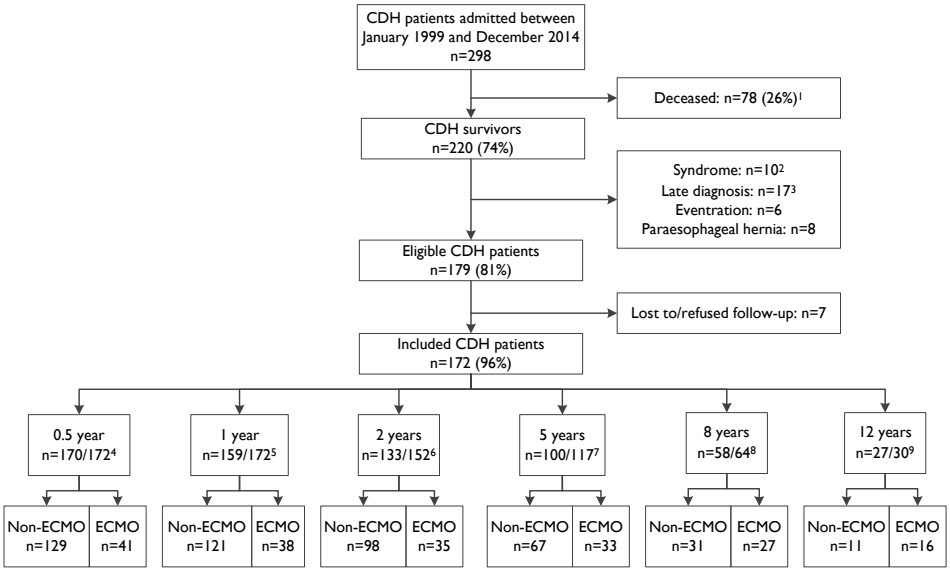
Table 1 shows patient characteristics. Of all 172 patients, 138 (80%) underwent 24-hour pH-metry at the median age of 2.9 months (interquartile range (IQR): 1.7-4.4), and 53 patients (38%) were diagnosed with GERD. Twenty-three patients were asymptomatic; the 30 others had the following symptoms: regurgitation (n=11), regurgitation with feeding difficulties (n=18), and oxygen saturation dips (n=1). Twenty patients (12%) underwent a Nissen fundoplication at the median age of 0.7 years (IQR: 0.3-2.6). Nineteen patients experienced regurgitation with feeding difficulties and one asymptomatic patient had histologically proven esophagitis despite adequately dosed drug therapy. Seventeen patients (10%) had a gastrostomy placement at the median age of 0.5 year (IQR: 0.3-0.9; 12 of these patients underwent Nissen fundoplication). Supplementary table 1 shows the numbers of patients who were tube fed at follow-up. None of the patients were tube fed at the age of 8 and 12 years.

Height measurements

The mean HFA was less than the norm at all ages in ECMO and non-ECMO patients (all $p<0.05$; figure 2). ECMO-treated patients were significantly shorter than non-ECMO patients at 2, 5 and 8 years of age ($p=0.048$, $p=0.004$ and $p=0.02$, respectively). In non-ECMO patients, the mean HFA declined from the ages of 2 to 5 years ($p=0.002$). In ECMO patients, the mean HFA declined from the ages of 1 year to 2 years and 2 years to 5 years ($p=0.01$ and $p<0.001$, respectively). From 8 to 12 years of age, the mean HFA significantly improved in the ECMO patients ($p=0.002$).

Target heights were available for 160 patients. In ECMO patients, the mean DTH was higher than the mean HFA at 2 years of age (table 2). In non-ECMO patients, the mean DTH was higher than the mean HFA from 6 months to 8 years of age. The mean DTH was still less than the norm from 2 to 12 years of age in both groups. The prevalence of stunting remained high in ECMO patients, but correcting for TH decreased stunting rates (figures 3A and 3B).

Figure 1. Flowchart of the study population



The enrollment of patients with CDH was n=172. For each routine visit, the actual number of patients of whom growth data were obtained and the number of patients who were eligible for follow-up are shown.

[1] ECMO patients (n=58); non-ECMO patients (n=20).

[2] 16p11.2 deletion syndrome (n=1); Cohen syndrome (n=1); Down syndrome (n=2); Loeys-Dietz syndrome (n=1); Ohtahara syndrome (n=1); Simpson-Golabi-Behmel syndrome (n=3); Wolf-Hirschhorn syndrome (n=1)

[3] CDH correction at the median age of 8.1 months (IQR: 1.5-19.9)

[4] No follow-up visit because of organizational reasons: n=2.

[5] No follow-up visit because of the following reasons: organizational reasons: n=9; refused follow-up: n=4.

[6] No follow-up visit because of the following reasons: organizational reasons: n=9; refused follow-up: n=6; emigration: n=2; lost to follow-up: n=1; died: n=1.

[7] No follow-up visit because of the following reasons: organizational reasons: n=2; refused follow-up: n=11; emigration: n=2; lost to follow-up: n=1; died: n=1.

[8] No follow-up visit because of the following reasons: refused follow-up: n=4; emigration: n=1; died: n=1.

[9] No follow-up visit because of the following reasons: refused follow-up: n=2; died: n=1.

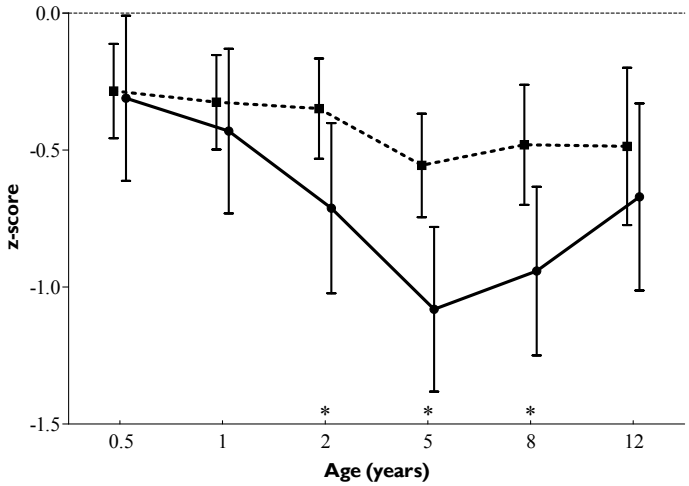
Abbreviations: CDH=congenital diaphragmatic hernia; ECMO=extracorporeal membrane oxygenation.

Table 1. Patient characteristics

	Total n=172	ECMO n=43	Non-ECMO n=129	p-value
Gestational age (weeks)	38.6±1.8	39.0±1.5	38.4±1.8	0.08
Birth weight (kilograms)	3.1±0.5	3.2±0.5	3.0 ±0.5	0.08
Male	98 (57%)	27 (63%)	71 (55%)	0.37
Ethnicity				0.72
Dutch	143 (83%)	35 (81%)	108 (84%)	
Other	29 (17%)	8 (19%)	21 (16%)	
Prenatal diagnosis	107 (62%)	21 (49%)	86 (67%)	0.04
Left sided hernia	151 (88%)	36 (84%)	115 (89%)	0.35
Age at surgery (days)	4 (3-6)	6 (4-13)	3 (2-4)	<0.001
Patch repair	126 (73%)	39 (91%)	87 (67%)	0.003
Days of mechanical ventilation	11 (6-23)	31 (19-51)	8 (5-15)	<0.001
Days of PICU stay	21 (13-43)	67 (33-99)	17 (10-29)	<0.001
Days of initial hospital stay	39 (21-70)	90 (58-143)	30 (20-51)	<0.001
Presence of chronic lung disease	76 (44%)	33 (77%)	43 (33%)	<0.001
Need for Nissen fundoplication	20 (12%)	12 (28%)	8 (6%)	<0.001
Gastrostomy placement	17 (10%)	9 (21%)	8 (6%)	0.01[1]

Data are presented as mean±standard deviation, median (interquartile range) or number (percentage), as appropriate.
[1] Fisher's exact test was used.
Abbreviations: ECMO=extracorporeal membrane oxygenation; PICU=pediatric intensive care unit.

Figure 2. Height-for-age in patients with congenital diaphragmatic hernia treated with and without extracorporeal membrane oxygenation until 12 years of age



Estimated marginal means with error bars representing 95% confidence intervals were plotted, and the presentation of data is based on a general linear model, with time-point, ECMO and their interaction effect as independent variables.
The solid line represents the ECMO patients; the dashed line represents the non-ECMO patients.
* The data indicate a significant difference between ECMO and non-ECMO patients.
Abbreviation: ECMO=extracorporeal membrane oxygenation.

Table 2. Height-for-age and distance-to-target height in patients with congenital diaphragmatic hernia treated with and without extracorporeal membrane oxygenation until 12 years of age

Age	ECMO patients			Non-ECMO patients		
	HFA z-score	DTH z-score	p-value[1]	HFA z-score	DTH z-score	p-value[1]
6 months	-0.31 (-0.61 to -0.10)	-0.11 (-0.44 to 0.21)	0.13	-0.29 (-0.46 to -0.11)	-0.13 (-0.32 to -0.05)	0.009
1 year	-0.43 (-0.73 to -0.13)	-0.28 (-0.59 to 0.04)	0.47	-0.33 (-0.50 to -0.15)	-0.17 (-0.34 to 0.01)	0.004
2 years	-0.71 (-1.02 to -0.40)	-0.58 (-0.90 to -0.27)	0.03	-0.35 (-0.53 to -0.17)	-0.19 (-0.37 to -0.01)	0.042
5 years	-1.08 (-1.38 to -0.78)	-0.94 (-1.23 to -0.66)	0.05	-0.56 (-0.75 to -0.37)	-0.41 (-0.59 to -0.23)	0.027
8 years	-0.94 (-1.25 to -0.63)	-0.84 (-1.12 to -0.57)	0.36	-0.48 (-0.70 to -0.26)	-0.33 (-0.53 to -0.14)	0.045
12 years	-0.67 (-1.01 to -0.67)	-0.57 (-0.89 to -0.25)	0.32	-0.49 (-0.77 to -0.20)	-0.38 (-0.66 to -0.10)	0.19

Data are presented as estimated marginal means (95% confidence intervals), and the presentation of data is based on a general linear model, with time-point, ECMO and their interaction effect as independent variables.

[1] P-values are based on a comparison of mean z-scores of HFA and DTH using paired sample t-tests.

Abbreviations: DTH=distance-to-target height; ECMO=extracorporeal membrane oxygenation ; HFA=height-for-age.

Weight measurements

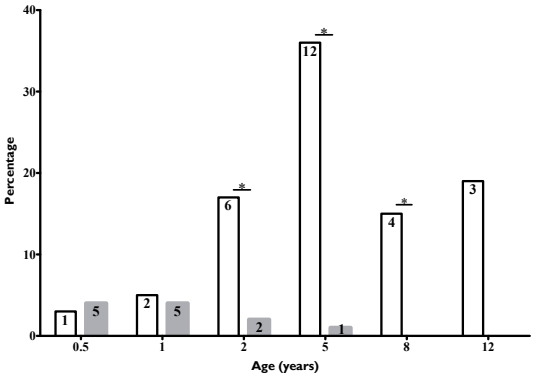
The mean WFH was less than the norm until the age of 5 years in non-ECMO patients and until the age of 8 years in ECMO patients (all $p<0.05$; figure 4). ECMO patients had lower mean WFH than non-ECMO patients from 6 months to 8 years of age (all $p<0.05$). The mean WFH declined from 6 months to 1 year of age in ECMO patients ($p=0.01$) and non-ECMO patients ($p=0.001$). In ECMO patients, the mean WFH improved from 8 to 12 years of age ($p<0.001$). In non-ECMO patients, the mean WFH improved from 5 to 8 years of age, and from 8 to 12 years of age ($p=0.03$ and $p=0.02$, respectively). The prevalence of wasting remained high in both groups (figure 3C).

Associations between growth measurements and clinical variables

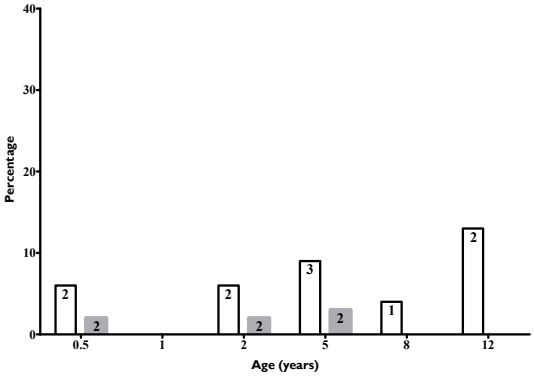
Associations between HFA, DTH, and WFH z-scores and clinical variables are shown in supplementary table 2. HFA was negatively associated with the duration of PICU stay and positively associated with gestational age. DTH was negatively associated with the duration of PICU stay and patch repair. There were significant interaction effects between time-point and ECMO, which showed that ECMO had a negative association with HFA and DTH from 1 to 8 years of age. Tube feeding at follow-up had a positive association with HFA, DTH, and WFH. There was a significant interaction effect between time-point and tube feeding on WFH, which showed the highest positive association between tube feeding and WFH at 6 months of age. WFH had a negative association with ECMO, patch repair, and the use of calorie-enriched feeds at follow-up.

Figure 3. Prevalence rates of wasting and stunting in patients with congenital diaphragmatic hernia treated with and without extracorporeal membrane oxygenation until 12 years of age

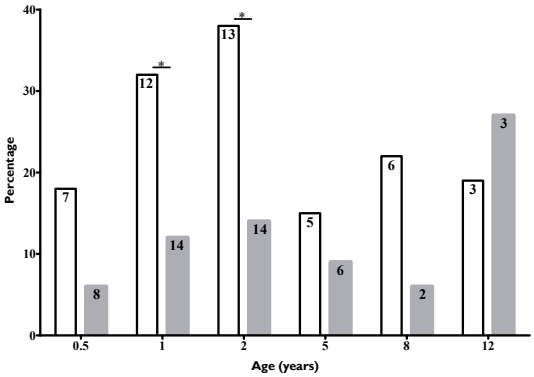
A. Prevalence rates of stunting (height-for-age z-score <-2)



B. Prevalence rates of stunting corrected for target height (distance-to-target height z-score <-2)

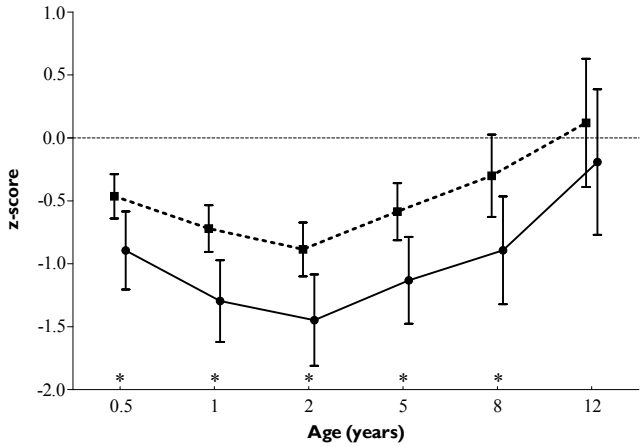


C. Prevalence rates of wasting (weight-for-height z-score <-2)



Bars show the percentages of stunting or wasting at follow-up, calculated as: the number of cases / the total number of patients seen at each time-point. The numbers within the bars represent the number of cases at each time-point.
The white bars represent the ECMO patients; the gray bars represent the non-ECMO patients.
* The data indicate a significant difference between ECMO and non-ECMO patients.
Abbreviation: ECMO=extracorporeal membrane oxygenation

Figure 4. Weight-for-height in patients with congenital diaphragmatic hernia treated with and without extracorporeal membrane oxygenation until 12 years of age



Estimated marginal means with error bars representing 95% confidence intervals were plotted, and the presentation of data is based on a general linear model, with time-point, ECMO and their interaction effect as independent variables.
The solid line represents the ECMO patients; the dashed line represents the non-ECMO patients.
* The data indicate a significant difference between ECMO and non-ECMO patients.
Abbreviation: ECMO=extracorporeal membrane oxygenation.

Pilot study: indirect calorimetry

At the median age of 8.2 years (IQR: 4.4-12.1), 11 patients underwent indirect calorimetry (supplementary table 3). REE was profoundly increased in 4 (36%) patients. Seven (64%) patients had a disturbed eating pattern (they could only eat small portions and/or it took them longer to eat compared to their peers).

Discussion

In this longitudinal study, we observed growth impairments in patients with CDH. High prevalence rates of wasting and stunting were found, especially in ECMO-treated patients. Furthermore, from 6 months to 1 year of age, the mean WFH z-score declined, which was followed by a decline in the mean HFA z-score until age 5 years. We assume that the decline in growth during infancy might be explained by inadequate nutritional intake to meet energy needs, and this possible cause should be explored as an initial step. In patients with CDH, feeding difficulties and increased resting and activity energy expenditure due to respiratory morbidity contribute to impaired growth. Although results seemed more favorable after correcting for TH, the mean DTH was less than normal at 12 years of age in both groups.

Recently, Terui et al.[10] retrospectively evaluated growth in 174 patients with CDH at 1.5, 3, and 6 years of age. They found wasting and/or stunting in 19.5% at 1.5 years of age. Weight improved from 1.5 to 3 years of age, although stunting remained in 13.5% at 6 years of age. Similar to what we found, chronic malnutrition seemed to develop via acute malnutrition. It is difficult to compare the prevalence rates of Terui et al.[10] with our prevalence rates because they used different definitions for wasting and stunting than we did. In addition, we had a higher number of ECMO-treated patients (6% versus 25%). We assume that feeding difficulties and increased energy expenditure occur especially in ECMO-treated patients who are the most critically ill and have the most hypoplastic lungs. Therefore, this subgroup of patients is more prone to persistent growth problems. Interestingly, we observed a catch-up of height in ECMO-treated patients from 8 to 12 years of age, which is reassuring. This catch-up growth may be explained by improvement of clinical problems over time, including lower rates of respiratory infections, hospitalizations, and feeding difficulties. Nevertheless, future studies should evaluate whether this catch-up growth is sustained into adolescence and adulthood.

Terui et al.[10] also summarized results of previous longitudinal studies that retrospectively evaluated growth in patients with CDH until 1 to 2 years of age.[5,6,9,11] Some studies reported catch-up growth from the age of 6 months to 1 year;[6,9] others from the age of 1 to 2 years.[5,11] However, it is difficult to compare our results with these studies because of differences in study populations, which included the selection of less severe[9] or more severe[5] patients with CDH, and retrospective study designs with potential selection bias due to relatively high numbers of patients lost to follow-up. In addition, nutritional management strategies differ among centers.

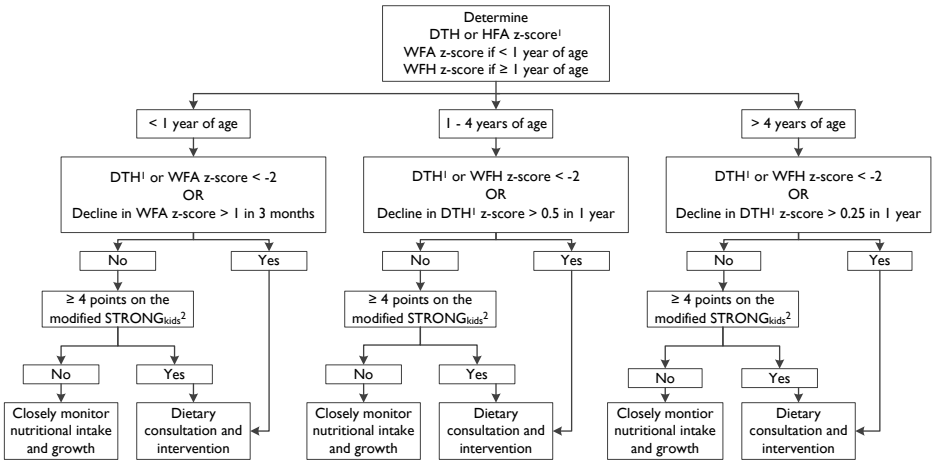
Multiple factors are thought to be involved in growth problems in patients with CDH, including feeding problems and an increased metabolism due to the increased work of breathing.[11] Previous studies identified different variables associated with impaired growth including lower birth weight,[6,10] prematurity,[6] ECMO treatment,[3,11] patch repair,[10] use of inhaled nitric oxide,[10] lower protein intake during PICU stay,[6] longer duration of mechanical ventilation[9,10] and hospital stay,[10] need for supplemental oxygen at discharge,[6,10,11] and GERD

therapy at discharge.[6] In our study, HFA and DTH were negatively associated with ECMO and the duration of PICU stay. WFH was negatively associated with ECMO, patch repair and the use of calorie-enriched feeds. ECMO treatment, patch repair, and a longer PICU stay are indicators of more severe CDH, which can explain the negative association between these clinical variables and growth. In addition, during PICU stay critically ill neonates are subjected to factors that may generate a poor growth status, including mechanical ventilation, surgery, feeding interruptions, and prolonged hospitalization.[21-23] A recent trial found that not starting parenteral nutrition up to one week in the PICU was superior to an early start of parenteral nutrition.[24] This means that one has to be cautious in administering parenteral feeding in the first week of PICU stay. Nevertheless, nutritional support is important for recovery and growth after the acute phase of critical illness. Growth and nutritional assessments and interventions should therefore be considered in the different phases of disease during PICU stay.[25]

Malnutrition has been associated with negative effects on long-term outcomes in different groups such as premature infants and children in developing countries. It is associated with immune dysfunction,[26] impaired neurodevelopment,[7, 8] behavioral problems,[27] lower educational achievement,[28] and lower economic status in adulthood.[28] In patients with CDH, lower weight and smaller head circumference at 2 years of age were associated with increased risk of borderline or delayed neurological outcome at this age.[29] Therefore, we think malnutrition should ideally be prevented, and, if present, should be treated at an early stage.

Tube feeding had a positive association with HFA, DTH and WFH z-scores. More liberal and longer use of tube feeding could be considered in patients with impaired growth. However, initiating oral feeding in an early phase is important to avoid feeding difficulties and oral aversion.[30] Therefore, a tailor made approach for each patient with CDH managed by a multidisciplinary nutritional support team is recommended. In addition to the American Academy of Pediatrics guideline recommendations of long-term follow-up,[4] we propose a risk stratification flowchart that can be used during follow-up to select patients who need dietary consultation and intervention (figure 5). This flowchart is based on the Screening Tool for Risk on Nutritional status and Growth ($STRONG_{kids}$).[31, 32]

Figure 5. Flowchart for dietary consultation and intervention in patients with congenital diaphragmatic hernia



[1] Use HFA if DTH is not available.

[2] Nutritional risk screening tool $STRONG_{kids}$ (screening tool for risk on nutritional status and growth)[31]

	Score if yes
Subjective clinical assessment: Is the patient in a poor nutritional status judged by subjective clinical assessment (diminished subcutaneous fat and/or muscle mass and/or hollow face)?	1 point
High risk disease state, are one of the following items present: -Extracorporeal membrane oxygenation treatment? -Chronic lung disease? -Recurrent (pulmonary) infections requiring antibiotics or hospitalization? -Recent major surgery? -High risk CDH judged by clinical assessment of physician?	2 points
Nutritional intake and losses, are one of the following items present: -Excessive diarrhea (≥5 times per day) and/or vomiting (>3 times per day) the last few days? -Pre-existing dietetically advised nutritional intervention (for example calorie-enriched feeds or tube feeding)? -Obvious reduced food intake during the last few days? -Inability to consume adequate intake (for example due to GERD)?	1 point
Weight loss or poor weight gain: Is there weight loss or no weight gain (infants <1 year) during the last weeks/months?	1 point

Abbreviations: CDH=congenital diaphragmatic hernia; DTH=distance-to-target height; ECMO=extracorporeal membrane oxygenation; GERD=gastroesophageal reflux disease; HFA=height-for-age; PICU=pediatric intensive care unit; WFA=weight-for-age; WFH=weight-for-height.

An increased REE and feeding difficulties may contribute to impaired growth. In a pilot study with REE measurements, we found an increased measured REE in four (36%) patients and a disturbed eating pattern in seven (64%). These results emphasize the importance of care by a multidisciplinary nutritional support team. Dietary consultation with REE measure-

ment could serve as a first step to identify possible causes of poor growth. All children with REE measurements were older than 3 years of age. REE may be even higher in the first 2 years of life because of an increased rate of breathing after correction of the defect. Indeed, Haliburton et al.[33] reported that 10 (59%) of 17 patients were hypermetabolic at the median age of 32 days. Longitudinal studies including more patients should be performed to assess metabolic needs in patients with CDH over time.

This is the largest prospective cohort study following growth of patients with CDH up to 12 years of age. Other study strengths are the high follow-up rate of 96% and the use of z-scores for growth by using recently established reference values for Dutch children.[16] In addition, this is the first study to report DTH z-scores in patients with CDH. Because adjusting for TH improved height z-scores, it seems important to calculate DTH z-scores when evaluating growth parameters in patients with CDH.

Our study has some limitations. Birth weight of most patients was estimated. Therefore, we used growth measurements at 6 months of age as the reference point in the general linear model analyses. Secondly, as this is a longitudinal study, not all children had yet reached the age of 12 years. Although general linear models can account for missing data values, the limited number of children assessed at 12 years of age may have contributed to not finding a significant difference in the mean HFA z-scores between ECMO and non-ECMO patients (type II error). Ongoing assessment of growth into adolescence is therefore recommended. In addition, management of CDH changed during the study period, which could have influenced our results. However, we did not find a significant association between HFA, DTH and WFH z-scores and the change in treatment protocol. In our pilot study, nutritional assessments were performed in a limited number of patients, however, our results support previous findings.[33] In addition, our study highlights the importance of early initiation of nutritional assessment and intervention by a multidisciplinary nutritional team.

Conclusions

We found impaired growth in patients with CDH, especially in those who had received ECMO treatment. A decline in weight gain was followed by a decline in linear growth suggestive of inadequate nutritional intake to meet energy needs during infancy. This study shows the importance for early risk stratification for poor growth to identify patients with a high risk, such as those who required ECMO treatment. Early risk stratification should be combined with nutritional assessments and interventions, as well as long-term follow-up of growth. Further studies are needed to determine the relation between growth problems and functional outcomes, both physical and neuropsychological.

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Supplements

Supplementary methods. Indirect calorimetry

Indirect calorimetry (Cosmed Quark RMR with Canopy, Tulipmed, Nieuwegein, the Netherlands) is an accurate method of estimating the basal metabolic rate in awake patients. The Cosmed Quark RMR measures resting energy expenditure (REE) using a canopy dilution technique. Before each measurement, gas and pressure calibrations were performed. All patients were spontaneously breathing without supplementary oxygen. Patients had to lay down for 5 minutes in advance to keep their breathing as calm as possible. After that, they had to breathe calmly for 20 minutes with an air-tight transparent plastic canopy hood over their head. The flow rate was directly measured with a digital turbine flowmeter. Expired air was extracted by a pump and analyzed by metabolic cart sensors. The oxygen consumption (VO_2 in ml/min) and carbon dioxide production (VCO_2 in ml/min) were measured by oxygen and carbon dioxide gas analyzers. The respiratory quotient was calculated: VCO_2/VO_2. Software used the Weir equation to calculate the REE from oxygen consumption and carbon dioxide production.[1, 2] REE was calculated with the Schofield equation for weight and height.[3] Increased energy expenditure was defined as an increase in measured REE of >10% compared with the calculated REE (($\frac{\text{measured REE}}{\text{calculated REE}}$ -1)×100%). Total energy need (TEN, kcal/day) was calculated using the following formula[4]:

$$\text{TEN} = \frac{(\text{measured REE})*(\text{activity factor} + \text{disease factor}-1)*\text{growth factor}}{\text{energy absorption coefficient}}$$

Total energy intake was calculated from the entries in a three-day food diary, which intake was compared with the estimated TEN. A total energy intake of ≥95% of TEN was considered as sufficient. The eating pattern was categorized as normal or disturbed (deviant from healthy peers) judged by the patients, parents and/or dietician.

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Supplementary table 1. Number of patients with tube feeding at follow-up

Age	ECMO	Non-ECMO	p-value
0.5 year	56% (23/41)	15% (19/129)	<0.001
1 year	53% (20/38)	13% (16/121)	<0.001
2 years	20% (7/35)	9% (9/98)	0.13[1]
5 years	9% (3/33)	1% (1/67)	0.10[1]

[1] Fisher's exact test was used.
Abbreviation: ECMO=extracorporeal membrane oxygenation.

Supplementary table 2. Associations between growth measurements and clinical variables

	Variable	Estimated coefficient	95% confidence interval	p-value
HFA z-score	*Follow-up time-point:*			
	0.5 year of age	Reference	Reference	-
	1 year of age	-0.12	-0.24 to -0.01	0.04
	2 years of age	-0.09	-0.25 to 0.07	0.26
	5 years of age	-0.32	-0.52 to -0.12	0.002
	8 years of age	-0.30	-0.56 to -0.05	0.02
	12 years of age	-0.47	-0.80 to -0.15	0.005
	ECMO	0.21	-0.19 to 0.61	0.30
	Patch repair	-0.22	-0.52 to 0.08	0.15
	Gestational age (weeks)	0.08	0.001 to 0.15	0.047
	Days of PICU stay	-0.005	-0.008 to -0.001	0.004
	Tube feeding at follow-up	0.25	0.06 to 0.44	0.009
	Calorie-enriched feeds at follow-up	-0.16	-0.34 to 0.02	0.08
	*Interaction term: ECMO*Follow-up time-point:*			
	0.5 year of age	Reference	Reference	-
	1 year of age	-0.38	-0.63 to -0.12	0.005
	2 years of age	-0.42	-0.76 to -0.08	0.02
	5 years of age	-0.63	-1.03 to -0.23	0.002
	8 years of age	-0.64	-1.10 to -0.17	0.008
	12 years of age	-0.45	-0.98 to 0.08	0.10
	*Interaction term: Days of PICU stay*Follow-up time-point:*			
	0.5 year of age	Reference	Reference	-
	1 year of age	0.004	0.002 to 0.006	<0.001
	2 years of age	0.002	-0.001 to 0.005	0.29
	5 years of age	0.003	-0.001 to 0.007	0.10
	8 years of age	0.005	0.0001 to 0.01	0.045
	12 years of age	0.009	0.003 to 0.01	0.004
DTH z-score	*Follow-up time-point:*			
	0.5 year of age	Reference	Reference	-
	1 year of age	-0.10	-0.22 to 0.01	0.07
	2 years of age	-0.07	-0.23 to 0.10	0.43
	5 years of age	-0.31	-0.51 to -0.11	0.002
	8 years of age	-0.30	-0.54 to -0.06	0.01
	12 years of age	-0.60	-0.91 to -0.29	<0.001
	ECMO	0.32	-0.11 to 0.75	0.14

Supplementary table 2. Continued

	Variable	Estimated coefficient	95% confidence interval	p-value
	Patch repair	-0.31	-0.60 to -0.02	0.04
	Gestational age (weeks)	0.05	-0.02 to 0.13	0.15
	Days of PICU stay	-0.006	-0.009 to -0.003	0.001
	Tube feeding at follow-up	0.29	0.09 to 0.49	0.004
	*Interaction term: ECMO*Follow-up time-point:*			
	0.5 year of age	Reference	Reference	-
	1 year of age	-0.35	-0.61 to -0.09	0.009
	2 years of age	-0.46	-0.81 to -0.11	0.01
	5 years of age	-0.65	-1.05 to -0.24	0.002
	8 years of age	-0.71	-1.15 to -0.27	0.002
	12 years of age	-0.42	-0.91 to 0.08	0.10
	*Interaction term: Days of PICU stay*Follow-up time-point:*			
	0.5 year of age	Reference	Reference	-
	1 year of age	0.004	0.002 to 0.006	0.001
	2 years of age	0.002	-0.002 to 0.005	0.33
	5 years of age	0.003	-0.0004 to 0.007	0.09
	8 years of age	0.005	0.001 to 0.01	0.02
	12 years of age	0.01	0.004 to 0.02	0.001
WFH z-score	*Follow-up time-point:*			
	0.5 year of age	Reference	Reference	-
	1 year of age	-0.17	-0.32 to -0.02	0.03
	2 years of age	-0.33	-0.52 to -0.13	0.001
	5 years of age	-0.01	-0.22 to 0.20	0.94
	8 years of age	0.24	-0.02 to 0.49	0.07
	12 years of age	0.80	0.45 to 1.14	<0.001
	ECMO	-0.57	-0.94 to -0.20	0.003
	Patch repair	-0.50	-0.81 to -0.20	0.001
	Days of PICU stay	0.002	-0.001 to 0.005	0.14
	Tube feeding at follow-up	0.66	0.35 to 0.96	<0.001
	Calorie-enriched feeds at follow-up	-0.30	-0.55 to -0.05	0.02
	New treatment protocol	0.21	-0.08 to 0.50	0.15
	*Interaction term: Tube feeding*Follow-up time-point:*			
	0.5 year of age	Reference	Reference	-
	1 year of age	-0.49	-0.80 to -0.17	0.003
	2 years of age	-0.48	-0.95 to -0.002	0.049
	5 years of age	-0.50	-1.26 to 0.26	0.19
	8 years of age	-	-	-
	12 years of age	-	-	-

Abbreviations: DTH=distance-to-target height; ECMO=extracorporeal membrane oxygenation; HFA=height-for-age; PICU=pediatric intensive care unit; WFH=weight-for-height.

Supplementary table 3. Results of dietary consultations and indirect calorimetry measurements

	Gender	ECMO	Nissen	Age (years)	HFA z-score	DTH z-score	WFH z-score
1	M	Yes	No	5.3	-2.36	-1.33	-1.23
2	F	Yes	No	3.3	-2.13	-1.51	-0.94
3	M	Yes	Yes	12.1	-2.88	-2.55	-1.96
4	M	Yes	No	15.6	-1.11	-1.02	-0.29
5	M	Yes	No	8.2	-2.10	-1.98	-0.94
6	F	No	No	12.3	-1.61	-0.85	-0.72
7	F	Yes	Yes	10.3	0.15	0.12	-4.36
8	M	No	No	4.4	-1.72	-2.33	-0.18
9	F	Yes	No	8.2	-2.81	-2.27	-1.82
10	M	Yes	Yes	12.1	-1.45	-2.26	-3.86
11	F	Yes	No	3.3	-2.20	-2.39	1.41

[1] Increased energy expenditure.

Abbreviations: DTH=distance-to-target height; ECMO=extracorporeal membrane oxygenation; F=female; HFA=height-for-age; M=male; REE=resting energy expenditure; TEN=total energy need; WFH=weight-for-height.

REE measured (kcal)	REE calculated (kcal)	Difference measured REE and calculated REE	TEN (kcal/day)	Caloric intake (% of TEN)	Eating pattern
881	824	+7%	1456	80	Disturbed
765	727	+5%	1509	93	Disturbed
1309	1090	+20%[1]	2680	60	Disturbed
2082	1585	+31%[1]	3500	96	Normal
1159	953	+22%[1]	2325	76	Normal
1136	1158	-2%	2000	102	Normal
1152	1086	+6%	2150	75	Disturbed
773	829	-7%	1840	98	Normal
896	862	+4%	1520	63	Disturbed
1207	1135	+6%	1776	89	Disturbed
993	770	+29%[1]	1260	94	Disturbed

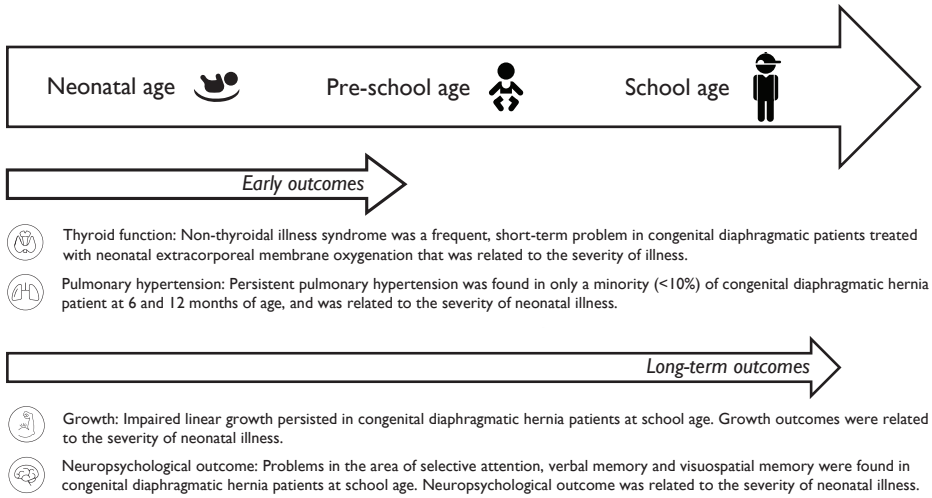
Chapter 9

General discussion

Background

The primary objective of this thesis was to evaluate the postnatal risks and outcomes of patients with congenital diaphragmatic hernia (CDH). We investigated early outcomes, including disturbed thyroid function during the neonatal period (chapters 2 and 3) and persistent pulmonary hypertension during the first year of life (chapter 4). In addition, we studied the long-term risks of growth failure (chapters 5 and 8) and impaired neurodevelopmental and neuropsychological outcomes (chapters 6 and 7) until school age. We also aimed to identify determinants of patient outcomes in these studies. Subjects were CDH patients treated in the Netherlands (chapters 2, 3, 4, 7 and 8) or Australia (chapters 5 and 6). Both countries have a standardized long-term follow-up program for CDH patients in place. The studies' most important conclusions are shown in figure 1. Here, we will discuss the main findings of our studies and relate these to the current literature. We also make recommendations for future research and implementation of our findings for patient care.

Figure 1. Main conclusions about the early and long-term outcomes of patients with congenital diaphragmatic hernia following the studies in this thesis



Early outcomes and risks

Thyroid function

From the first breath of life, neonates with CDH are at a high risk of mortality[1, 2] and morbidity.[3, 4] Difficulties with breathing start soon after birth due to pulmonary hypoplasia and pulmonary hypertension associated with the diaphragmatic defect. Although there is no univocal definition of neonatal or pediatric critical illness,[5] neonates with CDH can be considered critically ill from birth onwards as they have a life-threatening illness that requires support of vital organ functions to prevent death.[6] Those with the greatest severity of illness even require extracorporeal membrane oxygenation (ECMO) treatment. During critical illness, the body reacts with a stress response that causes endocrine, metabolic and immunologic changes.[6, 7]

In chapters 2 and 3, we focused specifically on the disturbed endocrine response of the hypothalamic-pituitary-thyroid (HPT) axis, which can occur in CDH patients during critical illness. This disturbed thyroid function during critical illness is called non-thyroidal illness syndrome (NTIS).[8-10] NTIS is characterized by low thyroxine (T4) and triiodothyronine (T3) concentrations, an increased reverse T3 concentration, and a normal or low thyroid-stimulating hormone (TSH) concentration.[8-10] One of the first occasions to find disturbed thyroid hormone levels is during the neonatal heel prick screening for congenital hypothyroidism. In chapter 2, we found that more than half of the ECMO-treated neonates had an aberrant screening result for congenital hypothyroidism before the start (73.1%) and during (67.3%) ECMO, which incidence was significantly higher than that of aberrant screening results after ECMO treatment (31.4%). Neonatal screening results showed a low total T4 concentration in combination with a low TSH concentration in almost all patients, which normalized in all survivors after recovery of critical illness. Eventually, none of the survivors with an aberrant screening result was diagnosed with congenital hypothyroidism. We, therefore, believe that these aberrant screening results are most likely due to NTIS.

Previous studies have also found disturbed thyroid hormone levels in other groups of critically ill neonates treated with[11] or without[12-14] ECMO. Other groups of critically ill neonates will presumably also have a high incidence of aberrant screening results for congenital hypothyroidism.[15] Clinicians should be aware that an aberrant congenital hypothyroidism screening result in a critically ill neonate is likely due to NTIS. Further, other contributing factors, including the use of medication such as dopamine, should be taken into account.[16] Neonatal screening for congenital hypothyroidism in such a patient can best be repeated after clinical recovery of critical illness. However, it is equally important to repeat screening before the age of 14 days as thyroxine treatment should be started before the first two weeks of life to prevent intellectual disabilities.[17]

To further characterize the disturbances in thyroid hormone concentrations during ECMO, we determined thyroid hormone concentrations in 21 neonates with CDH or meconium

aspiration syndrome at six fixed time-points before, during, and after ECMO (chapter 3). This study confirmed that low thyroid hormone concentrations, as is seen in NTIS, were already present in critically ill neonates before the start of ECMO. Immediately after the start of ECMO, thyroid hormone concentrations further declined, possibly due to hemodilution[11, 18] and the global inflammatory response occurring during the initial phases of cardiopulmonary bypass and ECMO.[19-21] Soon thereafter, first the TSH concentration significantly increased followed by an increase in total T4 and total T3 concentrations. This is in line with previous studies showing that restoration of the HPT axis after NTIS starts with an increase in TSH followed by increases in total T4 and total T3,[22, 23] which suggests that restoration of the HPT axis already occurs during neonatal ECMO.

Another potential factor contributing to the increase in TSH is the discontinuation of dopamine administration during ECMO as TSH is known to be suppressed by dopamine, and after dopamine is discontinued, TSH level again increases.[24] However, we think that this is not the most important factor involved, because dopamine levels were not associated with an aberrant screening result or with thyroid hormone concentrations in our studies (chapters 2 and 3).

In chapter 2, we found an association between an aberrant screening result for congenital hypothyroidism and the Pediatric Logistic Organ Dysfunction-2 (PELOD-2) score,[25] which is a score for the severity of illness. This suggests that the occurrence of NTIS is related to the severity of neonatal illness. On the other hand, we could not confirm an association between the PELOD-2 score and thyroid hormone concentrations in chapter 3. Explanations for this equivocal result are the low number of patients included and the limitations of the PELOD-2 score in assessing the severity of illness in this specific group of ECMO-treated neonates. Although the PELOD-2 score is validated for a broad group of pediatric intensive care unit (PICU) patients,[25, 26] its relative significance and performance in ECMO-treated neonates is currently unknown. Further, the score is affected by treatment such as ventilator settings and use of vasoactive drugs, and therefore it does not solely reflect the severity of illness.

Impaired neuropsychological outcomes have been found in CDH and ECMO-treated patients at school age (chapter 7).[27, 28] As thyroid hormones are essential for brain development during early life,[29, 30] disturbances of thyroid function during this period may have important consequences for neurodevelopmental outcome later in life. In the first 16 to 20 weeks of gestation, thyroid hormone influences neuronal proliferation and migration of neurons in the cerebral cortex, hippocampus and medial ganglionic eminence.[31-35] The second stage occurs during the remainder of pregnancy, when thyroid hormone is involved in neurogenesis, neuron migration, axonal outgrowth, dendritic branching and synaptogenesis, together with the initiation of glial cell differentiation and migration and the onset of myelination.[35-39] During the neonatal period, thyroid hormones influence migration of granule cells in the hippocampal dentate gyrus and cerebellum, migration of pyramidal cells in the cortex and migration of Purkinje cells in the cerebellum, and thyroid hormone-dependent gliogenesis and myelination continues.[35, 39-41] In chapter 3, we found normal cognitive outcomes in a

small sample of neonatal ECMO survivors (n=16) compared with the norm population at 2 years of age. Recent research has mainly focused on the effect of maternal thyroid function on children's neurodevelopment instead of neonatal thyroid function. Ghassabian et al.[42] found an adverse effect of maternal hypothyroxinemia on children's nonverbal intelligence quotient (IQ) at 6 years of age but no effect on brain morphology. Nevertheless, Korevaar et al.[43] showed that both low and high maternal free T4 were associated with lower child IQ and smaller grey matter and cortex volumes at school age. It is difficult to compare these results to ours as thyroid hormone-dependent neurological development differs between the fetal and neonatal periods.

The effect of temporarily low thyroid hormone levels during the neonatal period has been mostly studied in premature neonates with transient hypothyroxinemia.[44] Studies investigating the impact of hypothyroxinemia of prematurity on neurodevelopmental outcomes later in life have found contradictory results. One study found a significant association between thyroxine concentrations and psychomotor development at 2 years of age.[45] Another found that children with transient hypothyroxinemia had lower cognitive and verbal scores than children without transient hypothyroxinemia at 5 years of age.[46] However, a more recent study did not confirm this association between transient hypothyroxinemia of prematurity and adverse neurodevelopmental outcomes in young adults.[47] All three studies adjusted for possible confounders such as the degree of prematurity and the severity of neonatal illness. In contrast to low T4 levels, high T4 levels may also impair brain development. For example, Scratch et al.[48] found that higher T4 levels after very preterm birth were associated with lower scores on verbal learning and verbal memory at 7 years of age, whereas they did not find associations between T4 levels and brain volumes. As stated above, we did not find impaired cognitive scores in ECMO-treated neonates with NTIS at the age of 2 years. At school age, however, CDH and neonatal ECMO survivors experienced mainly problems in the areas of selective attention, verbal and visuospatial memory (chapter 7), which has been associated with injury in the area of the hippocampus.[49-54] As thyroid hormone concentrations are essential for the development of brain circuits in general, it is likely that other mechanisms are involved in determining neuropsychological outcome in survivors of neonatal critical illness later in life. Nevertheless, smaller hippocampal volumes and reduced memory functioning have also been found in children of mothers diagnosed with hypothyroidism,[55, 56] and in children and adolescents with congenital hypothyroidism.[57] From our studies, we cannot exclude an effect of thyroid hormone concentrations on neuropsychological outcomes at school age. Thus, future longitudinal studies assessing the impact of temporarily decreased thyroid hormone concentrations on specific neuropsychological outcome in the areas of attention and memory at school age as well as brain morphology should be performed to make a definite conclusion regarding this issue.

Apart from brain development, thyroid hormones are important for physical growth.[58] We did not investigate the relation between thyroid hormone concentrations and physical

growth in our studies. As thyroid hormone concentrations normalized after the period of neonatal critical illness, we assume that this temporarily phase of reduced thyroid hormone concentrations does not substantially affect growth in childhood.

Persistent pulmonary hypertension

Persistent pulmonary hypertension is an important determinant of mortality and morbidity in CDH patients.[59-61] It develops on account of a reduced number of pulmonary arteries in combination with altered vasoreactivity and abnormal pulmonary vascular remodeling.[62] Over the years, different definitions for and classifications of pulmonary hypertension have been used to study the risk of persistent pulmonary hypertension in CDH patients.[59-61, 63-65] One of the reasons for the use of these different definitions is the challenge of obtaining a well-performed echocardiogram as the heart of CDH patients is often in an abnormal position and no single reliable quantitative measure of pulmonary artery pressure and/or right ventricular function exists. To be able to compare study results and standardize pulmonary hypertension follow-up and treatment, we should avoid the use of different definitions. Therefore, a standardized protocol using validated echocardiography measures is needed for the echocardiographic diagnosis of pulmonary hypertension in CDH patients. In addition, echocardiography should be performed by ultrasound specialists who are well trained in echocardiography of CDH patients. In our study, all patients with persistent pulmonary hypertension were treated with sildenafil. Current guidelines advise to treat severe pulmonary hypertension with pulmonary vasodilators such as inhaled nitric oxide, sildenafil, and/or bosentan,[66] although there is no evidence for the use of these drugs from properly designed randomized controlled trials. Therefore, well-powered randomized controlled trials should be performed that investigate the effectiveness of these treatments in CDH patients. A multicenter, randomized controlled trial, the CoDiNOS trial, will start shortly to evaluate whether inhaled nitric oxide or intravenous sildenafil is superior in treating pulmonary hypertension in CDH patients.

Patients with persistent pulmonary hypertension during infancy had a longer duration of mechanical ventilation and hospital stay than patients without persistent pulmonary hypertension. This indicates that persistent pulmonary hypertension is associated with the severity of illness in early life, as previous studies also have shown. Lusk et al.[60] found an association between persistent pulmonary hypertension at 2 weeks of age and mortality or adverse respiratory outcomes (prolonged intubation (≥28 days) or prolonged respiratory support (≥56 days)). Wynn et al.[61] found that prenatal diagnosis, patch repair, post-operative complications, and ECMO were associated with worse pulmonary hypertension at 1 month of age. At 3 months of age, patch repair, non-isolated CDH, a genetic diagnosis and a lower birth weight were associated with worse pulmonary hypertension.[61]

Persistent pulmonary hypertension may also have important consequences for neurodevelopmental outcome later in life. Both pulmonary hypoplasia and pulmonary hypertension

can have important consequences for gas exchange. A lung with severe hypoplasia has fewer branching points and therefore fewer airways, arteries, veins and alveolar structures than a normal lung resulting in a fixed increased vascular resistance and decreased surface area for gas exchange,[67, 68] with a high risk of hypoxemia. This hypoxemia can lead to inadequate cerebral oxygenation, which may result in hypoxic-ischemic and eventually reperfusion injury in vulnerable areas such as the hippocampus.[69, 70] As stated above, this can lead to attention and memory problems later in life,[49-53] which we found in over 50% of CDH patients at 8 years of age (chapter 7). Still, we think that persistent pulmonary hypertension during infancy is not the main cause of these attention and memory problems in CDH patients at school age, seeing that more than 90% of CDH survivors in our study recovered from pulmonary hypertension during the first year of life. It may well be, however, that very early occurrence of pulmonary hypertension, which we found in 71% of the surviving CDH patients pediatric intensive care unit admission, has an effect on neuropsychological outcomes at school age.

An important long-term issue in patients with persistent pulmonary hypertension is ensuring adequate growth. These children may have an increased risk of growth failure due to a greater severity of illness in the neonatal period and possibly also due to other factors such as feeding difficulties due to an increased work of breathing, an increased resting energy expenditure and gastroesophageal reflux disease (GERD) (chapter 8). Indeed, we found that all patients with persistent pulmonary hypertension required tube feeding at follow-up (chapter 4). Therefore we think it is important to closely monitor these children's nutrition and growth.

We found that 71% of the CDH survivors had pulmonary hypertension on the first day of pediatric intensive care unit admission. However, more than 90% of the survivors recovered from pulmonary hypertension during the first year of life, and none showed relapse during follow-up after stopping medication for pulmonary hypertension. This is why we think that routine structured follow-up for pulmonary hypertension in CDH patients is unnecessary. Echocardiographic follow-up seems necessary only when pulmonary hypertension is still present at hospital discharge and/or patients still require treatment with sildenafil and/or bosentan at this time.

Long-term outcomes and risks

Growth and nutrition

In chapters 5 and 8, we focused on physical growth in CDH patients. After the infant period, CDH patients remain at risk for long-term morbidities including growth failure.[71] In the study presented in chapter 8, we found poor weight gain during the first 2 years of life, followed by a decline in linear growth. Although catch-up in weight occurred after 2 years of age, at 12 years of age linear growth was still below the norm. Therefore, the risk of growth failure seems especially high in the first years of life and incomplete catch-up growth occurs later in life. Previous studies have also reported growth problems in CDH survivors during the first years of life,[72-74] with prevalence rates of growth failure ranging from 8.2% to 68.8%.[72,73] This wide range is due to differences in study populations, retrospective study designs with potential selection bias on account of the relatively high numbers of patients lost to follow-up, and differences in nutritional management strategies. The longest follow-up study so far found that growth failure (stunting: height z-score <-2) persisted in 13.5% of patients at 6 years of age.[71] This study did not take into account parental heights. As there is a strong genetic determinant influencing growth,[75] we considered it important to also calculate children's target height and adjust for this when assessing linear growth. We then found significant differences in linear growth, with a higher height z-score after adjustment for target height. Therefore, we think it is important to calculate distance-to-target height z-scores when assessing linear growth in CDH patients, and possibly also in other patient groups. As in our study both height-for-age and distance-to-target height were still below the norm at 12 years of age, we think it is important to continue growth assessments in CDH patients. Studies in premature infants have found an increased risk for metabolic syndrome later in life as a consequence of catch-up growth.[76,77] Because catch-up growth also occurred in CDH patients, it is important to combine these growth assessments with assessment of the metabolic profile, including body composition measurements, in early adulthood.

We found that measures of severity of illness such as the need for ECMO, patch repair and the duration of PICU stay were negatively associated with growth (chapter 8). Additionally, mechanical ventilation was predictive of failure to thrive (weight-for-age and/or weight-for-length z-score <-2) at 6 months of age (chapter 5). This association with severity of illness and growth is in line with previous studies in which different indicators of severity of illness were associated with impaired growth. These included ECMO treatment,[74,78] patch repair,[71] use of inhaled nitric oxide,[71] longer duration of mechanical ventilation and hospital stay,[71,79] and need for supplemental oxygen at discharge.[71,72,74] In a recent study in 12 CDH patients, increased postnatal weight gain was associated with increased ipsilateral lung volume growth measured by infant pulmonary MRI.[80] This result in combination with our results of early growth failure highlights the importance of early start of nutritional assessment and interventions in high risk CDH patients. Therefore, early risk stratification to prevent growth

failure is indicated in CDH patients. In chapter 8, we propose a risk stratification flowchart that can be used to select patients who need early dietary consultation and intervention. Ideally, nutritional assessments and interventions should already be started in the PICU. It is also important to make parents aware of the risk of growth failure and involve them early in the nutritional care of their child. In the infant period, breastmilk feeding should be encouraged,[81] for example through family-centered care with kangaroo mother care. This may aid in establishing successful breastmilk feeding and improves parent-child attachment.[82-84] In CDH patients with a high risk of growth failure, calorie-enriched feeds should already be started during hospital admission to prevent early growth failure. Additionally, tube feeding should be considered if an increased work of breathing is causing feeding difficulties or stagnation in growth occurs. Still, it remains important to continue oral feeding to avoid feeding difficulties and oral aversion.[85]

Another frequent source of feeding difficulties is the presence of GERD, which occurs frequently in CDH patients.[86-89] Therefore in the Erasmus MC-Sophia Children's Hospital, all neonates with CDH are treated with antireflux medication until evaluation of GERD has been performed. In chapter 8, 53 (38%) patients were diagnosed with GERD using 24-hour pH-metry at the median age of 3 months and 20 (12%) patients underwent a Nissen fundoplication at the median age of 8 months. Additionally in chapter 5, 42% of CDH patients required treatment for GERD. Previous studies have also reported a high prevalence rate of gastroesophageal reflux, affecting up to 83% of CDH patients at 6 months of age.[90] It is difficult to compare the prevalence rates of GERD in the CDH population due to different definitions, different diagnostic methods used in studies including pH monitoring, upper gastrointestinal contrast studies or clinical diagnosis of GERD, as well as thresholds for initiating treatment. As GERD can contribute to growth failure in CDH patients, this should always be considered in CDH patients with feeding and/or growth problems. If present, it should be optimally treated using antireflux medication as a first step.

To unravel possible causes of poor growth, we performed indirect calorimetry measurements and nutritional assessments in a subgroup of 11 CDH patients with growth failure. Four (36%) of them had an increased energy expenditure, which suggests that increased energy expenditure plays a role in growth failure. Haliburton et al.[89] even found that 10 (59%) of 17 patients were hypermetabolic at the median age of 32 days. We assume that resting energy expenditure of CDH patients is higher in the first 2 years of life due to an increased rate of breathing after correction of the defect. Seven (64%) of the 11 patients with growth failure also had a disturbed eating pattern. Therefore, we think that both an increased resting energy expenditure and feeding difficulties were involved in growth failure. However, as these problems were not universal in patients with growth failure, the causes for poor growth in CDH patients are probably multifactorial also including the severity of illness during the neonatal period and presence of GERD. Limitations of the indirect calorimetry study were the small number of patients, the measurement of resting energy expenditure at

only one time-point, and the absence of activity energy expenditure measurements. Future studies should perform longitudinal resting and activity energy expenditure measurements in a large group of CDH patients, which can help in understanding the causes of growth failure.

Poor growth may negatively affect neurodevelopmental outcomes. Previous studies in premature infants[91] and CDH patients[92] have found that growth during infancy was negatively associated with neurodevelopmental outcomes later in life. The exact relation between poor growth and neurodevelopmental outcome in premature infants remains controversial, however, as many studies have not taken into account the severity of illness.[91] Additionally, no nutritional intervention trial that aimed to increase postnatal growth in premature infants has reported a long-term benefit of faster growth on neurodevelopmental outcome,[91] and a more recent study in children with extremely low birth weight showed no effect of catch-up-growth in the first 2 years of life on neurocognitive outcome at 11 years of age.[93] Although neuropsychological outcomes were not available for the patients included in our longitudinal growth study (chapter 8), we determined the association between growth at 1 year and neuropsychological outcome at school age in chapter 7. Only weight-for-height at 1 year of age was positively associated with IQ. In the multivariable analyses, however, only the maximum vasoactive-inotropic score (VIS), which is a measure of the dose of vasoactive medication needed and therefore also of the severity of illness, remained associated with verbal and visuospatial memory. These results suggest that the severity of neonatal illness has a greater impact on neurodevelopmental outcome than growth in the first year of life.

Neuropsychological outcome

In chapters 6 and 7, we found that neurodevelopmental and neuropsychological outcomes were impaired in a high number of CDH patients, and these problems varied with age. In line with the findings of Gischler et al.[78] and Danzer et al.,[94] gross motor skills were below average at one year of age (chapter 6). This is possibly the consequence of prolonged hospitalization with little physical activity and therefore fewer opportunities to obtain early gross motor skills in the first year of life. At 3 years of age, gross motor skills were normal in all patients indicating that CDH patients with delays in gross motor skills catch-up with their peers after the first year of life. However at 3 years of age, 27% of CDH patients experienced a mild delay on the expressive or receptive language scale. It is possible that subtle neurocognitive problems will become more evident with age, and therefore are only diagnosed at a later age. Similarly, previous studies found neuropsychological problems in the areas of attention and concentration in CDH and neonatal ECMO patients at 8 years of age, despite a normal IQ,[27, 28, 95] which is referred to as a growing-into-deficit phenomenon. To increase our understanding about the neuropsychological domains that are affected on the long-term, we performed extended neuropsychological assessments in eight-year-old CDH and neonatal ECMO patients. Further, we determined clinical factors that were associated with specific neuropsychological functions. In line with previous findings of our study

group,[27, 28, 95] we found that intelligence fell within the normal range at 8 years of age, except in CDH patients treated with neonatal ECMO (chapter 7). Further, sustained attention, verbal memory (immediate and delayed recall) and visuospatial memory (immediate and delayed recall as well as recognition) were below average compared to the general population. As these deficits in memory function can have a profound effect on everyday life and academic achievement,[50, 96] we think it is important to continue follow-up of neuropsychological outcome and academic achievement of CDH patients into adulthood.

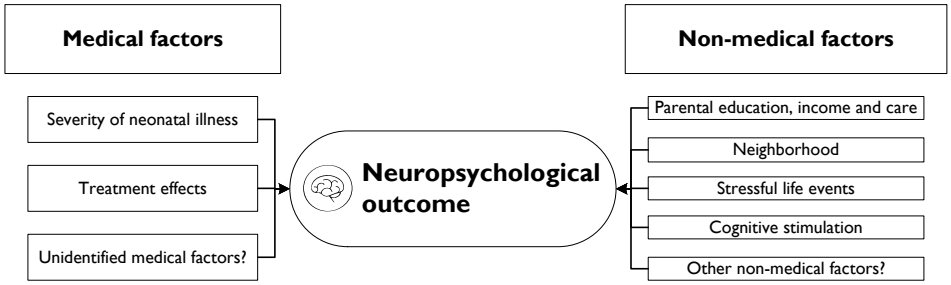
Neuropsychological outcome at 8 years of age was associated with the severity of neonatal illness indicated by the need for ECMO, treatment with veno-arterial ECMO, maximum VIS, ventilator-free days in the first 28 days of life, and duration of initial hospital stay. In the multivariable model, the maximum VIS remained the only predictor of delayed verbal and visuospatial memory scores. As mentioned above, brain areas susceptible for hypoxic-ischemic and reperfusion injury, such as the hippocampus and other limbic system regions, are altered following critical illness in early life and these areas are specifically related to impaired memory and attention.[49-54] Although our study does not show a causative effect of vasoactive medication on memory problems, the requirement of high levels of vasoactive medication in the first period of life could be an indirect marker of (regional) inadequate brain perfusion resulting in memory problems later in life. As monitor devices and clinical predictors for regional cerebral perfusion and oxygenation are not yet available for clinical use, the maximum VIS could be a useful component in estimating severity of illness and risk of memory problems in CDH and neonatal ECMO survivors at school age. Although the maximum VIS is a measure of pharmacologic cardiovascular support and therefore can be easily influenced by the treating physician, the VIS has already been validated to predict clinical outcomes in infants who require cardiac surgery.[97, 98] Future studies are needed to validate this score in other survivors of critical illness. We acknowledge that prediction of neuropsychological outcome after neonatal critical illness will remain challenging because multiple factors are involved in neuropsychological development (figure 2). These include medical factors such as severity of neonatal illness and treatment effects,[27, 28, 99, 100] as well as non-medical factors such as parental education,[95, 99-102] parental income,[100, 102-104] parental care,[102, 103, 105, 106] cognitive stimulation,[102, 105] stressful life events,[103] and neighborhood.[106] Ideally, all these factors are taken into account when predicting neuropsychological outcome in survivors of neonatal critical illness.

In addition to identification of predictors, it is important to design interventions to improve neuropsychological outcomes. Over the past years, there has been a growing interest in cognitive training programs that improve neuropsychological outcomes. However, only a few studies have assessed the effectiveness of these cognitive training programs in children. A working memory training program that has been studied most frequently is Cogmed,[107] which is a computerized training program designed to improve working memory by effectively increasing working memory capacity over a five-week training period (www.cogmed.

com). Its effectiveness is disputed, however, Klingberg et al.[108] found that Cogmed training had improved visuospatial and working memory in children with attention deficit hyperactivity disorder (ADHD) at 3 months follow-up. Nevertheless, a more recent study did not confirm improvements on neurocognitive tasks in ADHD children after Cogmed training.[109] Studies in very low birth weight preschoolers found positive effects of Cogmed on working memory, visual learning and verbal learning 4 weeks[110] and 7 months[111] after training. Furthermore, in ex-premature adolescents, Cogmed also improved verbal and visuospatial working memory immediately after training and at 6 months follow-up.[112] However, the authors of a recent meta-analytic review concluded that working memory training programs appear to produce short-term, specific training effects that do not generalize to measures of 'real world' cognitive skills.[113] Currently, the effectiveness of Cogmed is assessed in a randomized controlled trial of school aged survivors of neonatal ECMO and CDH (trial registration number: NTR4571).

To prevent neuropsychological problems in the future, it is important to identify the pathophysiological mechanism underlying neuropsychological problems in survivors of neonatal critical illness. Advanced neuroimaging techniques such as diffusion tensor imaging may increase our understanding of pathophysiological concepts of early brain development and injury. Therefore, studies using standardized advanced neuroimaging both at neonatal age and school age should be carried out. Furthermore, noninvasive neuromonitor devices that can monitor regional cerebral perfusion and oxygenation in vulnerable brain areas such as the hippocampus should be developed. Monitoring can result in early signaling of perfusion and oxygenation problems and thus enable early start and guidance of management, which can hopefully prevent adverse outcomes in the future. However, in addition to ischemia, the hippocampus is vulnerable for many other conditions such as neuroinflammation and chronic stress,[70] and therefore it might be hard to prevent hippocampal injury at all.

Figure 2. Multiple factors play a role in determining neuropsychological outcome of patients with congenital diaphragmatic hernia



Future perspectives

In this thesis we focused on non-respiratory morbidities in CDH patients. We found that all ECMO-treated neonates recovered from NTIS after critical illness. Pulmonary hypertension only persisted in a small subgroup of CDH patients with the greatest severity of illness. Therefore, NTIS and pulmonary hypertension appear to be mainly short-term problems. In contrast, we found persistent problems in the areas of physical growth and neuropsychological outcome at school age. Continuing longitudinal long-term follow-up could make clear whether these problems persist during adolescence and adulthood.

We found multiple determinants of outcomes including the need for ECMO treatment, the duration of mechanical ventilation, duration of PICU and initial hospital stay, and severity of illness scores such as the PELOD-2 score and the maximum VIS. These variables are all related to the severity of illness in the neonatal period, which suggests that this in itself is an important predictor of outcome in CDH patients. The building of a prediction model in our studies was hampered by the relatively small sample sizes, which is a common problem in follow-up studies including patients with rare diagnoses. Multicenter research could ensure sufficient patient enrollment to reach adequate statistical power in future studies. This will give opportunities to develop prediction models and improve patient care.

Multicenter research can be implemented by collaborations between multiple CDH treatment centers such as those collaborating within the CDH EURO Consortium. The CDH EURO Consortium is a collaboration between 22 tertiary European centers with an expertise in the treatment of CDH. In November 2007, the CDH EURO Consortium issued a protocol for standardized postnatal management of CDH patients.[114] Thereafter, the VICI-trial was the first randomized controlled trial in which these centers participated.[115] This trial has resulted in an update of the standardized postnatal management protocol.[66] It would be valuable to extend this agreement of compliance with standardized postnatal management to new long-term follow-up studies and further standardization of the long-term follow-up care of CDH patients. In these new long-term follow-up studies, the use of standardized clinical assessment and management plans (SCAMPs) would be valuable. A SCAMP is a dynamic clinical care algorithm designed to eliminate unnecessary resource utilization, decrease practice variation and improve patient outcomes, which undergoes iterative updates based on periodic data collection and review.[116, 117] SCAMPs have been found successful in pediatric cardiology,[118, 119] and we assume that they can also be beneficial in the care for CDH patients. The use of SCAMPs in the follow-up care of CDH patients is currently being implemented within the CDH Euro Consortium. In order to collaborate in long-term follow-up research and care, several aspects are important: standardization of data collection and data storage, availability of a good research infrastructure in the collaborating centers, the use of standardized definitions, standardized assessments, and standardized outcome measures with the use of population-appropriate reference data.[115, 120]

Another interesting research area is the development of intervention strategies that could lead to better outcomes. Intervention-based studies should be established for the different organ systems in which problems occur. Possible topics are the effectiveness of drugs for the treatment of pulmonary hypertension, the effectiveness of early nutritional interventions to improve physical growth, and the use of attention and memory training programs for neuropsychological outcomes. These intervention strategies should be investigated in well-powered randomized controlled trials.

Additionally, future studies should focus on identifying the pathophysiology behind adverse outcomes. This will help develop effective intervention strategies to improve outcomes, and hopefully in the future may prevent adverse outcomes. It has become apparent that adverse neuropsychological outcomes following neonatal critical illness are not limited to the group of CDH patients, as attention and memory problems have also been found in other groups of critically ill neonates such as ECMO-treated neonates,[28, 95, 121] premature neonates[122, 123] and neonates with congenital heart disease.[124-126] This suggests the existence of a common pathophysiologic mechanism underlying these neuropsychological problems. Inclusion of other groups of neonatal critical illness survivors in follow-up studies using advanced neuromonitoring and neuroimaging techniques could make clear whether there is a common pathophysiologic mechanism underlying these problems.

Conclusion

The studies in this thesis show that CDH is a complex condition, which not only affects the respiratory system. On the short-term, we found a high incidence of NTIS in the first week of life, with normalization of thyroid function after the period of critical illness. Pulmonary hypertension on the first day of hospital admission was common, but the risk of persistent pulmonary hypertension during the first year of life was low. On the long-term, we found impaired physical growth and neuropsychological problems in the areas of sustained attention, verbal and visuospatial memory at school age. These findings illustrate the need for long-term follow-up care for CDH patients, and continuing follow-up of these CDH through adolescence and adulthood. All outcomes were associated with variables related to the severity of neonatal illness. Therefore, it seems that the severity of illness in the neonatal period is the most important factor in determining outcomes of CDH patients. In the future, multicenter research using standardized treatment and outcome measures can lead to the development of multivariable prediction models. Additionally, multicenter collaborations with the use of SCAMPs may yield clues to improve the long-term follow-up care for CDH patients. Development of intervention strategies as well as identification of the underlying cause of adverse outcomes is also important to improve patient outcomes. Furthermore, it would be interesting to combine CDH patients with other groups of critically ill neonates in future follow-up studies to examine whether a common pathophysiologic mechanism exists that leads to similar outcomes of these patients.

Future research directions

-To investigate and improve long-term follow-up care for CDH patients, SCAMPs should be used in a large, multicenter cohort.
-To improve the prediction of adverse outcomes, multicenter studies should be instituted to design a multivariable prediction model, which can enable early risk stratification.
-To improve patient outcomes, intervention strategies, such as memory training programs, should be designed and investigated.
-To increase our understanding and possibly prevent adverse outcomes in the future, studies should also focus on the identification of pathophysiological mechanisms behind adverse outcomes.

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Chapter 10

Summary

Summary

Congenital diaphragmatic hernia (CDH) is a severe congenital anomaly of the diaphragm and both lungs, which occurs in approximately 1 per 2,500 births. Most CDH patients will have difficulty breathing directly after birth, and need immediate mechanical ventilatory support. Advanced postnatal treatment options with standardized treatment improved the survival outlook, even of CDH patients with most severe pulmonary hypoplasia and pulmonary hypertension. So far, previous studies have mainly focused on short-term and respiratory outcomes. Given the improved survival rate, it is important that research now also focuses on long-term outcomes including non-respiratory outcomes. This thesis describes the postnatal risks and short- and long-term outcomes of CDH patients from neonatal age until school age. Early outcomes include the risk of disturbed thyroid hormone concentrations during the neonatal period and the risk of persistent pulmonary hypertension in the first year of life. Long-term outcomes include the risk of growth failure and neuropsychological problems until school age. Six studies were performed in the Sophia Children's Hospital in Rotterdam, the Netherlands (**chapters 2, 3, 4, 7, and 8**), and two in the Westmead Children's Hospital in Sydney, Australia (**chapters 5 and 6**). Both countries use a structured program for the long-term follow-up of CDH patients.

Chapter 1

In **chapter 1**, we presented an overview of the current knowledge regarding postnatal management of CDH patients and the survivors' risk of developing short- and long-term morbidities. We gave a short overview of the history of CDH with the introduction of new treatment options over the years. New treatment options include delayed surgical repair after stabilization, gentle ventilation with permissive hypercapnia, and the use of inhaled nitric oxide, high frequency oscillation ventilation and extracorporeal membrane oxygenation (ECMO) as rescue therapies. These advances in treatment options and standardized evidence-based management have increased survival rates, which makes it now important to investigate long-term outcomes of CDH survivors.

Chapter 2

Chapter 2 described the results of a nationwide study among 168 ECMO-treated neonates screened by the newborn screening protocol. Aberrant screening results for congenital hypothyroidism were found in 73% of the neonates screened before ECMO, in 67% screened during ECMO, and in 31% screened after ECMO. Almost all (>98%) patients had a low total thyroxine concentration in combination with a low thyroid-stimulating hormone concentration. These thyroid hormone concentrations normalized in all survivors after recovery of critical illness, and none of the survivors with an aberrant screening result was diagnosed with congenital hypothyroidism. Therefore, aberrant screening results for congenital hypothy-

roidism in these neonates are likely due to non-thyroidal illness syndrome, which can occur during critical illness. Non-thyroidal illness syndrome is characterized by low thyroxine and low triiodothyronine concentrations, an increased reverse triiodothyronine concentration, and a normal or low thyroid-stimulating hormone concentration. Children screened before or during ECMO or those who had a higher Pediatric Logistic Organ Dysfunction-2 score (measure of severity of illness) had a higher risk of an aberrant screening result for congenital hypothyroidism. Mortality was not different between neonates with an aberrant screening result and neonates with a normal screening result.

Chapter 3

In **chapter 3**, we further investigated the disturbances in thyroid hormone concentrations occurring during neonatal ECMO. Thyroid hormone concentrations were measured at six fixed time-points before, during, and after ECMO in 10 neonates with CDH and 11 neonates with meconium aspiration syndrome. Before the start of ECMO, we found low concentrations of free thyroxine, total thyroxine, total triiodothyronine, and thyroid-stimulating hormone, with a high concentration of reverse triiodothyronine, which pattern is suggestive of non-thyroidal illness syndrome. One hour after the start of ECMO, all thyroid hormone concentrations, except free thyroxine, significantly declined. After this initial decline, first thyroid-stimulating hormone increased followed by increases in total thyroxine and total triiodothyronine. These changes suggest that restoration of the hypothalamic-pituitary-thyroid axis occurs during neonatal ECMO. In contrast, the concentration of free thyroxine remained constant within the normal range during the entire ECMO course, which suggests that thyroxine therapy is not required during neonatal ECMO. At the follow-up examination at 2 years of age, cognitive outcomes of survivors did not differ from that of the norm population.

Chapter 4

In **chapter 4**, we assessed the usefulness of routine follow-up for pulmonary hypertension by echocardiography and electrocardiography in 52 CDH patient during the first year of life. At 6 months of age, persistent pulmonary hypertension was found in three of them. At 12 months of age, two had persistent pulmonary hypertension. Patients with persistent pulmonary hypertension had been ventilated and admitted to the hospital for longer than patients without persistent pulmonary hypertension, and had all been treated with inhaled nitric oxide, sildenafil and bosentan. This indicates that patients with persistent pulmonary hypertension are more severely ill during the neonatal period than those without persistent pulmonary hypertension. Echocardiography was superior to electrocardiography in diagnosing pulmonary hypertension. Because over 90% of patients did not have persistent pulmonary hypertension at ages 6 and/or 12 months, we think follow-up for pulmonary hypertension should be reserved for CDH patients with echocardiographic signs of pulmo-

nary hypertension at hospital discharge and those treated with medication for pulmonary hypertension at hospital discharge.

Chapter 5

Chapter 5 described the physical growth and the presence of feeding problems in 38 CDH patients during the first year of life. Low weight, height, and head circumference were found at 3 months of age, with weight being most affected. However, catch-up growth for weight, height and head circumference occurred from 3 to 12 months of age. Duration of mechanical ventilation was the only clinical variable predictive of failure to thrive at 6 months of age. No predictors for failure to thrive were found at 12 months of age. One fifth of the patients had feeding problems, which included: poor suck-swallowing reflex, immature oral skills, feeding difficulties owing to increased work of breathing, oral aversion and gastroesophageal reflux disease.

Chapter 6

In **chapter 6**, we described the neurodevelopmental outcome of 18 CDH patients who were longitudinally assessed at 1 year and 3 years of age. Neurodevelopmental outcomes of CDH patients were compared with a healthy age- and gender-matched control group. At one year of age, gross motor skills were mildly delayed in one patient and severely delayed in three patients. At 3 years of age, expressive language scores were mildly delayed in three patients, and receptive language score was mildly delayed in one patient. All the other outcome scores were normal at 3 years of age. We found no difference in neurodevelopmental outcome between CDH patients and the control group at 1 year and 3 years of age.

Chapter 7

In **chapter 7**, we determined the neuropsychological outcome at 8 years of age in 40 patients with CDH (10 treated with neonatal ECMO) and 25 patients treated with neonatal ECMO following other diagnoses. Additionally, we aimed to identify variables associated with neuropsychological outcomes. We found specific impairments in the areas of sustained attention, verbal memory and visuospatial memory at 8 years of age in CDH and neonatal ECMO survivors, despite an overall average intelligence quotient (IQ). ECMO-treated CDH patients had the lowest IQ. We found that various severity of illness indicators were independently associated with neuropsychological outcome: treatment with ECMO, use of veno-arterial ECMO, a higher maximum vasoactive-inotropic score (maximum dose of vasoactive medication required), a lower number of ventilator-free days in the neonatal period, and a longer duration of initial hospital stay. Additionally, the number of anesthetic procedures and weight at 1 year of age were associated with neuropsychological outcome. However, only the maximum vasoactive-inotropic score remained negatively associated with delayed verbal and visuospatial memory when all contributing factors were taken into account in the multivariable model. This suggests that this measure of severity of illness, possibly indicating

(cerebral) hypoperfusion during early life, is related to specific memory problems at 8 years of age following CDH and/or neonatal ECMO.

Chapter 8

In **chapter 8**, we described the longitudinal physical growth of 172 CDH patients (43 treated with neonatal ECMO) until 12 years of age. Additionally, we wanted to find clinical variables associated with growth and unravel possible causes of growth failure. We found that weight gain declined from 6 months to 1 year of age, followed by a decline in linear growth until 5 years of age. This pattern of early deterioration of weight gain followed by a decline in linear growth is suggestive of inadequate nutrition during infancy. Overall, CDH patients treated with ECMO had a lower weight and height than CDH patients not treated with ECMO. At 12 years of age, height of CDH patients was still below the norm. Adjusting for target height improved height z-scores but not to normal. ECMO treatment, the need for patch repair and the duration of pediatric intensive care unit stay were negatively associated with height. ECMO treatment, the need for patch repair and the need for calorie-enriched feeds at follow-up were negatively associated with weight. Tube feeding at follow-up had a positive association with weight and height. We found an increased resting energy expenditure in four of the 11 patients with growth failure, and a disturbed eating pattern in seven of them. Therefore, both factors seem involved in growth failure. Our study results highlight the importance of early risk stratification in combination with nutritional assessments and interventions in preventing growth failure, as well as the importance of continuing long-term follow-up of growth.

Chapter 9

In **chapter 9**, we discussed the study results, put them into perspective, and made recommendations for future research and patient care. The studies in this thesis showed that CDH is a complex condition that does not only affect the respiratory system. In the short-term, there is a high risk of non-thyroidal illness syndrome and pulmonary hypertension. In the long-term, we found impaired linear growth and neuropsychological problems in the areas of sustained attention, verbal and visuospatial memory at school age. These findings illustrate the need for long-term follow-up care for CDH patients, and continuing follow-up of these children throughout adolescence and adulthood. Outcomes were associated with variables related to the severity of neonatal illness. Therefore, it seems that the severity of neonatal illness is the most important factor in determining outcomes. The development of multivariable prediction models should be further investigated in multicenter studies. Further, multicenter collaborations with the use of standardized clinical assessment and management plans may yield clues to improve the long-term follow-up and care of CDH patients. To improve patient outcomes, research should also focus on developing intervention strategies, such as memory training programs, as well as identifying underlying causes of adverse outcomes, which hopefully may lead to prevention of adverse outcomes in the future.

Chapter 11

Nederlandse samenvatting

Nederlandse samenvatting

Congenitale hernia diafragmatica ofwel een aangeboren middenrifbreuk, in het Engels 'congenital diaphragmatic hernia' (CDH), is een ernstige aangeboren afwijking van het middenrif en de longen die bij 1 op de 2.500 pasgeborenen voorkomt. De meeste kinderen met deze aandoening hebben direct na de geboorte moeite met ademen waardoor ze meteen beademd moeten worden. Dankzij nieuwe behandelmethoden en gestandaardiseerde behandeling zijn hun overlevingskansen in de afgelopen jaren echter sterk verbeterd. Het is nu dan ook belangrijk dat er verder onderzoek wordt gedaan naar hun uitkomsten op de langere termijn. Dit proefschrift beschrijft onderzoek naar diverse uitkomsten van CDH patiënten, waaronder de schildklierfunctie, de aanwezigheid van pulmonale hypertensie (verhoogde bloeddruk in de longvaten), de fysieke groei en de neuropsychologische ontwikkeling. Zes studies werden verricht in het Erasmus MC-Sophia Kinderziekenhuis in Rotterdam (**hoofdstukken 2, 3, 4, 7 en 8**) en twee studies in het Westmead Children's Hospital in Sydney (**hoofdstukken 5 en 6**). Zowel in Rotterdam als in Sydney worden CDH patiënten poliklinisch gevolgd tot aan de leeftijd van 18 jaar.

Hoofdstuk 1

In **hoofdstuk 1** wordt een overzicht gegeven van de geschiedenis en de huidige kennis omtrent de behandeling en de risico's van CDH. Na intubatie en opname op de kinder intensive care wordt gewacht met operatieve behandeling van de middenrifbreuk tot de patiënt klinisch stabiel is, waarbij beademd wordt met zo laag mogelijke piekdrukken. De meest zieke patiënten worden daarnaast behandeld met geïnhaleerde stikstofmonoxide, hoogfrequente beademing en extracorporele membraan oxygenatie (ECMO). ECMO is een behandeling waarbij een hart-longmachine het werk van de longen en het hart tijdelijk overneemt, waardoor deze organen kunnen herstellen. ECMO wordt alleen toegepast als uiterste redmiddel indien er zeer hoge kans is op overlijden als gevolg van ernstig longfalen. Eerdere studies hebben al laten zien dat longproblemen bij CDH patiënten langdurig aanwezig kunnen blijven. Op het ogenblik is er echter nog maar weinig bekend over de overige uitkomsten van CDH patiënten.

Hoofdstuk 2

In **hoofdstuk 2** beschreven we de incidentie van een afwijkende screeningsuitslag op congenitale hypothyreoïdie (aangeboren tekort aan schildklierhormoon) tijdens de hielprikscreening bij 168 ECMO-behandelde pasgeborenen. Als de screening werd verricht voor ECMO werd bij 73% een afwijkende uitslag gevonden; als dit tijdens ECMO werd gedaan bij 67%; en als dit na ECMO werd gedaan bij 31%. Bijna alle patiënten met een afwijkende screeningsuitslag hadden een lage concentratie totaal thyroxine in combinatie met een normale concentratie thyroïd stimulerend hormoon in hun bloed. Uiteindelijk bleek geen van de pasgeborenen met

een afwijkende screeningsuitslag congenitale hypothyreoïdie te hebben. Deze bevindingen duiden erop dat de afwijkende screeningsuitslagen bij ECMO-behandelde pasgeborenen het meest waarschijnlijk het gevolg zijn van het zogenoemde 'non-thyroidal illness syndroom', welke kan optreden bij ernstig zieke patiënten. Deze schildklierfunctiestoornis wordt gekenmerkt door lage concentraties thyroxine en triiodothyronine, een hoge concentratie inactief triiodothyronine in combinatie met een normale of lage concentratie thyroïd stimulerend hormoon. Screening voor en tijdens ECMO en een hogere 'Pediatric Logistic Organ Dysfunction-2' score, een maat voor de ziekte-ernst, waren geassocieerd met een hoger risico op een afwijkende screeningsuitslag op congenitale hypothyreoïdie. We vonden echter geen verschil in sterfte tussen pasgeborenen met een afwijkende screeningsuitslag en pasgeborenen met een normale screeningsuitslag.

Hoofdstuk 3

In **hoofdstuk 3** deden we verder onderzoek naar de veranderingen in schildklierhormoonconcentraties die optreden bij pasgeborenen tijdens ECMO. Op zes vaste tijdspunten (voor, tijdens en na ECMO) werden schildklierhormoonconcentraties in het bloed gemeten bij 10 pasgeborenen met CDH en 11 pasgeborenen met meconium aspiratie syndroom. De metingen voor ECMO wezen wederom op schildklierfunctiestoornissen passend bij het bovengenoemde 'non-thyroidal illness syndroom'. Na de start van de ECMO-behandeling, zagen we een verdere daling van de schildklierhormoonconcentraties, met uitzondering van de concentratie vrij thyroxine. Na deze daling namen de schildklierhormoonconcentraties gedurende de ECMO-behandeling weer toe. Allereerst steeg de concentratie thyroïd stimulerend hormoon, waarna ook de concentraties totaal thyroxine en totaal triiodothyronine toenamen. Dit patroon suggereert dat herstel van de hypothalamus-hypofyse-schildklier as optreedt tijdens de ECMO-behandeling. Omdat de vrij thyroxine concentratie stabiel en binnen de normaalwaarde bleef, lijkt behandeling met thyroxine tijdens ECMO niet nodig. Op de follow-up na 2 jaar, vertoonden overlevende kinderen normaal cognitief functioneren.

Hoofdstuk 4

In **hoofdstuk 4** onderzochten we of routinematige follow-up voor pulmonale hypertensie (verhoogde bloeddruk in de longvaten) zinvol was bij CDH patiënten gedurende het eerste levensjaar. Voor dit doel werd echocardiografie en elektrocardiografie uitgevoerd bij 52 CDH patiënten op de leeftijd van 6 maanden en/of 12 maanden. Drie patiënten hadden op de leeftijd van 6 maanden nog tekenen van pulmonale hypertensie bij echocardiografie, en twee op de leeftijd van 12 maanden. Deze patiënten met persisterende pulmonale hypertensie waren langer beademd tijdens de eerste ziekenhuisopname en verbleven langer in het ziekenhuis dan patiënten zonder persisterende pulmonale hypertensie. Ook waren al deze patiënten behandeld met geïnhaleerde stikstofmonoxide, sildenafil en bosentan, terwijl deze behandelingen niet bij alle patiënten zonder persisterende pulmonale hypertensie nodig

waren geweest. Dit duidt erop dat patiënten met persisterende pulmonale hypertensie ernstiger ziek zijn in de eerste levensweken. Omdat de diagnose pulmonale hypertensie bij twee van de drie patiënten gemist werd op het elektrocardiogram, lijkt elektrocardiografie niet geschikt voor de follow-up van pulmonale hypertensie bij CDH patiënten. Poliklinische follow-up van pulmonale hypertensie middels echocardiografie lijkt ons vooral zinvol bij patiënten met echocardiografische tekenen van pulmonale hypertensie bij ontslag naar huis en/of gebruik van medicatie voor pulmonale hypertensie ten tijde van ontslag.

Hoofdstuk 5

In **hoofdstuk 5** evalueerden we de fysieke groei en de aanwezigheid van voedingsproblemen bij 38 CDH patiënten gedurende het eerste levensjaar. Daarnaast onderzochten we mogelijke voorspellers voor het optreden van een groeiachterstand (failure to thrive). Op de leeftijd van 3 maanden hadden patiënten een lager gewicht, een kleinere lichaamslengte en een kleinere schedelomtrek dan normaal, waarbij het gewicht het meeste achterbleef. Op de leeftijd van 12 maanden was echter een inhaalgroei opgetreden, voor zowel gewicht, lengte als schedelomtrek. Een langere beademingsduur was de enige voorspeller voor een groeiachterstand op de leeftijd van 6 maanden. Eén vijfde van de kinderen had voedingsproblemen, waaronder onvoldoende ontwikkeling van de zuig- en slikreflex, onjuiste drinktechniek, voedingsproblemen door ademhalingsmoeilijkheden, orale aversie en gastro-oesofageale reflux.

Hoofdstuk 6

In **hoofdstuk 6** evalueerden we de ontwikkeling op het gebied van taal, motoriek en cognitie op 1-jarige en 3-jarige leeftijd bij 18 CDH patiënten, en vergeleken deze met een gezonde controlegroep. Op 1-jarige leeftijd, hadden twee patiënten een milde achterstand op het gebied van taalproductie of taalbegrip, en vier patiënten hadden een achterstand op het gebied van grove motoriek. Op 3-jarige leeftijd, hadden drie patiënten een milde achterstand op het gebied van taalproductie en één patiënt op het gebied van taalbegrip. De overige uitkomsten op het gebied van taal, motoriek en cognitie waren normaal op deze leeftijd. Er waren geen verschillen in ontwikkelingsuitkomsten (taal, motoriek en cognitie) tussen de CDH patiënten en de controlegroep op de leeftijd van 1 en 3 jaar.

Hoofdstuk 7

In **hoofdstuk 7** onderzochten we de neuropsychologische uitkomsten op 8-jarige leeftijd bij 40 CDH patiënten (van wie 10 behandeld waren met ECMO) en 25 patiënten zonder CDH die kort na de geboorte met ECMO behandeld waren. Daarnaast analyseerden we mogelijke voorspellers van neuropsychologische uitkomsten. Kinderen hadden problemen met volgehouden aandacht, verbaal geheugen en visueel geheugen, ondanks de aanwezigheid van een normaal intelligentie quotiënt (IQ). Het IQ van CDH patiënten die behandeld waren met ECMO was het laagst. Ziekte-ernst indicatoren waren de belangrijkste voor-

spellers voor neuropsychologische uitkomsten, wat erop wijst dat de meest ernstig zieke kinderen het hoogste risico lopen op een slechte neuropsychologische uitkomst. Meerdere ingrepen onder algehele narcose in het eerste levensjaar had ook een negatief effect op de neuropsychologische uitkomst. Daarnaast was er een positief verband tussen het gewicht op 1-jarige leeftijd en het IQ. Echter, na het invoeren van alle significante voorspellers in het voorspellingsmodel, bleef er alleen een negatief verband bestaan tussen de maximale 'vasoactive-inotropic' score, een maat voor de benodigde hoeveelheid vasoactieve medicatie en ziekte-ernst, en verbaal en visueel lange termijn geheugen. Dit duidt erop dat kinderen die het meest ernstig ziek waren in de eerste levensdagen, met daarbij mogelijk een hoger risico op cerebrale hypoperfusie, meer risico hadden op geheugenproblemen op 8-jarige leeftijd.

Hoofdstuk 8

In **hoofdstuk 8** beschreven we de longitudinale groei tot aan de leeftijd van 12 jaar bij 172 CDH patiënten (van wie 43 patiënten behandeld met ECMO). Daarnaast onderzochten we voorspellers voor fysieke groei en mogelijke oorzaken van een groeiachterstand. Een afbuiging van gewicht trad op van de leeftijd van 6 maanden tot 1 jaar, en hierna boog de lengtegroei af tot de leeftijd van 5 jaar. Dit groeipatroon met onvoldoende gewichtstoename gevolgd door vertraagde lengtegroei duidt mogelijk op chronische ondervoeding in het eerste levensjaar. ECMO-behandelde patiënten hadden een lager gewicht en een kleinere lengte dan patiënten die niet behandeld waren met ECMO. De eerder opgetreden groeiachterstand van patiënten werd niet geheel ingehaald, en op 12-jarige leeftijd waren zij nog steeds kleiner dan de normpopulatie. De lengtescores van patiënten verbeterden na correctie voor de berekende streeflengte maar zelfs na deze correctie bleven zij kleiner dan de normpopulatie. Lengte en gewicht werden beide negatief beïnvloed door ECMO-behandeling en correctie van de middenrifbreuk met een patch (kunststoflapje). Sondevoeding had een positief effect op zowel het gewicht als de lengte. Bij vier van de 11 CDH patiënten met een groeiachterstand, vonden we een verhoogd rustmetabolisme en bij zeven patiënten waren eetproblemen aanwezig. Beide factoren lijken dan ook een rol te spelen bij het ontstaan van een groeiachterstand. Ter voorkoming hiervan is vroegtijdige risicostratificatie nodig met regelmatige beoordeling van de voedingsstatus en het tijdig starten van voedingsinterventies.

Hoofdstuk 9

In **hoofdstuk 9** bespraken we de studieresultaten en gaven we aanbevelingen voor verder onderzoek. Uit ons onderzoek blijkt dat CDH een complexe aandoening is die niet alleen gepaard gaat met longproblemen. Op de korte termijn hadden kinderen een hoog risico op het 'non-thyroidal illness syndroom' en pulmonale hypertensie. Op de lange termijn vonden we groeiproblemen en problemen met volgehouden aandacht, verbaal geheugen en visueel geheugen. Deze problemen bevestigen dat het belangrijk is om CDH patiënten langdurig

te blijven volgen. De ziekte-ernst in de eerste levensweken was de belangrijkste voorspeller van uitkomsten in onze studies. Voor de ontwikkeling van betere voorspellingsmodellen in de toekomst, is meer multicenter onderzoek nodig. Daarnaast denken wij dat de invoering van 'standardized clinical assessment and management plans' waardevol kan zijn voor het verbeteren van de zorg voor CDH patiënten. Tot slot moet toekomstig onderzoek zich ook richten op het ontwikkelen van interventiestrategieën en het identificeren van de onderliggende oorzaken van problemen zodat deze mogelijk in de toekomst voorkomen kunnen worden.

Chapter 12

Appendices

List of abbreviations

List of publications

PhD portfolio

Dankwoord

Stellingen

About the author

List of abbreviations

BADS-C-NL	Behavioral assessment of the dysexecutive syndrome children-Dutch version
Bayley-III	Bayley scales of infant and toddler development-third edition
CDH	Congenital diaphragmatic hernia
CH	Congenital hypothyroidism
CI	Confidence interval
CO_2	Carbon dioxide
CPR	Cardiopulmonary resuscitation
DCT	Dot cancellation test
DTH	Distance-to-target height
ECG	Electrocardiograph
ECMO	Extracorporeal membrane oxygenation
FFP	Fresh frozen plasma
FiO_2	Inspiratory oxygen fraction
FT4	Free thyroxine
FTT	Failure to thrive
GERD	Gastroesophageal reflux disease
H_2O	Water
HFA	Height-for-age
HFOV	High frequency oscillation ventilation
HPT	Hypothalamic-pituitary-thyroid
iNO	Inhaled nitric oxide
IQR	Interquartile range
LHR	Lung-to-head ratio
MAS	Meconium aspiration syndrome
NTIS	Non-thyroidal illness syndrome
PAPVR	Partial anomalous pulmonary venous return
PEEP	Positive end expiratory pressure
PELOD-2	Pediatric Logistic Organ Dysfunction-2
PH	Pulmonary hypertension
PICU	Pediatric intensive care unit
PIP	Peak inspiratory pressure
PRISM III	Pediatric Risk of Mortality III
RAVLT	Rey auditory verbal learning test

RCFT	Rey complex figure test
REE	Resting energy expenditure
rT3	Reverse triiodothyronine
SCAMPS	Standardized clinical assessment and management plans
SD	Standard deviation
SDS	Standard deviation score
$STRONG_{kids}$	Screening Tool for Risk On Nutritional status and Growth
STROOP	Stroop color word test
T3	Triiodothyronine
T4	Thyroxine
TBG	Thyroxine-binding globulin
TEN	Total energy need
TH	Target height
TMT A	Trail making test section A
TMT B	Trail making test section B
TR	Tricuspid regurgitation
TSH	Thyroid-stimulating hormone or thyrotropin
TT4	Total thyroxine
TT3	Total triiodothyronine
VA	Veno-arterial
VCO_2	Carbon dioxide production
VIS	Vasoactive-inotropic score
VO_2	Oxygen consumption
VV	Veno-venous
WFH	Weight-for-height
WISC-III-NL	Wechsler intelligence scale for children-third edition-Dutch version
WNV	Wechsler nonverbal scale of ability

List of publications

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PhD portfolio

General information	
Name	Lisette Leeuwen
Department	Intensive care and pediatric surgery
PhD period	November 2014 - December 2017
Promotors	Prof. dr. D. Tibboel
	Prof. dr. R.M.H. Wijnen
Copromotor	Dr. H. IJsselstijn
Research school	Medical Genetic Center

1. PhD Training

General academic skills	Year	Workload
Systematical literature retrieval course	Jan. 2015	1.0 ECTS
Research integrity course	Apr. 2015	0.3 ECTS
Biomedical English writing and communication course	Apr. 2015	3.0 ECTS
BROK course	Sept. 2015	1.5 ECTS
Presenting skills for junior researchers	Oct. 2016	1.0 ECTS
Biomedical English writing course	June 2017	2.0 ECTS

Research skills	Year	Workload
Basic introduction on SPSS	May 2015	1.0 ECTS
Biostatistical methods I: basic principles	Oct. 2015	5.7 ECTS

Seminars and workshops	Year	Workload
Workshop: how to present your poster	Feb. 2015	0.5 ECTS
Amsterdam pediatrics symposium 2015	Feb. 2015	0.3 ECTS
CPO Course: patient oriented research	Apr. 2015	0.3 ECTS
Symposium: interdisciplinary care; an image of the future	Apr. 2015	0.2 ECTS
Workshop: grant writing	May 2015	0.1 ECTS
LNF conference 2015	June 2015	0.3 ECTS
Workshop: how to present your SLAM session	June 2015	0.2 ECTS
COEUR course: congenital heart disease	June 2015	1.5 ECTS
EUR course: presenting and pitching skills	Nov. 2015	0.3 ECTS
TULIPS: child health symposium	Mar. 2016	0.3 ECTS
Workshop: leading through conflict	Nov. 2016	0.1 ECTS

Erasmus MC PhD day	2015-2017	1.0 ECTS
Sophia Research Day	2015-2017	1.0 ECTS

Presentations	Year	Workload
'Growth and neurodevelopmental outcomes in congenital diaphragmatic hernia survivors during the first three years of life'	Apr. 2013	0.5 ECTS
'Deterioration of lung function and exercise capacity in congenital diaphragmatic hernia survivors over three decades'	Jan. 2015	0.5 ECTS
'High incidence of abnormal screening results for congenital hypothyroidism in ECMO-treated neonates'	Mar. 2015	0.5 ECTS
'Changes in thyroid hormone concentrations during neonatal ECMO'	Oct. 2016	0.5 ECTS
'Growth in congenital diaphragmatic hernia patients until 12 years of age'	Oct. 2016	0.5 ECTS
'Memory problems following neonatal critical illness'	Nov. 2016	0.5 ECTS

International and national conferences	Year	Workload
European Respiratory Society Congress, Barcelona (poster presentation)	Sept. 2013	2.0 ECTS
Pediatric Association of the Netherlands Congress, Veldhoven (oral presentation)	Nov. 2013	2.0 ECTS
International Student Congress of (bio)Medical Sciences, Groningen (oral presentation)	June 2014	2.0 ECTS
European Academy of Paediatric Societies Congress, Geneva (two oral presentations)	Oct. 2016	3.0 ECTS

Other activities	Year	Workload
Research meetings: department of respiratory medicine; department of intensive care and pediatric surgery; clinical PhD-students	2014-2017	1.5 ECTS
Organizing committee member Sophia Research Day	2014-2015	2.0 ECTS
Member of the Sophia Researchers Board (SOV)	2015-2017	4.0 ECTS

2. Teaching

Teaching activities	Year	Workload
Lecturing talented primary school children	2014	0.5 ECTS
Coaching first- to third-year medical students	2015-2017	2.0 ECTS
Tutoring first-year medical students	2015-2017	3.0 ECTS

Didactic courses and workshops	Year	Workload
Training 'Coaching of future Erasmusdoctors'	Feb. 2015	0.2 ECTS
E-learning symposium	June 2015	0.2 ECTS
Training 'Dealing with groups'	Aug. 2015	0.1 ECTS

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

Dankwoord

The breath of life, prana, ofwel onze ademhaling. Ademhalen begint onmiddellijk na de geboorte en is onlosmakelijk verbonden met het leven. Onze ademhaling ervaren we vaak als vanzelfsprekend maar dit is zeker niet voor iedereen het geval. In dit proefschrift heb ik laten zien dat ademhalen voor kinderen met een congenitale hernia diafragmatica vanaf het moment na de geboorte een probleem vormt. De problemen die optreden ten gevolge van het defect in hun diafragma gaan gepaard met een hoog risico op ziekte en zelfs sterfte. Vanaf de eerste ademhaling vechten deze kinderen met een buitengewone kracht voor hun leven. Het is ongelofelijk wat een herstel ze in deze vroege periode doormaken, maar hiervoor hebben ze een lange adem nodig. Ik heb enorm veel bewondering en respect voor de sterkte en veerkracht van deze patiënten en hun ouders. Allereerst wil ik dan ook alle patiënten en hun ouders bedanken. Zij zijn de reden dat ik ben begonnen met dit promotie onderzoek en ik hoop met mijn onderzoek te hebben bijgedragen aan het verbeteren van de zorg voor en uitkomsten van deze groep patiënten. Lieve patiënten en ouders, bedankt en ik wens jullie al het goede en heel veel geluk in de toekomst!

Het heeft even geduurd, maar nu is het eindelijk zover, het boekje is af! Zoals hierboven genoemd, hebben kinderen met een congenitale hernia diafragmatica een lange adem nodig voor hun herstel. Ik heb in de afgelopen jaren ervaren dat een lange adem ook essentieel is voor het schrijven van een proefschrift. Echter, ik had dit proefschrift nooit kunnen schrijven zonder de hulp van heel veel fantastische, mooie, lieve mensen die ik hier graag wil bedanken.

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with you and your family soon!

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nu al uit naar het eerste SOV Alumni Event!

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Namasté, heel veel liefs,

Lisette

Stellingen

1. The burden of congenital diaphragmatic hernia is far more extensive than only respiratory morbidity. - this thesis

2. The high incidence of aberrant screening results for congenital hypothyroidism in neonates treated with extracorporeal membrane oxygenation is caused by non-thyroidal illness syndrome. - this thesis

3. Routine screening of patients with congenital diaphragmatic hernia for pulmonary hypertension in the first year is a waste of money, time and effort. - this thesis

4. Neuropsychological outcome and physical growth of patients with congenital diaphragmatic hernia is determined by the severity of illness during the neonatal period. - this thesis

5. Patients with congenital diaphragmatic hernia should receive standardized long-term follow-up during childhood, and ideally, follow-up should be problem-oriented and a moving target with regular updates according to the latest research findings. - this thesis

6. Despite improved survival rates of critically ill children, pediatric intensive care has not become easier. - Rennick et al. Pediatrics. 2015

7. Physicians' 'gut feelings' are a valuable skill, and these 'gut feelings' should not be ignored but used in medical decision making as well as in life. - Van den Bruel et al. BMJ. 2012

8. An open, curious mind is the sine qua non of success and achievement in any field, and therefore should always be encouraged. - Werner et al. Med Educ. 2011

9. Work experience abroad is very beneficial and valuable for the holistic development of physicians. - Reardon et al. BMC Med Educ. 2015

10. A physician, though well versed in the knowledge and treatment of disease, who does not enter into the heart of the patient with the virtue of light and love, will not be able to heal the patient. - Charaka, 'Father of Indian Medicine'

11. Be the change you want to see in the world. - Mahatma Gandhi

Do you ever think about the importance of our breathing?

Life starts from the first breath we take in when we are born and continues until our last breath. Breathing is something we do naturally, most often without thinking about it, and without appreciating it. However, we cannot live without breathing: breath is life.

For children born with a diaphragmatic hernia, breathing is not so simple and self-evident. They will have difficulty breathing immediately after birth due to the lung problems associated with the defect in their diaphragm. Consequently, from the first breath of life, these children are at a high risk of mortality and morbidity. Fortunately, more and more of them now survive. Little is known, however, about the risks and consequences of this condition when children grow up.

This thesis describes the postnatal risks and outcomes of children with congenital diaphragmatic hernia from neonatal age until school age.