One Size Does Not Fit All

prognosis and therapy in congenital diaphragmatic hernia

Er is geen standaardaanpak

Prognose en therapie in congenitale hernia diafragmatica

Suzanne Cornelia Maria Cochius - Den Otter

One size does not fit all

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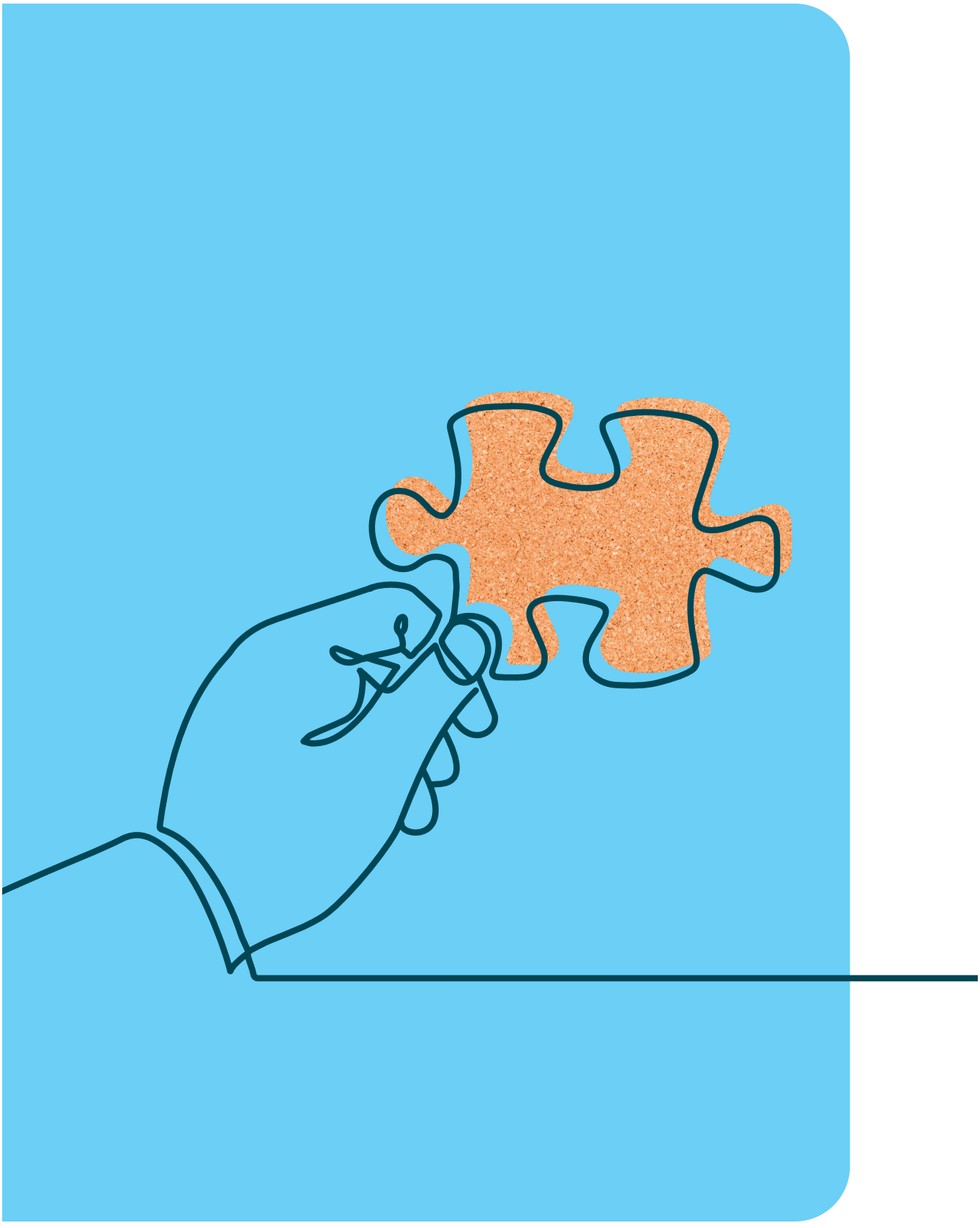
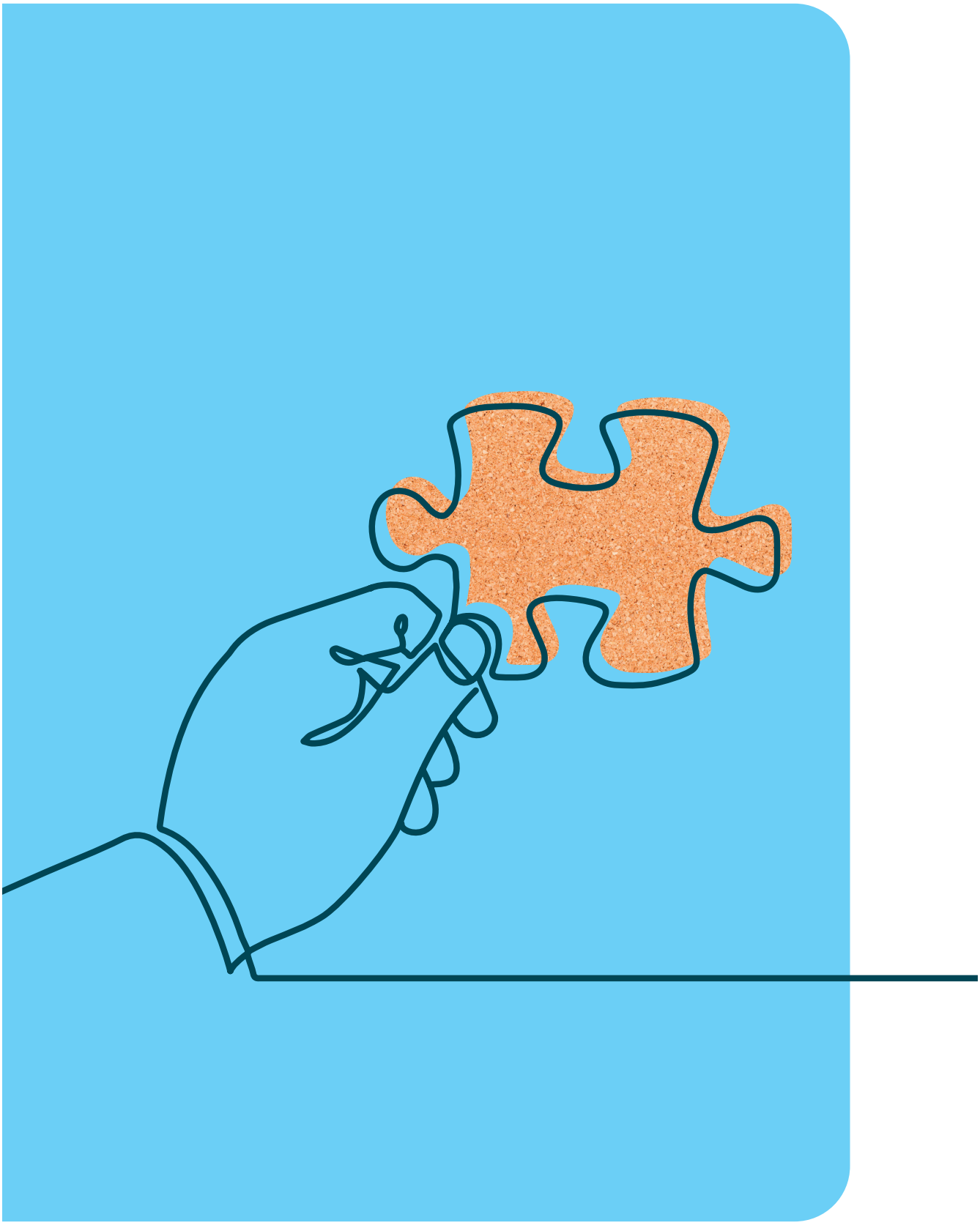
CONTENT



PART 1

Introduction



CHAPTER 1

Introduction

INTRODUCTION

Congenital diaphragmatic hernia (CDH) is a developmental defect of the lungs en diaphragm that occurs in 1 per 4000-4500 live births (1). In the Netherlands, approximately 40 patients with CDH are born alive each year.

The first description of diaphragmatic hernia was made by Ambroise Paré in 1575 (2). However, these cases were caused by trauma. CDH in a newborn was first reported in 1754 by George Macaulay, in an infant who died of respiratory failure soon after birth (3). In 1769 Giovanni Morgagni first described the parasternal hernia, now known as Morgagni hernia (4). Almost 100 years later, in 1848, Bochdalek described the posterolateral hernia; the Bochdalek hernia, and already recognized the importance of lung hypoplasia (5).

The Morgagni hernia is rare, consisting of only 3-5% of all CDH cases. Its diagnosis is often delayed, with patients presenting with nonspecific respiratory and gastrointestinal symptoms in infancy or even adulthood (6). However, herniation of the abdominal content with strangulation, bowel ischemia and perforation may occur (7).

The posterolateral, or Bochdalek hernia is seen in more than 90% of the cases, and can present left-sided, right-sided or bilateral. Also, instead of a true defect, an eventration or hernial sac can be present. From the early embryological phase onwards, the development of the diaphragm, both lungs and its vasculature is altered. This results in varies degrees of pulmonary hypoplasia and pulmonary hypertension (PH) (8). The defect in the diaphragm ranges from small defects to complete agenesis of mostly the left diaphragm. The exact etiology of CDH remains unknown and seems to be multifactorial (figure 1). It is also unknown why the diaphragm defects mainly occur on the left side, in a ratio of 8:1 compared to the right sided defects (9).

Mortality was very high before the introduction of surgery, and it was only at the beginning of the last century that surgery was considered a therapeutic option, decreasing mortality to approximately 85-50% (11, 12). Although Korns already recognized the importance of PH in 1921, it wasn't until the late 1980's that preoperative cardiopulmonary stabilization and delayed surgery became standard of care, further improving outcome (11, 13, 14). Nowadays, with the introduction of standardized care, mortality and morbidity has further decreased, with a survival of approximately 73% in well-established centers of expertise (15). Apart from the experience of the treatment teams, mortality and morbidity are now highly dependent on the severity of lung hypoplasia, the presence of PH, and the presence of associated anomalies, such as chromosomal and cardiac anomalies (1, 15, 16).

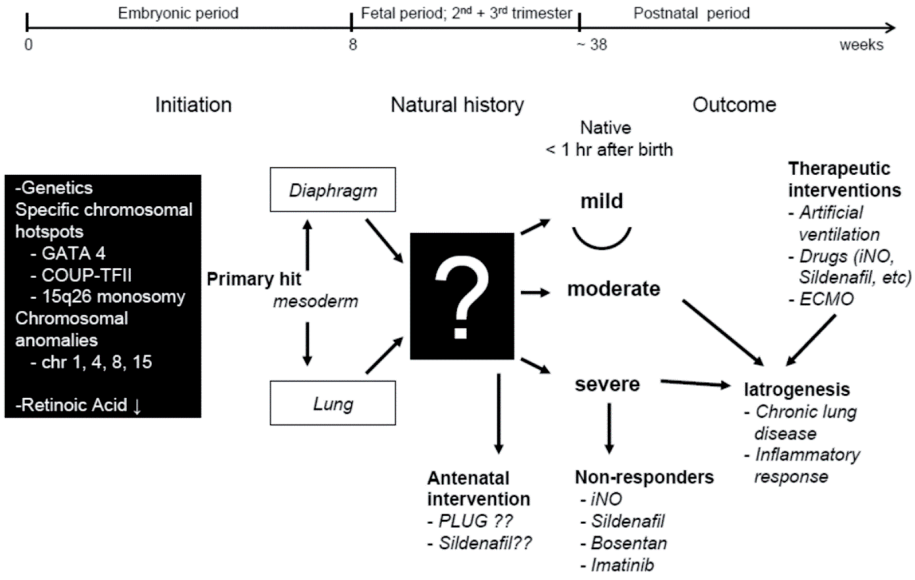


Figure 1. Schematic overview of the etiological factors, the natural history and therapeutic options of CDH (10).

PREDICTION

Prenatal parameters

In countries with routine prenatal ultrasound screening, CDH is most often diagnosed prenatally. The introduction of the second trimester ultrasound has increased the detection of congenital anomalies such as CDH considerably (17, 18). Several prenatal ultrasound parameters have been developed to prognosticate postnatal outcome in patients with CDH. Prenatal prediction can guide parents and caretakers in decisions regarding continuation of pregnancy and the use of prenatal therapy such as foetal endoscopic tracheal occlusion (FETO). It has also been used to compare patient populations and management strategies. One of the first ultrasound parameters that was used to predict lung hypoplasia and survival is the Lung-to-Head Ratio (LHR). To calculate the LHR, the contralateral lung is measured, using two perpendicular linear measurements. These measurements are multiplied and divided by the head circumference. However, the LHR was not found to be very reliable, as lung growth is not linear to head growth during pregnancy (19). Subsequently, the observed-to-expected Lung-to-Head Ratio (O/E LHR) was developed, comparing the observed LHR to the expected LHR appropriate for the age of the fetus. When using the tracing method, tracing the contours of the lung, this is a fairly reliable parameter with a small inter observer variability (20). When using MRI to evaluate the lung size, observed-to-expected total fetal lung volume (TFLV) is possibly a

more accurate predictor of survival, but operator experience in measurement of the lung volumes plays a role in its predicting value (21, 22).

Another prenatal predictor for postnatal outcome in patients with CDH is the position of the liver, evaluated on ultrasound or MRI. Intrathoracic position of the liver is associated with an increased risk of mortality and the need for extracorporeal membrane oxygenation (23, 24). Also the position of the stomach has been evaluated as a predictor for outcome (25, 26). However, one should be aware that all prenatal parameters can be used to predict lung hypoplasia, but do not reliably predict PH decreasing its sensitivity for adverse outcome (27, 28).

Also, to predict outcome, the presence of genetic or other major congenital anomalies plays a substantial role. Genetic anomalies can often be found with a micro-array or increasingly with next generation sequencing, for major anomalies the structural fetal ultrasound is used. The prevalence of these anomalies varies widely, depending on the population under evaluation. Prenatally, these anomalies are seen in approximately 34% of fetuses with CDH; 25% has associated anomalies such as cardiac, urinary tract, limb and central nervous system anomalies, 11% has chromosomal anomalies, genetic syndromes or microdeletions (1). A large part of the genetic anomalies are explained by de novo mutations (29). These anomalies are an important predictor for adverse outcome. In the prenatal period because of intrauterine fetal demise or termination of pregnancy, but also in the neonatal period mortality is high in this group of CDH patients (1, 30, 31).

Postnatal parameters

Postnatal models to predict morbidity and mortality has the potential to help care providers to start the right treatment in the right patient at the right time. It could prevent over and under treatment and early therapy can possibly prevent exacerbation of PH. For these postnatal predictions, there are several prediction models and variables such as SNAP II score, highest PaO2 minus highest PaCO2, and oxygenation index. However, many are based on relatively small groups of patients, are difficult to apply or have not been externally validated (32-36). Brindle et al, and the Congenital Diaphragmatic Hernia Study Group (CDHSG) have developed a simple and validated early clinical prediction rule in a large cohort of patients to identify low (<10%), intermediate (~20%), and high risk (~50%) of death. This model is based on birth weight, 5-minute Apgar score, severe PH, and the presence of cardiac and chromosomal anomalies (37).

The predicted value of this postnatal model has been favorable compared to prenatal predictors (38). One could argue that combining post- and prenatal risk factors within a single prediction model could further improve the significance of a prediction model.

However, prenatal and postnatal predictors have only been integrated in one prediction model in a small group of patients from a single center (39).

Biomarkers

Instead of clinical parameters, biochemical biomarkers might serve as better predictive markers of outcome. Biomarkers are defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" (40). Sensitive and specific biomarkers, preferably taken early, for instance plasma from the umbilical cord, could play a major role in the development of patient specific treatment algorithms. Although biomarkers are not routinely used, many have been tested in CDH animal models and some have been evaluated for its role in patients with CDH. An increased level of plasma vascular endothelial growth factor A (VEGFA), which is associated with embryonic vascular development amongst others, and a decreased level of placental growth factor, have been found to predict clinical severity of pulmonary vascular disease and mortality in CDH patients (41). The soluble receptor for advanced glycation end products (sRAGE) is a known marker for endothelial function, and Kipfmueller et al found it to be an early biomarker for the need of extracorporeal membrane oxygenation (ECMO) in CDH cases (42). Cytokines, involved in the systemic inflammatory response, have been proven to be elevated in patients with CDH, some already in utero, and its increase is directly related to disease severity (43, 44). Herrera-Rivero et al. evaluated the use of microRNA as biomarkers and found a dysregulation of microRNA participating in the transforming growth factor beta (TGF-β) signaling pathway in patients who developed CLD or died. This pathway plays an important role in lung development, especially in alveolarisation and tissue homeostasis. Also microRNA involved in the semaphoring signaling, important for development and regulation of immune responses, was dysregulated in this group of patients (45).

However, these potential biomarkers were only tested in small groups of patients in single centers and causality is hard to prove. Some biomarkers have been tested within a large multicenter trial of patients with CDH, the VICI trial, with less success (15). High-sensitivity troponin T (HsTnT) and N-terminal pro-brain natriuretic peptide (NT-proBNP) did not predict morbidity or mortality, although both have been proven to have a predictive value in cardiovascular diseases (46). Other presumed biomarkers such as tracheal sphingolipids, mediators involved in lung development, injury and repair, did not predict chronic lung disease or death in this population either (47).

TREATMENT

The CDH EURO Consortium is a well-established consortium of expertise centers in and outside Europe, that developed and revised standardized neonatal treatment guidelines based on clinical evidence and expert opinion (48, 49). They also collaborated in multicenter research such as the VICI trial (15). Since 2008, all patients with CDH, born in a center of the CDH EURO Consortium, are treated according to these guidelines. In 2018, the Canadian Congenital Diaphragmatic Hernia Collaborative developed a guideline for CDH care with similar conclusions, and similar low level of evidence (50).

Most children with CDH develop severe cardiorespiratory distress immediately after birth (51-53). As stated in the consensus guidelines, the key principle during the first days of life is avoiding high airway pressures whilst establishing adequate oxygenation and cardiovascular stability (48). Consequently, all infants are routinely intubated immediately after birth, followed by gentle ventilation to prevent ventilator induced lung injury (VILI). PH is treated with oxygen, sedation, blood pressure support and, if needed, with inhalational or systemic vasodilator agents (48, 50, 54, 55). Only after pulmonary and cardiovascular stabilisation, surgical repair of the diaphragmatic defect is performed. PH, severe lung hypoplasia and VILI are the most important risk factors for poor outcome (51, 56, 57). Therefore, it might be safer to apply an individualized and conservative approach, allowing the minority of newborns with good prenatal predictive parameters to breath spontaneously at birth, as prenatal parameters can predict the severity of lung hypoplasia, and VILI is associated with worse outcome in CDH patients.

The physiologic pulmonary vascular transition of the neonate after birth takes time, sometimes even weeks, to achieve normal values of pulmonary arterial pressure. In children with CDH the pulmonary vascular resistance often does not drop adequately due to altered development of the pulmonary vasculature and a reduced vascular bed. The pulmonary vasculature in CDH patients is characterized by increased medial and adventitial wall thickness, but also an increase in vasoconstriction and vascular reactivity (58). Three main pathways are known to influence the vascular reactivity and are in principle accessible for pharmacological therapy: the endothelin pathway, and the prostacyclin pathway, and the nitric oxide-cGMP pathway [12].

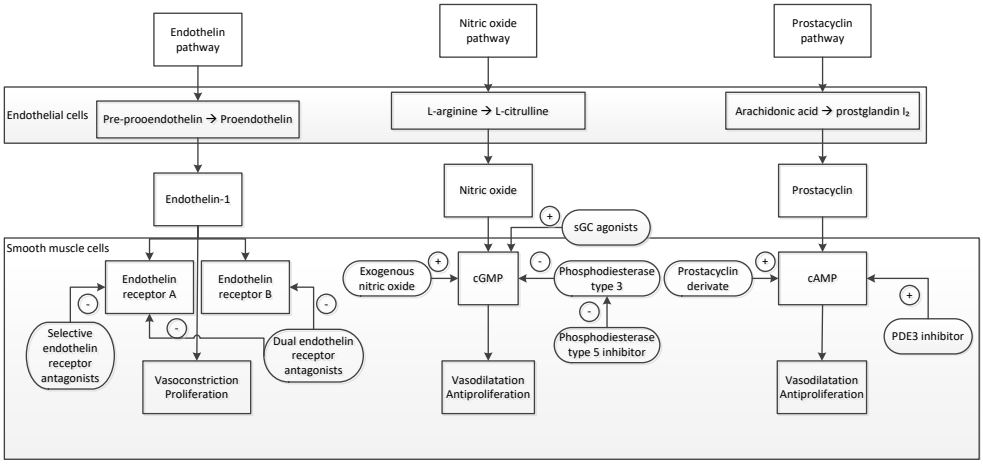


Figure 2. Three major pathways influencing pulmonary hypertension (59)

Targeted pharmacological therapy includes three classes of drugs based on these pathways. Drugs influencing the endothelin pathway are the endothelin receptor antagonists bosentan, ambrisentan and macitentan. These drugs only have an oral dosage form, because of their inability to be dissolved, and therefor are not suitable for the treatment of PH in newborns with CDH at birth. Pharmacotherapy influencing the prostacyclin pathway, such as prostacyclin derivates iloprost and treprostenil, can be given intravenously or via inhalation. Major disadvantage is their very short half-life with risk of rebound PH. Data are very limited but no randomized controlled trials (RCT) in infants with PH have shown superiority of these drugs, mainly compared to inhaled nitric oxide (iNO). Only retrospective data on small groups of CDH patients are available (59, 60). Currently, the selective phosphodiesterase type 3 (PDE3) inhibitor milrinone is investigated for its role in the treatment of PH in CDH patients (NCT02951130).

iNO and sildenafil both influence the nitric oxide-cGMP pathway. After inhalation, iNO diffuses rapidly across the alveolar-capillary membrane into the smooth muscle of pulmonary vessels and activates soluble guanylate cyclase. This enzyme mediates many of the biological effects of iNO and is responsible for the conversion of GTP to cGMP. The increase of intracellular concentrations of cGMP relaxes the smooth muscle via several mechanisms. iNO also causes bronchodilation, and has some anti-inflammatory and anti-proliferative properties (61). In patients with persistent pulmonary hypertension of the newborn (PPHN) iNO decreases the median duration of mechanical ventilation and reduces the need for ECMO. However, in the one available RCT in patients with CDH outcome did not improve, but was even slightly worse (62). Even though the positive pharmacodynamic effects in infants with CDH are much weaker than in infants with PPHN, in many centers iNO is standard of care in infants with CDH and PH (49, 63).

Sildenafil citrate is a selective phosphodiesterase type 5 (PDE5) inhibitor. PDE5 is an enzyme that specifically degrades cGMP. With the inhibitory effect of sildenafil on PDE5, it increases cGMP and enhances NO-mediated vasodilatation of the smooth muscles in vessels in and outside the lungs. Only 5 RCTs have been performed in a total of 166 newborns, all with PPHN. These studies showed a decrease in oxygenation index (OI) and mortality when sildenafil was compared to placebo, however, when compared to another drug or when added to another drug, there was no significant reduction in mortality (64). Despite the lack of extensive research in large groups of infants, the use of sildenafil in the neonatal intensive care has increased substantially over the last decade (65). In CDH patients only retrospective data are available. A decrease in pulmonary vascular resistance index and an increase in cardiac output was found in a small group of oral sildenafil-treated infants with CDH refractory to iNO (66). Intravenous sildenafil in CDH patients was associated with improved OI and the right-to-left shunt ratio over the PDA was reversed. However, a significant increase in vasopressor support was also seen (67, 68). This raises the question whether sildenafil is a better first line drug for the treatment for PH in CDH patients then iNO.

When a CDH patient with PH is treated with oxygen, sedation, blood pressure support and vasodilator agents without adequate effect, ECMO can be considered. However, the benefit of ECMO treatment in CDH patients remains debated, as randomized controlled data in the era of standardized care are lacking (69, 70). However, it will only be beneficial in patients with reversibility of respiratory failure and PH. At this time, there is no prognostic tool to predict reversibility. The best timing of surgery on ECMO, early versus late versus after ECMO decannulation, is also controversial. Observational trials showed contrasting results (71). However, Dao et al. showed in a large cohort study that early repair, with a median time to repair of 2 days, is associated with improved survival, mainly due to a decrease of non-repaired patients (72).

OUTCOME

Because survival in patients with CDH has improved substantially over time, morbidity and long-term follow-up has become a more important topic (73). Morbidity is influenced by the severity of lung hypoplasia and PH, but also by medical treatment and its potential iatrogenic sequelae. Although the relative contribution to iatrogenic damage is hard to quantify, infants with CDH are admitted to the intensive care and are subject to a multitude of invasive therapies. They are at risk for the development of chronic lung disease, chronic PH, but also gastroesophageal reflux disease, poor growth, recurrent infections and neurodevelopmental and neuropsychological sequelae (74). This underlines the important balance between the benefits of an invasive treatment and its burden.

CONCLUSION

Although mortality in patients with CDH has decreased significantly over time, it is still substantial. At the cost of increased survival multi organ morbidity is diagnosed increasingly, partly due to a more standardized long-term follow-up program that is in practice in many institutions nowadays (73). Better prenatal and postnatal prediction of outcome could help in developing a more individualized treatment plan, preventing under- and overtreatment and thus further improve outcome. Treatment options could then be tested in a specific subgroup of CDH patients. Right now, many treatment strategies in CDH patients are based on expert opinion, and iNO therapy as the first line treatment for PH in CDH patients might not be appropriate. In the search for a better therapeutic option the use of intravenous sildenafil might be promising.

AIMS AND OUTLINE OF THIS THESIS

Part I consists of the introduction of the disease CDH and explains the aim of this thesis to identify pre- and postnatal parameters and biochemical biomarkers to predict disease severity in CDH patients, and to evaluate different treatment modalities.

Part II focuses on the prediction of mortality and morbidity using pre- and postnatal parameters as well as biomarkers.

Chapter 2 describes the strength and weaknesses of preoperative chest radiographic thoracic area (CRTA) as a prediction tool, and evaluates its role compared to other prediction tools in the CDH population.

In **chapter 3** the CDHSG prediction rule is validated in the European population and additional prenatal predictive parameters are evaluated to further improve the model.

The novel biomarkers SIGLEC-14, BCAM and ANGPTL3, predictive for bronchopulmonary dysplasia in preterm infants, are tested in CDH patients in **chapter 4.**

Part III describes different treatment modalities and the possible individualization of treatment in CDH patients in the postnatal period.

In **chapter 5** a spontaneous breathing approach at birth in infants with good prenatal parameters is evaluated.

Chapter 6 describes the pharmacokinetic modelling of intravenous sildenafil in newborns with CDH.

The CoDiNOS trial protocol, an international randomized controlled trial comparing intravenous sildenafil with iNO for the treatment of PH in CDH patients, is reported in **chapter 7**.

In **part IV** the results of the studies are discussed and placed in a broader perspective in **chapter 8.** In **chapter 9** all results are summarized in English and Dutch.

LITERATURE

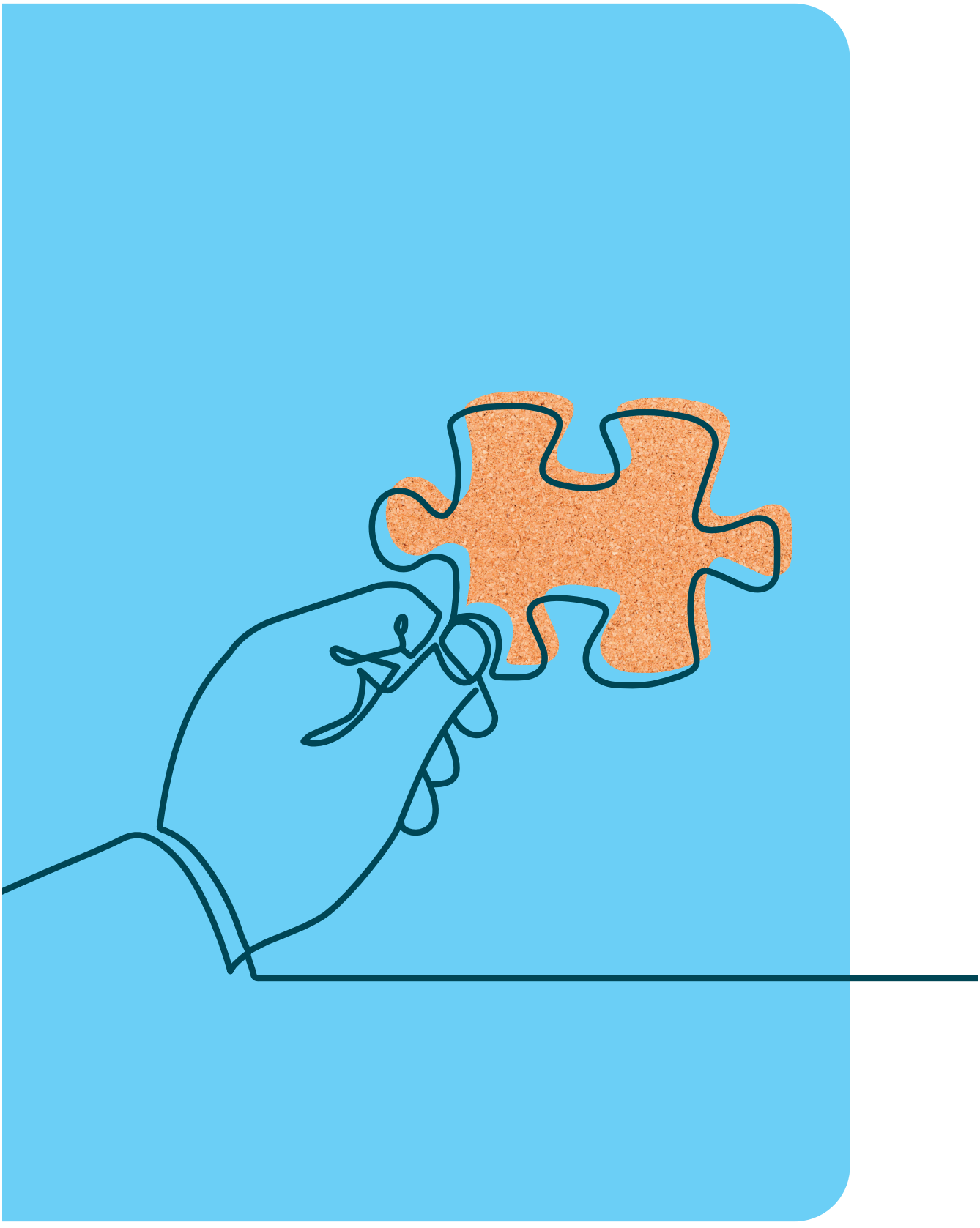
1. McGivern MR, Best KE, Rankin J, Wellesley D, Greenlees R, Addor MC, et al. Epidemiology of congenital diaphragmatic hernia in Europe: a register-based study. Arch Dis Child Fetal Neonatal Ed. 2015;100(2):F137-44.
2. Paré A. Les ouvres. Buon, Paris: 1579.
3. Macaulay G. An account of viscera herniation. Philos Trans Roy Coll Phys 1754;6:25-35.
4. Morgagni. Seats and causes of disease investigated by anatomy. London: 1769. Report No.
5. Bochdalek V. Einige Betrachtungen über die Entstehung des angeborenen Zwerchfellbruches. Als Beitrag zur pathologischen Anatomie der Hernien. Wochenschr Prakt Heilk. 1848;18:89-94.
6. Al-Salem AH. Congenital hernia of Morgagni in infants and children. J Pediatr Surg. 2007;42(9):1539-43.
7. Arora S, Haji A, Ng P. Adult Morgagni hernia: the need for clinical awareness, early diagnosis and prompt surgical intervention. Ann R Coll Surg Engl. 2008;90(8):694-5.
8. Keijzer R, Liu J, Deimling J, Tibboel D, Post M. Dual-hit hypothesis explains pulmonary hypoplasia in the nitrofen model of congenital diaphragmatic hernia. Am J Pathol. 2000;156(4):1299-306.
9. Burgos CM, Frenckner B, Luco M, Harting MT, Lally PA, Lally KP, et al. Right versus left congenital diaphragmatic hernia - What's the difference? J Pediatr Surg. 2017.
10. Sluiter I. Functional and structural studies of the pulmonary vasculature in congenital diaphragmatic hernia. Rotterdam: Erasmus Medical Center; 2012.
11. Golombek SG. The history of congenital diaphragmatic hernia from 1850s to the present. J Perinatol. 2002;22(3):242-6.
12. Abduljalil K, Jamei M, Rostami-Hodjegan A, Johnson TN. Changes in individual drug-independent system parameters during virtual paediatric pharmacokinetic trials: introducing time-varying physiology into a paediatric PBPK model. AAPS J. 2014;16(3):568-76.
13. Cartlidge PH, Mann NP, Kapila L. Preoperative stabilisation in congenital diaphragmatic hernia. Arch Dis Child. 1986;61(12):1226-8.
14. Tibboel D, Bos AP, Pattenier JW, Hazebroek FW, Madern GC, Molenaar JC. Pre-operative stabilisation with delayed repair in congenital diaphragmatic hernia. Z Kinderchir. 1989;44(3):139-43.
15. Snoek KG, Capolupo I, van Rosmalen J, Hout Lde J, Vijfhuize S, Greenough A, et al. Conventional Mechanical Ventilation Versus High-frequency Oscillatory Ventilation for Congenital Diaphragmatic Hernia: A Randomized Clinical Trial (The VICI-trial). Ann Surg. 2016;263(5):867-74.
16. Montalva L, Lauriti G, Zani A. Congenital heart disease associated with congenital diaphragmatic hernia: A systematic review on incidence, prenatal diagnosis, management, and outcome. J Pediatr Surg. 2019.
17. Stoll C, Tenconi R, Clementi M. Detection of Congenital Anomalies by Fetal Ultrasonographic Examination across Europe. Community Genet. 2001;4(4):225-32.
18. Rydberg C, Tunon K. Detection of fetal abnormalities by second-trimester ultrasound screening in a non-selected population. Acta Obstet Gynecol Scand. 2017;96(2):176-82.

19. Ba'ath ME, Jesudason EC, Losty PD. How useful is the lung-to-head ratio in predicting outcome in the fetus with congenital diaphragmatic hernia? A systematic review and meta-analysis. Ultrasound Obstet Gynecol. 2007;30(6):897-906.
20. Snoek KG, Peters NCJ, van Rosmalen J, van Heijst AFJ, Eggink AJ, Sikkel E, et al. The validity of the observed-to-expected lung-to-head ratio in congenital diaphragmatic hernia in an era of standardized neonatal treatment; a multicenter study. Prenat Diagn. 2017.
21. Kim AG, Norwitz G, Karmakar M, Ladino-Torres M, Berman DR, Kreutzman J, et al. Discordant prenatal ultrasound and fetal MRI in CDH: wherein lies the truth? J Pediatr Surg. 2019.
22. Dutemeyer V, Cordier AG, Cannie MM, Bevilacqua E, Huynh V, Houfflin-Debarge V, et al. Prenatal prediction of postnatal survival in fetuses with congenital diaphragmatic hernia using MRI: lung volume measurement, signal intensity ratio, and effect of experience. J Matern Fetal Neonatal Med. 2020:1-9.
23. Hedrick HL, Danzer E, Merchant A, Bebbington MW, Zhao H, Flake AW, et al. Liver position and lung-to-head ratio for prediction of extracorporeal membrane oxygenation and survival in isolated left congenital diaphragmatic hernia. Am J Obstet Gynecol. 2007;197(4):422 e1-4.
24. Oluyomi-Obi T, Kuret V, Puligandla P, Lodha A, Lee-Robertson H, Lee K, et al. Antenatal predictors of outcome in prenatally diagnosed congenital diaphragmatic hernia (CDH). J Pediatr Surg. 2017;52(5):881-8.
25. Basta AM, Lusk LA, Keller RL, Filly RA. Fetal Stomach Position Predicts Neonatal Outcomes in Isolated Left-Sided Congenital Diaphragmatic Hernia. Fetal Diagn Ther. 2016;39(4):248-55.
26. Cordier AG, Jani JC, Cannie MM, Rodo C, Fabietti I, Persico N, et al. Stomach position in prediction of survival in left-sided congenital diaphragmatic hernia with or without fetoscopic endoluminal tracheal occlusion. Ultrasound Obstet Gynecol. 2015;46(2):155-61.
27. Russo FM, Eastwood MP, Keijzer R, Al-Maary J, Toelen J, Van Mieghem T, et al. Lung size and liver herniation predict need for extracorporeal membrane oxygenation but not pulmonary hypertension in isolated congenital diaphragmatic hernia: systematic review and meta-analysis. Ultrasound Obstet Gynecol. 2017;49(6):704-13.
28. Wong M, Reyes J, Lapidus-Krol E, Chiang M, Humpl T, Al-Faraj M, et al. Pulmonary hypertension in congenital diaphragmatic hernia patients: Prognostic markers and long-term outcomes. J Pediatr Surg. 2018;53(5):918-24.
29. Longoni M, High FA, Qi H, Joy MP, Hila R, Coletti CM, et al. Genome-wide enrichment of damaging de novo variants in patients with isolated and complex congenital diaphragmatic hernia. Hum Genet. 2017;136(6):679-91.
30. Skari H, Bjornland K, Haugen G, Egeland T, Emblem R. Congenital diaphragmatic hernia: a meta-analysis of mortality factors. J Pediatr Surg. 2000;35(8):1187-97.
31. Burgos CM, Modee A, Ost E, Frenckner B. Addressing the causes of late mortality in infants with congenital diaphragmatic hernia. J Pediatr Surg. 2017;52(4):526-9.
32. Snoek KG, Capolupo I, Morini F, van Rosmalen J, Greenough A, van Heijst A, et al. Score for Neonatal Acute Physiology-II Predicts Outcome in Congenital Diaphragmatic Hernia Patients. Pediatr Crit Care Med. 2016;17(6):540-6.

33. Bruns AS, Lau PE, Dhillon GS, Hagan J, Kailin JA, Mallory GB, et al. Predictive value of oxygenation index for outcomes in left-sided congenital diaphragmatic hernia. J Pediatr Surg. 2018.
34. Congenital Diaphragmatic Hernia Study G. Estimating disease severity of congenital diaphragmatic hernia in the first 5 minutes of life. J Pediatr Surg. 2001;36(1):141-5.
35. Schultz CM, DiGeronimo RJ, Yoder BA, Congenital Diaphragmatic Hernia Study G. Congenital diaphragmatic hernia: a simplified postnatal predictor of outcome. J Pediatr Surg. 2007;42(3):510-6.
36. Baird R, MacNab YC, Skarsgard ED, Canadian Pediatric Surgery N. Mortality prediction in congenital diaphragmatic hernia. J Pediatr Surg. 2008;43(5):783-7.
37. Brindle ME, Cook EF, Tibboel D, Lally PA, Lally KP, Congenital Diaphragmatic Hernia Study G. A clinical prediction rule for the severity of congenital diaphragmatic hernias in newborns. Pediatrics. 2014;134(2):e413-9.
38. Akinkuotu AC, Cruz SM, Abbas PI, Lee TC, Welty SE, Olutoye OO, et al. Risk-stratification of severity for infants with CDH: Prenatal versus postnatal predictors of outcome. J Pediatr Surg. 2016;51(1):44-8.
39. Oh C, Youn JK, Han JW, Yang HB, Lee S, Seo JM, et al. Predicting Survival of Congenital Diaphragmatic Hernia on the First Day of Life. World J Surg. 2018.
40. Biomarkers Definitions Working G. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther. 2001;69(3):89-95.
41. Patel N, Moenkemeyer F, Germano S, Cheung MM. Plasma vascular endothelial growth factor A and placental growth factor: novel biomarkers of pulmonary hypertension in congenital diaphragmatic hernia. Am J Physiol Lung Cell Mol Physiol. 2015;308(4):L378-83.
42. Kipfmueller F, Heindel K, Geipel A, Berg C, Bartmann P, Reutter H, et al. Expression of soluble receptor for advanced glycation end products (sRAGE) is associated with disease severity in congenital diaphragmatic hernia. Am J Physiol Lung Cell Mol Physiol. 2019.
43. Schaible T, Reineke J, Gortner L, Monz D, Schaffelder R, Tutdibi E. Are Cytokines Useful Biomarkers to Determine Disease Severity in Neonates with Congenital Diaphragmatic Hernia? Am J Perinatol. 2017;34(7):648-54.
44. Schaible T, Veit M, Tautz J, Kehl S, Busing K, Monz D, et al. Serum cytokine levels in neonates with congenital diaphragmatic hernia. Klin Padiatr. 2011;223(7):414-8.
45. Herrera-Rivero M, Zhang R, Heilmann-Heimbach S, Mueller A, Bagci S, Dresbach T, et al. Circulating microRNAs are associated with Pulmonary Hypertension and Development of Chronic Lung Disease in Congenital Diaphragmatic Hernia. Sci Rep. 2018;8(1):10735.
46. Snoek KG, Kraemer US, Ten Kate CA, Greenough A, van Heijst A, Capolupo I, et al. High-Sensitivity Troponin T and N-Terminal Pro-Brain Natriuretic Peptide in Prediction of Outcome in Congenital Diaphragmatic Hernia: Results from a Multicenter, Randomized Controlled Trial. J Pediatr. 2016;173:245-9 e4.
47. Snoek KG, Reiss IK, Tibboel J, van Rosmalen J, Capolupo I, van Heijst A, et al. Sphingolipids in Congenital Diaphragmatic Hernia; Results from an International Multicenter Study. PLoS One. 2016;11(5):e0155136.

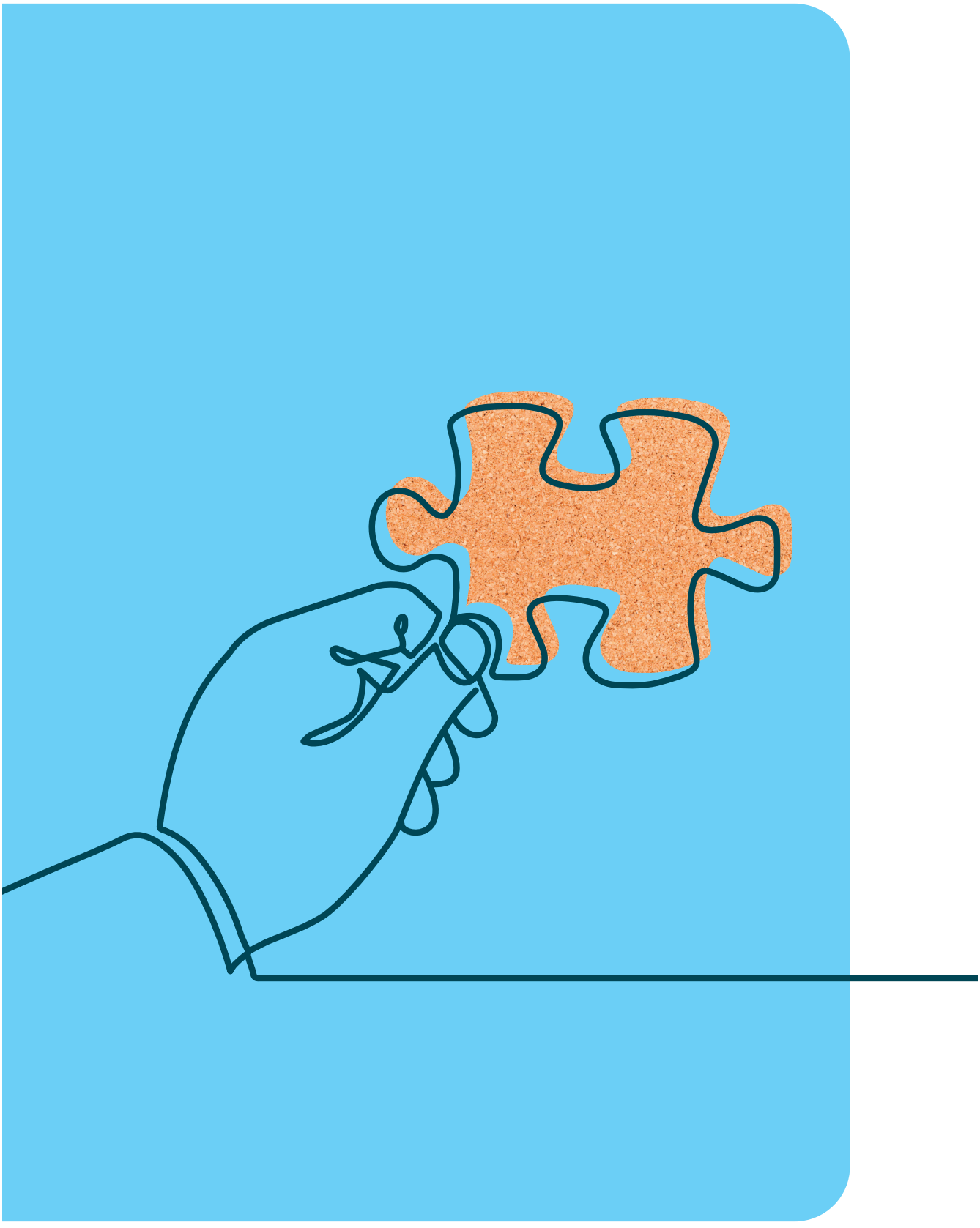
48. Snoek KG, Reiss IK, Greenough A, Capolupo I, Urlesberger B, Wessel L, et al. Standardized Postnatal Management of Infants with Congenital Diaphragmatic Hernia in Europe: The CDH EURO Consortium Consensus - 2015 Update. Neonatology. 2016;110(1):66-74.
49. Reiss I, Schaible T, van den Hout L, Capolupo I, Allegaert K, van Heijst A, et al. Standardized postnatal management of infants with congenital diaphragmatic hernia in Europe: the CDH EURO Consortium consensus. Neonatology. 2010;98(4):354-64.
50. Canadian Congenital Diaphragmatic Hernia C. Diagnosis and management of congenital diaphragmatic hernia: a clinical practice guideline. CMAJ. 2018;190(4):E103-E12.
51. Robinson PD, Fitzgerald DA. Congenital diaphragmatic hernia. Paediatr Respir Rev. 2007;8(4):323-34; quiz 34-5.
52. Frenckner B, Ehren H, Granholm T, Linden V, Palmer K. Improved results in patients who have congenital diaphragmatic hernia using preoperative stabilization, extracorporeal membrane oxygenation, and delayed surgery. J Pediatr Surg. 1997;32(8):1185-9.
53. Kays DW, Langham MR, Jr., Ledbetter DJ, Talbert JL. Detrimental effects of standard medical therapy in congenital diaphragmatic hernia. Ann Surg. 1999;230(3):340-8; discussion 8-51.
54. Storme L, Boubnova J, Mur S, Pognon L, Sharma D, Aubry E, et al. Review shows that implementing a nationwide protocol for congenital diaphragmatic hernia was a key factor in reducing mortality and morbidity. Acta Paediatr. 2017.
55. Jancelewicz T, Brindle ME, Guner YS, Lally PA, Lally KP, Harting MT, et al. Toward Standardized Management of Congenital Diaphragmatic Hernia: An Analysis of Practice Guidelines. J Surg Res. 2019;243:229-35.
56. Logan JW, Cotten CM, Goldberg RN, Clark RH. Mechanical ventilation strategies in the management of congenital diaphragmatic hernia. Semin Pediatr Surg. 2007;16(2):115-25.
57. Lally KP. Congenital diaphragmatic hernia. Curr Opin Pediatr. 2002;14(4):486-90.
58. Montalva L, Antounians L, Zani A. Pulmonary hypertension secondary to congenital diaphragmatic hernia: factors and pathways involved in pulmonary vascular remodeling. Pediatr Res. 2019.
59. Kraemer U, Cochius-den Otter S, Snoek KG, Tibboel D. Pharmacodynamic considerations in the treatment of pulmonary hypertension in infants: challenges and future perspectives. Expert Opin Drug Metab Toxicol. 2016;12(1):1-19.
60. Carpentier E, Mur S, Aubry E, Pognon L, Rakza T, Flamein F, et al. Safety and tolerability of subcutaneous treprostinil in newborns with congenital diaphragmatic hernia and life-threatening pulmonary hypertension. J Pediatr Surg. 2017;52(9):1480-3.
61. Ichinose F, Roberts JD, Jr., Zapol WM. Inhaled nitric oxide: a selective pulmonary vasodilator: current uses and therapeutic potential. Circulation. 2004;109(25):3106-11.
62. Inhaled nitric oxide and hypoxic respiratory failure in infants with congenital diaphragmatic hernia. The Neonatal Inhaled Nitric Oxide Study Group (NINOS). Pediatrics. 1997;99(6):838-45.
63. Putnam LR, Tsao K, Morini F, Lally PA, Miller CC, Lally KP, et al. Evaluation of Variability in Inhaled Nitric Oxide Use and Pulmonary Hypertension in Patients With Congenital Diaphragmatic Hernia. JAMA Pediatr. 2016.

64. Kelly LE, Ohlsson A, Shah PS. Sildenafil for pulmonary hypertension in neonates. Cochrane Database Syst Rev. 2017;8:CD005494.
65. Thompson EJ, Perez K, Hornik CP, Smith PB, Clark RH, Laughon M, et al. Sildenafil Exposure in the Neonatal Intensive Care Unit. Am J Perinatol. 2019;36(3):262-7.
66. Noori S, Friedlich P, Wong P, Garingo A, Seri I. Cardiovascular effects of sildenafil in neonates and infants with congenital diaphragmatic hernia and pulmonary hypertension. Neonatology. 2007;91(2):92-100.
67. Bialkowski A, Moenkemeyer F, Patel N. Intravenous sildenafil in the management of pulmonary hypertension associated with congenital diaphragmatic hernia. Eur J Pediatr Surg. 2015;25(2):171-6.
68. Kipfmueller F, Schroeder L, Berg C, Heindel K, Bartmann P, Mueller A. Continuous intravenous sildenafil as an early treatment in neonates with congenital diaphragmatic hernia. Pediatr Pulmonol. 2018.
69. Snoek KG, Greenough A, van Rosmalen J, Capolupo I, Schaible T, Ali K, et al. Congenital Diaphragmatic Hernia: 10-Year Evaluation of Survival, Extracorporeal Membrane Oxygenation, and Foetoscopic Endotracheal Occlusion in Four High-Volume Centres. Neonatology. 2018;113(1):63-8.
70. Rafat N, Schaible T. Extracorporeal Membrane Oxygenation in Congenital Diaphragmatic Hernia. Front Pediatr. 2019;7:336.
71. McHoney M, Hammond P. Role of ECMO in congenital diaphragmatic hernia. Arch Dis Child Fetal Neonatal Ed. 2018;103(2):F178-F81.
72. Dao DT, Burgos CM, Harting MT, Lally KP, Lally PA, Nguyen HT, et al. Surgical Repair of Congenital Diaphragmatic Hernia After Extracorporeal Membrane Oxygenation Cannulation: Early Repair Improves Survival. Ann Surg. 2019.
73. H IJ, Breatnach C, Hoskote A, Greenough A, Patel N, Capolupo I, et al. Defining outcomes following congenital diaphragmatic hernia using standardised clinical assessment and management plan (SCAMP) methodology within the CDH EURO consortium. Pediatr Res. 2018;84(2):181-9.
74. Kraemer US, Leeuwen L, Krasemann TB, Wijnen RMH, Tibboel D, H IJ. Characteristics of Infants With Congenital Diaphragmatic Hernia Who Need Follow-Up of Pulmonary Hypertension. Pediatr Crit Care Med. 2018;19(5):e219-e26.



PART II

Prediction



CHAPTER 2

Light at the horizon?: Predicting mortality in infants with congenital diaphragmatic hernia.

Suzan C.M. Cochius - den Otter, Dick Tibboel

Pediatr Crit Care Med. 2019;20(6):575-7

Congenital diaphragmatic hernia (CDH) is a developmental defect of the diaphragm and the lungs, resulting in pulmonary hypoplasia and abnormal pulmonary vasculature growth, causing pulmonary hypertension (PH) (1). CDH occurs in approximately one in 2500 live births and is associated with a reported mortality of approximately 27% in live-born patients (2). PH, severe lung hypoplasia and ventilator-induced lung injury are the most important risk factors for poor outcome in children with CDH (1). Prenatally, outcome prediction can guide parents and caretakers in decisions regarding termination of the pregnancy, the use of prenatal interventions such a temporary tracheal plugging (FETO), but also the use of specific postnatal therapy and the referral to high-volume centers for the delivery. Postnatally, an adequate early predictor can help parents to better understand the course of their child illness. Also, it can be used for severity based treatment, and standardized reporting and benchmarking between centers .

In this issue of *Pediatric Intensive Care Medicine*, Dassios et al (3) show elegantly that measurement of the preoperative chest radiographic thoracic area (CRTA) in CDH infants can help to predict mortality. The authors suggest that CRTA is an easy tool, with a low inter- and intra-observer variation. It has a significant correlation with functional residual capacity in CDH patients, revealing the presence or absence of lung hypoplasia . In this single-centre retrospective cohort study, chest x-rays of 84 infants were used to calculate the CRTA. Dassios et al. found that CRTA is a better predictor of survival then the prenatally measured lung-to-head ratio (LHR). Interestingly, they did not compare CRTA with observed-to-expected (O/E) LHR, although O/E LHR has been proven to be more reliable as a prenatal predictor than LHR alone, because it is a more stable variable during pregnancy (4). Also, it would be interesting to test its role in predicting the need for extracorporeal membrane oxygenation (ECMO), although in this centre no ECMO treatment was offered. CRTA is a predictor of lung hypoplasia, but does not take PH into account, the other important risk factor for mortality. To be able to truly value the CRTA, external validation will be needed, as this is a single centre cohort with a large percentage of infants treated with foetal endoscopic tracheal occlusion (FETO), potentially creating a selection bias.

Although its role is not completely clear yet, CRTA measurement adds to the large group of postnatal and prenatal tools to predict outcome in this vulnerable group of patients. Over the years, many have already looked for the "egg of Columbus", both for prenatal and postnatal measures. The size of the defect seen during surgery is a very reliable predictor for outcome, but not suitable as a marker due to the timing of surgery (5). As the CDH registry repeatedly showed, a significant number of CDH newborns with a so-called type 4 diaphragmatic defect, are never operated. Therefor an earlier predictor is needed. Many prenatal measures have been used as a prediction tool. The most reliable are the O/E LHR, MRI estimates of foetal lung volume (FLV) and position of the liver and stomach

(6). But these tools are not perfect either. For instance, the O/E LHR has an area under the curve (AUC) of only 0.77% for survival (4). Right now, different O/E LHR measurement techniques at different time points in gestation are being used between centers, and there is a learning curve in the examination of the O/E LHR (7). The longest diameter method overestimates the O/E LHR up to 34% and has a larger inter observer variability then the tracing method (8). Standardization of the measurement of prenatal variables such as O/E LHR, is essential. Measuring lung volumes on MRI seems promising (6). However, in many centers it is not possible to use MRI for this purpose, due to unavailability and costs. Also, the power of a prediction tool is depending on the presence of the information needed. Prenatal data are not always available, due to data transfer problems, differences in health care organization, long travel distances or other reasons.

A variety of postnatal tools have also been used to predict survival in CDH patients, such as APGAR score, SNAP II score, PaCO2 and oxygenation index. However, almost all are based on relatively small groups of patients, are difficult to apply or have not been validated (9-12).

Brindle et al, and the Congenital Diaphragmatic Hernia Study Group (CDHSG) have developed a simple early clinical prediction rule in a large cohort to identify low (<10%), intermediate (~20%), and high risk (~50%) of death in infants. This prediction model is based on birth weight, 5-minute Apgar score, severe PH on echocardiography, and the presence of cardiac and chromosomal anomalies (13). Validation of the prediction rule showed reasonable discrimination among these three groups, but an underestimation of mortality in the low risk group (13, 14).

Maybe the light at the horizon can be seen more clearly when incorporating prenatal and postnatal variables in one model. Prenatal variables seem to be able to adequately predict lung hypoplasia and the need for ECMO, but the prenatal assessment of O/E LHR or liver herniation as a marker for lung vascularization and postnatal PH seems less reliable (15). To evaluate the effect of PH on mortality, postnatal variables are still essential for the accurate prediction of outcome in these infants. However, it is not easy to develop such a tool. Oh et al. made a predictionmodel using polyhydramnion, gestational age at diagnosis, O/E LHR, best oxygenation index and tricuspid regurgitation, in a small group from a single center(16). They used tricuspid regurgitation on the first day of life as definition for PH. Unfortunately the reporting of PH on echocardiography is not standardized and different definitions are being used. In Europe, the presence of PH is often defined as pulmonary pressures higher than >2/3 of the systemic pressures instead of supra-systemic pulmonary pressures as often used in other centers (13, 17). Furthermore, the timing of the measurement is very different between centers (13, 14). Furthermore, when using a voluntary database, or a database based on coded diagnosis, the accuracy of the

data is difficult to interpret. More consistent measuring techniques and reporting would probably make these different variables more suitable for the use of outcome prediction. And last but not least, the treatment of these patients needs to be standardized for the accurate prediction of outcome. This will minimize variation in outcome due to treatment differences. Right now, the same treatment protocol is being used in most centers in Europe, initiated and guided by the CDH-EURO Consortium guidelines (17). Also, more recently in Canada, standardized guidelines have been developed (18).

CDH continues to be a birth defect with a high mortality and morbidity.The work of Dassios and collagues (3) is a next step in the identification of mortality risk at an early time after birth. Although many tools have been developed to predict mortality, none of them is perfect. With standardized measurement of prenatal and postnatal variables, incorporated in one model, prediction might become more accurate in the future.

LITERATURE

1. Robinson PD, Fitzgerald DA. Congenital diaphragmatic hernia. Paediatr Respir Rev. 2007;8(4):323-34; quiz 34-5.
2. Snoek KG, Capolupo I, van Rosmalen J, Hout LJ, Vijfhuize S, Greenough A, et al. Conventional Mechanical Ventilation Versus High-frequency Oscillatory Ventilation for Congenital Diaphragmatic Hernia: A Randomized Clinical Trial (The VICI-trial). Ann Surg. 2015.
3. Dassios T AK, Makin E, Bhat R, Krokidis M, Greenough A. Prediction of mortality in newborn infants with severe congenital diaphragmatic hernia using the chest radiographic thoracic area. Pediatr Crit Care Med. 2019.
4. Snoek KG, Peters NCJ, van Rosmalen J, van Heijst AFJ, Eggink AJ, Sikkel E, et al. The validity of the observed-to-expected lung-to-head ratio in congenital diaphragmatic hernia in an era of standardized neonatal treatment; a multicenter study. Prenat Diagn. 2017.
5. Congenital Diaphragmatic Hernia Study G, Lally KP, Lally PA, Lasky RE, Tibboel D, Jaksic T, et al. Defect size determines survival in infants with congenital diaphragmatic hernia. Pediatrics. 2007;120(3):e651-7.
6. Russo FM, Cordier AG, De Catte L, Saada J, Benachi A, Deprest J, et al. Proposal for standardized prenatal ultrasound assessment of the fetus with congenital diaphragmatic hernia by the European reference network on rare inherited and congenital anomalies (ERNICA). Prenat Diagn. 2018;38(9):629-37.
7. Cruz-Martinez R, Figueras F, Moreno-Alvarez O, Martinez JM, Gomez O, Hernandez-Andrade E, et al. Learning curve for lung area to head circumference ratio measurement in fetuses with congenital diaphragmatic hernia. Ultrasound Obstet Gynecol. 2010;36(1):32-6.
8. Jani JC, Peralta CF, Nicolaides KH. Lung-to-head ratio: a need to unify the technique. Ultrasound Obstet Gynecol. 2012;39(1):2-6.
9. Snoek KG, Capolupo I, Morini F, van Rosmalen J, Greenough A, van Heijst A, et al. Score for Neonatal Acute Physiology-II Predicts Outcome in Congenital Diaphragmatic Hernia Patients. Pediatr Crit Care Med. 2016;17(6):540-6.
10. Bruns AS, Lau PE, Dhillon GS, Hagan J, Kailin JA, Mallory GB, et al. Predictive value of oxygenation index for outcomes in left-sided congenital diaphragmatic hernia. J Pediatr Surg. 2018.
11. Congenital Diaphragmatic Hernia Study G. Estimating disease severity of congenital diaphragmatic hernia in the first 5 minutes of life. J Pediatr Surg. 2001;36(1):141-5.
12. Patel MJ, Bell CS, Lally KP, Lally PA, Katakam LI, Congenital Diaphragmatic Hernia Study G. Lowest PaCO2 on the first day of life predicts mortality and morbidity among infants with congenital diaphragmatic hernia. J Perinatol. 2018.
13. Brindle ME, Cook EF, Tibboel D, Lally PA, Lally KP, Congenital Diaphragmatic Hernia Study G. A clinical prediction rule for the severity of congenital diaphragmatic hernias in newborns. Pediatrics. 2014;134(2):e413-9.
14. Bent DP, Nelson J, Kent DM, Jen HC. Population-Based Validation of a Clinical Prediction Model for Congenital Diaphragmatic Hernias. J Pediatr. 2018.

15. Russo FM, Eastwood MP, Keijzer R, Al-Maary J, Toelen J, Van Mieghem T, et al. Lung size and liver herniation predict need for extracorporeal membrane oxygenation but not pulmonary hypertension in isolated congenital diaphragmatic hernia: systematic review and meta-analysis. Ultrasound Obstet Gynecol. 2017;49(6):704-13.
16. Oh C, Youn JK, Han JW, Yang HB, Lee S, Seo JM, et al. Predicting Survival of Congenital Diaphragmatic Hernia on the First Day of Life. World J Surg. 2018.
17. Snoek KG, Reiss IK, Greenough A, Capolupo I, Urlesberger B, Wessel L, et al. Standardized Postnatal Management of Infants with Congenital Diaphragmatic Hernia in Europe: The CDH EURO Consortium Consensus - 2015 Update. Neonatology. 2016;110(1):66-74.
18. Canadian Congenital Diaphragmatic Hernia C. Diagnosis and management of congenital diaphragmatic hernia: a clinical practice guideline. CMAJ. 2018;190(4):E103-E12.

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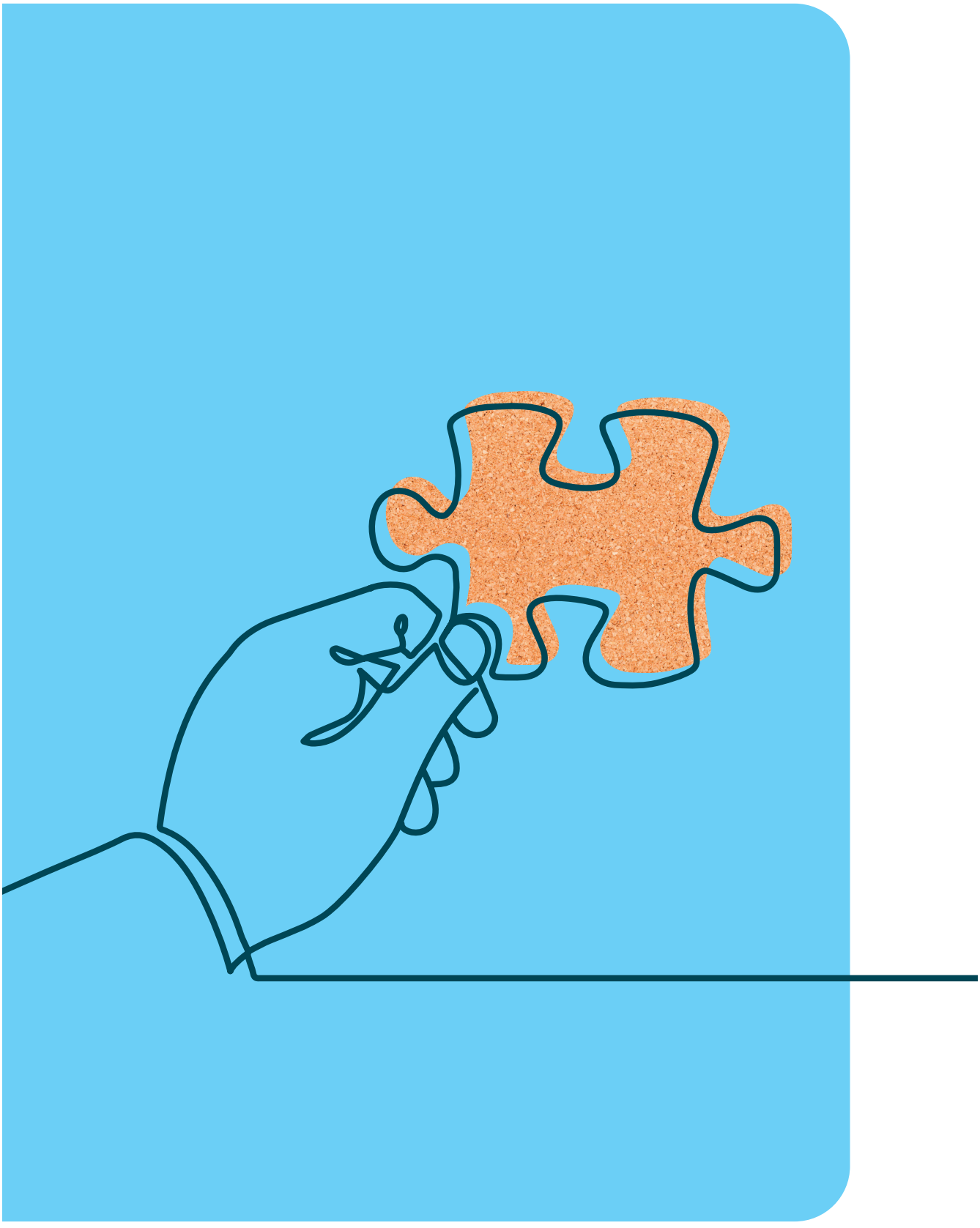

CHAPTER 3

Validation of a Prediction Rule for Mortality in Congenital Diaphragmatic Hernia

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Nina C.J. Peters, Titia E. Cohen – Overbeek,
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Pediatrics. 2020:145(4)

ABSTRACT

Background: Congenital diaphragmatic hernia (CDH) is a rare congenital anomaly with a mortality of approximately 27%. The Congenital Diaphragmatic Hernia Study Group (CDHSG) developed a simple postnatal clinical prediction rule to predict mortality in newborns with CDH. The aim of the study is to externally validate the CDHSG rule in the European population and to improve its prediction of mortality by adding prenatal variables.

Methods: We performed a European multicenter retrospective cohort study and included all newborns diagnosed with unilateral CDH, born between 2008 and 2015. Newborns born from November 2011 onwards were included for the external validation of the rule (n=343). To improve the prediction rule, we included all prenatally diagnosed patients born between 2008 and 2015 (n=620) and collected pre- and postnatal variables. We build a logistic regression model and performed bootstrap resampling and computed calibration plots.

Results: With our validation dataset the CDHSG rule had an area under the curve (AUC) of 79.0% showing a fair predictive performance. For the new prediction rule prenatal herniation of the liver was added and absent 5 minute Apgar score was taken out. The new prediction rule showed good calibration and with an AUC of 84.6%, it had good discriminative abilities.

Conclusion: In this study, we externally validated the CDHSG rule for the European population, which showed fair predictive performance. The modified rule, with prenatal liver herniation as an additional variable, appears to further improve the model's ability to predict mortality in a population of patients with prenatally diagnosed CDH.

INTRODUCTION

Congenital diaphragmatic hernia (CDH) is a severe developmental defect of the diaphragm causing lung hypoplasia and pulmonary hypertension (PH), leading to a mortality of 27% in live-born patients (1). Identification of risk factors that prognosticate outcome in patients with CDH is essential to accurately counsel parents and to compare patient populations and management strategies.

Prenatally, outcomes are predicted using observed-to-expected lung-to-head ratio (O/E LHR), MRI calculations of lung volumes and position of the liver and stomach (2-7). These prenatal parameters can be used to predict lung hypoplasia, but do not seem to reliably predict PH (8, 9).

For the postnatal prediction of survival, there are several prediction models and variables such as SNAP II score and oxygenation index. However, many are based on relatively small groups of patients, are difficult to apply or have not been externally validated (10-14).

Brindle et al, and the Congenital Diaphragmatic Hernia Study Group (CDHSG) have developed a simple early clinical prediction rule in a large cohort of patients to identify low (<10%), intermediate (~20%), and high risk (~50%) of death in the postnatal period. This prediction model is based on birth weight, 5-minute Apgar score, severe PH, and the presence of cardiac and chromosomal anomalies (15). Validation of the prediction rule showed reasonable discrimination between groups (15, 16).

This postnatal model has been favorably compared to prenatal predictors (17). However, there is potential value in combining post- and prenatal risk factors within a single prediction model. Prenatal and postnatal predictors have only been integrated in one prediction model in a small group of patients from a single center (18). The aim of our study was to externally validate the CDHSG clinical prediction rule in a European population and incorporate additional prenatal variables to further improve the rule.

PATIENTS AND METHODS

The data were collected from four high-volume CDH centers, treating ten or more patients with CDH per year (19). These centers are part of the CDH Euro Consortium; Erasmus University Medical Center, Rotterdam, Radboudumc Amalia Children's Hospital, Nijmegen, The Netherlands, University Hospital Mannheim, Mannheim, Germany and Bambino Gesu' Children's Hospital, Rome, Italy. The CDH Euro Consortium is a voluntary collaboration of European institutions, that works together in research. This collaborative group also

developed the CDH EURO Consortium management guidelines that are implemented in all participating centers (1, 20). Institutional review board approval was obtained from the Medical Ethics Committee Erasmus MC in Rotterdam (MEC2016-109).

For the *external validation* of the CDHSG prediction rule, patients born before November 2011 were excluded, because these patients were included in the CDHSG database and used for the development of the original CDHSG prediction rule (15). We included all live-born infants with CDH, born between November 2011 and 2015. We reviewed the data of these patients from the local CDHSG database and added missing data from the medical files if available. The collected data were in accordance with the definitions used by Brindle et al; low birth weight (<1500 gram), low Apgar score <7 at 5 minutes or the absence of an Apgar score, severe PH defined as right to left shunt or estimated supra-systemic pulmonary pressures on the first echocardiography, chromosomal anomalies, defined as any abnormalities in the chromosomal array, and major cardiac anomalies, classified as all anomalies other than patent foramen ovale, patent ductus arteriosus, atrial septum defect and ventricular septum defect (15).

The data of each patient were entered in the CDHSG prediction rule to calculate a total CDH risk score, ranging from 0 to 8 (table 1). This score was used to stratify the patients into one of the 3 risk groups; low (0), intermediate (1-2) and high risk (3-8).

For the *implementation of prenatal variables in the CDHSG prediction rule*, we included all live-born infants with prenatally diagnosed CDH, born between 2008 and 2015. The predictors in the CDHSG prediction rule were reviewed. Most of the variables were used as binary variables. However, to further improve the model, birth weight was also tested as a continuous variable and low Apgar score was defined as <5 at 5 minutes or <7 at 5 minutes. Missing Apgar score was left out, as one of the centers never calculates an Apgar score for CDH patients. Also, after discussion with an expert group, consisting of pediatric intensivists, neonatologists and prenatal specialists across participating centers, we decided that the variable chromosomal anomalies should always be in the model, because of its major significance in the decision to start and continue treatment.

Additionally, candidate pre- and postnatal predictors were selected by the expert group. The first measured O/E LHR after 18 weeks of gestation was included as a continuous variable. The presence of intrathoracic liver herniation on the last prenatal ultrasound was used as a binary variable. Also, the side of the hernia, fetal endotracheal occlusion (FETO), the presence of polyhydramnios (21), gestational age at diagnosis and gestational age at birth were selected.

STATISTICAL ANALYSES

To describe the baseline characteristics of the patients with CDH, medians and IQRs were used for continuous variables and percentages for categorical variables. Comparisons between baseline characteristics and death before discharge were made using the chi-square test for categorical variables and the Mann-Whitney test for continuous variables. Comparisons between centers were made using Kruskal-Wallis and chi-square tests.

For the external validation of the CDHSG prediction rule, multiple imputation was performed for missing data (table 2), creating 100 databases using fully conditional specification. Because the available data between centers were heterogeneous, we used "center" as a covariate in the multiple imputation. Then, the CDHSG prediction score was calculated for each individual using the final prediction rule as used by Brindle et al (15) as well as the original equation, which was used to develop the CDHSG prediction rule (table 1). The predictive performance was assessed using calibration plots and the c-statistic (i.e. the area under the receiver-operating-characteristic curve). Also, the predicted outcome of the final equation was compared with the observed outcome in the study cohort from the pooled database.

For the new model, predictors were tested using univariate analysis, assessing if a variable was associated with increased mortality. We corrected for center. The selected variables were put into a multivariable logistic regression model using the stepwise backward method. In every step the variable with the highest p-value was excluded if its p-value was >0.1, and this was repeated until all variables included in the model had $p<0.1$. The model was evaluated with a calibration plot, assessing the discriminatory abilities of the model, followed by bootstrapping to correct for the optimism of the model. We then calculated the predicted risk per patient and plotted the ROC curves to determine cut-off values of the predicted risk for 3 risk groups; low, intermediate and high risk. SPSS Statistics version 24 and R version 3.6.1 with the packages rms and mice were used for the statistical analyses.

RESULTS

753 patients were diagnosed with CDH between January first, 2008 and December 31st, 2015. Eight patients were excluded, because there were no patient characteristics available. Fourteen pregnancies resulted in an intra-uterine fetal demise. 343 patients were born between 2011 and 2015, and their data were used for the validation of the original prediction rule. 620 patients were included to develop the new rule. In 111 patients the

diagnosis was not prenatally known and therefore they were excluded for the new rule. This postnatally diagnosed group had a mortality of 9%.

Table 1 CDHSG prediction rule and the new model

Original CDHSG prediction equation	**1/(1 + exp(2.65** **- log(2.634)*(low birth weight)** **- log(2.718)*(low 5 min Apgar score <7)** **- log(4.678)*(missing 5 minute Apgar score)** **- log(4.073)*(severe PHT)** **- log(5.22)*(MCA)** **- log(3.928)*(chromosomal anomaly))**	**%**
		Value
Final CDHSG prediction rule (15)	Low birth weight (<1500 g)	1
	Low 5 minute Apgar score (<7)	1
	Missing 5 minute Apgar score	2
	Severe PHT	2
	MCA	2
	Chromosomal anomaly	1
	Total CDHSG score (sum values)	0-8
New prediction model with additional prenatal variable	1/(1 + exp(-0.6735 + 0.0013*(birth weight (g)) – 1.7150*(low 5 minute Apgar score <7) – 1.4871*(severe PHT) – 0.9471*(MCA) – 0.8754*(chromosomal anomaly) – 0.7235*(intrathoracic liver herniation on prenatal US))	**%**

PHT = pulmonary hypertension; MCA = major cardiac anomalies; US = ultrasound

Baseline characteristics of both patient groups are shown in table 2. In 70.3% of the patients in the cohort used for the validation, the first echocardiogram was performed within the first 24 hours of life. The overall mortality was 18%. In the group used for the new rule, 76.9% of the patients, had their first echocardiogram performed within the first 24 hours of life. Their overall mortality was 23%. In both groups, the baseline characteristics of the patients that survived were significantly different from those who died, except for sex (table 2). Also, these characteristics also differed significantly between centers the, as presented in table 3.

Table 2 Patient characteristics for the validation of the CDHSG rule and the new model

	Patients for validation (n=343)	No. missing data	Survivors (n=280)	Non-Survivors (n=63)	p-value	Patients new model (n=620)	No. missing data	Survivors (n= 478)	Non-Survivors (n=142)	p-value
Male	193 (56%)	0	156 (56%)	37 (59%)	0.663	366 (59%)	0	288 (60%)	78 (55%)	0.258
First measured O/E LHR	40.0 (IQR 33.4-49.5)	99 (29%)	41.5 (IQR 34.0-50.1)	37 (IQR 25.8-47.1)	<0.05	39.8 (IQR 32.3-49.5)	127 (20%)	41.3 (IQR 33.9-50.5)	34.9 (IQR 27.0-42.6)	<0.05
Intra-thoracic liver herniation	176 (51%)	44 (13%)	127 (45%)	49 (78%)	<0.05	335 (54%)	18 (3%)	234 (49%)	101 (71%)	<0.05
Prenatal surgery	25 (7%)	0	17 (6%)	8 (13%)	0.068	62 (10%)	0	34 (7%)	28 (20%)	<0.05
Gestational age (wk)	38.0 (IQR 37.0-38.6)	3 (<1%)	38.0 (IQR 37.1-38.6)	37.1 (IQR 35.6-38.6)	<0.05	38.0 (IQR 36.6-38.4)	4 (1%)	38.0 (IQR 37.0-38.6)	37.0 (IQR 35.0-38.8)	<0.05
Left sided defect	292 (85%)	0	242 (86%)	50 (79%)	0.135	536 (87%)	4 (1%)	428 (90%)	108 (76%)	<0.05
Apgar score at 5 min	8 (IQR 7-9)	58 (17%)	8 (IQR 7-9)	7 (IQR 6-8)	<0.05	8 (IQR 7-8)	131 (21%)	8 (IQR 7-9)	7 (IQR 6-8)	<0.05
Apgar score <7 at 5 min	56 (16%)	58 (17%)	34 (12%)	22 (35%)	<0.05	86 (14%)	131 (21%)	39 (8%)	47 (33%)	<0.05
Birth weight (kg)	3.00 (IQR 2.67-3.33)	1 (<1%)	3.00 (IQR 2.70-3.35)	2.80 (IQR 2.15-3.20)	<0.05	2.97 (IQR 2.56-3.25)	3 (<1%)	3.00 (IQR 2.69-3.30)	2.63 (IQR 2.04-3.00)	<0.05
Chromo-somal anomalies	19 (6%)	0	11 (4%)	8 (13%)	<0.05	23 (4%)	5 (1%)	13 (3%)	10 (7%)	<0.05
Pulmonary hyper-tension	79 (23%)	66 (19%)	47 (17%)	32 (51%)	<0.05	169 (27%)	123 (20%)	99 (21%)	70 (49%)	<0.05
ECMO treatment	110 (32%)	2 (<1%)	74 (26%)	36 (57%)	<0.05	190 (31%)	0	122 (26%)	68 (48%)	<0.05
Survival	280 (82%)	0				478 (77%)	0			

Patient characteristics specified for survivors and non-survivors. Patients used for the validation of the CDHSG rule on the left side and patients used for the new model on the right side. O/E LHR = observed to expected lung-to-head ratio; GA = gestational age; ECMO = extra-corporeal membrane oxygenation

Table 3 Patient characteristics specified per center for the new model (n=620)

Center	First measured O/E LHR (%)	Intra-thoracic liver hernia-tion on prenatal US	Prenatal surgery	Left sided hernia	5 minute Apgar score <7	Birth weight (kg)	Major cardiac anomalies	Chromo-somal Anoma-lies	Severe PHT	ECMO	Survival
Rotter-dam	43.2 (34.1-53.4)	32%	5%	86%	18%	3.0 (2.5-3.2)	4%	2%	16%	30%	72%
Nijmegen	42.3 (36.6-50.4)	40%	11%	84%	19%	2.9 (2.6-3.3)	2%	7%	58%	44%	66%
Mann-heim	37.8 (30.6-46.1)	68%	13%	88%	15%	2.9 (2.6-3.3)	5%	5%	21%	37%	83%
Rome	45.1 (35.6-58.6)	45%	4%	84%	0%*	3.0 (2.5-3.3)	8%	0%	45%	0%	70%
p-value	<0.01	<0.01	<0.01	0.835	0.151	0.963	0.382	0.087	<0.01	<0.01	<0.01

O/E LHR = observed to expected lung-to-head ratio; US = ultra sound; PHT = pulmonary hypertension;
ECMO = extra-corporeal membrane oxygenetion *Apgar score is never calculated in this center

The outcome of the CDHSG prediction rule after multiple imputations is shown in table 4. 46% of the patients was grouped in the low-risk group (score 0), with an observed mortality of 4%, and 38% was grouped in the intermediate group (score 1-2) with a mortality of 22%. The high-risk group (score 3-8) was smaller, containing 16% of the patients with a mortality of 66%. The discrimination of the model was moderately strong with a c-statistic of 0.784 for the original equation and 0.790 for the final CDHSG prediction rule.

Table 4 CDHSG prediction rule: predicted and observed mortality risk after multiple imputation

CDHSG score	0 (n=157.0)	1 (n=34.5)	2 (n=96.9)	3 (n=34.3)	4 (n=17.8)	5 (n=1.3)	6 (n=1.3)
Predicted mortality	6.6%	17.1%	24.1%	45.5%	60.1%	87.9%	87.5%
Observed mortality	4.0%	18.5%	22.9%	42.7%	63.6%	87.5%	100%

Subsequently, to develop a new rule, the original prediction rule was modified. First, logistic regression was performed within the large dataset using a backwards elimination algorithm. Missing data were imputed. O/E LHR, side of the hernia, gestational age at birth, FETO, polyhydramnios, Apgar score <5 at 5 minutes, and gestational age at diagnosis were excluded from the model with backward elimination. Although chromosomal anomalies had a p-value >0.1, we forced it into the model (table 5).

The new model contains birth weight as a continuous variable, and intrathoracic herniation of the liver, major cardiac anomalies, chromosomal anomalies, Apgar score <7 at 5 minutes and severe PH as binary variables (table 1). Evaluation of the model in a calibration plot showed good discrimination of the model with a c-statistic of 0.859. Correcting for the optimism of the model, estimated around 1.4%, the c-statistic is 0.846. Supplement figure 1 shows the ROC curve of the new model. We then stratified the patients into one of the 3 groups; low, intermediate and high risk of mortality. When using <10% (mild), 10-50% (moderate) and >50% (severe) risk of mortality as cut-off points, the cut-off between the mild group and the moderate group showed a sensitivity of 90.8% and a specificity of 55.4%, whereas the cut-off between the moderate and the severe group showed a sensitivity of 49.3% and a specificity of 93.5%.

Table 5 Odds ratios for mortality for variables in the new model

Variable	Adjusted OR	95% Confidence interval
Intercept	1.9611	0.5570 – 6.9048
Birth weight (gram)	0.9987	0.9983 – 0.9991
Intrathoracic liver herniation	2.0616	1.2300 – 3.4555
MCA	2.5781	0.9631 – 6.9020
Chromosomal anomalies	2.3998	0.8277 – 6.9579
Severe PHT	4.4242	2.6159 – 7.4826
Apgar score <7	5.5567	3.0719 – 10.0513

OR = odds ratio; MCA = major cardiac anomalies; PHT = pulmonary hypertension

The disease severity using the rules per center is presented in table 1 and 2 of the supplement.

DISCUSSION

In this study we externally validated the CDHSG rule in the European population. We found the rule had fair discrimination, but also room for optimization, comparable to the internal validation of Brindle et al (15). Bent et al also validated the rule in a large group of patients with CDH born in California, and found an underestimation of mortality in the patients with a score of 1 (16). We did not find this in our population. This might be explained by the difference in health care systems in Europe and the United States. In Europe, centralized care is more common and many patients with CDH are born in high volume centers. It is increasingly recognized that centralized care improves outcome in these patients (19). This might also explain the lowest mortality in patients born in the largest center of our

study. Furthermore, in Europe many CDH centers collaborate in the CDH Euro Consortium, which has developed a standardized treatment protocol, increasing survival from 67% to 88% (20, 22).

Even though it seems valid to use the model, some variables were not useful or difficult to apply in our population. In one of the four centers, Apgar scores were never measured because the medical team feels it is not a useful tool in this patient group. Patients with CDH will have a lower Apgar score as they are intubated directly after birth (20). Brindle et al theorized that the absence of an Apgar score implies a sicker infant, but this was not applicable to our cohort. Also, the measurement of PH on echocardiography is not standardized and different definitions for PH are being used. Brindle et al used right to left shunting or estimated supra-systemic pulmonary pressures. In Europe, the presence of PH is often defined as pulmonary pressures higher than >2/3 of the systemic pressures (20, 23, 24). Furthermore, the timing of the measurement differs between centers. Brindle et al used the earliest echocardiography in the model, Bent et al used PH at discharge (16). Presumably, the incidence of severe PH would be underestimated in Bent's study, as many patients with PH have already died and in others pulmonary pressures have decreased (25). The registered incidence of PH in non-survivors is only 33.5% in Bent's study, while it is over 50% in our cohort, and over 60% in the CDHSG population, supporting this assumption (15, 16).

To improve the power of the original prediction rule, combining prenatal and postnatal variables is presumably superior. Prenatal variables have been found to adequately predict lung hypoplasia and the need for ECMO but are less reliable as a marker for PH (8, 9). To predict mortality postnatal variables are still essential. Different prenatal variables were tested in the model and eventually only the position of the liver was a significant variable. Surprisingly, O/E LHR was not of additional value to the model, while in earlier studies O/E LHR did have a role in predicting survival. It is a more reliable prenatal predictor than the lung-to-head-ratio, as it is a stable variable during pregnancy (6, 26). However, different O/E LHR measurement techniques are being used between centers, and there is a learning curve in the examination of the O/E LHR (27, 28). In two of the centers, the tracing method was always used, while in the others also the longest axis diameter method was used. The longest axis diameter method overestimates the O/E LHR up to 34% and has a larger inter-observer variability (29-31). In addition, the O/E LHR can be calculated with multiple calculators (i.e. www.totaltrial.eu or www.perinatology.com) which results in different ratio's. In our study, the method of calculation varied. Measuring lung volumes on MRI holds promise (32). However, in many centers it is not possible to use MRI for this purpose, due to costs and lack of availability. For this study, analysis of fetal lung volume resulted in too many missing data. Another prenatal predictor is stomach position (7). This measure, however, is not implemented in standard prenatal care and

could therefore not be analyzed for this study. More consistent measuring techniques and reporting would probably make these different prenatal variables more suitable for use in outcome prediction.

For the modified model, we also made some changes to the original variables. "Missing Apgar score" was taken out. On the other hand, we kept chromosomal anomalies in the model, although its association with mortality was not significant (p-value was >0.1). Possibly some patients had a clinically insignificant abnormality on array, however, as these data are retrospective, it was not possible to reliably select only clinically relevant cases. Therefor we choose to include all patients with abnormalities on chromosomal array. CDH is associated with numerous chromosomal anomalies, and often the associated anomaly has a major impact on mortality (33, 34).

The strength of our study is the large population of patients with CDH and the amount of prenatal data available. Often, the implementation of prenatal data in a prediction model is difficult due to the amount of missing data (15). Also, when using a voluntary database, or a database based on coded diagnosis, the accuracy of the data is difficult to interpret. We were able to go back to the original patient files when needed. However, we did have some missing data as shown in table 2, which we corrected for using multiple imputation, a statistical tool often used in this setting. However, possibly some predictors were not significant in the model due to the large amount of missing data, such as the presence of polyhydramnios and the gestational age at birth. Furthermore, as all 4 centers are part of the CDH Euro Consortium, postnatal clinical management is similar in all centers, increasing the reliability of an early prediction model, but it also potentially limits the generalizability of our study and may contribute to an optimistic assessment of model performance. Therefor our new model needs additional external validation in a more heterogeneous group like the patients in the CDHSG database, to prove its generalizability. Also, as prenatal data are necessary for the new rule, other settings with imperfect prenatal care provision may not benefit as much from this new model. The original CDHSG model is very easy to apply at the bedside. Although this is not true for our model, a more complicated calculation is necessary.

In a population with a rare congenital defect with high mortality and morbidity, it is very important to reliably predict outcomes. Prenatally, this can guide parents and clinicians in decisions regarding perinatal management, and the referral to high-volume centers for the delivery in areas with a low density population. Postnatally, adequate prediction can help parents to better understand the course of their child's illness. In addition, it can also be used for standardized reporting and benchmarking between centers. A good postnatal prediction model can potentially improve care for specific groups of patients with CDH. This model can act as a practical tool when stratifying patients into risk groups, such as

mild, moderate and severe. The mild group of patients, identified with a highly sensitive threshold, could receive less aggressive treatment, such as a spontaneous breathing trial at birth (35). On the other hand, the severe group, identified with a highly specific threshold, could potentially benefit most from more aggressive experimental therapies, and the true benefit of these therapies could be detected earlier as there would be no dilution of effect due to the inclusion of patients with lower risk. For all these reasons, there is a need for a reliable prediction model, that can be applied on the first day of life (36). The power of a prediction model is dependent on the availability of the information needed for the calculation. Prenatal data are not readily available in some areas in the world. However, the only prenatal parameter significant in our model, the prenatal position of the liver on ultrasound, is reasonably easy to evaluate.

CONCLUSION

We have successfully validated the CDHSG prediction rule within a European population. We also developed a modification of the original rule, implementing prenatal variables, with apparent improvement of the predictability of mortality. Standardization of the measurements of prenatal variables such as O/E LHR and the postnatal variable PH, could potentially increase their predicting value and further improve these models. Validation of this modified rule is needed to evaluate its generalizability.

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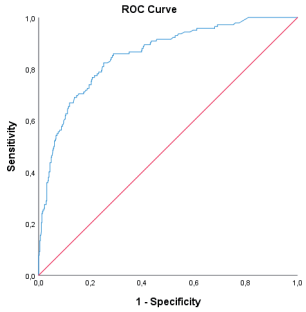
LITERATURE

1. Snoek KG, Capolupo I, van Rosmalen J, Hout Lde J, Vijfhuize S, Greenough A, et al. Conventional Mechanical Ventilation Versus High-frequency Oscillatory Ventilation for Congenital Diaphragmatic Hernia: A Randomized Clinical Trial (The VICI-trial). Ann Surg. 2016;263(5):867-74.
2. Hedrick HL, Danzer E, Merchant A, Bebbington MW, Zhao H, Flake AW, et al. Liver position and lung-to-head ratio for prediction of extracorporeal membrane oxygenation and survival in isolated left congenital diaphragmatic hernia. Am J Obstet Gynecol. 2007;197(4):422 e1-4.
3. Jani J, Keller RL, Benachi A, Nicolaides KH, Favre R, Gratacos E, et al. Prenatal prediction of survival in isolated left-sided diaphragmatic hernia. Ultrasound Obstet Gynecol. 2006;27(1):18-22.
4. Kilian AK, Busing KA, Schuetz EM, Schaible T, Neff KW. Fetal MR lung volumetry in congenital diaphragmatic hernia (CDH): prediction of clinical outcome and the need for extracorporeal membrane oxygenation (ECMO). Klin Padiatr. 2009;221(5):295-301.
5. Oluyomi-Obi T, Kuret V, Puligandla P, Lodha A, Lee-Robertson H, Lee K, et al. Antenatal predictors of outcome in prenatally diagnosed congenital diaphragmatic hernia (CDH). J Pediatr Surg. 2017;52(5):881-8.
6. Snoek KG, Peters NCJ, van Rosmalen J, van Heijst AFJ, Eggink AJ, Sikkel E, et al. The validity of the observed-to-expected lung-to-head ratio in congenital diaphragmatic hernia in an era of standardized neonatal treatment; a multicenter study. Prenat Diagn. 2017.
7. Cordier AG, Jani JC, Cannie MM, Rodo C, Fabietti I, Persico N, et al. Stomach position in prediction of survival in left-sided congenital diaphragmatic hernia with or without fetoscopic endoluminal tracheal occlusion. Ultrasound Obstet Gynecol. 2015;46(2):155-61.
8. Russo FM, Eastwood MP, Keijzer R, Al-Maary J, Toelen J, Van Mieghem T, et al. Lung size and liver herniation predict need for extracorporeal membrane oxygenation but not pulmonary hypertension in isolated congenital diaphragmatic hernia: systematic review and meta-analysis. Ultrasound Obstet Gynecol. 2017;49(6):704-13.
9. Wong M, Reyes J, Lapidus-Krol E, Chiang M, Humpl T, Al-Faraj M, et al. Pulmonary hypertension in congenital diaphragmatic hernia patients: Prognostic markers and long-term outcomes. J Pediatr Surg. 2018;53(5):918-24.
10. Snoek KG, Capolupo I, Morini F, van Rosmalen J, Greenough A, van Heijst A, et al. Score for Neonatal Acute Physiology-II Predicts Outcome in Congenital Diaphragmatic Hernia Patients. Pediatr Crit Care Med. 2016;17(6):540-6.
11. Bruns AS, Lau PE, Dhillon GS, Hagan J, Kailin JA, Mallory GB, et al. Predictive value of oxygenation index for outcomes in left-sided congenital diaphragmatic hernia. J Pediatr Surg. 2018.
12. Congenital Diaphragmatic Hernia Study G. Estimating disease severity of congenital diaphragmatic hernia in the first 5 minutes of life. J Pediatr Surg. 2001;36(1):141-5.
13. Schultz CM, DiGeronimo RJ, Yoder BA, Congenital Diaphragmatic Hernia Study G. Congenital diaphragmatic hernia: a simplified postnatal predictor of outcome. J Pediatr Surg. 2007;42(3):510-6.
14. Baird R, MacNab YC, Skarsgard ED, Canadian Pediatric Surgery N. Mortality prediction in congenital diaphragmatic hernia. J Pediatr Surg. 2008;43(5):783-7.

15. Brindle ME, Cook EF, Tibboel D, Lally PA, Lally KP, Congenital Diaphragmatic Hernia Study G. A clinical prediction rule for the severity of congenital diaphragmatic hernias in newborns. Pediatrics. 2014;134(2):e413-9.
16. Bent DP, Nelson J, Kent DM, Jen HC. Population-Based Validation of a Clinical Prediction Model for Congenital Diaphragmatic Hernias. J Pediatr. 2018.
17. Akinkuotu AC, Cruz SM, Abbas PI, Lee TC, Welty SE, Olutoye OO, et al. Risk-stratification of severity for infants with CDH: Prenatal versus postnatal predictors of outcome. J Pediatr Surg. 2016;51(1):44-8.
18. Oh C, Youn JK, Han JW, Yang HB, Lee S, Seo JM, et al. Predicting Survival of Congenital Diaphragmatic Hernia on the First Day of Life. World J Surg. 2018.
19. Bucher BT, Guth RM, Saito JM, Najaf T, Warner BW. Impact of hospital volume on in-hospital mortality of infants undergoing repair of congenital diaphragmatic hernia. Ann Surg. 2010;252(4):635-42.
20. Snoek KG, Reiss IK, Greenough A, Capolupo I, Urlesberger B, Wessel L, et al. Standardized Postnatal Management of Infants with Congenital Diaphragmatic Hernia in Europe: The CDH EURO Consortium Consensus - 2015 Update. Neonatology. 2016;110(1):66-74.
21. Sandlin AT, Chauhan SP, Magann EF. Clinical relevance of sonographically estimated amniotic fluid volume: polyhydramnios. J Ultrasound Med. 2013;32(5):851-63.
22. Reiss I, Schaible T, van den Hout L, Capolupo I, Allegaert K, van Heijst A, et al. Standardized postnatal management of infants with congenital diaphragmatic hernia in Europe: the CDH EURO Consortium consensus. Neonatology. 2010;98(4):354-64.
23. Patel N, Kipfmueller F. Cardiac dysfunction in congenital diaphragmatic hernia: Pathophysiology, clinical assessment, and management. Semin Pediatr Surg. 2017;26(3):154-8.
24. Patel N, Mills JF, Cheung MM. Assessment of right ventricular function using tissue Doppler imaging in infants with pulmonary hypertension. Neonatology. 2009;96(3):193-9; discussion 200-2.
25. Keller RL, Tacy TA, Hendricks-Munoz K, Xu J, Moon-Grady AJ, Neuhaus J, et al. Congenital diaphragmatic hernia: endothelin-1, pulmonary hypertension, and disease severity. Am J Respir Crit Care Med. 2010;182(4):555-61.
26. Jani J, Nicolaides KH, Keller RL, Benachi A, Peralta CF, Favre R, et al. Observed to expected lung area to head circumference ratio in the prediction of survival in fetuses with isolated diaphragmatic hernia. Ultrasound Obstet Gynecol. 2007;30(1):67-71.
27. Kehl S, Siemer J, Brunnemer S, Weiss C, Eckert S, Schaible T, et al. Prediction of postnatal outcomes in fetuses with isolated congenital diaphragmatic hernias using different lung-to-head ratio measurements. J Ultrasound Med. 2014;33(5):759-67.
28. Cruz-Martinez R, Figueras F, Moreno-Alvarez O, Martinez JM, Gomez O, Hernandez-Andrade E, et al. Learning curve for lung area to head circumference ratio measurement in fetuses with congenital diaphragmatic hernia. Ultrasound Obstet Gynecol. 2010;36(1):32-6.
29. Jani JC, Peralta CF, Nicolaides KH. Lung-to-head ratio: a need to unify the technique. Ultrasound Obstet Gynecol. 2012;39(1):2-6.

30. Jani J, Peralta CF, Benachi A, Deprest J, Nicolaides KH. Assessment of lung area in fetuses with congenital diaphragmatic hernia. Ultrasound Obstet Gynecol. 2007;30(1):72-6.
31. Abbasi N, Ryan G, Johnson A, Cortes MS, Sangi-Haghpeykar H, Ye XY, et al. Reproducibility of fetal lung-to-head ratio in left diaphragmatic hernia across the North American Fetal Therapy Network (NAFTNet). Prenat Diagn. 2019;39(3):188-94.
32. Kastenholz KE, Weis M, Hagelstein C, Weiss C, Kehl S, Schaible T, et al. Correlation of Observed-to-Expected MRI Fetal Lung Volume and Ultrasound Lung-to-Head Ratio at Different Gestational Times in Fetuses With Congenital Diaphragmatic Hernia. AJR Am J Roentgenol. 2016;206(4):856-66.
33. Slavotinek AM. The genetics of common disorders - congenital diaphragmatic hernia. Eur J Med Genet. 2014;57(8):418-23.
34. Zaiss I, Kehl S, Link K, Neff W, Schaible T, Sutterlin M, et al. Associated malformations in congenital diaphragmatic hernia. Am J Perinatol. 2011;28(3):211-8.
35. Morini F, Capolupo I, van Weteringen W, Reiss I. Ventilation modalities in infants with congenital diaphragmatic hernia. Semin Pediatr Surg. 2017;26(3):159-65.
36. Daodu O, Brindle ME. Predicting outcomes in congenital diaphragmatic hernia. Semin Pediatr Surg. 2017;26(3):136-9.



Supplemental digital content figure 1 Receiver operating characteristic curve of the new prediction model

Supplemental digital content table 1 Severity of CDH per center based on the CDHSG prediction rule

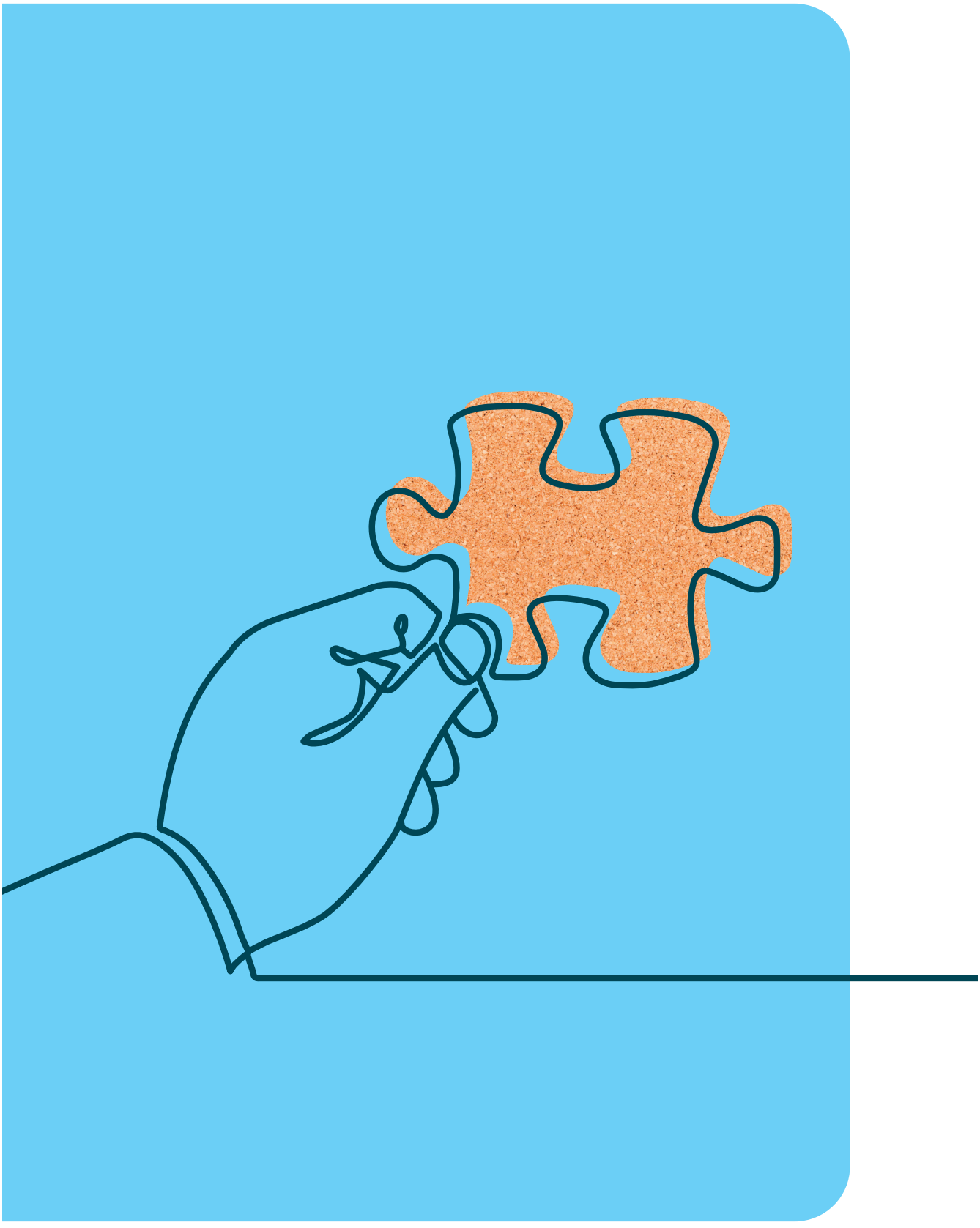
Center	Patients (pooled n)	Mortality (%)	Score: 0 (low risk)	Score: 1 - 2 (intermediate risk)	Score: 3 - 8 (high risk)
Rotterdam	79	20.3%	36.4%	52.0%	11.6%
Nijmegen	59	27.1%	32.2%	44.6%	23.2%
Mannheim	179	13.4%	61.1%	27.5%	11.4%
Rome	26	26.9%	0.0%	56.5%	43.5%

After multiple imputation of missing data for CDHSG prediction rule (n=100 databases), the CDHSG prediction rule score was calculated for each patient. Data presented in the table are from the pooled database.

Supplemental digital content table 2 Severity of CDH per center based on the new prediction rule

Center	Patients (pooled n)	Mortality (%)	Overall mean mortality risk	Mild (mortality risk < 10%)	Moderate (mortality risk 10 – 50%)	Severe (mortality risk >50%)
Rotterdam	129	27.9%	17.9%	53.8%	35.7%	10.5%
Nijmegen	62	33.9%	26.9%	31.4%	49.2%	19.3%
Mannheim	336	17.0%	22.1%	48.2%	37.1%	14.7%
Rome	93	30.1%	27.6%	43.6%	33.7%	22.7%

After multiple imputation of missing data for the new prediction rule (n=100 databases), the new prediction rule was used to calculate the mortality risk for each patient. Data presented in the table are from the pooled database.



CHAPTER 4

Implementing disease-specific biomarkers for the early detection of bronchopulmonary dysplasia

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ABSTRACT

Objective: We herewith demonstrate the comprehensive quality assessment of our previously published biomarkers (BCAM, SIGLEC-14, ANGPTL-3) that sensitively predict bronchopulmonary dysplasia (BPD) in the first week of life to enable their implementation into routine diagnostics.

Methods: We analyzed first week of life plasma samples of n=61 preterm infants <32 weeks gestational age (GA) on two proteomic platforms (SomaLogic®, Olink Proteomics®) and confirm the predictive value of the biomarkers BCAM, SIGLEC-14 and ANGPTL-3 for BPD. We then demonstrate the robustness of our biomarkers towards the protein measurement technique using different detection platforms as well as towards cohort characteristics, i.e. size and disease or comorbidity incidence.

Results: We identify the best model for disease prediction that combines log2 transformed protein concentrations with GA and early onset infection in a generalized additive model using BPD as binary outcome (AUC=0.82 (leave-1-out cross-validation), AIC=45.01 (GA and EOI: AIC=53.03; null model: AIC=75.00)). The biomarkers furthermore successfully identify functional (duration of mechanical ventilation) and structural (Magnetic Resonance Imaging (MRI), T1 relaxation times) disease characteristics. Disease specificity is demonstrated using a neonatal and an adult cohort including patients with congenital diaphragmatic hernia, chronic obstructive pulmonary disease (COPD) and lung fibrosis.

Conclusion: Our findings strongly support the potential of the identified biomarkers and support their stepwise integration into clinical routine, currently realized in the design of a clinical trial.

INTRODUCTION

Biomarkers that early and sensitively identify preterm infants with neonatal chronic lung disease (CLD), internationally known as bronchopulmonary dysplasia (BPD), are urgently needed to inform postnatal clinical decision-making and allow for the design of personalized follow-up strategies. Previous approaches aiming at the identification of such markers have been largely limited by the use of targeted protein analysis and non-sensitive detection techniques (1, 2) combined with standard analysis tools rather than statistical modelling including clinical variables (1).

In order to overcome these limitations - and in contrast to diagnostics solely relying on clinical criteria (3) -we employed an unbiased proteome screening together with statistical modelling and identified a combination of plasma markers, which sensitively predicts BPD development as early as in the first week of life (4).

Implementation of these markers into clinical routine requires the thorough assessment of critical quality criteria relevant for the diagnostic process and the design of a clinical study including disease specificity as well as robustness of the results towards analysis technique and cohort characteristics.

We herewith i) confirm the predictive value of the identified biomarkers for BPD in a large, prospective cohort of preterm infants, ii) demonstrate the robustness of our findings towards the protein analysis technique by the use of two different proteomic platforms, iii) show the biomarkers' clinical value by predicting functional and structural disease characteristics and iv) prove disease specificity in a neonatal and an adult CLD cohort, *i.e.* congenital diaphragmatic hernia (CDH), idiopathic pulmonary fibrosis (IPF) and chronic obstructive pulmonary disease (COPD).

The results of our comprehensive analysis enable the initiation of a clinical study to implement the identified biomarkers into today's diagnostic routine.

PATIENTS AND METHODS

Patients. Preterm infants: We analyzed plasma samples obtained from preterm infants born <32 weeks gestational age (GA) in the first week of life. Preterm infants were prospectively included in the study at the Perinatal Center in Munich (cohort #1 (EC #195-07): n=53, GA 27.7 (23.2-30.6) weeks (mean), 43.4% males) after informed parental consent following the in- and exclusion criteria published previously (4). Clinical variables were comprehensively monitored from birth to discharge (see table 1). Mild, moderate and severe BPD was

diagnosed at 36 weeks postmenstrual age (3) (no BPD n=24 (45.3%), mild BPD n=15 (28.3%), moderate BPD n=6 (11.3%), severe BPD n=8 (15.1%)). To assess structural and functional disease characteristics, infants underwent lung Magnetic Resonance Imaging (MRI) at 36 weeks postmenstrual age (5). In an additional step, preterm infants recruited at a different study site according to the same in- and exclusion criteria (EC #AZ 15-304; n=8, GA 26.1 weeks (mean), 75% males, n=4 no BPD, n=4 moderate/severe BPD) were added to cohort #1, in order to mimic sampling conditions of a clinical trial, *i.e.* ongoing recruitment of small sample sets with random distribution of clinical characteristics.

Table 1. Patient characteristics

Cohort #1 (Munich)	N=53	
GA (weeks)	27.7	(23.2-30.6)
Birth weight (g)	896	(510-1590)
IUGR	6	11.3%
Gender (femaie / male)	30 / 23	
ANCS	47	88.7%
Chorioamnionitis	28	52.8%
Early onset infection (EOI)	14	26.4%
RDS≥III°	12	22.6%
Days of mechanical ventilation	43	0-109
Days of oxygen supplementation	42	0-186
PDA	24	45.3%
Postnatal steroids	23	43.4%
ROP≥III°	16	30.2%
IVH≥III°	6	11.3
ICU days	67	9-116
BPD		
- None	24	45.3%
- Mild	15	28.3%
- Moderate	6	11.3%
- Severe	8	15.1%
PH (from 2/3 of syst pressure)	0	0%
Additional patients study site Luebeck	N=8	
GA (weeks)	26.1	24.1-29.0
Birth weight (g)	852	520-1470
Gender (femaie / male)	2/6	
Early onset infection (EOI)	2	25%
BPD		
- None	4	50%
- Mild	0	0%
- Moderate	4	50%
- Severe	0	0%

Data are given as median and range or number and percent of total in group respective range. GA, gestational age; NS-pH, umbilical cord pH; ANCS, antenatal corticosteroids; RDS, respiratory distress syndrome; ROP, retinopathy of prematurity; IVH, intraventricular hemorrhage; ICU, intensive care unit; BPD, bronchopulmonary dysplasia. NA (not available): PDA n=7, ICU days n=1. Intrauterine growth restriction was defined as birth weight below the 10th percentile. Postnatally, diagnosis and severity of respiratory distress syndrome (RDS) were scored on anterior-posterior (a.-p.) chest radiographs according to Couchard et al (6). Chorioamnionitis was defined as inflammatory alterations of the chorionic plate (histologic examination) or signs of maternal and fetal signs of infection (7). Systemic infections (EOI) were diagnosed according to Sherman et al. (8) based on one or more clinical and laboratory signs of infection. BPD was defined according to Jobe and Bancalari (3) and graded as mild (oxygen supplementation at 28 days postnatally), moderate (oxygen supplementation < 30% and/or ventilator support at 36 weeks postmenstrual age), and severe (oxygen supplementation < 30% and/or ventilator support at 36 weeks postmenstrual age).

Term neonates with CDH: To assess disease specificity of the biomarkers, measurements were repeated in a cohort of neonates with CDH with and without CLD, defined as the need for mechanical ventilation and/or oxygen supplementation beyond day 28 of life, prospectively included in the VICI-trial (9) after informed parental consent (n=21, cohort #2, ErasmusMC, Rotterdam (EC #MEC-2006-260): GA 38.0 weeks (mean), 33.3% males, n=6 deceased, n=6 no CLD, n=9 CLD. See table S2, online data supplement).

Adult patients with CLD: Biomarker specificity was next addressed in a cohort of adult CLD patients (cohort #3, CPC-M bioArchive, Munich, EC #19-629) comprising samples from patients with idiopathic pulmonary fibrosis (IPF, n=30, 58.3 years of age (mean), 76.7% males), chronic obstructive pulmonary disease (COPD, n=26, 47.8 years of age (mean), 58% males) and subjects free of lung disease according to clinical history (n=25, KORA cohort (10): 59.6 years of age (mean), 52% males).

Proteomic platforms and sample analysis. Serial whole blood samples (200µl minimum each) obtained during routine laboratory blood drawings were collected using Ethylenediaminetetraacetic acid (EDTA) neonatal collection tubes. After pseudonymization samples were processed for proteomic screening by centrifugation (1000g, 5 minutes) before supernatants were aliquoted and stored at -80 °C. Samples were analyzed by two analysis platforms: The **SOMAscan**™ assay (SomaLogic®, Boulder, USA) uses 1124 individual high affinity molecules (SOMAmer® - slow off-rate modified DNA aptamer - reagents) quantified on a custom Agilent hybridization array (11, 12). The **Proximity Extension Assay (PEA)** (Olink Proteomics®, Uppsala, Sweden) employs a matched pair of antibodies linked to unique oligonucleotides detected in multiplexed fashion in a high throughput fluidic chip system measuring 630 unique proteins (13). Both techniques are designed for the accurate quantification of human plasma proteins present in concentrations below picogram per milliliter using even low-amount samples. For PEA measurements, SIGLEC5&14 was detected in the identical aliquot by ELISA (R&D Systems, MN, USA). Samples were analyzed on either platform: cohort #1: SOMAscan™

(SomaLogic®) (n=34 samples) and PEA (Olink-Proteomics®) (n=19 samples, n=8 additional samples (Luebeck)); cohort#2 PEA (Olink-Proteomics®) (n=21); cohort #3 SOMAscan™ (SomaLogic®) (n=72 (including healthy individuals)).

MRI analysis

Lung MRI was performed at the time of BPD diagnosis, *i.e.* 36 weeks postmenstrual age in spontaneously breathing infants (cohort #1, n=20, fraction of inspired oxygen (FiO_2)=0.21) using a 3-Tesla whole-body scanner. The protocol included pulse sequences for assessment of quantitative relaxation parameters: (i) ECG-triggered T_2-weighted single-shot fast spin echo (ssFSE) sequences in three orthogonal orientations (echo time (TE): 57 ms; repetition time (TR): 2 RR intervals), (ii) single-slice ssFSE T_2 mapping (TR 2000 ms, 4 TEs 26–92 ms) and T_1 (ms) mapping (TR/TE 3000 ms/26 ms, six inversion times TI (slice-selective): 25–2600 ms and no inversion) acquiring eight averages (total acquisition time ≈5 min). For T_2- and T_1-mapping analysis, manual segmentation into four lung quadrants was performed (Osirix MD). T_2 and T_1 relaxation times were calculated by non-linear exponential signal fitting for four lung quadrants separately and the total lung.

Statistical analysis.

Analysis was performed in all samples after technical quality control and identification of outliers by principal component analysis (prcomp function, R framework, log2-transformed data). Cohort #1: n=53 (measured by SOMAscan™ (SomaLogic®), PEA (Olink-Proteomics®)) was merged using the sva package (version 3.36) in R (version 4.0)); in a second step additional samples (n=8, PEA (Olink-Proteomics®)) were included into cohort #1; cohort #2: n=21 (Olink-Proteomics®); cohort #3: n=72 (SOMAscan™ (SomaLogic®)). AUCs were calculated using a leave-one-out cross-validation with a generalized additive model that included BPD as binary outcome and log2 transformed protein expression data together with the clinical variables gestational age (GA) and EOI (early onset of infection) as covariates (Equation 1, i.e. the best model to explain BPD: $\boldsymbol{BPD \sim BCAM + SIGLEC14 + ANGPTL3 + GA + EOI}$). Predicted values for mechanical ventilation [days] and T_1 relaxation times (ms) were calculated using a leave-1-out cross-validation with a linear regression model using the same covariates with continuous outcomes. The clinical covariates in the model for CDH were GA and gender, while for adult CLD the covariates were age and gender.

RESULTS

Protein markers sensitively detect BPD in preterm infants. We confirm the predictive power of the three previously identified biomarkers (4) in additional samples obtained in the first week of life and measured on two proteomic platforms: Plasma protein levels

reflect disease severity (cohort #1, **Fig. 1A**) and predict disease development (**Fig. 1B**, no BPD vs. mild, and moderate and severe BPD, ANOVA p-value=1.2x10^{-5} (leave-1-out cross-validation)) with significant differences between the disease groups: no BPD vs. mild BPD (t-test p-value=0.023), no BPD vs. moderate and severe BPD (t-test p-value=9x10^{-6}), mild BPD vs. moderate and severe BPD (t-test p- value=0.011). Significant predictive power is furthermore demonstrated using count variables that indicate i) functional, *i.e.* need for mechanical ventilation [days] (**Fig. 1C**; observed vs. predicted: r=0.77, p-value=1.8x10^{-6}, MAE=11.19, RMSE=14.03) and ii) structural, *i.e.* decreased T_1 relaxation times [ms] (**Fig. 1D**; r=0.49, p-value=0.0075, MAE=61.19, RMSE=77.88) changes in the BPD lung.

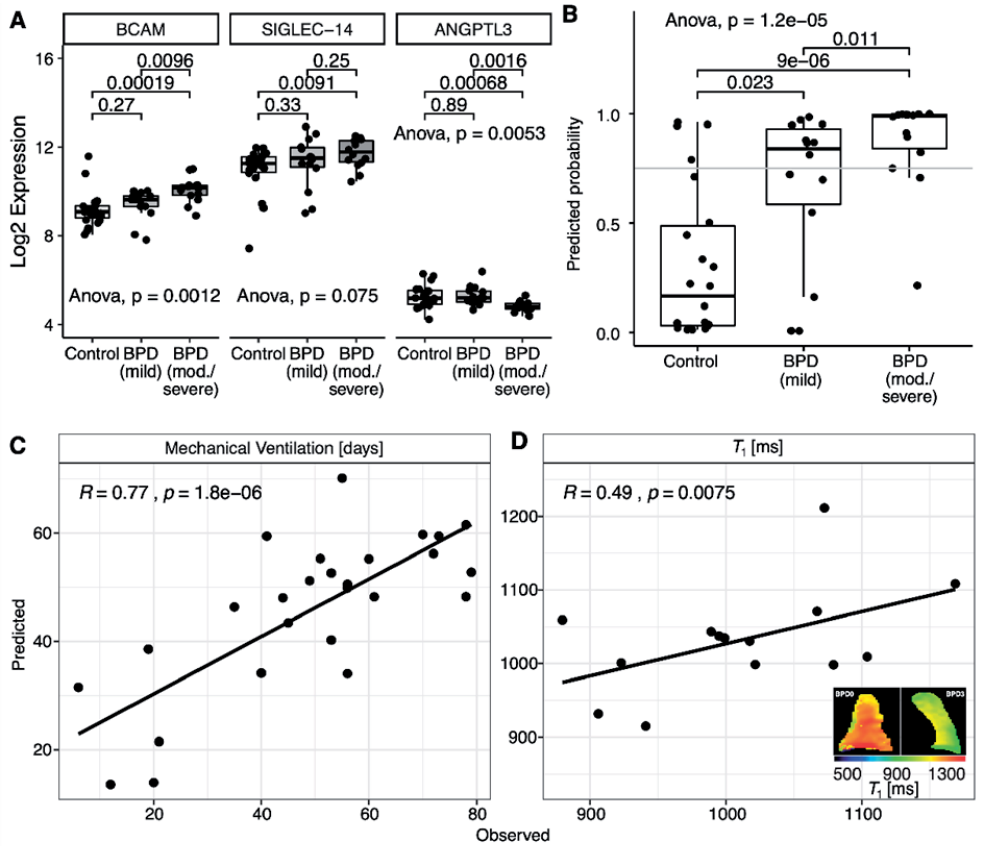


Figure 1: A) Log2 transformed protein expression of BCAM, SIGLEC-14 and ANGPTL-3 in cohort #1, samples obtained in the first week of life and grouped by disease grade (ANOVA and t-test as indicated); medians, 25 and 75% quartiles, whiskers represent 95% of the data. B) Predicted probability of no BPD vs. BPD (all grades) resulting from leave-1-out cross-validation (cohort #1) including log2 transformed protein data, gestational age (GA) and early onset infection (EOI) (ANOVA p=1.2x10-5); t-test p-values for: no BPD vs. BPD mild

p=0.023; no BPD vs. BPD (mod./severe) p=9.3x10-6; BPD (mild) vs. BPD (mod./severe) p=0.011. Prediction analysis (leave- 1-out model, cohort #1, Munich) for C) mechanical ventilation [days] (r=0.77, p=1.8x10-6) and D) structural changes, i.e. T1 relaxation times [ms] obtained by lung MRI at term (r=0.49; p=0.0075). Insert: Representative images of T1 relaxation times [ms] in an infant with severe BPD (BPD3) compared to an infant without the disease (BPD0).

Significant power and robustness of the selected model to predict BPD. The log2 transformed protein expression levels for BCAM, SIGLEC-14 and ANGPTL-3 were corrected for the analysis on two different proteomic platforms. PCA plots the separation by clinical features such as BPD, mechanical ventilation and gestational age that significantly outweighs the separation by analysis platform (**Fig. 2A**). A comparison of sixteen models, that included protein expression and clinical covariates significantly associated with BPD development, identified the model using protein levels, GA and early onset infection (EOI) as the ideal model to predict disease with optimized AUC (0.82) and AIC (45.01 compared to the baseline model without proteins but with GA and EOI: AIC=53.03, and the null model: AIC=75.00; **Fig. 2B**). Application of the selected model demonstrates sensitive prediction of all BPD cases (AUC=0.82 (cohort #1)) and the most severe BPD cases (AUC=0.85 (cohort #1)). Predictive power remains high after integration of additional measurements at a different study site (AUC=0.75 (cohort #1 plus n=8 (Luebeck))).

Biomarker specificity demonstrated in neonatal and adult CLD cohorts. Analysis in a cohort of neonatal patients with chronic lung disease of different origin as well as in adult CLD patients indicated specificity for BPD: Protein levels do not discriminate neonatal CLD CDH survivors from no-CLD CDH (age-matched, AUC 0.63, **Fig. 2C**), but only separate fatal outcome in CLD CDH (AUC 0.78, **Fig. 2C**). Accounting for the most prevalent complication in severe CLD CDH, *i.e.* pulmonary hypertension (PH), cases with advanced PH are identified by the biomarkers with most pronounced effects for SIGLEC-5/14 (cohort #2, p=0.03, data not shown). In adult patients (cohort #3), protein levels do not separate cases with COPD or IPF (**Fig. 2D**) from pulmonary healthy controls with sufficient discriminative power.

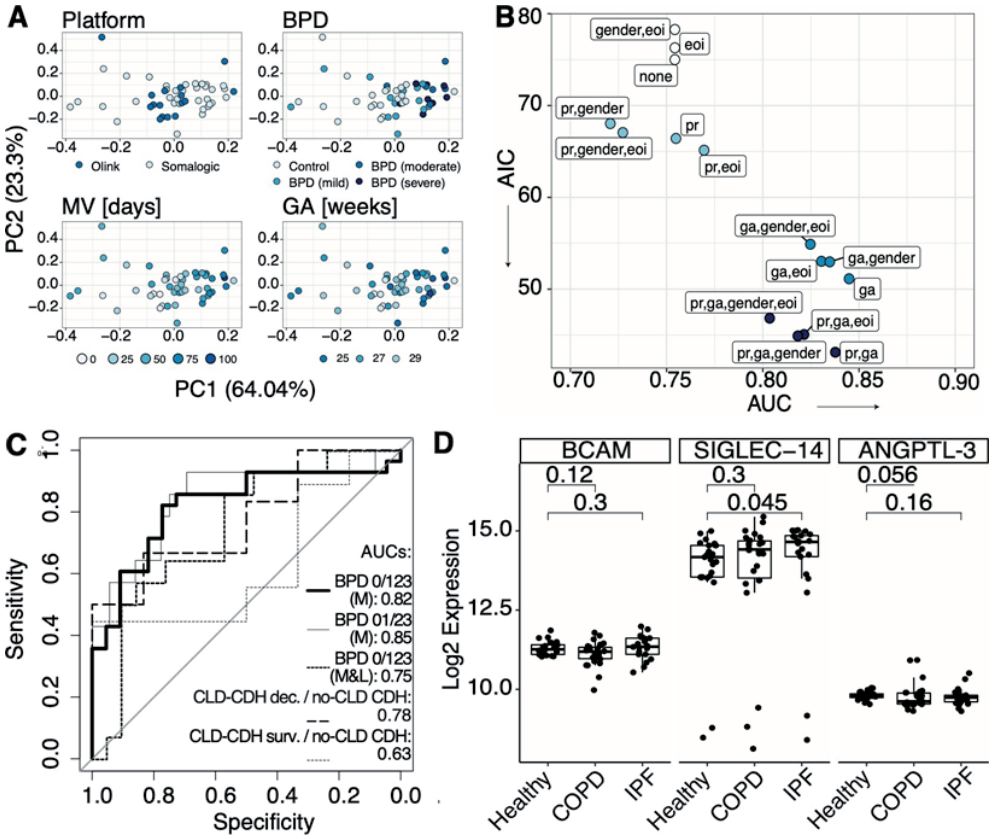


Figure 2: A) PCA plot for cohort #1 after correction for analysis technique, i.e. proteomic platform (SOMAscanTM (SomaLogic®) (light blue); PEA (Olink-Proteomics®) (black)), showing significant separation for clinical variables such as BPD, mechanical ventilation (MV) [days], gestational age (GA) [weeks] in contrast to a lack of separation by analysis platform. B) Akaike's Information Criterion (AIC) vs. AUC for 15 different models (n=15) including log2 transformed protein expression (pr) as well as gestational age (GA), gender and early onset infection (EOI); the 16th model (gender only) was excluded as a severe outlier (AUC=1, AIC=76.95) C) AUC values calculated for protein expression, GA and EOI: bold line (cohort #1): no BPD (0) vs. mild (1), moderate (2) and severe (3) BPD, AUC=0.82; thin line (cohort #1): no and mild BPD (0,1) vs. moderate and severe BPD (2,3), AUC=0.85; dotted line (multi-center analysis; cohort #1 and additional samples (n=8, Luebeck): no BPD (0) vs. mild, moderate and severe BPD (1,2,3), AUC=0.75; dashed line (cohort #2): CLD-CDH deceased vs. no-CLD CDH infants AUC=0.78; thin dotted line (cohort #2): CLD-CDH survivors vs. no-CLD CDH infants, AUC=0.63. D) Boxplots for log2 transformed protein expression levels in cohort #3: adult chronic lung disease (CLD), i.e. IPF and COPD vs. healthy subjects. Wilcoxon Rank Sum test; median, 25 and 75% quartiles indicated; whiskers represent 95% of all data.

DISCUSSION

Bronchopulmonary dysplasia is a multifactorial disease and remains the most serious lung condition of prematurity associated with significant mortality and morbidity. The heterogeneous BPD phenotype driven by matrix remodeling, vascular disease and airway pathology in combination with a diagnostic process solely relying on clinical criteria at 36 weeks of post- menstrual age highlights both the immediate need to establish new diagnostic markers for clinical practice early in life and the challenging conditions that accompany this process (1, 2). Prediction models based on clinical parameters such as birth weight, gestational age or initial respiratory illness did not show adequate sensitivity and specificity (14-16) and studies that targeted risk factor identification, modification of postnatal treatment strategies or the implementation of new therapeutic approaches (17-21) were challenged by the lack of early disease detection.

The demand for early disease indicators led to the analysis of protein-based markers in different body fluids, extended by the search for metabolites or miRNAs (2). Most studies, however, investigated only a restricted panel of markers and used classical statistical methods that did not allow for the design of diagnostic algorithms. To overcome these limitations, we involved high-end, unbiased screening approaches and advanced statistical modelling to successfully identify three markers that sensitively predict BPD as early as in the first week of life.

As clinical implementation of these markers requires addressing critical quality criteria, we performed comprehensive follow-up studies that allow us to present the confirmation of the three protein biomarkers (BCAM, SIGLEC-14, ANGPTL-3) and their ability to predict BPD in the early postnatal course: We demonstrate that the measurements are robust towards the analysis technique applied, *i.e.* different proteomic platforms where data separation is driven by BPD and its associated clinical characteristics rather than proteomic platform, and towards the inclusion of different cohorts with varying size and distribution of clinical characteristics. This is in line with previous studies that showed positive correlation of protein expression levels measured by PEA or SOMAscan^{TM}, respectively) (22, 23).

The assessment of 16 models incorporating proteins expression levels alone or in different combinations with relevant clinical parameters identified the model that best predicts BPD while including a clinical covariate that consistently associates with disease and protein expression levels, *i.e.* log2 transformed protein concentrations in combination with GA and EOI.

In order to further support the clinical relevance of the three biomarkers identified, we demonstrate the predictive strength of our model to identify functional and structural

disease characteristics, indicated by the prolonged need for mechanical ventilation and also sensitive imaging markers obtained by lung MRI at term (5). Next to detecting pulmonary immaturity, prediction of required ventilation times by the biomarkers could enable the establishment of counteractive measures in the future. To demonstrate specificity of the model for BPD, we investigated two additional CLD cohorts: In neonates suffering from congenital diaphragmatic hernia (CDH), the model identifies only fatal cases while further discrimination according to disease severity is not achieved. In adult patients with CLD, *i.e.* emphysema (COPD) or lung fibrosis (IPF), the biomarkers did not allow to separate patients from healthy individuals with sufficient discriminative power.

We thereby present the significant potential of these biomarkers to improve clinical decision making for the preterm infant at risk for BPD in the first week of life. The ability to perform these measurements in easily accessible, low volume samples (13) further supports their clinical implementation even in low birth weight infants. The potential of these biomarkers to enable risk stratification by the identification of disease subtypes, *e.g.* a vascular phenotype (24, 25) and/or according to characteristic pathophysiologic changes such as pulmonary inflammation or matrix remodeling needs to be investigated further and could be of value for the design of future treatment strategies. The relevance of a pro- angiogenic factor, *i.e.* ANGPTL-3 for disease prediction is in line with the study of Arjaans et al., that associated early postnatal changes in circulating angiogenic peptides with later development of BPD under special consideration of vascular disease, *i.e.* pulmonary hypertension (PH). The imbalance between the immune-activating SIGLEC-14 (26, 27) and the inhibitory SIGLEC-5 (28, 29) has been associated with invasive infections in human newborns (30) involving MAPK and AKT signaling. Its early presence in the preterm circulation could reflect the degree of pulmonary inflammation, known to characterize BPD ranging from the recruitment of neutrophils to macrophage influx (31-34) and the disturbed balance of pro- and anti-inflammatory factors (33) in the BPD lung. Levels of SIGLEC-14 could hold potential for the prediction of long-term perspectives in infants with BPD, known to suffer from recurrent pulmonary infections in the first years of life (35). In line with this, previous studies suggested a role of SIGLEC-14 in the host response to viral airway infections (36). The protein might be of relevance for other forms of chronic lung diseases as shown by our findings in CDH cases with underlying severe PH and adult patients with COPD. The laminin receptor BCAM could mirror the process of tissue remodeling and its associated cellular cross- talk (32, 37), thereby indicating a relevant disease process with individual expression (38).

Taken together, our findings strongly support the potential of the biomarkers for their stepwise integration into clinical decision-making, currently realized by the preparation of a clinical trial. Further studies will evaluate the biomarker's role to guide monitoring strategies, underlined by our finding that biomarker expression remains stable at day 28 of life (4).

LITERATURE

1. Lal CV, Ambalavanan N. Biomarkers, Early Diagnosis, and Clinical Predictors of Bronchopulmonary Dysplasia. Clin Perinatol. 2015;42(4):739-54.
2. Rivera L, Siddaiah R, Oji-Mmuo C, Silveyra GR, Silveyra P. Biomarkers for Bronchopulmonary Dysplasia in the Preterm Infant. Front Pediatr. 2016;4:33.
3. Jobe AH, Bancalari E. Bronchopulmonary dysplasia. Am J Respir Crit Care Med. 2001;163(7):1723-9.
4. Forster K, Sass S, Ehrhardt H, Mous DS, Rottier RJ, Oak P, et al. Early Identification of Bronchopulmonary Dysplasia Using Novel Biomarkers by Proteomic Screening. Am J Respir Crit Care Med. 2018;197(8):1076-80.
5. Forster K, Ertl-Wagner B, Ehrhardt H, Busen H, Sass S, Pomschar A, et al. Altered relaxation times in MRI indicate bronchopulmonary dysplasia. Thorax. 2020;75(2):184-7.
6. Couchard M, Polge J, Bomsel F. [Hyaline membrane disease: diagnosis, radiologic surveillance, treatment and complications] Maladie des membranes hyalines. diagnostic et surveillance radiologiques, traitement, complications etude radioclinique de 589 cas. Ann Radiol (Paris). 1974;17(7):669-83.
7. Franz AR, Steinbach G, Kron M, Pohlandt F. Interleukin-8: a valuable tool to restrict antibiotic therapy in newborn infants. Acta Paediatr. 2001;90(9):1025-32.
8. Sherman MP, Goetzman BW, Ahlfors CE, Wennberg RP. Tracheal asiration and its clinical correlates in the diagnosis of congenital pneumonia. Pediatrics. 1980;65(2):258-63.
9. Snoek KG, Capolupo I, van Rosmalen J, Hout Lde J, Vijfhuize S, Greenough A, et al. Conventional Mechanical Ventilation Versus High-frequency Oscillatory Ventilation for Congenital Diaphragmatic Hernia: A Randomized Clinical Trial (The VICI-trial). Ann Surg. 2016;263(5):867-74.
10. Holle R, Happich M, Lowel H, Wichmann HE, Group MKS. KORA--a research platform for population based health research. Gesundheitswesen. 2005;67 Suppl 1:S19-25.
11. Gold L, Ayers D, Bertino J, Bock C, Bock A, Brody EN, et al. Aptamer-based multiplexed proteomic technology for biomarker discovery. PLoS One. 2010;5(12):e15004.
12. Rohloff JC, Gelinas AD, Jarvis TC, Ochsner UA, Schneider DJ, Gold L, et al. Nucleic Acid Ligands With Protein-like Side Chains: Modified Aptamers and Their Use as Diagnostic and Therapeutic Agents. Mol Ther Nucleic Acids. 2014;3:e201.
13. Assarsson E, Lundberg M, Holmquist G, Bjorkesten J, Thorsen SB, Ekman D, et al. Homogenous 96-plex PEA immunoassay exhibiting high sensitivity, specificity, and excellent scalability. PLoS One. 2014;9(4):e95192.
14. Hentschel J, Friedel C, Maier RF, Bassir C, Obladen M. Predicting chronic lung disease in very low birthweight infants: comparison of 3 scores. J Perinat Med. 1998;26(5):378-83.
15. Sinkin RA, Cox C, Phelps DL. Predicting risk for bronchopulmonary dysplasia: selection criteria for clinical trials. Pediatrics. 1990;86(5):728-36.
16. Yoder BA, Anwar MU, Clark RH. Early prediction of neonatal chronic lung disease: a comparison of three scoring methods. Pediatr Pulmonol. 1999;27(6):388-94.

17. Askie LM, Henderson-Smart DJ, Irwig L, Simpson JM. Oxygen-saturation targets and outcomes in extremely preterm infants. N Engl J Med. 2003;349(10):959-67.
18. Bhandari V. The potential of non-invasive ventilation to decrease BPD. Semin Perinatol. 2013;37(2):108-14.
19. Carlo WA, Stark AR, Wright LL, Tyson JE, Papile LA, Shankaran S, et al. Minimal ventilation to prevent bronchopulmonary dysplasia in extremely-low-birth-weight infants. J Pediatr. 2002;141(3):370-4.
20. Davis PG, Schmidt B, Roberts RS, Doyle LW, Asztalos E, Haslam R, et al. Caffeine for Apnea of Prematurity trial: benefits may vary in subgroups. J Pediatr. 2010;156(3):382-7.
21. Engle WA, Kominiarek MA. Late preterm infants, early term infants, and timing of elective deliveries. Clin Perinatol. 2008;35(2):325-41, vi.
22. Graumann J, Finkernagel F, Reinartz S, Stief T, Brodje D, Renz H, et al. Multi-platform Affinity Proteomics Identify Proteins Linked to Metastasis and Immune Suppression in Ovarian Cancer Plasma. Front Oncol. 2019;9:1150.
23. Sun BB, Maranville JC, Peters JE, Stacey D, Staley JR, Blackshaw J, et al. Genomic atlas of the human plasma proteome. Nature. 2018;558(7708):73-9.
24. Baker CD, Alvira CM. Disrupted lung development and bronchopulmonary dysplasia: opportunities for lung repair and regeneration. Curr Opin Pediatr. 2014;26(3):306-14.
25. Camenisch G, Pisabarro MT, Sherman D, Kowalski J, Nagel M, Hass P, et al. ANGPTL3 stimulates endothelial cell adhesion and migration via integrin alpha vbeta 3 and induces blood vessel formation in vivo. J Biol Chem. 2002;277(19):17281-90.
26. Angata T, Hayakawa T, Yamanaka M, Varki A, Nakamura M. Discovery of Siglec-14, a novel sialic acid receptor undergoing concerted evolution with Siglec-5 in primates. FASEB J. 2006;20(12):1964-73.
27. Yamanaka M, Kato Y, Angata T, Narimatsu H. Deletion polymorphism of SIGLEC14 and its functional implications. Glycobiology. 2009;19(8):841-6.
28. Crocker PR, Paulson JC, Varki A. Siglecs and their roles in the immune system. Nat Rev Immunol. 2007;7(4):255-66.
29. Varki A, Angata T. Siglecs--the major subfamily of I-type lectins. Glycobiology. 2006;16(1):1R-27R.
30. Ali SR, Fong JJ, Carlin AF, Busch TD, Linden R, Angata T, et al. Siglec-5 and Siglec-14 are polymorphic paired receptors that modulate neutrophil and amnion signaling responses to group B Streptococcus. J Exp Med. 2014;211(6):1231-42.
31. Bhandari V. Hyperoxia-derived lung damage in preterm infants. Semin Fetal Neonatal Med. 2010;15(4):223-9.
32. Blackwell TS, Hipps AN, Yamamoto Y, Han W, Barham WJ, Ostrowski MC, et al. NF-kappaB signaling in fetal lung macrophages disrupts airway morphogenesis. J Immunol. 2011;187(5):2740-7.
33. Bose CL, Dammann CE, Laughon MM. Bronchopulmonary dysplasia and inflammatory biomarkers in the premature neonate. Arch Dis Child Fetal Neonatal Ed. 2008;93(6):F455-61.

34. Wallace MJ, Probyn ME, Zahra VA, Crossley K, Cole TJ, Davis PG, et al. Early biomarkers and potential mediators of ventilation-induced lung injury in very preterm lambs. Respir Res. 2009;10:19.
35. Bhandari A, Panitch HB. Pulmonary outcomes in bronchopulmonary dysplasia. Semin Perinatol. 2006;30(4):219-26.
36. Angata T, Ishii T, Motegi T, Oka R, Taylor RE, Soto PC, et al. Loss of Siglec-14 reduces the risk of chronic obstructive pulmonary disease exacerbation. Cell Mol Life Sci. 2013;70(17):3199-210.
37. Bland RD, Ertsey R, Mokres LM, Xu L, Jacobson BE, Jiang S, et al. Mechanical ventilation uncouples synthesis and assembly of elastin and increases apoptosis in lungs of newborn mice. Prelude to defective alveolar septation during lung development? Am J Physiol Lung Cell Mol Physiol. 2008;294(1):L3-14.
38. Thibeault DW, Mabry SM, Ekekezie, II, Zhang X, Truog WE. Collagen scaffolding during development and its deformation with chronic lung disease. Pediatrics. 2003;111(4 Pt 1):766-76.

Supplement 1 Study population

Preterm infants with or without later development of BPD and a gestational age (GA) of ≤32 weeks were prospectively included in the study.

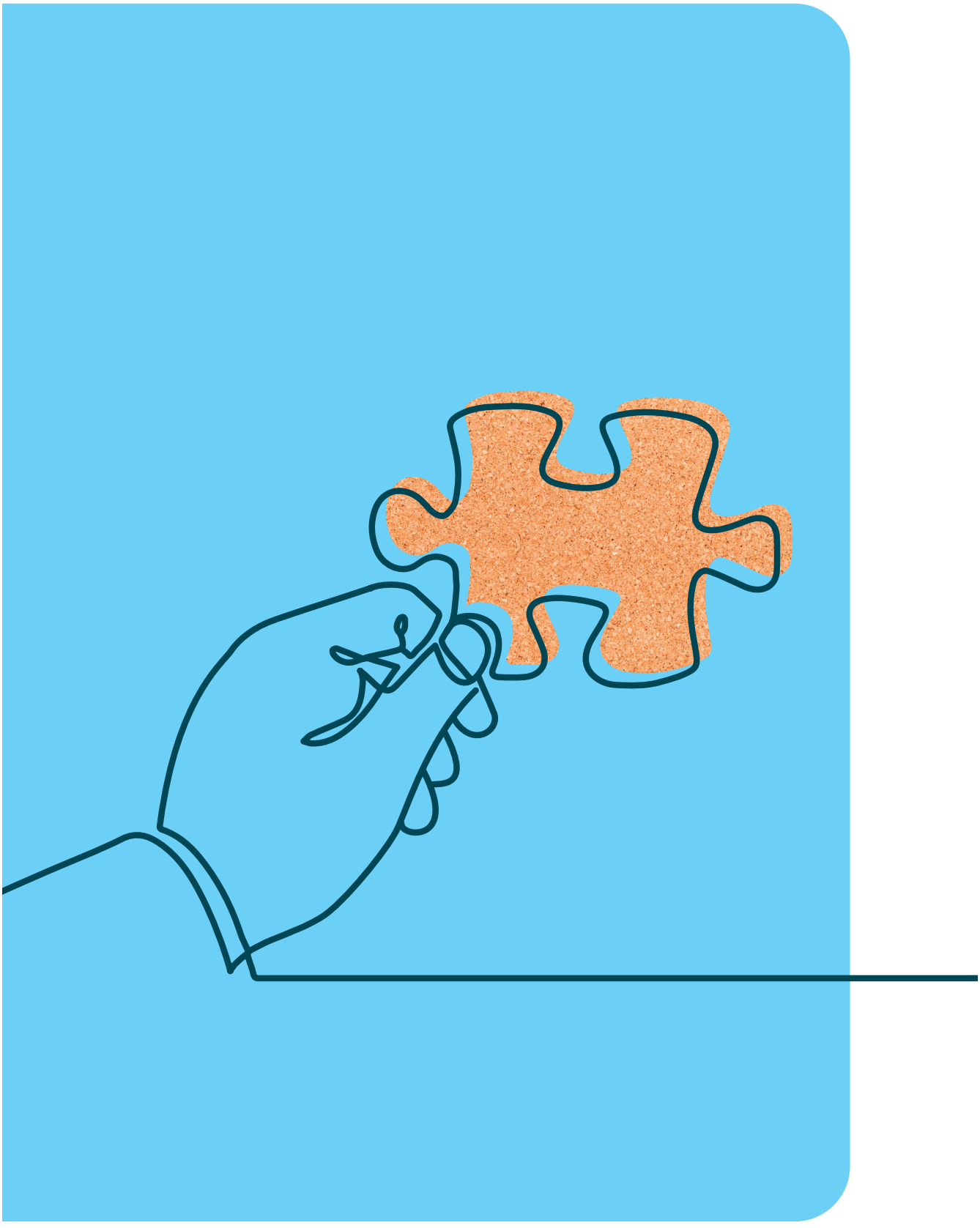
Exclusion criteria were severe congenital malformations (e.g. hypoplastic left-heart syndrome, severe hypoplasia of the lungs or congenital diaphragmatic hernia), chromosomal abnormalities (e.g. trisomy 13 or 18), inborn errors of metabolism, and decision for palliative therapy directly after birth.

The following definitions were applied: BPD was defined according to Jobe and Bancalari and graded as follows: mild (oxygen supplementation at 28 days postnatally), moderate (need for < 30% oxygen at 36 weeks PMA or discharge, whichever comes first), or severe (need for ≥ 30% oxygen and/or ventilator support, i.e. positive pressure ventilation (PPV) or nasal continuous positive airway pressure (NCPAP)) at 36 weeks postmenstrual age or discharge, whichever comes first) BPD. Days with ventilator support were recorded as endotracheal (invasive) mechanical ventilation (IPPV), nasal intermittent mandatory or intermittent positive pressure ventilation (NIPPV) and/or nasal continuous positive airway pressure (CPAP) in days. A course of antenatal corticosteroids was recorded as "complete" if two doses of betamethasone were given >24 hours before birth with the last dose administered no later than 7 days before birth. Chorioamnionitis was defined as the presence of inflammatory alterations of the chorionic plate at histologic examination or signs of infection in both mother and infant. Intrauterine growth restriction was defined as birth weight below the 10th percentile. Postnatally, diagnosis and severity of RDS (respiratory distress syndrome) was scored on anterior-posterior (a.-p.) chest radiographs according to Couchard et al. Systemic infections were diagnosed according to Sherman et al. with one or more clinical and laboratory signs of infection.

Table Supplement 2. Patient characteristics cohort #2 (CDH patients, Rotterdam)

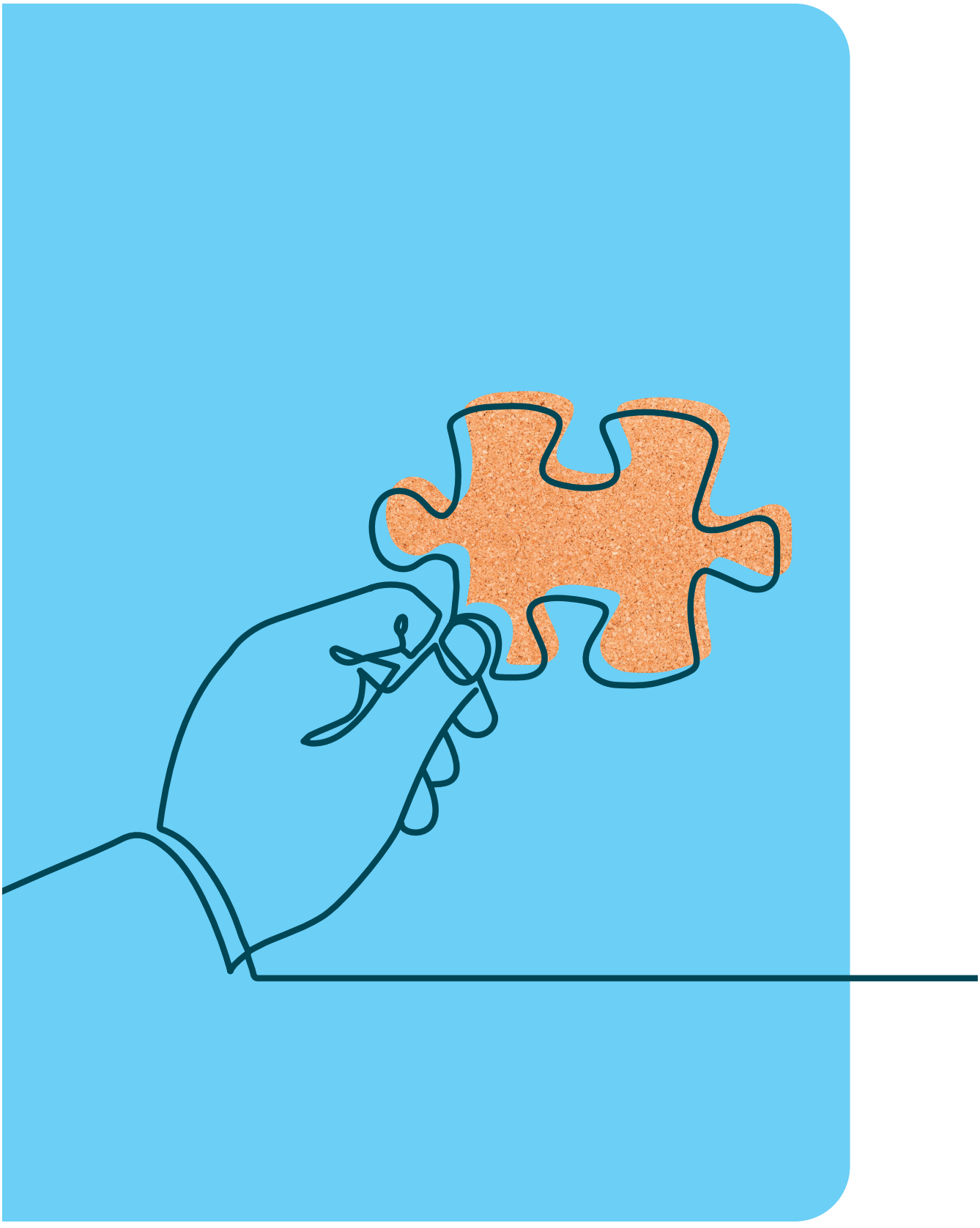
n		21	
GA (weeks)		38.0	(33.6-41.3)
Gender (female/male)		(14/7)	
Death		6	(28.6%)
EOI	no	17	(81%)
	yes	4	(19%)
Mechanical Ventilation	no	8	(38.1%)
	yes	10	(47.6%)
	NA	3	(14.3%)
O_2 on day 28	no	6	(28.6%)
	yes	10	(47.6%)
	NA	2	(9.5%)
Severity CLD	no CLD	6	(28.6%)
	mild CLD	8	(38.1%)
	severe CLD	1	(4.8%)
PH (first echocardiogram)	no	6	(28.6%)
	PH (from 2/3 of syst. pressure)	14	(66.7%)
	NA	1	(4.8%)
ECMO	no	13	(61.9%)
	yes	8	(38.1%)
iNO	no	11	(52.4%)
	yes	10	(47.6%)

Data are given as median and range or number and percent of total in group respective range. CLD, chronic lung disease; ECMO, Extra Corporal Membrane Oxygenation; EOI, early onset infection; GA, gestational age; iNO, inhalation nitric oxide; NA, not available; PH, pulmonary hypertension.



PART III

Therapy



CHAPTER 5

Routine intubation in newborns with congenital diaphragmatic hernia; reconsidering the paradigm

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ABSTRACT

Background and objective: International consensus guidelines advice routine intubation for all neonates with congenital diaphragmatic hernia (CDH) at birth. However, risk of ventilator induced lung injury and the impact on perinatal transition should not be underestimated. We introduced a more personalized perinatal management strategy. We hypothesized that a spontaneous breathing approach (SBA) in the delivery room is justified for newborns with CDH and a low risk of respiratory failure.

Method: An SBA was prenatally planned in patients born after 35 weeks of gestation with an isolated left-sided CDH and an observed-to-expected lung-to-head ratio >50% without liver herniation. At birth these patients were respiratory supported as needed, with a low threshold for intubation. Between December 2014 and July 2019 16/72 (22%) patients received an SBA. We retrospectively evaluated feasibility and safety of an SBA.

Results: The SBA was successful in 7 (44%) patients, 9 (56%) patients were intubated due to respiratory failure and needed mild ventilator support with maximum inspiratory pressures of median 23cmH2O for a median of 7 days. 1 patient developed pulmonary hypertension with need for nitric oxide for 4 days. Survival was 100%.

Conclusions and Relevance: In this relevant subgroup of infants with CDH and favorable prenatal parameters, an SBA is feasible and safe. This personalized perinatal approach avoids overtreatment with potential adverse side effects.

INTRODUCTION

Congenital diaphragmatic hernia (CDH) is a developmental defect of both the diaphragm and the developing lungs, resulting in the herniation of the abdominal organs in the chest cavity. Pulmonary hypoplasia and pulmonary hypertension (PH) often result in respiratory insufficiency immediately at birth (1). CDH occurs in 1 in every 2500 live births, and is associated with an improved survival of approximately 70-80% in live-born patients since the implementation of international treatment guidelines (2-4). According to these consensus guidelines, the key principle during the perinatal stabilization period is avoiding high airway pressures whilst establishing adequate oxygenation and cardiovascular stability (2). Consequently, infants are routinely intubated immediately at birth and gentle ventilation strategies are adopted to prevent ventilator induced lung injury (VILI) (2, 5-7). However, with the improvement of prenatal diagnostics, such as prenatal ultrasound and fetal magnetic resonance imaging (MRI), the severity of pulmonary hypoplasia can be estimated more precisely (8, 9). This enables composing personalized perinatal management strategies. In newborns with left-sided CDH, an observed-to-expected lung-to-head (O/E LHR) over 50% and an intra-abdominal liver position (mild group) assessed by prenatal ultrasound, survival rates exceed 90% (10, 11). For these patients, the current guidelines potentially result in overtreatment, and the risk of VILI and the impact on perinatal transition should not be underestimated (12).

We hypothesized that in this group with relative mild disease, a spontaneous breathing approach (SBA) is feasible and safe during fetal to neonatal transition. In this paper we describe our experience after adjusting our local protocol, allowing a planned SBA.

STUDY DESIGN AND METHODS

We performed a retrospective single center study in newborns with CDH, born at Erasmus University Medical Center Rotterdam, a national and level 3 referral center with extracorporeal membrane oxygenation. This study was approved by the local IRB (MEC2019-714) and informed consent was waived due to the retrospective design of the study.

Our local protocol is based on the adjusted CDH EURO Consortium guidelines (2). Accordingly we modified our protocol in December 2014, allowing planned SBA in patients with mild CDH born >35 weeks of gestation(2). We used the O/E LHR measured between 24 and 38 weeks gestational age(8). Congenital anomalies were defined as anatomic anomalies on prenatal ultrasound or genetic mutations (microarray). We included all consecutive patients born between December 2014 and July 2019 who met these criteria.

The SBA was classified as failed if the infant required intubation any time before elective intubation for surgery. Surgery was planned electively with an experienced CDH operating team.

In our perinatal center, a perinatal treatment plan is made for all patients with CDH in a multidisciplinary team meeting around 32 weeks of gestation, attended by obstetricians, fetal medicine specialists, neonatologists, pediatric intensivists and surgeons. Prior to this, both a fetal medicine specialist and a postnatal specialist counsel the parents and discuss the treatment strategies, including an SBA if applicable. Postnatal resuscitation is executed according to CDH guidelines(2). The newborn is positioned on the resuscitation table and a Replogle tube, 10 French, is inserted for continuous stomach decompression. In case of planned SBA, the infant is supported with oxygen if necessary (Neopuff™ infant T-piece resuscitator, Fisher&Paykel Healthcare Ltd), aiming for preductal saturations >85%(2). Continuous positive airway pressure (CPAP) is allowed. The infant is intubated if insufflation breaths or ventilation are needed, since positive pressure ventilation via mask increases the air in the digestive tract, subsequently compressing the lungs, resulting in hypoxia and PH.

Maximum oxygen need on the ventilator was defined as oxygen need after stabilization in the delivery room. During the initial treatment in the delivery room, often 100% oxygen is given, and per protocol this is decreased slowly. Therefore we used a cut-off point of 3 hours after delivery for the highest oxygen need.

Patient characteristics and outcome parameters were described as either absolute numbers or percentages for categorical data, or median (interquartile range; IQR) for continuous data. The Mann Whitney U test was used to compare patients in whom SBA was successful with patients who required intubation.

RESULTS

During the study period 71 newborns with CDH were treated in our hospital. Of those, 18 (25%) patients fulfilled the criteria for an SBA, but in 3 patients this was not prenatally planned and therefore not performed (figure1). Patient characteristics of the 16 patients that received an SBA are shown in table 1. At a median of 32 weeks and 1 day (IQR 30+4-34+1), the O/E LHR used in the multidisciplinary team meeting, was measured. This O/E LHR was median 57% (52-74-9).

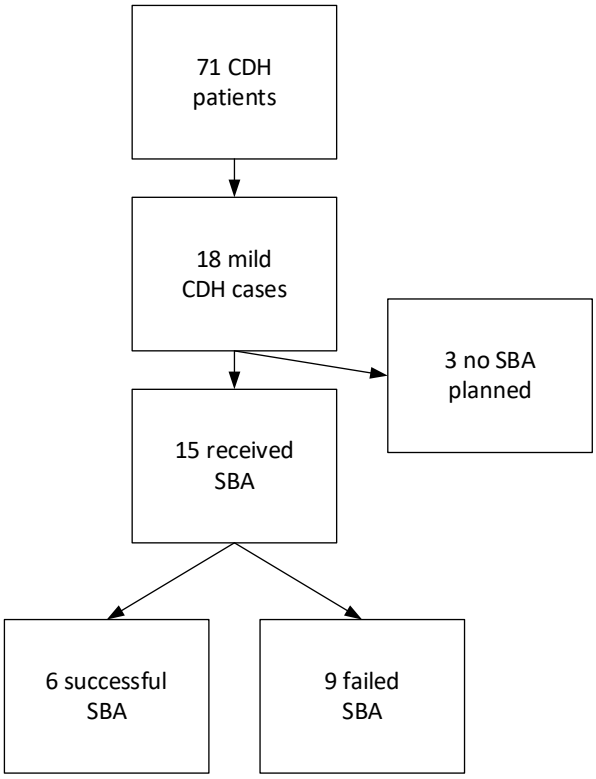


Figure 1 Patient flow chart

CDH= congenital diaphragmatic hernia, SBA= spontaneous breathing approach

SBA was successful in 6 out of 15 patients (40%); 3 required CPAP for several minutes, and 5 were transferred to the unit with binasal cannulae (Intersurgical), 1-2 liters FiO2 30-40%. All were electively intubated for surgery. 9 out of 15 patients required intubation after birth: 7 at birth, 2 several hours after birth in the intensive care unit. 8 of these intubated patients received relatively mild ventilation with a median peak pressure of 23cmH2O (IQR 19.5-25), they were extubated shortly after the surgical procedure with a median of 3.5 days (IQR 1.5-5). Only one patient (O/E LHR 57%) developed PH and was treated with inhaled nitric oxide for 4 days and oxygen supplement therapy for 28 days. Apart from the anticipated difference in ventilation days and duration of oxygen therapy, there were no clinical differences between patients with successful and failed SBA (table 1). The overall survival was 100%.

Table 1 Patients with and without successful SBA

	Successful SBA (n=6) Median (IQR) / %	Failed SBA (n=9) Median (IQR)	p-value
Male gender	50%	78%	
Birth weight (kg)	2.78 (2.38-3.22)	3.0 (2.85-3.20)	0.24
Apgar score 1 minute	7.5 (5.8-8)	6.5 (4.3-7.8)	0.41
Apgar score 5 minutes	8 (8-9.3)	7 (7-8.8)	0.18
Gestational age at birth (weeks)	37.8 (37.0-38.5)	38.3 (37.9-38.6)	0.37
O/E LHR (%)	66 (49.8-82.3)	55 (52-64.5)	0.56
Peak ventilator pressure* (cm H2O)	23.5 (21.5-27)	23 (19.5-25)	0.37
VIS score*	0 (0-18.8)	4.6 (0-15.5)	0.57
Days on ventilator	1 (1-2.5)	7 (4-10)	**<0.05**
Day of surgery	3 (2-4)	3 (2-5)	1.0
Defect type			1.0
A	0	2 (22%)	
B	2 (33%)	5 (56%)	
C	1 (17%)	0	
D	0	0	
missing	3 (50%)	2 (22%)	
Patch repair	2 (33%)	5 (56%)	0.53
Days on ventilator after surgery	1 (1-2.5)	4 (2-5.5)	**0.05**
Total oxygen therapy (days)	4.5 (2.5-7)	15 (5-17)	**<0.05**
Discharge from ICU in days	6 (5-10.75)	18 (7.5-25)	**<0.05**
Discharge home in days	18 (9-31.5)	28 (13.5-45)	0.44
Medical support at discharge**			
None	4 (66%)	4 (44%)	0.7
G-tube feeding	2 (33%)	5 (56%)	

Abbreviations: ICU= intensive care unit, O/E LHR= observed-to-expected lung-to-head ratio, SBA= spontaneous breathing approach, VIS= vasoactive inotropic support score, IQR= interquartile range *recorded continuously during ICU admission, **defined as ventilator, oxygen, pharmaceutical, G-tube feeding

DISCUSSION

In this study we showed that, in a selected but relevant (20-25 %) group of newborns with CDH, an SBA is feasible and safe. To our knowledge, this is the first study to report a more conservative approach in this subset of infants as suggested by Snoek *et al* and Morini *et al* (2, 13). This fits in a more personalized approach in the delivery room and improves postnatal parent-infant interaction.

The lungs of newborns with CDH are known to be hypoplastic, characterized by decreased airway branches, fewer alveoli, and excessive muscularization of the arterioles (14, 15). As a result, most newborns with CDH are respiratory insufficient directly after birth. Mortality and morbidity in patients with CDH is still substantial, but has decreased considerably since the introduction of international consensus guidelines (2, 5, 16). In these guidelines all patients with CDH are immediately intubated and ventilated after birth. One of the keystones of these guidelines is gentle ventilation to prevent lung damage of the hypoplastic lung and to decrease the negative impact of positive intrathoracic pressure on pulmonary perfusion (2, 5-7). Despite the improvement of care, the prognosis of patients with CDH is still highly variable, depending on the amount of lung hypoplasia and the presence of PH. Prenatal parameters, such as O/E LHR and liver position, are considered useful predictors for the severity of lung hypoplasia and mortality in these patients (11).

In patients with an O/E LHR of 50% or higher, and an abdominal position of the liver, survival is high (11, 17). For this subgroup of patients with mild CDH, intubation and mechanical ventilation immediately after birth has potential unnecessary side effects. Positive pressure ventilation can cause lung injury due to a combination of barotrauma, volutrauma, atelectrauma and hyperoxia (13).

It has long been recognized that gentle ventilation strategies are essential to prevent barotrauma. Already in 1981, Srouji *et al* showed that the incidence of pneumothorax and interstitial emphysema in patients with CDH was highly associated with ventilation (18). With gentle ventilation, using lower peak pressures, this has decreased substantially (19). However, in preterm infants, the prevention of invasive ventilation altogether, decreases the incidence of bronchopulmonary dysplasia (BPD) and mortality substantially (20). In addition, the duration of ventilation in these patients is directly associated with BPD (21). In patients with CDH the incidence of chronic lung disease (CLD), defined as oxygen dependence beyond 28 days of life, is approximately 30% (4). Although the etiology of BPD in preterm infants and CLD in children with CDH is different, both patient groups have vulnerable lungs, and gentle ventilation has improved outcome considerably (13). Hence, we speculated that in infants with mild disease, mechanical ventilation potentially inflicts damage in lungs that actually have sufficient capacity for adequate gas-exchange. An SBA has the potential to prevent CLD in patients with mild patients. In our cohort only one patient developed mild CLD, with oxygen need until day 28, and overall survival was 100%. Although these are promising results, our results should be interpreted with caution as our cohort consists of a small selected population with a high a-priori chance of survival without sequelae.

Lung injury can also occur when the alveoli and bronchioles collapse and reopen with every breath as the patient ventilates below the alveolar opening pressure. This has

been described in premature infants and is associated with the absence of surfactant (22). Although the lungs of patients with CDH are abnormal, there is no surfactant deficiency at birth (23). However, insufficient respiration can enhance the development of atelectasis. In our cohort, we prevented atelectrauma by intubating the patients as soon as their breathing pattern was insufficient. In case of dyspnea, PEEP was given for several minutes to guarantee adequate airway opening, and if needed the patient was intubated subsequently.

Oxygen induced lung injury can be caused by hyperoxia, since high alveolar oxygen concentrations cause pulmonary edema, and increase inflammatory markers and cell necrosis (24). In term infants the use of oxygen in the delivery room has decreased remarkably, since the use of FiO_2 1.0 during neonatal resuscitation has been associated with increased mortality (25). High oxygen concentrations are still often administered in patients with CDH, although the target range for preductal SpO_2 in the delivery room is 80-95% (2). There are no prospective trials in patients with CDH, evaluating oxygen management in the delivery room. A small retrospective trial in CDH patients compared the use of FiO_2 1.0 with the use of FiO_2 0.5 at the start of resuscitation and found no adverse effects using less oxygen. However, the need to increase the FiO_2 was associated with an adverse outcome (26). In lambs the use of FiO_2 1.0 in initial ventilation reduced the vasodilator response to iNO (27). iNO is the therapy of first choice in CDH patients with PH, but the use of FiO_2 1.0 potentially reduces its effectiveness (2, 28). Therefore, the role of oxygen in the resuscitation of newborns with CDH needs to be evaluated. In our population, oxygen was administered via the Neopuff or via nasal prongs with FiO_2 1.0. Although the Neopuff is probably very sufficient in delivering oxygen, actual FiO_2 via nasal prongs is substantially lower, preventing hyperoxia (29, 30).

Patients with CDH not only have a highly variable degree of lung hypoplasia, but also have a higher risk of PH due to abnormal vascularization of the lungs, causing a delayed transition from the fetal to the neonatal circulation and an increase in vascular reactivity. After birth, lung aeriation is the main trigger to decrease pulmonary vascular resistance and subsequently increase pulmonary blood flow (31). Hypoplastic lungs have a low compliance and an impaired clearance of liquid resulting in a delay in lung aeration(31). As such, this often results in transient hypoxia and hypotension after birth, only to be mitigated by the immediate initiation of respiratory support. Therefore, only patients with favorable prenatal parameters, suggesting adequate lung size, are potential candidates for an SBA. In these patients an SBA potentially leads to a more physiological fetal to neonatal transition. Immediate intubation after birth might trigger PH as this is a stressful event, and although it is preferable to adequately sedate the infant prior to intubation, this is not always feasible (2). Also, positive intrathoracic pressure has a negative impact on pulmonary perfusion, and the need for sedation has an adverse effect on the systemic

blood pressure, potentially increasing the right-to-left shunt over the open ductus arteriosus and further decreasing pulmonary perfusion. On the other hand, airway collapse is known to trigger PH (32). Therefore it is essential to closely monitor respiratory function when performing an SBA to recognize early signs of progressive insufficiency. In our cohort, only one patient developed PH, for which iNO was given for a limited number of days. Other therapeutic strategies in the delivery room, such as physiological based cord clamping, might also play an important role in the reduction of pulmonary vascular resistance directly after birth (33, 34). A clinical trial to investigate the role of physiological cord clamping in newborns with CDH is on its way (NTR7853).

In this study we selected CDH patients with the most favorable predicted outcome. Both right-sided CDH and an intra-thoracic position of the liver decreases survival substantially (35, 36). Other associated abnormalities, such as cardiac anomalies and chromosomal anomalies, independently influence outcome of patients with CDH negatively, and were therefore also excluded from the SBA (37). However, in more than half of our patients the SBA was not successful, even though prenatal parameters were equal in both the successful and the unsuccessful group. Also, one patient developed PH even though the prenatal parameters were reassuring. Although the prenatal prediction of lung hypoplasia seems reliable, the prediction of PH is not (38). In our study we used the tracing method to calculate the O/E LHR on prenatal ultrasound for prediction of pulmonary hypoplasia. This is the most reliable measurement technique of O/E LHR with a relatively low inter-observer-variability (39, 40). Possibly, the use of total fetal lung volume measurement on MRI can increase the accuracy of predicting lung hypoplasia and PH (41). However, in our setting, no MRI images were made. Although we could not reliably predict the success of an SBA, its failure did not seem to negatively affect the outcome in our cohort, since only one of the patients developed mild CLD and none died. However, a larger prospective trial is needed to validate these results. We believe it is essential for a multidisciplinary team to prenatally plan the delivery and for the delivery to take place in an experienced center. Close monitoring of the newborn, evaluating the respiratory effort and supporting breathing invasively when necessary, are essential. In 3 patients, no SBA was planned and therefore it was not performed in the delivery room. Although the reason for this was not reported, we hypothesize that this was due to the recent change of the protocol in 2015.

CONCLUSION

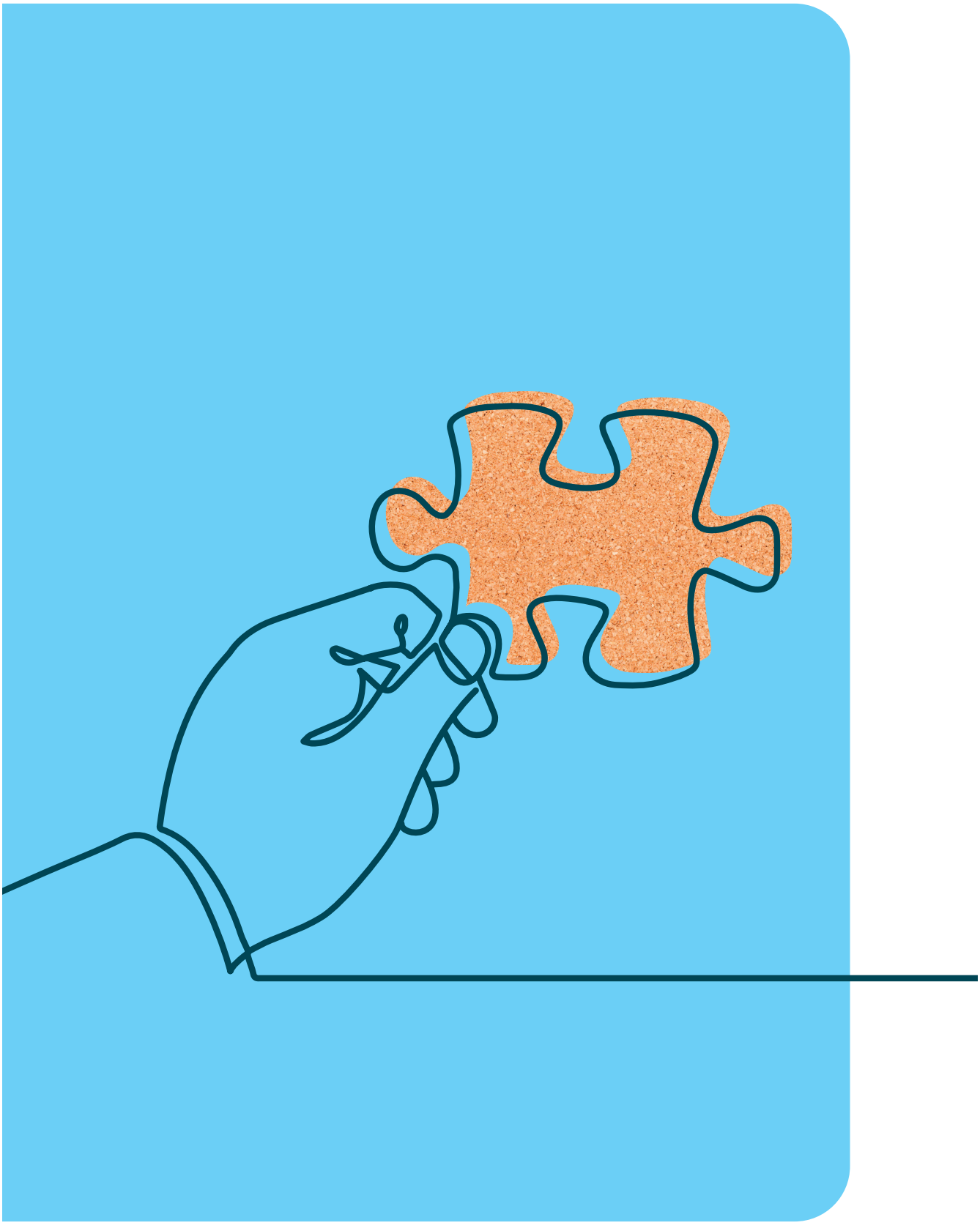
In a selected subgroup of patients with CDH and favorable prenatal parameters, a planned SBA is feasible and fits in a more personalized approach in the delivery room. In the setting of an experienced multidisciplinary team, an SBA safe and avoids overtreatment with potential adverse side effects.

LITERATURE

1. Robinson PD, Fitzgerald DA. Congenital diaphragmatic hernia. Paediatr Respir Rev. 2007;8(4):323-34; quiz 34-5.
2. Snoek KG, Reiss IK, Greenough A, Capolupo I, Urlesberger B, Wessel L, et al. Standardized Postnatal Management of Infants with Congenital Diaphragmatic Hernia in Europe: The CDH EURO Consortium Consensus - 2015 Update. Neonatology. 2016;110(1):66-74.
3. Reiss I, Schaible T, van den Hout L, Capolupo I, Allegaert K, van Heijst A, et al. Standardized postnatal management of infants with congenital diaphragmatic hernia in Europe: the CDH EURO Consortium consensus. Neonatology. 2010;98(4):354-64.
4. Snoek KG, Capolupo I, van Rosmalen J, Hout Lde J, Vijfhuize S, Greenough A, et al. Conventional Mechanical Ventilation Versus High-frequency Oscillatory Ventilation for Congenital Diaphragmatic Hernia: A Randomized Clinical Trial (The VICI-trial). Ann Surg. 2016;263(5):867-74.
5. Storme L, Boubnova J, Mur S, Pognon L, Sharma D, Aubry E, et al. Review shows that implementing a nationwide protocol for congenital diaphragmatic hernia was a key factor in reducing mortality and morbidity. Acta Paediatr. 2017.
6. Canadian Congenital Diaphragmatic Hernia C. Diagnosis and management of congenital diaphragmatic hernia: a clinical practice guideline. CMAJ. 2018;190(4):E103-E12.
7. Jancelewicz T, Brindle ME, Guner YS, Lally PA, Lally KP, Harting MT, et al. Toward Standardized Management of Congenital Diaphragmatic Hernia: An Analysis of Practice Guidelines. J Surg Res. 2019;243:229-35.
8. Snoek KG, Peters NCJ, van Rosmalen J, van Heijst AFJ, Eggink AJ, Sikkel E, et al. The validity of the observed-to-expected lung-to-head ratio in congenital diaphragmatic hernia in an era of standardized neonatal treatment; a multicenter study. Prenat Diagn. 2017;37(7):658-65.
9. Oluyomi-Obi T, Kuret V, Puligandla P, Lodha A, Lee-Robertson H, Lee K, et al. Antenatal predictors of outcome in prenatally diagnosed congenital diaphragmatic hernia (CDH). J Pediatr Surg. 2017;52(5):881-8.
10. Deprest J, Brady P, Nicolaides K, Benachi A, Berg C, Vermeesch J, et al. Prenatal management of the fetus with isolated congenital diaphragmatic hernia in the era of the TOTAL trial. Semin Fetal Neonatal Med. 2014;19(6):338-48.
11. Snoek KG, Peters NCJ, van Rosmalen J, van Heijst AFJ, Eggink AJ, Sikkel E, et al. The validity of the observed-to-expected lung-to-head ratio in congenital diaphragmatic hernia in an era of standardized neonatal treatment; a multicenter study. Prenat Diagn. 2017.
12. Dumpa V, Bhandari V. Surfactant, steroids and non-invasive ventilation in the prevention of BPD. Semin Perinatol. 2018;42(7):444-52.
13. Morini F, Capolupo I, van Weteringen W, Reiss I. Ventilation modalities in infants with congenital diaphragmatic hernia. Semin Pediatr Surg. 2017;26(3):159-65.
14. George DK, Cooney TP, Chiu BK, Thurlbeck WM. Hypoplasia and immaturity of the terminal lung unit (acinus) in congenital diaphragmatic hernia. Am Rev Respir Dis. 1987;136(4):947-50.

15. Mous DS, Buscop-van Kempen MJ, Wijnen RMH, Tibboel D, Rottier RJ. Changes in vasoactive pathways in congenital diaphragmatic hernia associated pulmonary hypertension explain unresponsiveness to pharmacotherapy. Respir Res. 2017;18(1):187.
16. Snoek KG, Greenough A, van Rosmalen J, Capolupo I, Schaible T, Ali K, et al. Congenital Diaphragmatic Hernia: 10-Year Evaluation of Survival, Extracorporeal Membrane Oxygenation, and Foetoscopic Endotracheal Occlusion in Four High-Volume Centres. Neonatology. 2018;113(1):63-8.
17. Deprest JA, Flemmer AW, Gratacos E, Nicolaides K. Antenatal prediction of lung volume and in-utero treatment by fetal endoscopic tracheal occlusion in severe isolated congenital diaphragmatic hernia. Semin Fetal Neonatal Med. 2009;14(1):8-13.
18. Srouji MN, Buck B, Downes JJ. Congenital diaphragmatic hernia: deleterious effects of pulmonary interstitial emphysema and tension extrapulmonary air. J Pediatr Surg. 1981;16(1):45-54.
19. Wung JT, Sahni R, Moffitt ST, Lipsitz E, Stolar CJ. Congenital diaphragmatic hernia: survival treated with very delayed surgery, spontaneous respiration, and no chest tube. J Pediatr Surg. 1995;30(3):406-9.
20. Subramaniam P, Ho JJ, Davis PG. Prophylactic nasal continuous positive airway pressure for preventing morbidity and mortality in very preterm infants. Cochrane Database Syst Rev. 2016(6):CD001243.
21. Jensen EA, DeMauro SB, Kornhauser M, Aghai ZH, Greenspan JS, Dysart KC. Effects of Multiple Ventilation Courses and Duration of Mechanical Ventilation on Respiratory Outcomes in Extremely Low-Birth-Weight Infants. JAMA Pediatr. 2015;169(11):1011-7.
22. Fujiwara T, Maeta H, Chida S, Morita T, Watabe Y, Abe T. Artificial surfactant therapy in hyaline-membrane disease. Lancet. 1980;1(8159):55-9.
23. Boucherat O, Benachi A, Chailley-Heu B, Franco-Montoya ML, Elie C, Martinovic J, et al. Surfactant maturation is not delayed in human fetuses with diaphragmatic hernia. PLoS Med. 2007;4(7):e237.
24. Davis JM, Penney DP, Notter RH, Metlay L, Dickerson B, Shapiro DL. Lung injury in the neonatal piglet caused by hyperoxia and mechanical ventilation. J Appl Physiol (1985). 1989;67(3):1007-12.
25. Saugstad OD, Ramji S, Soll RF, Vento M. Resuscitation of newborn infants with 21% or 100% oxygen: an updated systematic review and meta-analysis. Neonatology. 2008;94(3):176-82.
26. Riley JS, Antiel RM, Rintoul NE, Ades AM, Waqar LN, Lin N, et al. Reduced oxygen concentration for the resuscitation of infants with congenital diaphragmatic hernia. J Perinatol. 2018.
27. Lakshminrusimha S, Russell JA, Steinhorn RH, Swartz DD, Ryan RM, Gugino SF, et al. Pulmonary hemodynamics in neonatal lambs resuscitated with 21%, 50%, and 100% oxygen. Pediatr Res. 2007;62(3):313-8.
28. Putnam LR, Tsao K, Morini F, Lally PA, Miller CC, Lally KP, et al. Evaluation of Variability in Inhaled Nitric Oxide Use and Pulmonary Hypertension in Patients With Congenital Diaphragmatic Hernia. JAMA Pediatr. 2016.
29. Wettstein RB, Shelledy DC, Peters JI. Delivered oxygen concentrations using low-flow and high-flow nasal cannulas. Respir Care. 2005;50(5):604-9.

30. Ellsworth L, Meerkov M, Doglioni N, Trevisanuto D, Weiner G. Oxygen supplementation in the delivery room: T-piece resuscitator cap open or occluded? J Perinatol. 2019;39(8):1078-80.
31. Flemmer AW, Thio M, Wallace MJ, Lee K, Kitchen MJ, Kerr L, et al. Lung hypoplasia in newborn rabbits with a diaphragmatic hernia affects pulmonary ventilation but not perfusion. Pediatr Res. 2017;82(3):536-43.
32. Creamer KM, McCloud LL, Fisher LE, Ehrhart IC. Ventilation above closing volume reduces pulmonary vascular resistance hysteresis. Am J Respir Crit Care Med. 1998;158(4):1114-9.
33. Kashyap AJ, Hodges RJ, Thio M, Rodgers KA, Amberg BJ, McGillick EV, et al. Physiologically based cord clamping improves cardiopulmonary haemodynamics in lambs with a diaphragmatic hernia. Arch Dis Child Fetal Neonatal Ed. 2019.
34. Lefebvre C, Rakza T, Weslinck N, Vaast P, Houfflin-Debarge V, Mur S, et al. Feasibility and safety of intact cord resuscitation in newborn infants with congenital diaphragmatic hernia (CDH). Resuscitation. 2017;120:20-5.
35. DeKoninck P, Gomez O, Sandaite I, Richter J, Nawapun K, Eerdekens A, et al. Right-sided congenital diaphragmatic hernia in a decade of fetal surgery. BJOG. 2015;122(7):940-6.
36. Cordier AG, Russo FM, Deprest J, Benachi A. Prenatal diagnosis, imaging, and prognosis in Congenital Diaphragmatic Hernia. Semin Perinatol. 2019:51163.
37. Slavotinek AM. The genetics of common disorders - congenital diaphragmatic hernia. Eur J Med Genet. 2014;57(8):418-23.
38. Russo FM, Eastwood MP, Keijzer R, Al-Maary J, Toelen J, Van Mieghem T, et al. Lung size and liver herniation predict need for extracorporeal membrane oxygenation but not pulmonary hypertension in isolated congenital diaphragmatic hernia: systematic review and meta-analysis. Ultrasound Obstet Gynecol. 2017;49(6):704-13.
39. Jani JC, Peralta CF, Nicolaides KH. Lung-to-head ratio: a need to unify the technique. Ultrasound Obstet Gynecol. 2012;39(1):2-6.
40. Abbasi N, Ryan G, Johnson A, Cortes MS, Sangi-Haghpeykar H, Ye XY, et al. Reproducibility of fetal lung-to-head ratio in left diaphragmatic hernia across the North American Fetal Therapy Network (NAFTNet). Prenat Diagn. 2019;39(3):188-94.
41. Kastenholz KE, Weis M, Hagelstein C, Weiss C, Kehl S, Schaible T, et al. Correlation of Observed-to-Expected MRI Fetal Lung Volume and Ultrasound Lung-to-Head Ratio at Different Gestational Times in Fetuses With Congenital Diaphragmatic Hernia. AJR Am J Roentgenol. 2016;206(4):856-66.



CHAPTER 6

Pharmacokinetic modeling of intravenous sildenafil in newborns with congenital diaphragmatic hernia

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ABSTRACT

Purpose: We developed a pharmacokinetic model of intravenous sildenafil in newborns with congenital diaphragmatic hernia (CDH) to achieve a target plasma concentration of over 50 µg/l.

Methods: 23 CDH newborns with pulmonary hypertension (64 blood samples) received intravenous sildenafil. Patients received a loading dose of 0.35mg/kg (IQR 0.16mg/kg) for 3hours, followed by a continuous infusion of 1.5mg/kg/day (IQR 0.1mg/kg/day). For model development Non Linear Mixed Modelling was used. Inter-individual variability (IIV) and inter-occasion variability were tested. Demographic and laboratory parameters were evaluated as covariates. Normalized prediction distribution errors (NPDE) and Visual Predictive Check (VPC) were used for model validation.

Results: A two-compartment disposition model of sildenafil and a one-compartment disposition model of desmethylsildenafil (DMS) was observed with IIV in sildenafil and DMS clearance, and volume of distribution of sildenafil. NPDE and VPC revealed adequate predictability. Only postnatal age increased sildenafil clearance. This was partly compensated by a higher DMS concentration, which also has a therapeutic effect. In this small group of patients sildenafil was tolerated well.

Conclusions: This model for sildenafil in CDH patients shows that concentration targeted sildenafil dosing of 0.4mg/kg in 3 hours, followed by 1.6mg/kg/day continuous infusion achieves appropriate sildenafil plasma levels.

INTRODUCTION

Congenital diaphragmatic hernia (CDH) is a developmental defect of the diaphragm with abnormal lung development and pulmonary vasculature growth, resulting in pulmonary hypertension (PH) (1). CDH is associated with a reported mortality of approximately 27% in live-born patients since the implementation of international treatment guidelines (2-4). PH is one of the most important risk factors for poor outcome in infants with CDH (1, 5, 6).

During fetal life, high resistance in the pulmonary circulation is normal and causes most of the blood flow to bypass the lungs through the ductus arteriosus. As part of normal transition, the pulmonary vascular resistance drops immediately after birth, and the blood flow through the lung vasculature increases significantly. Normal values of pulmonary vascular pressures, similar to healthy adults, are usually reached around the age of two months (7). In infants with CDH, the pulmonary vascular resistance often does not drop adequately, due to increased vascular reactivity, excessive muscularization of the pulmonary arterioles and increased thickness of the arterial media and adventitia (8).

Intravenous sildenafil, a phosphodiesterase 5 (PDE5) inhibitor that increases cyclic guanosine monophosphate (cGMP) resulting in pulmonary vasodilation, is increasingly used in infants with CDH, with promising results (9-11). In newborns with persistent pulmonary hypertension of the newborn (PPHN) without CDH, sildenafil improves oxygenation index (OI) and survival (12). However, data on optimal dosing, pharmacokinetics (PK) and dynamics are scarce in newborns, and CDH patients are often excluded from trials. PK of intravenous sildenafil in term neonates with PPHN, using a two-compartment model, shows a threefold increase in sildenafil clearance and its active metabolite, desmethylsildenafil (DMS) in the first week of life (13). DMS has a 50% potency compared to sildenafil (14). Volume of distribution was fourfold higher than in adults, resulting in a longer half-life (13).

Also, long term safety data are scarce (15). In a study evaluating safety using a dose-escalating strategy, intravenous sildenafil was well tolerated and it improved oxygenation when using a higher infusion dose. With a 3 hours loading dose of 0.4mg/kg and a continuous infusion of 1.6mg/kg/day, target plasma concentration was achieved without causing hypotension (16).

As PH is a major determent of survival in patients with CDH, data on the PK and pharmacodynamics of sildenafil are urgently needed. The objective of this study was to develop a PK model and dosing regimen for sildenafil in CDH patients.

METHODS

Study design

This open label study was conducted in two level 3 referral centers for CDH in Germany and the Netherlands between November 2013 and September 2015. Local ethical review boards approved the protocol. Twenty-three newborns with CDH and clinical signs of PH were treated with intravenous sildenafil. Patients received a loading dose of 0.35mg/kg (IQR 0.16mg/kg) for 3 hours, followed by a continuous infusion of 1.5mg/kg/day (IQR 0.1mg/kg/day). However, many different regimens were being used; ten patients received a loading dose of 0.4mg/kg in 3 hours, followed by continuous infusion of 1.6mg/kg/day, in one patient the infusion was very slowly increased over time, starting with 0.2mg/kg/day, in others no loading dose was given and a continuous infusion was started, ranging from 1.4mg/kg/day to 4mg/kg/day. Target plasma sildenafil levels over 50 µg/l were aimed for, assuming that the same target range is applicable in CDH patients compared to other causes of neonatal PH (16). DMS was not added to calculate this target range, because the target range for DMS is unknown and DMS was also not taken into account in the study of Steinhorn et al (16). The patients were treated as per protocol according to international consensus (3). Patient and baseline characteristics of the 23 patients are provided in the supplemental table 1. All patients received inotropic drug support and were treated with inhaled nitric oxide (iNO). One patient started treatment with bosentan in the second week of life. Thirteen patients needed extracorporeal membrane oxygenation (ECMO) (median time of start 8:15h after birth, IQR 9:39h), of whom two were already on ECMO before sildenafil initiation, overall mortality was 26%.

Primary outcomes were the PK of sildenafil and its active metabolite, DMS. Safety data were collected as secondary outcome variables. Oxygenation Index (OI) was used to evaluate the clinical effect of sildenafil on PH. Hypotension was defined as mean blood pressure lower then gestational age in weeks. The Vasoactive Inotropic Score (VIS) was used to evaluate cardiovascular tolerance. This is a scoring system used for the amount of inotropic support needed and is negatively associated with long term outcome (17). OI and VIS were analyzed using linear regression analysis.

Laboratory analysis

We used 50 µl EDTA blood and 200 µl internal standard solution (vardenafil in methanol) was added. This solution was vortexed for 10 seconds and centrifuged for 5 minutes. 200 µl supernatant was added to the insert vial, which was used in the Thermo TQS Vantage LC-MS/MS. Column 2.1x100 mm Waters Acquity CSH C18 1.7um. The mobile phase A consisted of 2mM ammonium acetate in 0.1% formic acid in water. The mobile phase B consisted of 2mM ammonium acetate in 0.1% formic acid in LC-MS methanol. Flow rate was 0.5ml/min. The mobile phase composition changed linearly during analysis in a

percentage mobile phase A (from 80% to 0) and B (from 2% to 100%). Total analysis time was 4 minutes. The injected volume was 10µl. The method was validated according to FDA guidelines between 2-1000 µg/l for sildenafil and 2-500 µg/l for DMS (18).

Population PK modelling

PK analysis was conducted with non-linear mixed effects modelling using NONMEM® version 7.2 (ICON Development Solutions, Ellicott City, MD) and PsN® (version 3.7.6). Pirana software was used as an interface between NONMEM, R (version 3.2.2) and Xpose (version 4). Using NONMEM we could estimate average PK parameters for the population as well as Inter individual variability (IIV), inter occasion variability (IOV), and residual error.

Base model development

One- two- and three-compartment models were tested for sildenafil and DSM, using the first-order conditional estimation method with interaction (FOCE+I). First a structural model for sildenafil was developed. IIV and IOV, with occasion defined per day, were assessed on each parameter using an exponential model. Residual variability was first tested with an additive and proportional error for each component. The parameters for the base model for sildenafil were fixed when the model for the metabolite was developed. In the combined model we estimated all parameters.

Model selection was based on minimum objective function values, parameter precision, error estimates, shrinkage values and visual inspection of the goodness of fit plots. Shrinkage was calculated for all model parameters. A shrinkage value below 20% was considered acceptable (19).

Covariate model development

Demographic and laboratory characteristics including postnatal age, gender, creatinine, urea, aspartate transaminase (ASAT) and alanine transaminase (ALAT), were evaluated as potential model covariates. Allometric scaling was used to account for variability in PK parameters owing to differences in bodyweight (20). Covariates that significantly improved the model in univariate analysis, defined as $p \leq 0.05$, were added to the full model. A backward elimination process was subsequently performed with statistical significance indicated by $p \leq 0.001$. Continuous covariates were normalised to the population median values and incorporated as power model functions (Eq.1). Categorical covariates were transformed to binary covariates and incorporated as shown in Eq.2.

$$\theta_i = \theta_{pop} * \left(\frac{cov_i}{cov_m}\right)^{\theta cov} \quad (1)$$

$$\theta_i = \theta_{pop} * \theta_{cov}{}^{cov_i} \quad (2)$$

With θi being the individual model predicted PK parameter (e.g. clearance) for an individual with covariate value cov_i, θpop being the population estimate for that parameter, cov_m representing the median covariate value and θcov the covariate effect. In the equation for categorical covariates cov_i is either 1 or 0.

Model evaluation

We used multiple procedures to assess the robustness of the parameter estimates and to validate the final model. First of all, a bootstrap resampling method was applied (21). Thousand bootstrap datasets were generated by sampling randomly from the original dataset with replacement. The validity of the model was evaluated by comparing the median values and their corresponding 95% confidence intervals of the bootstrap replicates with the estimates of the original dataset.

Subsequently, the model was validated using both visual predictive check (VPC) by simulating 500 datasets, and a normalized prediction distribution errors (NPDE) analysis (22, 23). The VPCs were prediction corrected and stratified for the covariates that are included in the final model. NPDE is a simulation-based diagnostic which can be used to evaluate models developed on datasets with variable dosing regimens. The analytical value of this method has been previously described by Comets et al (23).

Concentration effect relationship

Drug concentrations were simulated on the time points of blood pressure measurement to find a relationship between the concentration of sildenafil, DMS and hypotension (mean blood pressure lower than gestational age), assuming a 50% activity of DMS compared with sildenafil. The relationship was tested using Mann-Whitney statistical testing.

RESULTS

Sixty-four samples were taken at different time points between 1 and 385 hours after the start of the infusion, 34 of these samples were taken during ECMO. None of the patients received hemofiltration. Median sildenafil level of all patients was 200 (range 42-262) µg/l at 3 hours and increased to 366 (19-506) µg/l at 12 hours. Infants receiving a loading dose of 0.4mg/kg in 3 hours achieved sildenafil levels ranging between 190 and 262µg/l at 3 hours and between 346 and 506µg/l after 12 hours. Median DMS level within the first 12 hours was 20 (range 9-85) µg/l with a slow increase to a median of 65 (range 17-92) µg/l between 24 and 48 hours.

To evaluate the effect of sildenafil on OI and VIS, numbers were too small. However, in only one patient sildenafil infusion was temporarily stopped after one hour, due to

hypotension that could not be sufficiently treated with inotropes. One patient had two episodes of hypotension, after 7 hours and 7 days, for which the sildenafil was decreased for the same reason. This patient also developed pulmonary haemorrhage after 6 days of sildenafil infusion. In both patients, no correlation with high sildenafil levels was seen. No other adverse events were seen.

Base model and covariate analysis

The model included two-compartment disposition of sildenafil and one-compartment disposition of DMS with IIV in both clearance of sildenafil and DMS, and in volume of distribution of sildenafil. The residual error was described with a proportional error model. Allometric scaling with fixed exponents (0.75 CL and Q and 1 for Vd) improved the model. Estimation of the exponent did not result in further improvement.

The base two-compartment model with allometric scaling was used as reference for the covariate analysis. After graphical analysis, the univariate analysis resulted in the following covariates: ECMO, postnatal age, urea, ALAT and weight. With a median age of 2.4 days, postnatal age was the only significant covariate after backward elimination. Increase in age resulted in increased sildenafil clearance, as can be seen in Figure 1. Age is presented in 3 groups: 2, 6 and 10 days. When age increases, the clearance highly increases. If age is increased from 2.4 to 10 days, clearance is increased with a factor of 4 (Table 1). Shrinkage (residual error in the model) is good with regards to clearance. Shrinkage is high for distribution volume, resulting in a large residual error.

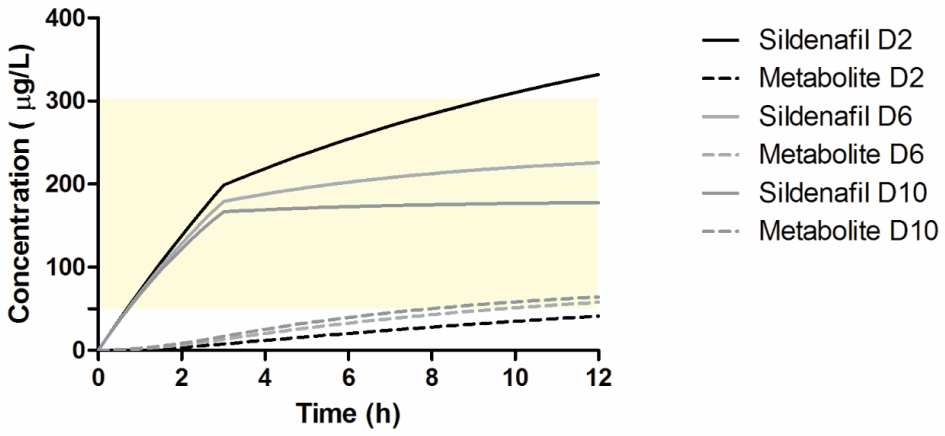


Figure 1: Simulation of the current dose in which 0,4 mg/kg was given in 3 hours, followed by 1.6 mg/kg/day. This leads to concentrations within the therapeutic range. However, in the group with the youngest age (2 days) the concentration is in higher target range. The therapeutic range of 50-300 ug/l is marked in yellow.

Table 1: Parameter estimates of the base model, final model and bootstrap analysis

	Base model	Final model (rse I %)	Bootstrap results Median [95% CI]
OFV	1191	1160	1154 [918-1372]
CL sildenafil (L/h)	6.7	5.2 (19)	5.1 [3.7-7.0]
Age*(days)		0.58 (26)	0.58 [0.3-0.9]
V1 sildenafil (L)	75.1	115 (30)	118 [61-159]
V2 sildenafil (L)	10	10 (na)	NA
Q sildenafil (L/h)	44.3	221 (3)	330 [1.9-13719]
Cl metabolite (L/h)	24.2	25.9 (12)	26.6 [21.2-32.6]
V3 metabolite	1040	366(83%)	287 [79-999]
IIV (%)			
Cl sildenafil	50.3	51.3 (24%)	24 [5-65]
V1 sildenafil	32.2	21.0 (55%)	5 [0-35]
Cl metabolite	37.8	40.5 (25%)	15 [2-51]
Residual variability			
Proportional Cl sildenafil	0.60	0.50(10)	0.5 [0.4-0.6]
Proportional metabolite	0.57	0.44 (10)	0.4 [0.4-0.5]

Cl=Cl(sildenafil)(weight in kg/70)**0.75*EXP(IIV sildenafil)*(AGE/2.4)**0.58, Shrinkage: Base model Cl sildenafil 12%, Cl metabolite 26%, V1 61%; Final model Cl sildenafil 11%, V1 59 % and Cl metabolite 19%

Evaluation of the final model

All estimates were within the limits, given the criteria as defined. Goodness-of-fit plots of the final model showed that the population predictions and individual predictions were evenly distributed around the line of unity when compared with observed concentrations, and the conditional weighted residuals were normally distributed over time (figure 2). A bootstrap analysis with 1000 bootstrap replicates was performed to obtain 95% confidence intervals for all PK parameters.

Owing to minimization and boundary errors, the bootstrap results were recomputed without filtering the samples (1000 runs computed: 495 runs successful). Results of the bootstrap are shown in table 1.

In the VPC a large variation was present. However, the median and the variability were within the corresponding simulations (figure 3). This demonstrates the good predictive performance of the final model in the internal validation. Evaluation of the predictive performance with NPDE analysis showed adequate predictive ability, with distribution of the NPDEs not significantly deviating from a normal distribution (figure 4 in supplement).

Concentration effect relationship

To investigate the relationship between blood pressure and drug concentration, simulations of drug concentrations were performed on the time points of blood pressure measurement. No correlation was seen.

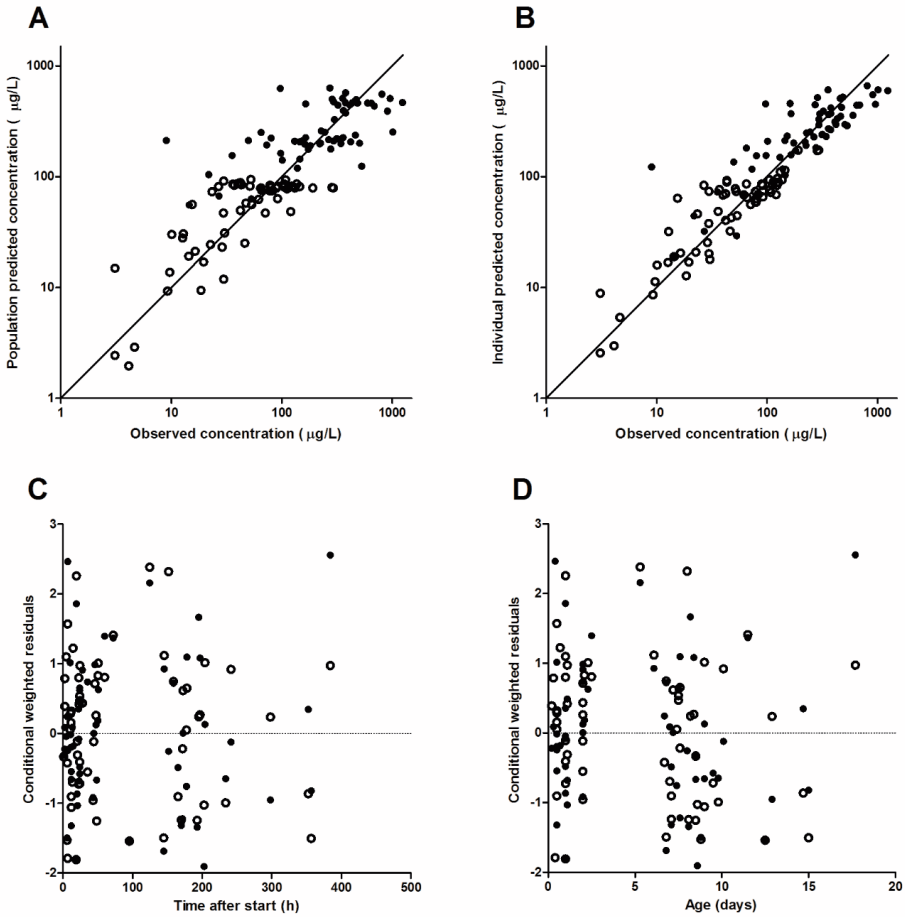


Figure 2: Goodness-of-fit plots of the final model. a) Observed concentration (DV) plotted against predicted concentration (PRED). b) DV plotted against individual predicted concentration (IPRED). C) Conditional weighted residuals versus time after start. D) Conditional weighted residuals versus age.

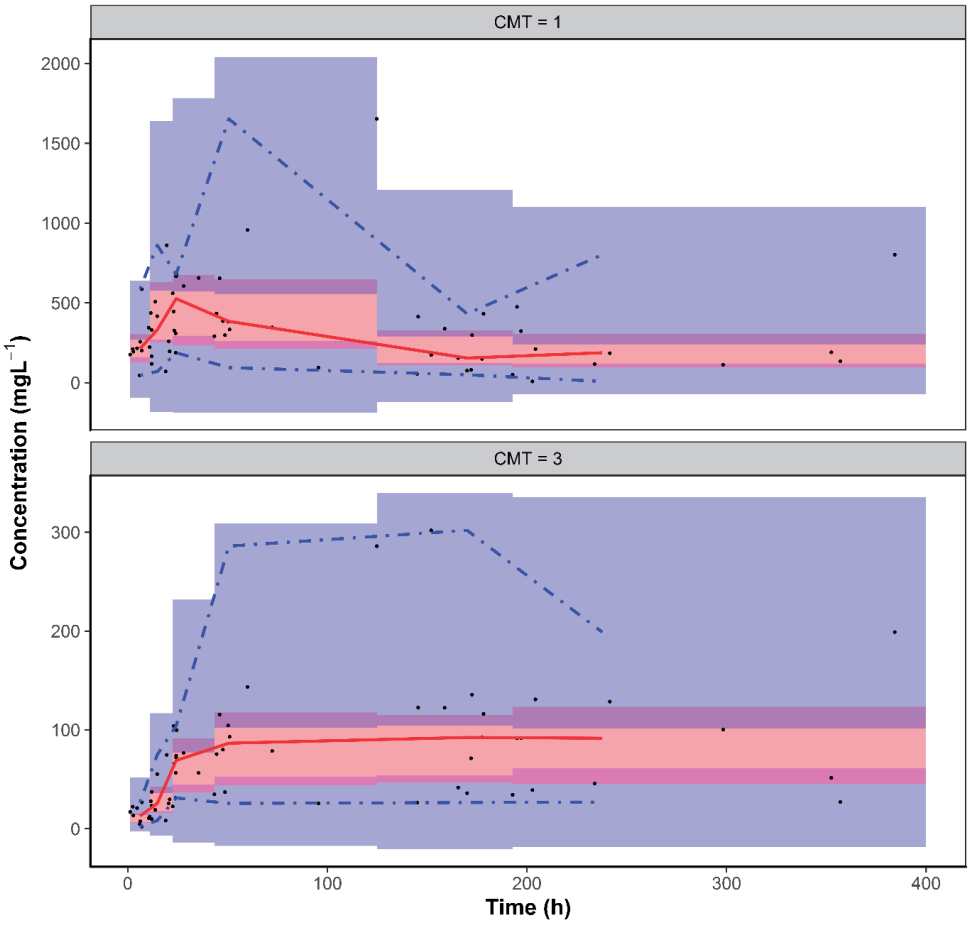


Figure 3: Visual Predictive Check of sildenafil (CMT/compartment 1) and DMS (CMT/compartment 3), showing how well the average trend of the observations (solid line) and how well the variability of the observed data (two dashed lines) fall within the model-simulated average trend (red shaded area) and the model-simulated variability (blue shaded areas) represented as a 95% confidence interval (CI). The average and the variability of the observed data both fall within the corresponding simulations.

DISCUSSION

Although intravenous sildenafil is increasingly used in CDH patients, a structured analysis of PK data was lacking. Therefore, the objective of the study was to develop a PK model and dosing regimen for intravenous sildenafil in newborns with CDH. A loading dose of

0.4mg/kg in 3 hours followed by continuous infusion of 1.6mg/kg/day achieve target sildenafil plasma levels within 3 hours.

Only 5 RCTs with a total of 166 patients, have evaluated the effect of sildenafil in newborns, all excluding patients with congenital anomalies, including CDH. When sildenafil was compared with placebo or MgSO4, a decrease in OI and mortality was seen. When sildenafil was added to inhaled NO there was no difference in outcome (24-28). In CDH patients only retrospective data are available. A decrease in pulmonary vascular resistance index and an increase in cardiac output were found in a small group of oral sildenafil-treated infants with CDH refractory to iNO (9). However, there is a large interpatient variability in pharmacokinetics of oral sildenafil (29). Intravenous sildenafil in CDH patients was associated with improved OI and the reversal of the right-to-left shunt ratio over the ductus arteriosus. However, a significant increase in vasopressor support was also observed (10, 30).

We found that postnatal age increases sildenafil clearance suggesting maturation of the expression of hepatic CYP enzymes involved, as also observed by Mukherjee et al. Sildenafil metabolism is mostly mediated by two enzymes; CYP3A4 and CYP2C9 (31). The activity of both enzymes is very low at birth and increases substantially during the first weeks of life (32-34). Also, the improvement of the clinical condition possibly increases the metabolic activity of the liver (32, 35, 36). The increase in clearance shows a non-linear increase over time and is stronger in younger age as commonly seen in infants (13, 35). This increase in clearance lowers sildenafil plasma levels which is partly compensated with the increase of DMS concentrations in time (figure 1 and figure 5 in supplement).

A loading dose of 0.4mg/kg in 3 hours followed by continuous infusion achieved target sildenafil plasma levels within 3 hours (figure 1). This dosing regime was first described by Steinhorn et al. in a dose-escalating trial in newborns with PPHN in the absence of CDH. Is this small group of 4 patients, sildenafil plasma levels reached 107.78µg/l at 3 hours and 246.28µg/l at 24 hours, and it was well tolerated and improved the OI (16). Our data suggest that there is no major difference in the PK of sildenafil in CDH patients, although our patients reached therapeutic plasma levels earlier, after approximately one hour. As the clinical condition of CDH patients can deteriorate quickly, the ideal dosing regimen reaches therapeutic plasma levels as soon as possible with the least possible side effects. This seems feasible with the dosing regimen we propose. Evaluation of the clinical effects of sildenafil and DMS in CDH patients suggested relative cardiovascular tolerance but a more clear effect could not be seen. With the amount of patients and samples, we could develop a PK model, however, for the secondary outcome parameter numbers were too low. Also, 57% of patients needed ECMO treatment, at that point making the OI invalid to analyse the effect of sildenafil on PH. As these are retrospective data, echocardiography

was not performed at predefined time points, making it impossible to evaluate the effect of sildenafil on PH in these patients. However, in this selected group of critically ill CDH patients with PH mortality was 26%, whereas reported overall mortality for CDH patients is 27%.

There are some limitations of the study. As CDH is a rare disease, 23 patients is a relatively large group, but to test the model and to better achieve patient targeted dosing, prospective external validation is needed. A multicenter RCT, the CoDiNOS trial, has started in Europe, comparing iNO with intravenous sildenafil as first line treatment of PH in CDH patients (NTR6982). In this trial sildenafil and DMS plasma levels will be collected to externally validate the model.

We were not able to retain a significant effect of ECMO on our model. However, considering the increase in circulating volume and the use of an oxygenator one would assume ECMO to be of substantial influence(37, 38). We probably did not find this due to the relatively few samples taken in only 23 patients. We observed both lowered sildenafil levels as well as increased levels when on ECMO. Ahsman et al evaluated the PK of oral sildenafil in infants on ECMO and post-ECMO, and also found contradicting results on the influence of ECMO (29, 39). In comparison to the work of Ahsman, new ECMO systems are used with smaller tubes and filters. We expect that the effect of ECMO can be better evaluated as part of the external validation performed in the CoDiNOS trial (NTR6982). Bosentan is known to decrease sildenafil plasma concentrations, but it was not a significant covariate in our study, because only one patient was co-treated with bosentan (40). Sildenafil loading dose and continuous infusion seems to be tolerated well as in only two patients sildenafil had to be temporarily decreased or interrupted due to hypotension. However, because of the retrospective character of the study, further analysis of the cardiovascular tolerance of sildenafil in this patient group, using VIS, was not possible. Kipfmueller et al found an acute improvement of OI but also the need to increase inotropic support in patients with CDH (30). In a prospective setting these secondary outcome parameters can be evaluated much more reliable.

This is the first study on the PK of intravenous sildenafil in a representative population of CDH patients in need for PH treatment. Using population PK modelling, a sildenafil plasma concentration model was developed with sparse sampling. In conclusion, intravenous sildenafil loading dose of 0.4mg/kg for 3 hours followed by continuous infusion of 1.6mg/kg/day achieves adequate sildenafil plasma levels. Only postnatal age influences its clearance. This dosing regimen was well tolerated in this small group of CDH patients. The current PK model is the first step towards concentration targeted sildenafil dosing in CDH patients. The model will be further validated in the CoDiNOS trial (NTR6982).

LITERATURE

1. Robinson PD, Fitzgerald DA. Congenital diaphragmatic hernia. Paediatr Respir Rev. 2007;8(4):323-34; quiz 34-5.
2. Snoek KG, Reiss IK, Greenough A, Capolupo I, Urlesberger B, Wessel L, et al. Standardized Postnatal Management of Infants with Congenital Diaphragmatic Hernia in Europe: The CDH EURO Consortium Consensus - 2015 Update. Neonatology. 2016;110(1):66-74.
3. Reiss I, Schaible T, van den Hout L, Capolupo I, Allegaert K, van Heijst A, et al. Standardized postnatal management of infants with congenital diaphragmatic hernia in Europe: the CDH EURO Consortium consensus. Neonatology. 2010;98(4):354-64.
4. Snoek KG, Capolupo I, van Rosmalen J, Hout Lde J, Vijfhuize S, Greenough A, et al. Conventional Mechanical Ventilation Versus High-frequency Oscillatory Ventilation for Congenital Diaphragmatic Hernia: A Randomized Clinical Trial (The VICI-trial). Ann Surg. 2016;263(5):867-74.
5. Logan JW, Cotten CM, Goldberg RN, Clark RH. Mechanical ventilation strategies in the management of congenital diaphragmatic hernia. Semin Pediatr Surg. 2007;16(2):115-25.
6. Lally KP. Congenital diaphragmatic hernia. Curr Opin Pediatr. 2002;14(4):486-90.
7. Vali P, Lakshminrusimha S. The Fetus Can Teach Us: Oxygen and the Pulmonary Vasculature. Children (Basel). 2017;4(8).
8. Kool H, Mous D, Tibboel D, de Klein A, Rottier RJ. Pulmonary vascular development goes awry in congenital lung abnormalities. Birth Defects Res C Embryo Today. 2014;102(4):343-58.
9. Noori S, Friedlich P, Wong P, Garingo A, Seri I. Cardiovascular effects of sildenafil in neonates and infants with congenital diaphragmatic hernia and pulmonary hypertension. Neonatology. 2007;91(2):92-100.
10. Bialkowski A, Moenkemeyer F, Patel N. Intravenous sildenafil in the management of pulmonary hypertension associated with congenital diaphragmatic hernia. Eur J Pediatr Surg. 2015;25(2):171-6.
11. Kipfmueller F, Schroeder L, Berg C, Heindel K, Bartmann P, Mueller A. Continuous intravenous sildenafil as an early treatment in neonates with congenital diaphragmatic hernia. Pediatr Pulmonol. 2018;53(4):452-60.
12. Kelly LE, Ohlsson A, Shah PS. Sildenafil for pulmonary hypertension in neonates. Cochrane Database Syst Rev. 2017;8:CD005494.
13. Mukherjee A, Dombi T, Wittke B, Lalonde R. Population pharmacokinetics of sildenafil in term neonates: evidence of rapid maturation of metabolic clearance in the early postnatal period. Clin Pharmacol Ther. 2009;85(1):56-63.
14. Cheitlin MD, Hutter AM, Jr., Brindis RG, Ganz P, Kaul S, Russell RO, Jr., et al. ACC/AHA expert consensus document. Use of sildenafil (Viagra) in patients with cardiovascular disease. American College of Cardiology/American Heart Association. J Am Coll Cardiol. 1999;33(1):273-82.
15. Samiee-Zafarghandy S, Smith PB, van den Anker JN. Safety of sildenafil in infants*. Pediatr Crit Care Med. 2014;15(4):362-8.

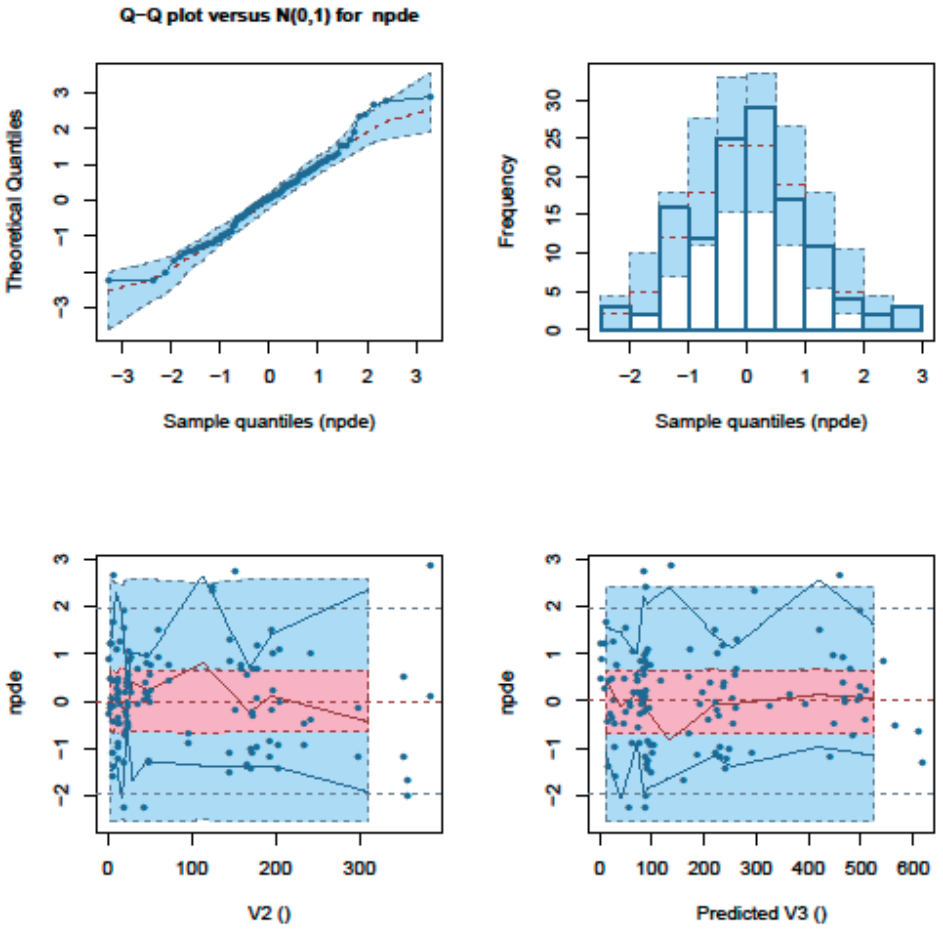
16. Steinhorn RH, Kinsella JP, Pierce C, Butrous G, Dilleen M, Oakes M, et al. Intravenous sildenafil in the treatment of neonates with persistent pulmonary hypertension. J Pediatr. 2009;155(6):841-7 e1.
17. Leeuwen L, Schiller RM, Rietman AB, van Rosmalen J, Wildschut ED, Houmes RJM, et al. Risk Factors of Impaired Neuropsychologic Outcome in School-Aged Survivors of Neonatal Critical Illness. Crit Care Med. 2018;46(3):401-10.
18. Guidance of Industry Bioanalytical Method Validation FDA; 2001 [
19. Karlsson MO, Savic RM. Diagnosing model diagnostics. Clin Pharmacol Ther. 2007;82(1):17-20.
20. Sharma V, McNeill JH. To scale or not to scale: the principles of dose extrapolation. Br J Pharmacol. 2009;157(6):907-21.
21. Ette EI. Stability and performance of a population pharmacokinetic model. J Clin Pharmacol. 1997;37(6):486-95.
22. Bergstrand M, Hooker AC, Wallin JE, Karlsson MO. Prediction-corrected visual predictive checks for diagnosing nonlinear mixed-effects models. AAPS J. 2011;13(2):143-51.
23. Comets E, Brendel K, Mentre F. Computing normalised prediction distribution errors to evaluate nonlinear mixed-effect models: the npde add-on package for R. Comput Methods Programs Biomed. 2008;90(2):154-66.
24. Baquero H, Soliz A, Neira F, Venegas ME, Sola A. Oral sildenafil in infants with persistent pulmonary hypertension of the newborn: a pilot randomized blinded study. Pediatrics. 2006;117(4):1077-83.
25. Uslu S, Kumtepe S, Bulbul A, Comert S, Bolat F, Nuhoglu A. A comparison of magnesium sulphate and sildenafil in the treatment of the newborns with persistent pulmonary hypertension: a randomized controlled trial. J Trop Pediatr. 2011;57(4):245-50.
26. Al Omar S, Salama H, Al Hail M, Al Rifai H, Bunahia M, El Kasem W, et al. Effect of early adjunctive use of oral sildenafil and inhaled nitric oxide on the outcome of pulmonary hypertension in newborn infants. A feasibility study. J Neonatal Perinatal Med. 2016;9(3):251-9.
27. Vargas-Origel A, Gomez-Rodriguez G, Aldana-Valenzuela C, Vela-Huerta MM, Alarcon-Santos SB, Amador-Licona N. The use of sildenafil in persistent pulmonary hypertension of the newborn. Am J Perinatol. 2010;27(3):225-30.
28. Herrera TR CG, Holberto CJ, Loera GRG, Rodriguez BI. . Oral sildenafil as an alternative treatment in the persistent pulmonary hypertension in newborns [Sildenafil oral como alternativo en el tratamiento de recien nacidos con hipertension pulmonar persistente}. . Revista Mexicana de Pediatria 2006;73(4):159-63.
29. Ahsman MJ, Witjes BC, Wildschut ED, Sluiter I, Vulto AG, Tibboel D, et al. Sildenafil exposure in neonates with pulmonary hypertension after administration via a nasogastric tube. Arch Dis Child Fetal Neonatal Ed. 2010;95(2):F109-14.
30. Kipfmueller F, Schroeder L, Berg C, Heindel K, Bartmann P, Mueller A. Continuous intravenous sildenafil as an early treatment in neonates with congenital diaphragmatic hernia. Pediatr Pulmonol. 2018.
31. Hyland R, Roe EG, Jones BC, Smith DA. Identification of the cytochrome P450 enzymes involved in the N-demethylation of sildenafil. Br J Clin Pharmacol. 2001;51(3):239-48.

32. Kearns GL, Abdel-Rahman SM, Alander SW, Blowey DL, Leeder JS, Kauffman RE. Developmental pharmacology--drug disposition, action, and therapy in infants and children. N Engl J Med. 2003;349(12):1157-67.
33. Ince I, de Wildt SN, Wang C, Peeters MY, Burggraaf J, Jacqz-Aigrain E, et al. A novel maturation function for clearance of the cytochrome P450 3A substrate midazolam from preterm neonates to adults. Clin Pharmacokinet. 2013;52(7):555-65.
34. Treluyer JM, Gueret G, Cheron G, Sonnier M, Cresteil T. Developmental expression of CYP2C and CYP2C-dependent activities in the human liver: in-vivo/in-vitro correlation and inducibility. Pharmacogenetics. 1997;7(6):441-52.
35. Abduljalil K, Jamei M, Rostami-Hodjegan A, Johnson TN. Changes in individual drug-independent system parameters during virtual paediatric pharmacokinetic trials: introducing time-varying physiology into a paediatric PBPK model. AAPS J. 2014;16(3):568-76.
36. Brussee JM, Vet NJ, Krekels EHJ, Valkenburg AJ, Jacqz-Aigrain E, van Gerven JMA, et al. Predicting CYP3A-mediated midazolam metabolism in critically ill neonates, infants, children and adults with inflammation and organ failure. Br J Clin Pharmacol. 2018;84(2):358-68.
37. Wildschut ED, Ahsman MJ, Allegaert K, Mathot RA, Tibboel D. Determinants of drug absorption in different ECMO circuits. Intensive Care Med. 2010;36(12):2109-16.
38. Raffaeli G, Allegaert K, Koch B, Cavallaro G, Mosca F, Tibboel D, et al. In Vitro Adsorption of Analgosedative Drugs in New Extracorporeal Membrane Oxygenation Circuits. Pediatr Crit Care Med. 2018;19(5):e251-e8.
39. Ahsman M. J. WED, Witjes B.C.M., Tibboel D., Mathot R.A.A. . Population pharmacokinetics of oral sildenafil during and after extracorporeal membrane oxygenation in neonates. . Determinants of pharmacokinetic variability during extracorporeal membrane oxygenation A roadmap to rational pharmacotherapy in children Rotterdam, The Netherlands: Erasmus Medical Center Rotterdam; 2010. p. 133-49.
40. Burgess G, Hoogkamer H, Collings L, Dingemanse J. Mutual pharmacokinetic interactions between steady-state bosentan and sildenafil. Eur J Clin Pharmacol. 2008;64(1):43-50.

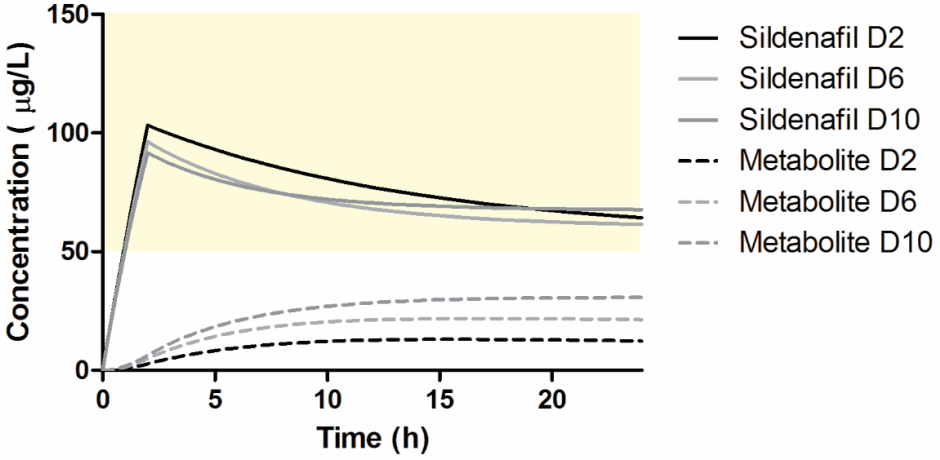
Supplemental digital content table 1 Patient characteristics and baseline characteristics (t=0)

	Median	IQR	%
Gestational age (weeks)	37.5	1.7	
Weight (kg)	2.95	0.8	
O/E LHR (%)	37	17	
Liver			
Up			61% (14)
Down			35% (8)
Unknown			4% (1)
Side hernia			
Left			87% (20)
Right			9% (2)
Bilateral			4% (1)
FETO			22% (5)
Associated anomalies			13% (3)
Surgical repair			
Primary closure			30% (7)
Patch			61%(14)
No repair			9% (2)
SNAP score	27	24	
Postnatal age (days)	0.1	0.5	
VIS	45	37	
OI	21.5	32.3	
Hypotension			17% (4)
ECMO at start sildenafil			9% (2)
Urea (mmol/l)	2.5	0.8	
Creatinine (umol/l)	53	10	
ASAT (U/l)	38	16	
ALAT (U/l)	12.5	8	
ECMO during admission			57% (13)
Total days on oxygen	20.6	21.4	
Death			26% (6)

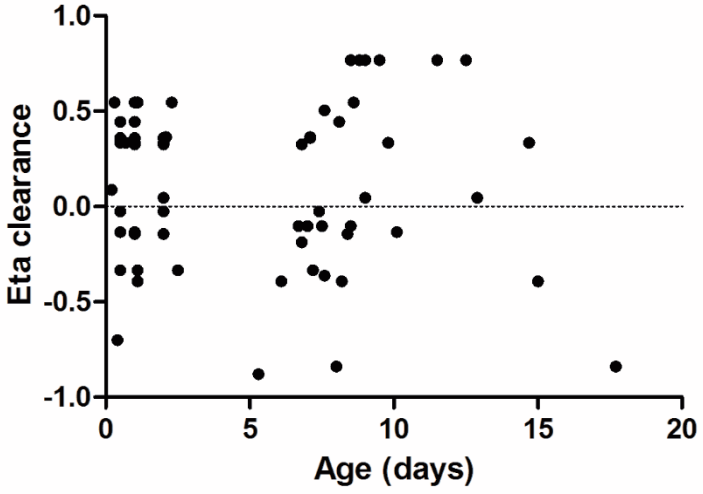
O/E LHR = observed to expected lung to head ratio; FETO = fetal endotracheal occlusion; SNAP score = score for neonatal acute physiology; VIS = vasoactive inotropic score; OI = oxygenation index; ECMO = extracorporeal membrane oxygenation



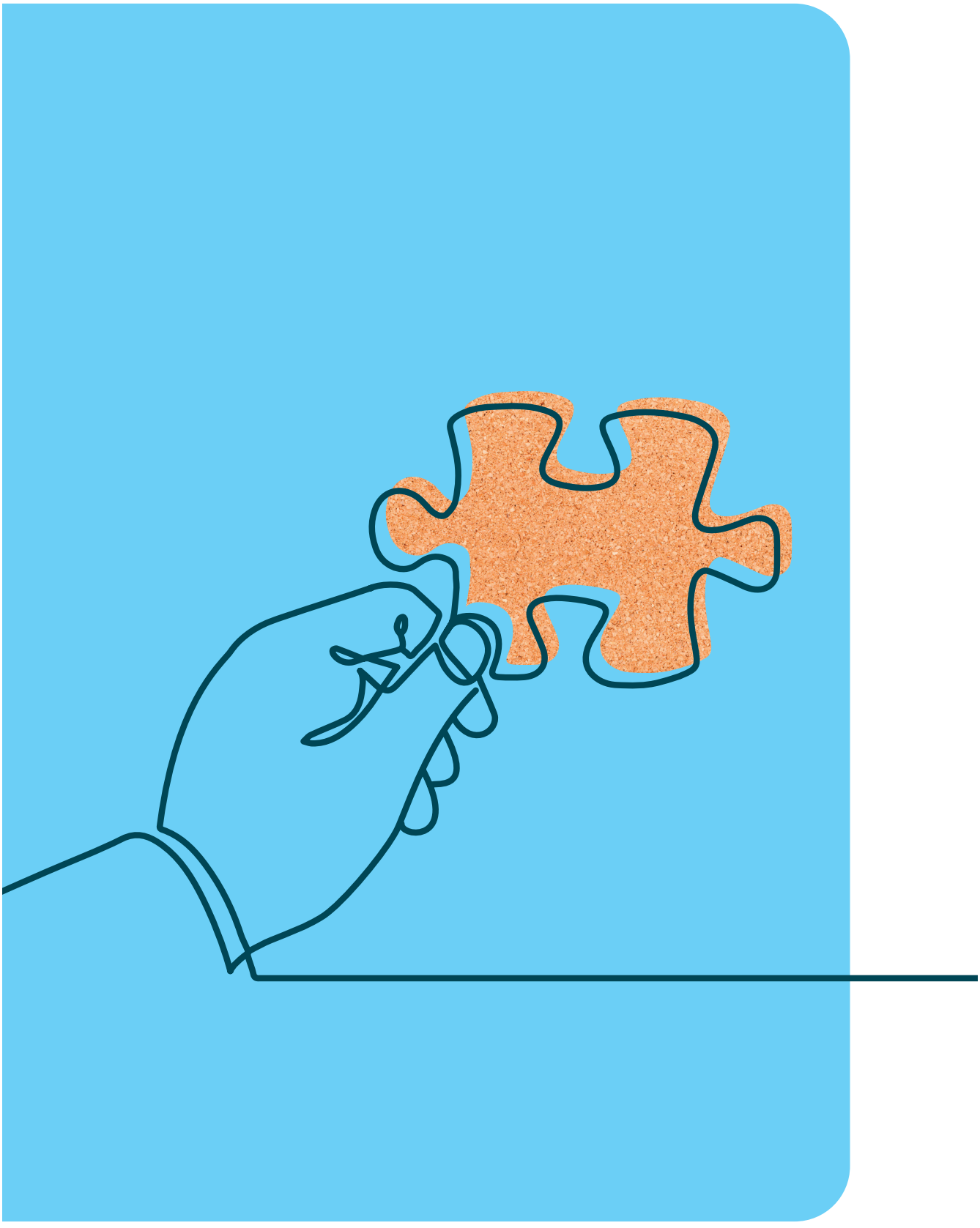
Supplemental digital content figure 4: The graphs in the top row, QQ plots and histogram, show the normality of the NPDEs distribution. The graphs in the bottom row, scatter plots vs. concentration predictions, show the distribution of the variance. V2 = Time (hours), V3 = Predicted concentrations (ug/L)



Supplemental digital content figure 5: Simulation of a loading dose of 0,2 mg/kg in 2 hours followed by individualized dose 0,2 mg/kg/day in the youngest children (2 days), 0,4 mg/kg/day in the middle category (6 days) and 0,6 mg/kg/days in the group of 10 days. With these regimen a lower therapeutic level was anticipated. The therapeutic range is marked in yellow, only 50-150ug/l is shown to make the figure more clear.



Supplemental digital content figure 6: ETA clearance versus age in days. There is no trend visible as all points are nicely distributed over the graph.



CHAPTER 7

The CoDiNOS trial protocol: An international randomized controlled trial of intravenous sildenafil versus inhaled nitric oxide for the treatment of pulmonary hypertension in neonates with congenital diaphragmatic hernia

Suzan CM Cochius - den Otter, Thomas Schaible, Anne Greenough, Arno van Heijst, Neil Patel, Karel Allegaert, Joost van Rosmalen, Dick Tibboel on behalf of the CDH EURO Consortium

BMJ Open. 2019;9(11):e032122

ABSTRACT

Introduction: Congenital diaphragmatic hernia (CDH) is a developmental defect of the diaphragm that impairs normal lung development, causing pulmonary hypertension (PH). PH in CDH newborns is the main determinant for morbidity and mortality. Different therapies are still mainly based on "trial and error". Inhaled nitric oxide (iNO) is often the drug of first choice. However, iNO does not seem to improve mortality. Intravenous (iv) sildenafil has reduced mortality in newborns with PH without CDH, but prospective data in CDH patients are lacking.

Methods and analysis: In an open label, multicenter, international randomized controlled trial in Europe, Canada and Australia, 330 newborns with CDH and PH are recruited over a four-year period (2018-2022). Patients are randomized for iv sildenafil or iNO. Sildenafil is given in a loading dose of 0.4 mg/kg in 3 hours; followed by continuous infusion of 1.6 mg/kg/day, iNO is dosed at 20 ppm.

Primary outcome is absence of PH on day 14 without pulmonary vasodilator therapy and/or absence of death within the first 28 days of life. Secondary outcome measures include clinical and echocardiographic markers of PH in the first year of life.

We hypothesize that sildenafil gives a 25% reduction in the primary outcome from 68% to 48% on day 14, for which a sample size of 330 patients is needed. An intention-to-treat analysis will be performed. A p-value (two-sided) < 0.05 is considered significant in all analyses.

Ethics and dissemination: Ethics approval has been granted by the ethics committee in Rotterdam (MEC-2017-324) and the central Committee on Research Involving Human Subjects (NL60229.078.17) in the Netherlands. The principles of the Declaration of Helsinki, the Medical Research Involving Human Subjects Act, and the national rules and regulations on personal data protection will be used. Parental informed consent will be obtained.

Registration: Trial registration number NTR6982 (Trial NL6796).

INTRODUCTION

Congenital diaphragmatic hernia (CDH) is a severe developmental defect of the diaphragm with an incidence of approximately 1 in 3000 live births and a mortality of 27% (1). Because of this defect, the abdominal organs herniate into the chest causing pulmonary hypoplasia and abnormal pulmonary vasculature growth, resulting in pulmonary hypertension (PH) (2). In adults and children, PH is defined as mean pulmonary artery pressure (mPAP) exceeding 25 mmHg with a pulmonary capillary wedge pressure of minimal 15 mmHg (3).

The normal pulmonary vascular transition of the neonate takes around two months to achieve these low values of mPAP. During fetal life, there is high resistance in the pulmonary circulation which results in most of the blood flow to bypass the lungs through the ductus arteriosus and oval foramen. Immediately after birth, the pulmonary vascular resistance drops and the blood flow to the lungs significantly increases (4). In contrast, the pulmonary vascular resistance often does not drop adequately in children with CDH due to a decreased vascular bed associated with lung hypoplasia, and an altered development of the pulmonary vasculature with excessive muscularization of the arterioles, with increased thickness of the arterial media and adventitia. Although the presence of lung hypoplasia can be predicted with prenatal parameters, reliable predictors for PH in CDH patients are lacking (5). The incidence of PH in CDH patients is 68-79% and causes considerable morbidity and mortality (1, 2, 6). Therapy in newborns with PH , such as inhaled nitric oxide (iNO) and sildenafil, has improved outcomes in general. However, trials in infants with CDH are sparse.

Inhaled nitric oxide (iNO) diffuses rapidly across the alveolar-capillary membrane into the smooth muscle of pulmonary vessels to activate soluble guanylate cyclase. This enzyme mediates many of the biological effects of iNO, and is responsible for the conversion of guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP). The increase of intracellular cGMP relaxes smooth muscles via several mechanisms. iNO also causes bronchodilation and has anti-inflammatory and anti-proliferative effects (7). In term and near term infants with persistent pulmonary hypertension of the newborn (PPHN), iNO decreases the median duration of mechanical ventilation and reduces the need for extracorporeal membrane oxygenation (ECMO). However, in the two available randomized controlled trials (RCT) with a small number of patients with CDH, mortality did not improve and more ECMO treatment was needed despite short-term improved of oxygenation in some treated patients (8, 9). In the centers of the CDH EURO Consortium, iNO is standard of care in infants with CDH and PH although the positive pharmacodynamic effects in these infants are less convincing then in infants with PPHN (6, 10). The pathophysiological mechanism of this difference is not understood. In resource poor settings iNO is often

unavailable. In the search to find another treatment option, trials to evaluate the effect of sildenafil in newborns with PPHN have been conducted (11).

Sildenafil is a selective phosphodiesterase type 5 (PDE5) inhibitor. PDE5 is an enzyme that specifically degrades cGMP. Sildenafil inhibits PDE5, increasing cGMP and NO-mediated vasodilatation of the smooth muscles in arteries. Only five RCTs have been performed in newborns, all non-CDH patients with PPHN. Four of these studies showed a decrease in oxygenation index (OI) and mortality in a setting where iNO was not available, while one trial showed no additional benefit of sildenafil when added to iNO (11). Although sildenafil is increasingly used in CDH patients, only retrospective data are available (12). A decrease in pulmonary vascular resistance index and an increase in cardiac output were found in a small group of oral sildenafil-treated infants with CDH refractory to iNO (13). Intravenous sildenafil improved OI and reversed the right-to-left shunt ratio over the PDA, but it also increased the need for inotropic support (14, 15). However, its effect on outcome is unknown.

We hypothesize that intravenous sildenafil is superior to iNO. iNO is the therapy of first choice in most centers despite the lack of evidence, and sildenafil is the most promising drug for the treatment of PH in CDH patients and is increasingly being used (6, 12, 16). However, no studies have been performed comparing iNO with intravenous sildenafil in newborns with CDH and PH or PH alone. Based on the current knowledge, there is equipoise for both treatment modalities.

METHODS AND ANALYSIS

Design

The CoDiNOS (Congenital Diaphragmatic hernia Nitric Oxide versus Sildenafil) trial is a prospective, multicenter, international randomized controlled trial conducted in high volume pediatric surgical centers in Europe, Canada and Australia. The members of the CDH Euro Consortium participating in the trial are listed in the Appendix.

Objectives

The primary objective of the study is to determine whether the incidence of PH is lower in CDH patients treated with intravenous sildenafil than in patients treated with iNO, with the primary outcome defined as the absence of PH on echocardiography on day 14 without pulmonary vasodilator therapy and without treatment failure and/or death within the first 28 days after birth. PH is defined as systolic pulmonary arterial pressure> 2/3 systolic systemic pressure and/or right ventricular (RV) dilatation/septal displacement and RV dysfunction +/- left ventricular dysfunction.

The secondary outcomes are:

(1) change in OI after 12 and 24 hours of therapy

(2) overall mortality

(3) the incidence of treatment failure which is defined as:

- inability to maintain preductal saturations above 85% (± 7 kPa or 52 mmHg) or postductal saturations above 70% (±5.3 kPa or 40 mmHg)
- and/or increase in CO_2 > 70 mmHg (9.3 kPa) despite optimization of ventilator management
- and/or inadequate oxygen delivery with metabolic acidosis defined as lactate ≥ 5 mmol/l and pH < 7.15 and/or hypotension resistant to fluid therapy and adequate inotropic support resulting in a urine output < 0.5 ml/kg/hour
- and/or lactate ≥ 5 mmol/l and pH < 7.15
- and/or OI consistently ≥ 40

(4) time on intervention drug, defined as intervention drug free days after initiation of the intervention, calculated on day 14

(5) need for ECMO

(6) ventilator free days on day 28

(7) the use of drugs for PH treatment during the hospital admission

(8) the use of pulmonary and/or cardiac medication at discharge and its total duration of administration

(9) short-term and long-term PH on echocardiography at 24 hours, 28 days/discharge and 6 and 12 months

(10) the incidence of chronic lung disease

(11) the development of neurological abnormalities evaluated with ultrasound of the brain before the start of the trial, after surgery and before discharge

(12) the external validation of the sildenafil (pharmacokinetic – pharmacodynamic) PKPD model for the pharmacokinetics and the pharmacodynamic effects of sildenafil

Safety outcomes include adverse events due to the study drugs and the vasoactive-inotropic support score (VIS).

Patients

Infants diagnosed with CDH who have PH in the first week after birth, are eligible for the trial if born at or after a gestational age of 34 weeks. The diagnosis of PH is defined as at least two of the following four criteria: (I) systolic pulmonary arterial pressure> 2/3 systolic systemic pressure estimated by echocardiography. (II) RV dilatation/septal displacement, RV dysfunction +/- left ventricular dysfunction. (III) Pre-post ductal SpO_2 difference > 10%. (IV) OI >20. Exclusion criteria are a severe chromosomal anomaly which may imply a decision to stop or not to start life-saving medical treatment, severe cardiac anomaly expected to need corrective surgery in the first 60 days of life, renal anomalies associated with oligohydramnios, severe orthopedic and skeletal deformities, which are likely to influence thoracic, and / or lung development and severe anomalies of the central nervous system. Patients who are born in another center and transported with iNO are also excluded from the trial. Patients who received fetal interventions (trachea balloon placement) are not excluded.

Following antenatal diagnosis, the parents are counselled and informed about the study by the clinician or research coordinator. Also, they receive a patient information letter and an informed consent form . If the patient is not born in a participating center or the diagnosis of CDH was not known, parents are counselled after the diagnosis of CDH and are informed about the study. Also, they receive written information and an informed consent form. This informed consent form contains consent for the trial and for collection of data and material for future research.

For the development of the protocol the SPIRIT reporting guidelines have been used (17). This publication is based on protocol version 4, June 13th 2018.

Patient and public involvement

Patients and the public were not involved in the development of the trial protocol. However, CDH UK Sparks, as a parent organization, has assessed and commented on the protocol and as provided start-up funding as also mentioned in the funding statement. This organization is and will be regularly informed on progress and results of the trial.

Study procedures

Baseline assessment

Antenatal ultrasound data about the characteristics of the CDH are collected. These data include the observed/expected lung-head ratio, position of the liver and stomach and the amniotic fluid index. An MRI or an ultrasound is performed depending on local experience and possibilities. If an MRI is performed, the observed/expected fetal lung volume will be calculated. Also data on prenatal interventions are collected. In all mothers, a planned vaginal or caesarean delivery is pursued.

Randomization, intervention and blinding

Participants will be randomized using ALEA, which is an online, central randomization service (https://www.aleaclinical.eu). Allocation concealment will be ensured, as the service will not release the randomization code until the patient has been recruited into the trial, which takes place after all baseline characteristics have been added. ALEA randomizes the patient with a computer-generated randomization list, made by the independent statistician of the Data Safety and Monitoring Board. Blocked randomization, with variable block sizes and stratification by center, is used to achieve equal distribution of the two interventions among the participants.

Postnatally, infants are treated according to a standardized protocol for patients with CDH, which is implemented in all participating centers. This protocol was developed with the available evidence and consensus between the participating centers and was updated in June 2016 (10, 16). If the patient is diagnosed with PH in the first week of life, the patient will be allocated to one of the two study drugs (figure 1). iNO is provided by a tank connected to a ventilator. Different devices are used in different centers. Some centers use integrated systems, making it impossible to disconnect the iNO tank and replace it with another gas to facilitate a blinded intervention. Therefore, the study is open label. iNO is given with a starting dose of 20 ppm, which is the maximum dose (18, 19). Sildenafil is given intravenously, using a loading dose of 0.4mg/kg in 3 hours, followed by continuous infusion of 1.6mg/kg/day (20, 21). To wean the study drugs a standard protocol is followed (figure 2). The allocated drugs will be restarted as per protocol if criteria for its use are met again before the age of 14 days. To further standardize care, an inotropic support flow chart is included in the study protocol (figure 3). After day 14 treatment of PH will be at the discretion of the local medical team and the study drug can be changed to, for instance, sildenafil orally. The use of bosentan, milrinone and prostin next to the study treatment is allowed. The use of bosentan as add on therapy is allowed and is considered as PH treatment on day 14.The intervention will be prematurely stopped when the patient meets one or more of the defined failure criteria, described in point three of the secondary outcomes. Further treatment will then be at the discretion of the medical team and will be according to the standardized protocol(16). INO and sildenafil can both be given outside

the study protocol. An ECMO-procedure may then be started in centers where ECMO is available. Data of all patients are used in the intention-to-treat analysis.

Follow up

After day 14, additional clinical data, such as time on ventilator support (days) and the use of drugs for the treatment of PH, are collected to answer the secondary outcome questions. Also, echocardiographic measurements are taken at 6 and 12 months to evaluate the presence of chronic PH (table 1)

Data collection

Echocardiography parameters are measured by local physicians, centrally collected and reviewed by two blinded independent physicians to reduce inter-observer variation. Demographic and neonatal characteristics as well as data on the clinical course of all patients are entered in a password protected web-based database in Rotterdam (OpenClinica). Upon request the collected data will be available. All centers will keep a logbook of the number of non-participants, including the reasons for not participating. Study documents are securely stored at each study site for 15 years.

Laboratory testing

Blood, urine and tracheal samples are collected in most centers during the trial. Blood samples are collected before the start of the study and at different time points until day 14. Some samples will be used to externally validate a NONlinear Mixed Effects Modeling (NONMEM) prediction model for sildenafil. The other samples will be used in future research on biomarkers to predict severity and outcome of PH in CDH patients. The samples are centrifuged for 6 minutes at 3000 rpm (22). Thereafter, the plasma is removed and stored at –20 degrees Celsius or colder. The total amount of blood taken is maximal 2.5 % of the circulating volume. Blood sampling will only be done if a central or peripheral line is still present and/or in combination with routine laboratory measurements. This way blood sampling is a minimal burden for the patient.

Tracheal aspirate for proteomic analysis is also collected at different time points during routine tracheal suctioning in ventilated patients. Protein profiling with proteomics is used to identify specific groups of proteins that are involved in the pathogenesis of PH. The tracheal aspirates is centrifuged for 6 minutes at 3000 rpm and stored at –80 degrees Celsius (23).

Also, 8-hour urine is collected at different time points. Two samples of 5 ml are taken and stored at –20 degrees Celsius or colder.

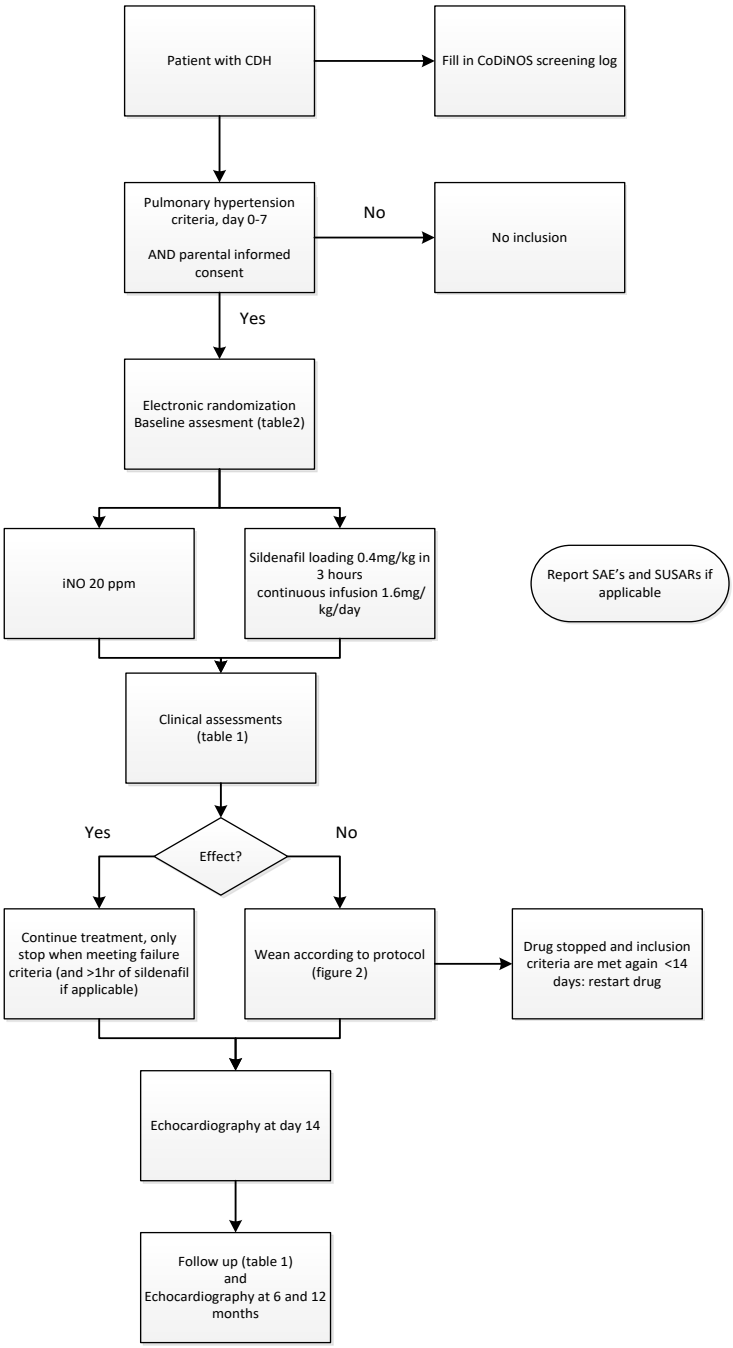


Figure 1 Trial flow chart

Flow chart showing the steps of the trial, from birth until 12 months. CDH: congenital diaphragmatic hernia; SAE: serious adverse event; SUSAR: suspected unexpected serious adverse event

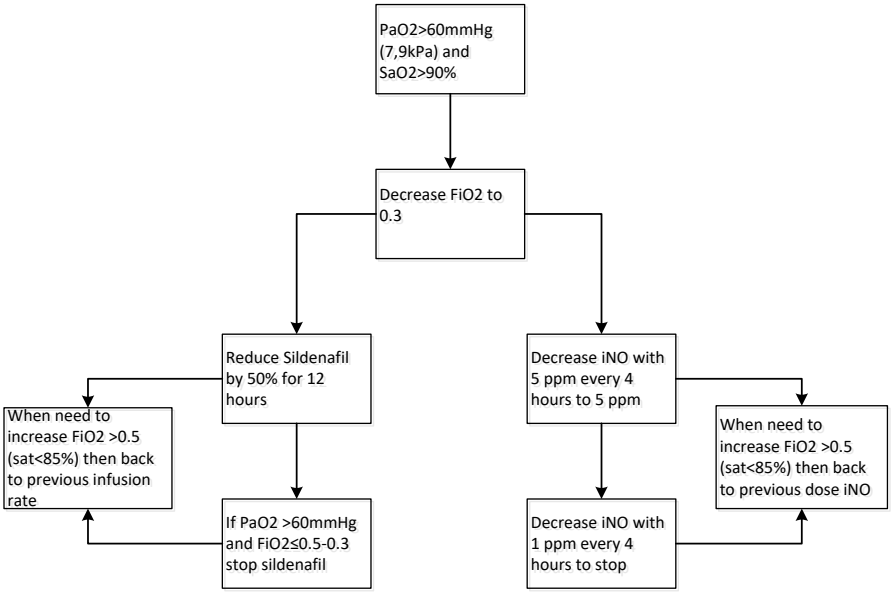


Figure 2 Protocol to wean study drug

Flow chart showing the protocol to wean off inhaled nitric oxide or intravenous sildenafil. iNO: inhaled nitric oxide; ppm: parts per million

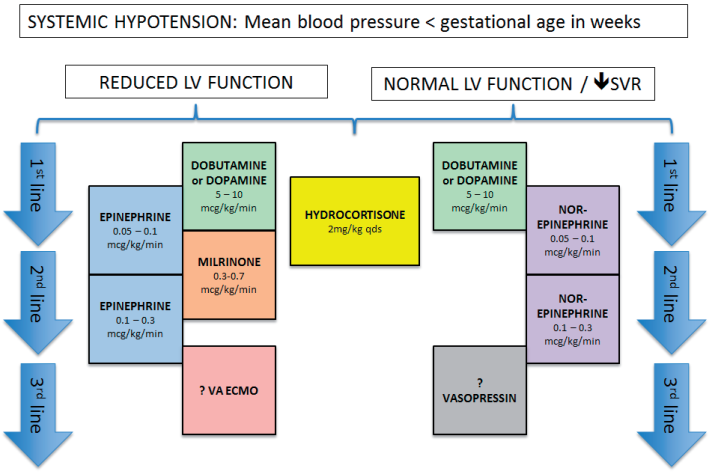


Figure 3 Treatment flow chart of systemic hypotension

Flow chart that is added to the treatment protocol, showing the treatment plan for systemic hypotension. VA ECMO: veno-arterial extracorporeal membrane oxygenation

Table 1 Procedures and measurements

	Day 0-7 before start thera-py	3 hrs after start silde-nafil	12hrs after start	8 am after start	24hrs after start	Day of surgery, pre-opera-tively	Day after sur-gery	Day of ECMO, pre-cannula-tion	8 am after start ECMO	Day 14	Day 28 / before dis-charge	Day 56	6 mnth	12 mnth
Echocardio-graphy	X				X					X	X		X	X
Calculation OI	X		X		X									
Calculation VIS score	X		X		X									
Blood sample	X			X		X	X	X	X	X				
Tracheal aspirate	X			X		X	X	X	X	X				
Urine sample	X			X		X	X	X	X	X				
Severity of CLD											X	X		
Ultrasound brain	X						X							
Sildenafil plasma level		X		X		X	X		X					

OI: oxygenation index; VIS score: vasoactive-inotropic support score; CLD: chronic lung disease; ECMO: extracorporeal membrane oxygenation

Withdrawal of participants

Parents may decide to withdraw from the study at any time without any consequences. The investigator can decide to withdraw a patient from the study for urgent medical reasons. In some cases, there may be exclusion criteria, which were not known before randomization. If this is the case, the patient will be withdrawn from the study after contacting the study coordinator. With consent of the parents data will still be collected, stored and analyzed to perform an intention-to-treat analysis. These children will be treated according to standard practice (10, 16).

Sample size calculation

The sample size calculation is based on a power analysis for the primary outcome, using previously published data on PH. . Lusk et al. showed that PH, defined as >2/3 systemic blood pressure measured on echocardiography, in CDH patients on day 14 has a positive predictive value of 0.8 for death, death or ventilation, and death or ventilator support. PH on day 14 is observed in 64% of CDH patients (24).

Even though the definition of the primary outcome is not the same, we assume a similar outcome percentage of 64% for failing the primary outcome in our trial, the absence of PH on day 14 without pulmonary vasodilator therapy and/or absence of death within the first 28 days of life, in the iNO group. Our aim is to promote practice change, therefor we aim for a clinically significant difference For a 25% relative reduction to 48%, a sample size of 300 patients (150 patients per group) is needed to obtain a power of 80%. This will match a number needed to treat of 6.25. Taking missing data and the effects of correction for covariates into account, we adjust this sample size to 330 patients. In the collaborating centers 550 patients will be born in three years. Based on our earlier trial (CMV Versus HFO in Congenital Diaphragmatic Hernia; VICI trial) we expect to have an inclusion rate of 60%. Therefore, the inclusion of 330 patients should be reached in three years.

Data analysis

The patients will be analyzed according to the group they are randomized to (intention-to-treat analysis). A p-value (two-sided) < 0.05 is considered significant in all analyses. The primary endpoint will be analyzed using multiple logistic regression with randomization arm, center, observed/expected head-lung ratio, position of the liver, side of the defect, defect size and ventilation modality as independent variables (25). If necessary, multiple imputation using the fully conditional specification method will be used to account for missing data in the independent variables. We will perform a sensitivity analyses with adjustment for the use of prostin and milrinone, to account for the effects of these vasodilators on PH.

The following analyses will be performed for the secondary outcomes. The distribution of VIS score in all study participants will be compared between t=0 and t=12 hours after initiation of drug administration using a Wilcoxon signed rank test. The distribution of changes in OI and VIS score from t=0 to t=12 and t=24 hours will be compared between the randomization groups with a Mann-Whitney test. The overall mortality in the first year of life will be compared between the randomization groups with Kaplan-Meier curves and the log-rank test. The number of treatment failures, the need for ECMO (in ECMO centers), and the need for medication for PH or chronic lung disease at discharge, and during the first year of life, will be compared between randomization groups with chi-square tests. The number of study drug free days at day 14, the number of ventilation-free days until day 28, the fraction of days with need for medical treatment (excluding the study drug) for PH during the hospital admission, and the severity of chronic lung disease using the Bancalari definition, will be compared between randomization groups with Mann-Whitney tests. Deaths will be counted as the worst outcome in these analyses, in accordance with the intention-to-treat principle. The presence of PH at 28 days/discharge, 6 and 12 months according to the echocardiographic parameters will be compared between randomization groups with a chi-square test.

To externally validate the pharmacokinetic model of sildenafil and it active metabolite (in NONMEM) Normalized prediction distribution errors (NPDE) and Visual Predictive Check (VPC) will be used. Furthermore, the model will be used to predict the drug concentrations from the new data set using simulations, in which we expect that the difference will be less than 20%. To assess whether there is a relationship between the concentration of sildenafil, its active metabolite and the clinical effects, such as OI, VIS score and echocardiography measures, a Mann-Whitney or Student's t-test will be used.

Safety reporting and trial oversight

All severe adverse events (SAEs) and suspected unexpected serious adverse reactions (SUSARs) are reported from the enrolment until 12 month follow-up. Persistent or significant disability or incapacity that was not expected with the given observed to expected lung to head ratio (O/E LHR) is evaluated as an SAE. An elective hospital admission is not a SAE. All SAEs and SUSARs are reported to the approving ethics committees in accordance with their requirements. We will report the SAEs and SUSARs that result in death or are life threatening within 7 days of first knowledge. All other SAEs and SUSARs will be reported within a period of maximum 15 days. Once a year throughout the clinical trial, we will submit a safety report to the approving ethics committees and competent authorities of the countries involved.

The trial will be monitored by qualified, independent monitors. The trial is classified as a trial with moderate risk and a specific monitoring plan is in place.

The data safety monitoring board will monitor the incidence of mortality on a continuous basis. If at some point a large difference in mortality, defined as an absolute risk increase of 25%, between the two treatment groups is noticed, the data safety monitoring board may recommend ending the study.

Insurance will cover compensation to patients who suffer harm from trial participation.

Ethics and dissemination

Ethics approval has been granted by the local ethics committee in Rotterdam (MEC-2017-324) and by the central Committee on Research Involving Human Subjects (NL60229.078.17) in the Netherlands. The trial will be submitted to the regulatory bodies and the local institutional review boards (IRB's) in all participating countries. Important amendments will be communicated to all relevant parties. The study will be conducted according to the principles of the Declaration of Helsinki, in accordance with the Medical Research Involving Human Subjects Act, and national rules and regulations on personal data protection. Parental informed consent will be obtained. The results of this study will be disseminated via peer-reviewed publications and implemented in the international guidelines for the treatment of newborns with CDH.

Funding statement

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LITERATURE

1. Snoek KG, Capolupo I, van Rosmalen J, Hout LJ, Vijfhuize S, Greenough A, et al. Conventional Mechanical Ventilation Versus High-frequency Oscillatory Ventilation for Congenital Diaphragmatic Hernia: A Randomized Clinical Trial (The VICI-trial). Ann Surg. 2015.
2. Robinson PD, Fitzgerald DA. Congenital diaphragmatic hernia. Paediatr Respir Rev. 2007;8(4):323-34; quiz 34-5.
3. Galie N, Hoeper MM, Humbert M, Torbicki A, Vachiery JL, Barbera JA, et al. Guidelines for the diagnosis and treatment of pulmonary hypertension: the Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS), endorsed by the International Society of Heart and Lung Transplantation (ISHLT). Eur Heart J. 2009;30(20):2493-537.
4. Vali P, Lakshminrusimha S. The Fetus Can Teach Us: Oxygen and the Pulmonary Vasculature. Children (Basel). 2017;4(8).
5. Russo FM, Eastwood MP, Keijzer R, Al-Maary J, Toelen J, Van Mieghem T, et al. Lung size and liver herniation predict need for extracorporeal membrane oxygenation but not pulmonary hypertension in isolated congenital diaphragmatic hernia: systematic review and meta-analysis. Ultrasound Obstet Gynecol. 2017;49(6):704-13.
6. Putnam LR, Tsao K, Morini F, Lally PA, Miller CC, Lally KP, et al. Evaluation of Variability in Inhaled Nitric Oxide Use and Pulmonary Hypertension in Patients With Congenital Diaphragmatic Hernia. JAMA Pediatr. 2016.
7. Ichinose F, Roberts JD, Jr., Zapol WM. Inhaled nitric oxide: a selective pulmonary vasodilator: current uses and therapeutic potential. Circulation. 2004;109(25):3106-11.
8. Inhaled nitric oxide and hypoxic respiratory failure in infants with congenital diaphragmatic hernia. The Neonatal Inhaled Nitric Oxide Study Group (NINOS). Pediatrics. 1997;99(6):838-45.
9. Clark RH, Kueser TJ, Walker MW, Southgate WM, Huckaby JL, Perez JA, et al. Low-dose nitric oxide therapy for persistent pulmonary hypertension of the newborn. Clinical Inhaled Nitric Oxide Research Group. N Engl J Med. 2000;342(7):469-74.
10. Reiss I, Schaible T, van den Hout L, Capolupo I, Allegaert K, van Heijst A, et al. Standardized postnatal management of infants with congenital diaphragmatic hernia in Europe: the CDH EURO Consortium consensus. Neonatology. 2010;98(4):354-64.
11. Kelly LE, Ohlsson A, Shah PS. Sildenafil for pulmonary hypertension in neonates. Cochrane Database Syst Rev. 2017;8:CD005494.
12. Lakshminrusimha S, Keszler M, Kirpalani H, Van Meurs K, Chess P, Ambalavanan N, et al. Milrinone in congenital diaphragmatic hernia - a randomized pilot trial: study protocol, review of literature and survey of current practices. Matern Health Neonatol Perinatol. 2017;3:27.
13. Noori S, Friedlich P, Wong P, Garingo A, Seri I. Cardiovascular effects of sildenafil in neonates and infants with congenital diaphragmatic hernia and pulmonary hypertension. Neonatology. 2007;91(2):92-100.

14. Bialkowski A, Moenkemeyer F, Patel N. Intravenous sildenafil in the management of pulmonary hypertension associated with congenital diaphragmatic hernia. Eur J Pediatr Surg. 2015;25(2):171-6.
15. Kipfmueller F, Schroeder L, Berg C, Heindel K, Bartmann P, Mueller A. Continuous intravenous sildenafil as an early treatment in neonates with congenital diaphragmatic hernia. Pediatr Pulmonol. 2018.
16. Snoek KG, Reiss IK, Greenough A, Capolupo I, Urlesberger B, Wessel L, et al. Standardized Postnatal Management of Infants with Congenital Diaphragmatic Hernia in Europe: The CDH EURO Consortium Consensus - 2015 Update. Neonatology. 2016;110(1):66-74.
17. Chan AW, Tetzlaff JM, Altman DG, Laupacis A, Gotzsche PC, Krleza-Jeric K, et al. SPIRIT 2013 statement: defining standard protocol items for clinical trials. Ann Intern Med. 2013;158(3):200-7.
18. Tworetzky W, Bristow J, Moore P, Brook MM, Segal MR, Brasch RC, et al. Inhaled nitric oxide in neonates with persistent pulmonary hypertension. Lancet. 2001;357(9250):118-20.
19. Barrington KJ, Finer N, Pennaforte T, Altit G. Nitric oxide for respiratory failure in infants born at or near term. Cochrane Database Syst Rev. 2017;1:CD000399.
20. Steinhorn RH, Kinsella JP, Pierce C, Butrous G, Dilleen M, Oakes M, et al. Intravenous sildenafil in the treatment of neonates with persistent pulmonary hypertension. J Pediatr. 2009;155(6):841-7 e1.
21. Mukherjee A, Dombi T, Wittke B, Lalonde R. Population pharmacokinetics of sildenafil in term neonates: evidence of rapid maturation of metabolic clearance in the early postnatal period. Clin Pharmacol Ther. 2009;85(1):56-63.
22. Snoek KG, Kraemer US, Ten Kate CA, Greenough A, van Heijst A, Capolupo I, et al. High-Sensitivity Troponin T and N-Terminal Pro-Brain Natriuretic Peptide in Prediction of Outcome in Congenital Diaphragmatic Hernia: Results from a Multicenter, Randomized Controlled Trial. J Pediatr. 2016;173:245-9 e4.
23. Bowler RP, Ellison MC, Reisdorph N. Proteomics in pulmonary medicine. Chest. 2006;130(2):567-74.
24. Lusk LA, Wai KC, Moon-Grady AJ, Steurer MA, Keller RL. Persistence of pulmonary hypertension by echocardiography predicts short-term outcomes in congenital diaphragmatic hernia. J Pediatr. 2015;166(2):251-6 e1.
25. Lally KP, Lasky RE, Lally PA, Bagolan P, Davis CF, Frenckner BP, et al. Standardized reporting for congenital diaphragmatic hernia--an international consensus. J Pediatr Surg. 2013;48(12):2408-15.

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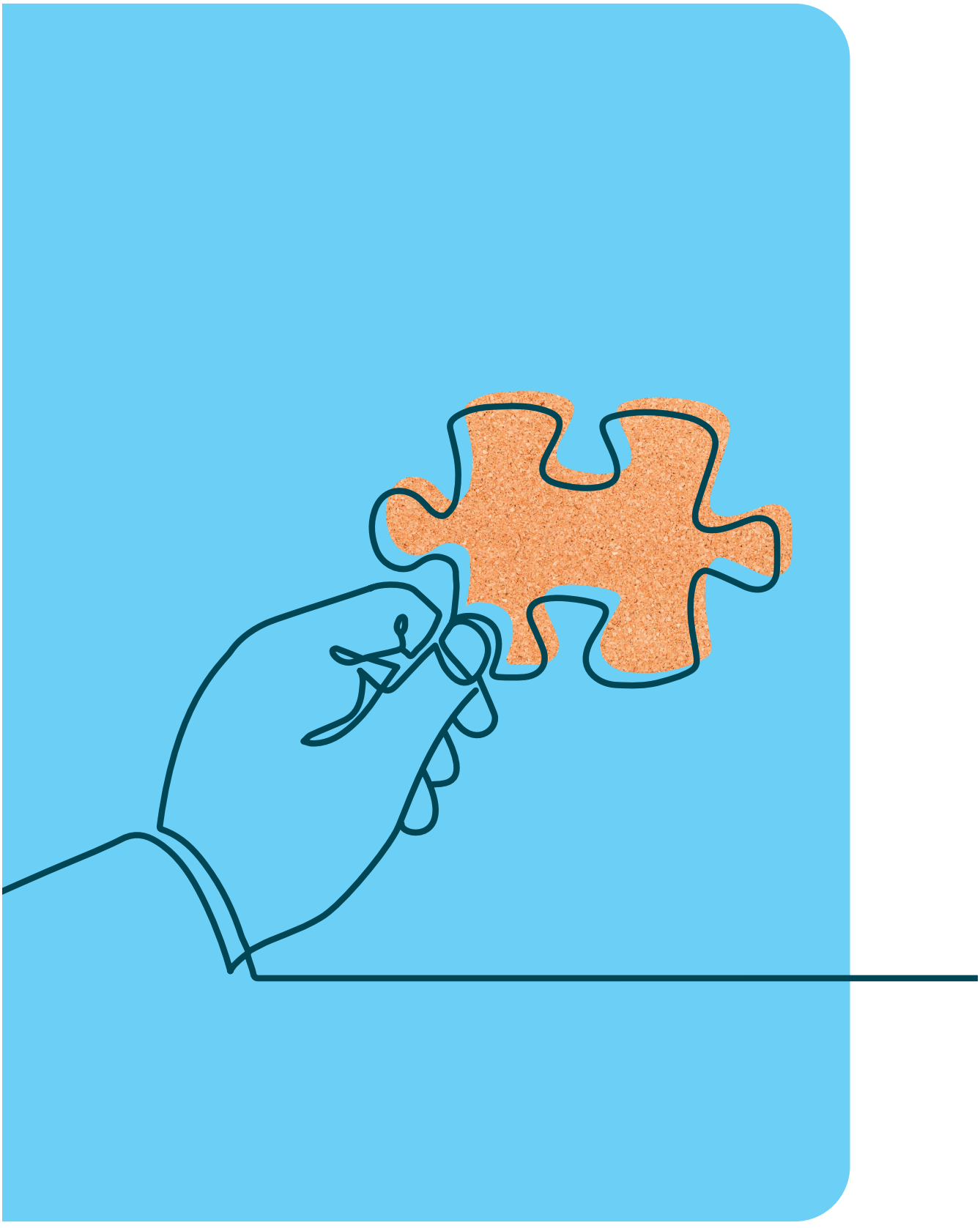
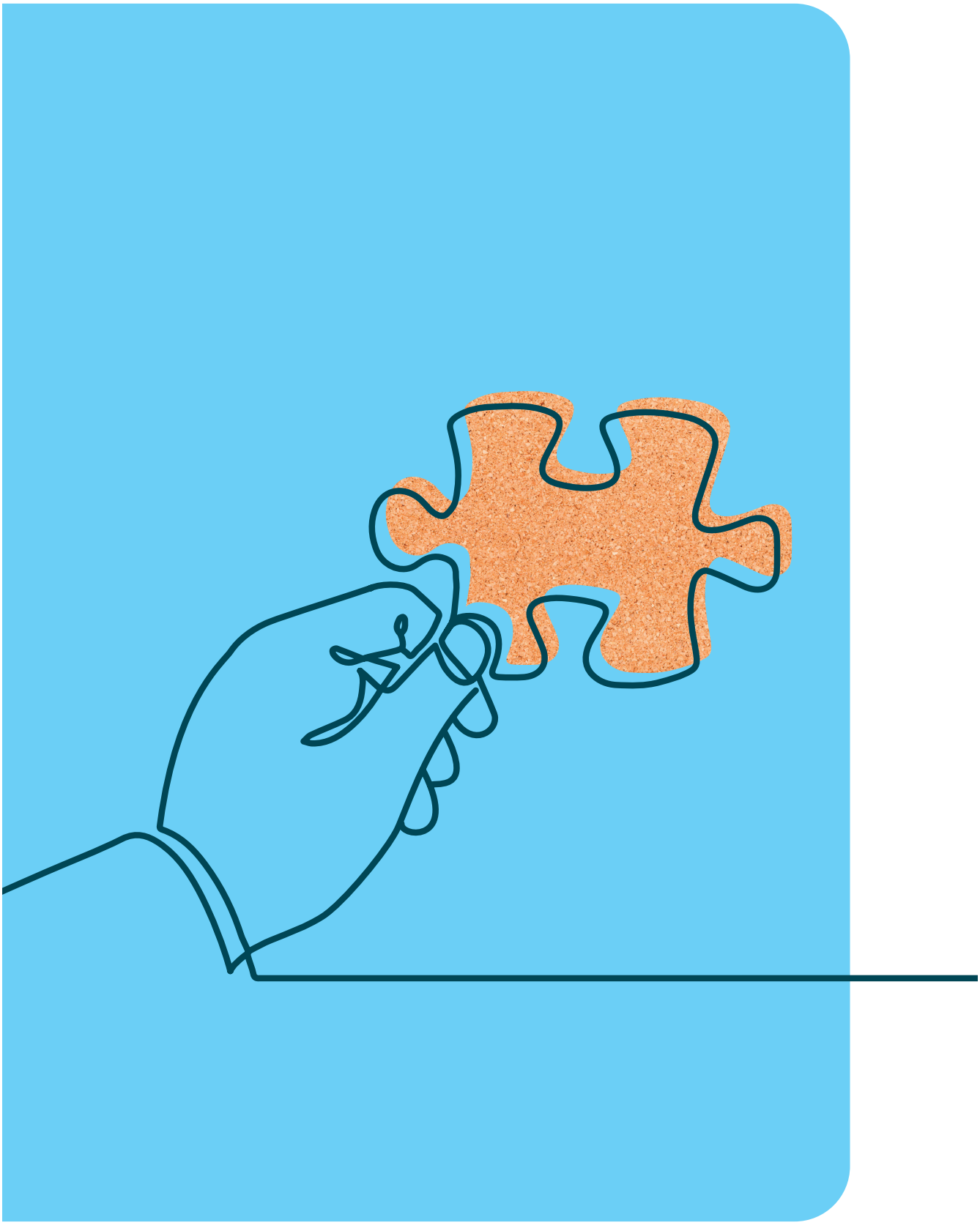


PART IV

Discussion and summary



CHAPTER 8

General discussion

GENERAL DISCUSSION

The research in this thesis describes various aspects of the clinical care for newborns with CDH, with emphasis on aspects of prediction, biomarkers and treatment. Most research has been conducted in a multicenter context within the CDH EURO Consortium, in the era after the widespread implementation of the CDH EURO Consortium guidelines (1). The consortium consists of 22 tertiary clinical centers inside and outside Europe with expertise in the treatment of patients with CDH. These centers collaborate to enhance the clinical care for these patients by developing clinical guidelines for the perinatal treatment of CDH patients, which are mostly based on expert opinion up till now, but also by developing an uniform and standardized international follow-up program (1, 2). Another important aspect of the mission of the consortium is to contribute to evidence based care by performing leading-edge research, such as the VICI trial and more recently the CoDiNOS trial (3)(chapter 7). CDH is a rare disease, making international and multidisciplinary collaboration essential to further improve clinical care for this patient group, as mortality remains around 25% even in the most experienced centers

PREDICTION

Clinical prediction models

The severity of illness in CDH patients is highly variable and is dependent on the severity of lung hypoplasia and pulmonary hypertension, but also on the presence of additional anomalies. For both parents and caretakers early outcome prediction is essential to get insight in the prognosis of the individual patient. Prenatally, outcome prediction can guide parents and care providers in decisions regarding continuation of the pregnancy, the use of prenatal interventions such a temporary tracheal plugging (FETO), the referral to high-volume centers for the delivery, and the use of specific postnatal therapy. Postnatally, an adequate early predictor can help parents to better understand the course of their child's illness. Also, it can be used for a more individualized treatment strategy, and standardized reporting and benchmarking between centers. The Congenital Diaphragmatic Hernia Study Group (CDHSG) is a collaboration of centers from all over the world that voluntarily contribute data from CDH patients. These data are compiled to form an international registry and can be used for the development of prediction tools, among other things.

To predict mortality a new easy tool, the preoperative chest radiographic thoracic area (CRTA) has been developed (chapter 2) (4). This tool seems to accurately correlate with the extent of lung hypoplasia and is a more reliable predictor of survival then the lung-to-head ratio (LHR), but was not when compared to the more reliable observed-to-expected LHR. The CRTA adds to a variety of postnatal tools that have been developed to predict

survival in CDH patients (table 1). However, most are based on relatively small groups of patients, are difficult to apply or have not been validated (5-8). Brindle et al, and the CDHSG have developed and validated a simple early postnatal clinical prediction rule in a large cohort to identify low (<10%), intermediate (~20%), and high risk (~50%) of death in infants. This prediction model is based on birth weight, 5-minute Apgar score, severe PH on echocardiography, and the presence of cardiac and chromosomal anomalies (9). We found that in our group of CDH patients with standardized postnatal care the CDHSG-rule was a fair predictor of survival (chapter 3) (9). This was in line with the validation of the prediction rule by Bent et al, showing a reasonable discrimination, but in their group an underestimation of mortality in the low risk group was seen (9, 10). This might be explained by the difference in health care systems in Europe and the United States. In Europe, centralized and standardized care is more common and many patients with CDH are increasingly born in high volume centers, which improves outcome (11). This might also explain the lowest mortality in patients born in the largest center of our study, although the variability in survival per year remains high even in the centers with the highest experience

We investigated the possibility to improve the model by incorporating both prenatal and postnatal variables in one model. Prenatal variables are able to adequately predict lung hypoplasia and the need for ECMO, but the prenatal assessment of O/E LHR or liver herniation as a marker for lung vascularization and postnatal PH seems less reliable (12). Although these prenatal variables have a role in predicting lung hypoplasia, in almost all postnatal prediction tools prenatal parameters are not taken into account. Interestingly, we found that liver herniation was the only prenatal variable that added to the power of the CDHSG model. This might be explained by collinearity and the absence of uniformity in the measurement of the O/E LHR. Different O/E LHR measurement techniques at different time points in gestation are being used between centers, and there is a learning curve in the measurement of the O/E LHR (13). The longest diameter method even overestimates the O/E LHR up to 34% and has a larger inter observer variability compared to the tracing method (14). The standardization of measurement techniques such as O/E LHR, is essential to improve prediction. However, when using the tracing method the O/E LHR still only has an area under the curve (AUC) of 0.77 for survival (15). International training and standardization is needed and should be implemented within the context of one of the European Reference Networks, ERNICA. Measuring lung volumes on MRI seems promising (16). However, in many centers it is not possible to use MRI for this purpose, due to unavailability and costs. Unfortunately, not only prenatal variables lack standardization. Also the reporting of PH by echocardiography is not standardized and the echocardiographs are performed by a variety of medical specialists with significant differences in experiences. Furthermore, different definitions are being used. In Europe, the presence of PH is often defined as pulmonary pressures higher than 2/3 of the

systemic pressures instead of supra-systemic pulmonary pressures as often used in other centers (1, 9). Furthermore, the timing of measurement is very different between centers (9, 10). Although many European centers perform an echocardiography in the first 24 hours of life, the Canadian centers perform the first echocardiography within 48 hours of life (1, 17). During the first days of life, pulmonary pressures decrease substantially due to physiological transition to extra uterine life. The timing of the echocardiography therefore influences its results. However, even with more consistent measuring techniques, timing and standardized reporting, outcome is also dependent on the later postnatal clinical course and therapy used in the individual patient. Standardized therapy improves outcome, and increases accurate prediction of outcome (11). Right now, the same treatment protocol is being used in most centers in Europe, initiated and guided by the CDH-EURO Consortium guidelines (1, 18). Also, more recently in Canada, standardized guidelines have also been developed (17). These guidelines are fairly similar, although the Canadian guidelines use stricter saturation and pCO2 limits and make a more explicit choice in inotropic support. However, even with standardized care there is a difference in outcome between centers. High-volume CDH centers have better outcomes as they are more experienced (19)(chapter 3). In conclusion, perfect outcome prediction is impossible. Nevertheless, clinical prediction scores like the CDHSG rule and our modified rule are useful for risk stratification. This can lead to a more individualized treatment plan, as shown in chapter 5, describing a spontaneous breathing approach in the delivery room in mild CDH cases.

Lessons learned:

Many predictors for disease severity in CDH exist, but only a few have been externally validated.

Integrating the prenatal liver position and postnatal parameters increases the predictive power of the CDHSG model for mortality in CDH patients.

Predictive prenatal and postnatal parameters and postnatal care are dependent variables

Future perspective:

Standardization of measuring techniques and reporting for several parameters is urgently needed to increase their predictive power.

Although the role of prediction models in the postnatal treatment of CDH patients is limited at the moment, patient selection based on prediction scores, initiating individualized care, should be further investigated as it can potentially improve outcome. It can prevent overtreatment in mild cases and might have a role in prevention and early treatment of PH and cardiac failure in severe cases.

Table 1 Prenatal and early postnatal outcome prediction in CDH patients

Predictors and models	Factors in the model	Prediction	Type of research
Prenatal			
LHR (12)		ECMO	Meta-analysis
O/E LHR (12, 15)		Survival, CLD, ECMO	Two centre study, meta-analysis
Position of the liver (12, 20)		ECMO, survival	Meta-analysis, systematic review
Position of the stomach (21)		Survival, ECMO, mechanical ventilation, respiratory support, patch repair	Single centre study
Mediastinal shift angle (22)		Survival	Single centre study
O/E TFLV (12, 20)		ECMO, survival	Meta-analysis, systematic review
Quantitative lung index		Survival	Single centre study
Foetal risk score (23)	O/E LHR, liver position, stomach position, side, malformations	Unsatisfactory outcome (mortality or LOS>180 days)	Japanese registry
CDH-CPI (24)	Genetic or syndromic anomaly, cardiac anomaly, LV/RV ratio, McGoon index, presence of sac, liver position, LHR, TFLV	Survival	Single centre study
Postnatal			
CRTA (4)		Survival	Single centre study
SNAP II Score (5)	pH, PaO2:FiO2, temperature, diuresis, seizures, blood pressure	Survival, need for ECMO	Multicentre (VICI trial)
Oxygenation Index (6, 25)		Survival, LOS, timing of surgery	Single centre study
Tidal volumes at birth (26)		Survival, CLD	Single centre prospective study
Lowest PaCO2		Survival, ECMO, respiratory support day 30	CDHSG registry
WHSRpf (27)	Highest PaO2 minus highest PaCO2	Survival	Single centre study
PF-PCO2 tool (28)	(PaO2/FiO2 ratio) minus PaCO2	Survival	Single centre study
CDH-PS (7)	Birth weight, 5 min Apgar score	Survival	CDHSG registry
CDHSG prediction rule (9)	Birth weight, 5 min Apgar score, PH, cardiac and chromosomal anomalies	Survival	CDHSG registry
ECMO risk stratification (29)	1 and 5 min Apgar score, highest and lowest post-ductal PaCO2	ECMO	CDHSG registry
Mixed			
Perinatal prognostic index (30)	Polyhydramnios, gestational age at diagnosis, O/E LHR, OI, tricuspid regurgitation	30-day mortality	Single centre study
Modified CDHSG prediction rule (chapter 3)	Birth weight, 5 min Apgar score, PH, cardiac and chromosomal anomalies, liver position	Survival	4 centres of CDH EURO Consortium

LHR= lung-to-head ratio, O/E LHR= observed-to-expected lung-to-head ratio, CLD= chronic lung disease, ECMO= extracorporeal membrane oxygenation, TFLV= total foetal lung volume, RV= right ventricle, LV= left ventricle, CRTA= chest radiographic thoracic area, LOS= length of stay, PH= pulmonary hypertension, OI= oxygenation index

Biomarkers

Biochemical biomarkers, solely or in a panel, are potentially useful for the prediction of CLD, PH and mortality in CDH patients. However, the ideal biomarker in CLD patients is yet to be found. We tested SIGLEC-5/14, BCAM and ANGPTL3, a panel of biomarkers which were found to be a sensitive prediction tool, at least for BPD in preterm infants (chapter 4) (31). Although the panel was not predictive for CLD in CDH, it did predict mortality in our population with an AUC of 0.76. Siglecs help immune cells to distinguish between "self" and "non-self". SIGLEC-5/14 has a role in the regulation of the immune response in the lung and is associated with Group B Streptococcus infections in preterm infants and newborns, but also with the degree of tissue damage and the amount of inflammatory exacerbations in COPD in adults (32, 33). BCAM is associated with impaired lung development and airway branching (31). ANGPTL3 has a regulatory role in the angiogenesis of the lung (34). Although these biomarkers did have an association with mortality in CDH patients, it was not as strong as the association with BPD in preterm infants. Both BPD and CLD reflect an alteration in lung development, unlike chronic lung disease in adults such as COPD. However, BPD in preterm infants and CLD in CDH patients differ substantially. Although the definition of BPD is still controversial, to diagnose BPD by definition the infants are born preterm in contrast to the older definition in which BPD could also be diagnosed in term infants who needed oxygen supplementation on day 28 postnatal age (35). The substrate for BPD in preterm infants is a combination of a structurally immature lung and lung injury and subsequent repair. In the preterm infant the injury can be caused by inflammation, due to chorioamnionitis, oxygen exposure and mechanical ventilation. Developmental disruptions, such as growth restriction and nicotine exposure, play a role too, impeding the alveolarization of the lung (36). BPD is associated with pulmonary vascular remodelling causing PH. Although BPD and PH partly have the same risk factors, there is growing evidence that PH in preterm infants has a foetal "origin" that is caused in part by maternal vascular disease during pregnancy (37). In contrast to BPD and PH in BPD, a structural lung defect in early foetal development, possibly due to changes in the retinoic acid signalling pathway, causes pulmonary hypoplasia and PH in CDH patients. This is characterized by thickened alveolar walls, reduced alveolar air spaces and an increase in interstitial tissue (38). The differences between BPD and PH are underlined by the effect of surfactant on the initial lung pathology at birth. In contrast to the premature infant, surfactant does not improve outcome in CDH patients because surfactant maturation in CDH lungs is not delayed (39, 40). Pulmonary hypertension in CDH is characterized by hypermuscularization of the midsized and large vessels and neomuscularization of the small capillaries in an incomplete capillary bed. These changes, as well as the expression of markers associated with more contractile smooth muscles, are already seen early in gestation, and can be explained by altered behaviour and maturation of pericytes, the precursors of smooth muscle cells (41, 42). Although inflammation has a clear role in BPD, this is not obvious in CDH patients. Interestingly, not ANGPTL3 with a

role in angiogenesis, but SIGLEC-5/14, a marker involved in the immune response, could identify mortality. We evaluated these markers on day 1 and 3, but the exact moment of sampling on day 1 could be soon after birth or at 24 hours. All infants with CDH received intensive care treatment, consisting of intubation and ventilation with 100% of oxygen initially, parenteral nutrition and if needed inotropic support, antibiotics and vasodilating drugs. One could argue that these early postnatal influences could have already triggered inflammation. Earlier biomarkers, such as samples taken from the umbilical cord, might be more suitable. However, the finding of SIGLEC-5/14 as a biomarker for mortality in CDH raises the question how much harm is done with the current treatment strategies. This endorses the importance of individualized care, balancing the benefit and the harm. These inflammatory biomarkers might also guide us towards a more restrictive approach to oxygen therapy, since high alveolar oxygen concentrations cause pulmonary edema, and increase inflammatory markers and cell necrosis (43).

SIGLEC-5/14, BCAM and ANGPTL3 were derived from proteomic screening in preterm infants (31). Instead of analyzing a single biomarker or a group of known biomarkers, proteomics might also increase our knowledge in which proteins are relevant in the clinical course of CDH. Proteomic profiling offers complementary information to genomics and can be used without any preliminary hypotheses. Protein function and abundance is dependent on genomic expression, but also on multiple posttranscriptional and posttranslational mechanisms (44). By using whole proteome screening at different time points, relevant pathways and proteins might give a more detailed insight in the pathophysiology of CDH and the effects of therapy. Using proteomic screening in tracheal aspirate, CDH patients could be separated from healthy controls, intrauterine via amniotic fluid, as well as in the first 48 hours of life (45, 46). This may be the first step towards the identification of different disease phenotypes and subsequently a protein profile-specific treatment plan. But tracheal aspirate sampling is an invasive procedure. Exhaled breath condensate (EBC) analysis, collected by cooling exhaled breath, might be a reliable non-invasive method. However, there is no standardization in EBC collection for intubated infants at this moment (47).

Lessons learned

BPD in preterm infants and CLD in CDH patients are different entities. The panel of biomarkers SIGLEC-5/14, BCAM and ANGPTL3 can predict mortality but not CLD in CDH patients. SIGLEC-5/14, an inflammatory biomarker, was the strongest predictor of mortality.

Future perspectives

With proteomic screening at different time points, derangements in relevant pathways might give a more detailed insight in the pathophysiology of CDH and the effects of therapy.

Umbilical cord sampling might be useful for baseline and CDH specific sampling, eliminating postnatal influences.

TREATMENT

Pulmonary hypertension

Over time the focus of therapy in CDH has changed. Until the 80's CDH was considered a surgical emergency (figure 1). Thereafter preoperative stabilization because mainstream, focusing on correcting acidosis and hypoxia (48). However, aggressive ventilation strategies cause barotrauma in the hypoplastic lungs. In 1995 Wung et al reported a respiratory strategy focusing on the prevention of hyperventilation and hyperinflation to minimize iatrogenic lung injury and exacerbation of pulmonary hypertension (49). Since then this has been adopted worldwide and surgical closure has been transformed into an elective procedure in "stable" patients. However, it wasn't until the late 2000 though, that the VICI trial started, the first RCT in CDH patients, comparing conventional ventilation with high frequency ventilation (50). Primary outcome was chronic lung disease and/or mortality on day 28. However, inclusion rates of 400 patients were never met, the study was halted after 171 patients and a study period of 5 years. The study showed no difference in primary outcome between ventilation modes, but was heavily underpowered. Other outcome parameters, including ventilation time, inotropic support and need for ECMO, favored conventional ventilation (3). Also prenatal interventions, first open surgery and tracheal clipping and later fetal endoscopic tracheal occlusion (FETO), were developed to increase lung growth. The first RCT did not show improvement of survival or morbidity in fetuses with severe CDH, and more infants were born prematurely in the intervention group (51). The disappointing results of the trial at that time resulted in a moratorium on tracheal plugging at least in the USA. As technical progress made the procedure more safe, the so-called TOTAL trial was created and conducted mainly by a small number of expertise centers in Europe; Leuven, Barcelona and London. Currently, the TOTAL trial, an international RCT is investigating FETO. In the moderate (NCT00763737) CHD group the trial is completed and we are awaiting results. In the severe (NCT01240057) CDH group, the trial is still recruiting. Nowadays the focus has shifted from the hypoplastic lung towards PH. At this time two RCTs are recruiting CDH patients in the search for the best initial therapy for PH, the CoDiNOS trial (chapter 7) and the "Milrinone in Congenital Diaphragmatic Hernia" trial (NCT02951130). There are also a few trials on their way that focus on physiological based cord clamping, such as the PinC trial (NCT04373902) and the CHIC trial (NCT04429750), with the aim to decrease the incidence of PH. (table 2)

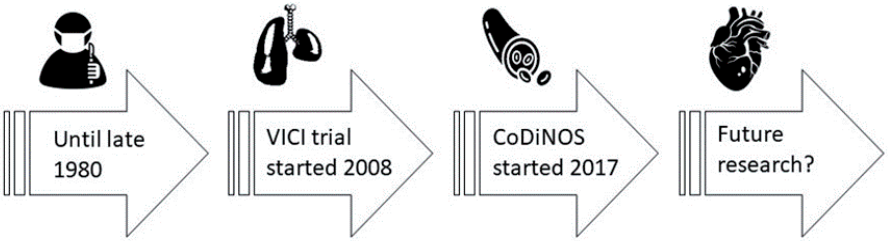


Figure 1. Focus of research and therapy in CDH patients "shifting the paradigm"

Table 2. Randomized controlled trials in CDH currently recruiting

RCT	Started in	Intervention	Primary outcome
FETO	2008	Fetoscopic endoluminal tracheal occlusion with a balloon versus expectant management	to increase lung size
Milrinone in CDH	2016	Milrinone versus placebo	treatment of PH
CDH Optimisation of Neonatal Ventilation	2016	Ventilation with different tidal volumes	Change in Pressure Time Product of the diaphragm
CoDiNOS	2017	Sildenafil versus iNO	treatment of PH
PinC	2020	Physiological based cord clamping versus direct cord clamping	to prevent development of PH
CHIC	2020	Physiological based cord clamping versus direct cord clamping	to prevent development of PH

In Chapter 7 we describe the CoDiNOS trial protocol, comparing intravenous sildenafil with iNO in the treatment of PH in CDH patients. When performing an RCT in infants using a pharmacological intervention, it is essential to first establish an adequate dosing regimen to be able to evaluate efficacy. Although pharmacokinetic drug testing in adults is very common, in infants dosing regimen are often an extrapolation from adult data, only corrected for body size (52). This assumes that fractioning of the dose will lead to similar plasma drug levels as it also assumes that, besides body composition, children have similar renal, gastrointestinal and hepatic function. This can result in over- and under dosing, leading to toxicity or reduced efficacy (53). Before starting the CoDiNOS trial we developed a pharmacokinetic model of sildenafil in infants with CDH (chapter 6). We found that the dosing regimen commonly used in newborns with PPHN was also applicable to

CDH patients. A loading dose of 0.4mg/kg in 3 hours followed by a continues infusion of 1.6mg/kg/day achieved adequate sildenafil plasma levels. Steinhorn et al found this dosing regimen in a dose-escalation trial in infants with persistent pulmonary hypertension of the newborn (54). Although numbers were too low to find a strong correlating between plasma concentration and clinical effects, plasma concentrations over 50µg/l seemed to decrease the oxygenation index. In our cohort plasma concentrations were 190 µg/l and higher after 3 hours. However, numbers were too low to find any correlation between these concentrations and the oxygenation index. Samples collected during the CoDiNOS trial will give more insight in the dose-response correlation of sildenafil in CDH patients as well as other pharmacodynamic effects. Pharmacodynamic evaluation is not only important for its effect but also for its side effects. Currently, sildenafil is given with a loading dose over 3 hours, because of the risk of hypotension. Consequently, the first clinical effect of sildenafil can probably only be seen after 1 hour of infusion, which is a long time for this instable group of patients with CHD and PH. Unintentionally, the loading dose of sildenafil was given as a bolus to one of our CDH patients. Subsequently the saturation improved from 84% to 98% with a only a slight decrease of blood pressure from mean 48mmHg to mean 44mmHg (figure 2). Possibly the positive effect of sildenafil on PH and therefor cardiac output compensates the negative effect on blood pressure due to vasodilatation in CHD patients with good cardiac function.

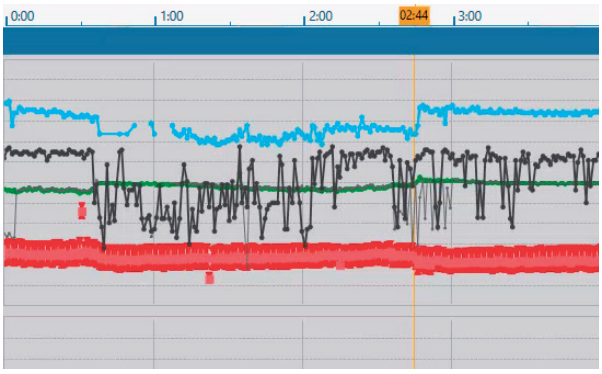


Figure 2. Bolus of sildenafil in newborn with CDH

Blue line = oxygen saturation, black line = respiratory rate, green line = heart rate, red line = blood pressure

Although the CoDiNOS trial is still recruiting patients and evidence suggests that sildenafil can play a role in the treatment of PH in CDH, one could argue that, from a pathophysiological standpoint, it would be more logical to compare drugs that act on different pathways, instead of comparing drugs that both act on the nitric oxide-cGMP pathway. Also, there seems to be no apparent alteration of this pathway in CDH patients, decreasing the change of finding an effective drug (55). Drugs that affect the endothelin

pathway might be more successful. CDH patients show an increase in endothelin A and B receptor expression and ECE-1 enzyme. This enzyme is responsible for the conversion of endothelin-1 to its active form (55). CDH patients with PH have higher endothelin-1 plasma levels than CDH patients without PH (56). However, endothelin receptor antagonists are still only available in oral form, making them unsuitably for the treatment of CDH patients. The prostacyclin pathway seems to be altered in CDH patients too. Mous et al found a decrease of prostaglandin-I_2 receptor expression, which would explain the negative effect of prostacyclin derivates on PH in CDH patients, although results are conflicting (55, 57, 58). All drugs available for PH in infants, children and adults influence one of the three pathways. However, the etiology of PH differs widely between age groups but also between different diseases and none of these drugs can resolve PH as a whole (one drug fits all). The focus of new drugs might need to be outside these pathways. Possibly proteomic screening will give us a new entry for drug development. For instance, in adults with pulmonary arterial hypertension bromodomain-containing protein 4 (BRD4) has been identified as driver for hyperpoliferative, apoptosis-resistant and inflammatory phenotypes of endothelial cells and smooth muscle cells. BRD4 is a member of the bromodomain and extra-terminal motif (BET) family. RVX208, an BET inhibitor, reverses these phenotypes in vitro and in rat models. A clinical trial has not been performed though (59). Proteomic screening can also give more insight in the effects and side effects of treatment.

It is to be questioned if an RCT as we know it in its present form, in orphan diseases such as CDH, is the optimal tool to collect evidence based information. The VICI trial had recruiting issues due to the lower than anticipated recruitment rate and a lack of financial resources, which affected the organization of the research infrastructure in participating centers. Also in the CoDiNOS trial serious recruiting issues exist. We planned to include 330 patients in a time frame of 3 years. However, at the time of writing, we have included 11 patients in 3 years. This is partly caused by lower than anticipated recruitment rates due to strict inclusion criteria. It is very important to select the right patients when performing an RCT. The potential benefit and harm of an intervention should be taken into account and including mild cases will dilute the effect of an intervention. Although echocardiogram is often perceived to be the best diagnostic tool in newborns, the incidence of PH on echocardiogram on day 1 of life overestimates the incidence of clinical relevant PH, because high pulmonary pressures are still part of the physiological transition. We only include infants with clinically relevant PH, defined as PH on echocardiogram and clinical signs of PH, decreasing the incidence of PH from 60% to around 30%. Other important recruiting issues are a delay in National Drug Authority approval in participating countries, issues with legislation and insurance, and limited funding. Performing an RCT in pediatric critical care is challenging, especially when a high number of centers is needed due to low volumes, like in the CoDiNOS trial. Lack of funding and time are known barriers to conduct an RCT in pediatric critical care. Collaborating in a research network, such as the CDH EURO

Consortium, increases the chance of success (60). However, regulations to conduct an RCT in pediatrics, especially drug interventions, are increasingly stringent. For example, many countries and health care institutions force researchers to use a Clinical Trial Organization when conducting a trial. However, these organizations are often very expensive, swallowing a major part of the budget of investigator-led trials. Interestingly enough, investigator-initiated research is significantly more frequently cited than industry-led trials in critical care medicine (61). This proves that investigator-initiated research is very important as it is more clinically relevant and has more impact on clinical practice. Legislation should not further complicate investigator-initiated research but should focus on stimulating these relevant trials. This was acknowledged in the revision of Directive of the European Commission in 2014, but does not seem to have resulted in a substantial practice change (62). However, in 2017 European Reference Networks were launched in response to a European Commission call. The goal of these networks is to improve healthcare for rare diseases in Europe, focusing on medical training and research, information dissemination and evaluation. One of these networks is ERNICA, the European Reference Network for rare Inherited and Congenital (digestive and gastrointestinal) Anomalies. Although the CDH EURO Consortium is a robust network, affiliating with ERNICA can potentially increase funding and logistical support for RCTs in the future by merging funding and resources. To further increase the clinical relevance of trials, patient organizations are increasingly involved in the funding and development of trials, especially in investigator-initiated trials. The CoDiNOS trial is funded for an important part by CDH-UK Sparks, the CDH patient organization from the United Kingdom.

New statistical approaches might also increase the feasibility of controlled trials. For instance, one could add real-world controls to a trial, decreasing the number of included patients needed to achieve statistical significance. The real-world controls consist of patients whose parents either chose not to participate in the trial or were not included due to logistical and organizational issues. Looking at the VICI-trial, more than 425 of the 619 patients who were treated in the VICI trial centres during the study period were not included in the trial. These real-world controls should be highly comparable to the VICI patients, because they share the same treatment period, the same treatment guideline (i.e. the CDH EURO consortium guidelines) and they were treated in the same centres. Most of the patients not participating were initially treated with conventional mechanical ventilation, as this was the standard of care at the time. When combining data from the randomized VICI trial with the observational data from patients who did not participate in the trial, one needs to account for potential differences in baseline patient characteristics and other biases that may arise from the inclusion of non-randomized data. Different statistical techniques such as dynamic borrowing can be used for this purpose (63). This approach would lead to revised estimates of the treatment effect of ventilation mode on the primary endpoint with greater statistical power and precision. Using real-world

controls can increase the feasibility of RCTs in many rare patient population substantially. However, to our knowledge it has not been used in clinical research.

One could also decrease the number of patients needed by changing the primary endpoint from a dichotomous and cross-sectional endpoint to a more sensitive endpoint, incorporating repeated measurements or having a more informative scale (e.g., ordinal or continuous). This increases the statistical power to test the impact of an intervention on the patient's outcome. This is especially relevant for CLD, which was defined in the VICI trial as the need for any respiratory support on day 28, without taking into account the amount and the duration of respiratory support. Several additional measurements collected from VICI trial patients could be used to define more informative endpoints. This can include ordinal endpoints such as the level of ventilation support, continuous variables such as amount of oxygen support, and derived endpoints such as time to discharge or time to reduction of ventilator support. Based on these more informative endpoints, multiple hypothesis testing with improved statistical properties compared to the original primary analysis can be applied. One can combine the endpoints in a single composite endpoint, for instance by defining a score that incorporates information from several endpoints and also accounts for mortality, but one can also test each endpoint separately. Specific statistical approaches to account for multiplicity for the testing of multiple, repeatedly measured endpoints will be needed, such as the multiple marginal generalized estimating equation models (GEE) method (64). The GEE method can incorporate endpoints on different scales (e.g. death and oxygen support), repeated measurements, and measurements recorded at a single time point only, while accounting for the correlation between endpoints to maximize the power of statistical tests.

Lessons learned

Pharmacokinetics of sildenafil and its metabolite is similar in newborns with PPHN and CDH and only postnatal age influences its clearance.

Controlled trials in children with orphan diseases are difficult to perform for various reasons, although these trials have a large clinical impact. Trials executed by a consortium have the largest change to succeed.

Future perspectives

Although the pharmacokinetic profile of intravenous sildenafil in patients with CDH is more clear, little is known of its pharmacodynamic profile. The results of the CoDiNOS trial will give more insight in the PKPD model of sildenafil and it metabolite.

New drug trials should focus on the endothelin pathway as this pathway seems altered the most in CDH patients. However, an intravenous dosage form needs to be developed

first. Also, proteomic screening will potentially reveal new pathways that can be used for drug development.

Newly developed statistical tools are currently not implemented in clinical research. However, these tools can decrease the number of patients needed to answer the research question.

Respiratory support

Therapy in CDH patients is standardized in European centers since the introduction of the CDH EURO Consortium guidelines in 2010. This has increased survival from 67% to 88% (1, 18). Most of the content of the guidelines is based on expert opinion, not on evidence. With reliable prediction models one can select specific patient groups to change common practice, because one size probably does not fill all. According to these guidelines all CDH patients are immediately intubated and ventilated with 100% oxygen after birth. One of the keystones of the guidelines is gentle ventilation to prevent lung damage of the hypoplastic lung and to decrease the negative impact of positive intrathoracic pressure on pulmonary perfusion by limiting inspiratory peak pressures (1, 17, 65, 66). We have introduced a spontaneous breathing approach (SBA) in the delivery room for patients with mild CDH, using the prenatal prediction criteria for isolated left sided CDH, O/E LHR of 50% or higher, and an abdominal position of the liver (chapter 5). For this subgroup of CDH patients, intubation and mechanical ventilation immediately after birth has potential unnecessary side effects. Positive pressure ventilation can cause lung injury due to a combination of barotrauma, volutrauma, atelectrauma and hyperoxia (67). Also, with a successful SBA, stress, pain and the need for sedation is reduced and consequently postnatal parent-infant interaction is improved. We found that in this group with relatively mild disease, SBA is feasible. In 40% the SBA was successful, in the other 60% it did not seem to cause harm. More research is needed to explore the effect of an unsuccessful SBA, to evaluate the patient selection criteria and to externally validate these results. Although an RCT would be the most powerful tool to prove the role of an SBA in mild CDH patients, this does not seem feasible due to low patient numbers.

Lessons learned

Patient selection to make an individualized treatment plan is feasible and resulted in an SBA in patients with mild CDH. The SBA was successful in 40% of the patients.

Future perspectives

The effect of an unsuccessful SBA should be further investigated, as well as the patient selection criteria. The results also need external validation.

The role of the heart

Using prediction to tailor therapy as we did (chapter 5), could now become a logical step in the optimization of the treatment of CDH patients. In pediatric oncology risk stratification is an important tool to individualize the treatment protocol for patients (68). However, it is important to use the right tools to stratify the patient. In CDH patients, the focus has been on the lungs and the vascular bed. However, growing evidence shows that we should not overlook the heart (figure 1). In a large group of CDH patients 39% had some degree of ventricular dysfunction, right, left or both on the initial echocardiogram. Especially left ventricular (LV) dysfunction and dysfunction of both ventricles was associated with increased mortality (69). Even in the "low-risk"- group, defined as defect size A/B without other structural anomalies, diminished LV function, as well as severe PH, were strong independent predictors of outcome (70). PH is partly responsible for cardiac dysfunction in CDH patients. The right ventricle dilates and shows diastolic dysfunction due to the increase in afterload. Septal displacement impairs the left ventricle function. But in left-sided CDH patients the left ventricle is often also hypoplastic, further increasing its dysfunction (71). Also in right-sided CDH patients the right ventricle is decreased in size (72). Possibly immediate cord clamping in the delivery room also contribute to cardiac dysfunction. Before cord clamping, oxygenated blood in the umbilical veins shunts via the ductus venosus and foramen ovale to the left atrium, guaranteeing venous return to the LV. Cord clamping separates the newborn from its oxygen source and from the blood flow required to maintain LV preload. At the same time, LV afterload increases as the low-resistance circulation of the placenta is removed (73, 74). As a result, cardiac output decreases. Various trials, such as the PinC trial (NCT04373902), are currently recruiting, to investigate the effect of physiological based cord clamping on PH and cardiac function (table 2).

CDH patients with PH and a diminished LV function are more often unresponsive to intravenous sildenafil and iNO as shown by Kipfmueller et al and Lawrence at al (75, 76). It is assumed that, although pulmonary vascular resistance decreases, the failure of the LV is exacerbated by the increase in pulmonary venous return (77). Milrinone, a phosphodiesterase 3 inhibitor, improves diastolic function and possibly has a direct effect on PH, potentially making it a very suitable drug for the treatment of both right ventricular (RV) and LV dysfunction in CDH patients. However, in a small group of CDH patients milrinone did not improve outcome (78). Although the RCT "Milrinone in Congenital Diaphragmatic Hernia" trial (NCT02951130) focusses on the effect of milrinone on oxygenation index, it will also increase our knowledge in the role of milrinone in supporting the heart in CDH. Also vasopressors, such as vasopressin, could potentially play a role in LV dysfunction. Vasopressin has shown to improve hemodynamics in CDH patients (79). The mechanism of improvement is unclear, but may be explained by improved biventricular function in response to an increase in LV afterload without adverse effects on pulmonary vascular

resistance. When LV dysfunction persists, ECMO can be used as a last resort. Later on, in childhood, an increased ejection fraction and a reduction of the stroke volume of the LV is seen in CDH patients, without decreased perception of fitness (80).

The severity of early RV diastolic dysfunction is correlated with length of ventilator support, length of stay and mortality (81). Interestingly enough, pulmonary artery pressure is not associated with the same outcome, suggesting that the right ventricle itself plays an important role in disease severity (82). RV diastolic dysfunction often improves after 2 to 3 days, but can deteriorate after surgery (81). First step in the treatment of RV dysfunction is reducing afterload by treating PH. The next step might be a cardiotrope such as milrinone. If severe RV dysfunction persists ECMO treatment might be needed (71). RV function in CDH patients seems to be altered years after the surgical repair (83). The clinical impact of this alteration into adulthood is unclear. The trial "Growing up after surgery for Congenital Diaphragmatic Hernia (CDH): Problems for life?" is currently recruiting, evaluating cardiac function, among other things, in young adults with CDH (NTR NL7688).

Although more research is needed, one could argue that in the treatment of CDH, the cardiac function should also be assessed to stratify the patient and incorporate the cardiac function in the treatment plan. Also, surgery in CDH patients is typically planned after hemodynamic stabilization, possibly one should take diastolic dysfunction into account before performing surgery (84). However, Tanaka et al showed that early surgery, within 24 hours, improved diastolic dysfunction in CDH patients, increasing the preload of the left ventricle, which increases its contractibility (85).

However, like in PH, the assessment of cardiac function on echocardiogram is not standardized. Different measurement techniques with various degrees of difficulty to measure and analyze are being used. Also, some of these measurements are load or heart rate dependent (71). Therefore, in the CoDiNOS trial we use a standardized panel of measurements to assess the pulmonary pressures and the heart. One could argue that clinical parameters should also be incorporated in the assessment of cardiac function as the pattern of cardiac dysfunction can change and an echocardiogram will not be readily available any time of the day and is fairly invasive for a newborn in intensive care. Although these parameters are surrogate markers for cardio-vascular status, integrating parameters such as pre- and postductal saturation, blood pressure and heart rate and its variation in a real-time multimodality model might help us to see very early signs of PH or cardiac dysfunction. Multimodality monitoring is currently being investigated in CDH patients for a different purpose, in the NEMO trial (NTR7160). The NEMO trial focusses on peri-operative neuromonitoring and growth and neurodevelopment at the age of 2 years. Although a single neuromonitor, such as NIRS, was not related to clinical outcome or prognosis, the integration of several monitors into a multimodality model

using high frequent measurements might substantially increase its clinical relevance (86). This concept of multimodality monitoring might also be useful for other clinical outcome parameters such as PH and cardiac dysfunction. In neonatal intensive care attempts have been made to develop multimodality hemodynamic monitoring. However there are still some important limitations, such as the use of surrogate measurements because direct measurement of hemodynamic measurements is impossible in newborns, but also limitations in technology and costs (87). High density monitoring might also give more insight in the effect of oxygen in CDH patients, because one could use an area-under-the-curve analyses instead of a cutoff analyses (88). This longitudinal and cumulative approach probably better addresses the effect of hyperoxia on the lung and pulmonary vasculature.

Lessons learned

Ventricular dysfunction of both the RV and LV can be seen in CDH patients and is predictive for outcome.

Future perspectives

First, a golden standard for echocardiographic assessment of cardiac dysfunction should be developed. As this is not a continues monitor, a multimodality model might help to evaluate cardiac function and PH in real-time.

The treatment of the ventricular dysfunction needs to be further investigated, with and without the presence of PH as the treatment of PH can further exacerbate LV dysfunction.

LITERATURE

1. Snoek KG, Reiss IK, Greenough A, Capolupo I, Urlesberger B, Wessel L, et al. Standardized Postnatal Management of Infants with Congenital Diaphragmatic Hernia in Europe: The CDH EURO Consortium Consensus - 2015 Update. Neonatology. 2016;110(1):66-74.
2. H IJ, Breatnach C, Hoskote A, Greenough A, Patel N, Capolupo I, et al. Defining outcomes following congenital diaphragmatic hernia using standardised clinical assessment and management plan (SCAMP) methodology within the CDH EURO consortium. Pediatr Res. 2018;84(2):181-9.
3. Snoek KG, Capolupo I, van Rosmalen J, Hout Lde J, Vijfhuize S, Greenough A, et al. Conventional Mechanical Ventilation Versus High-frequency Oscillatory Ventilation for Congenital Diaphragmatic Hernia: A Randomized Clinical Trial (The VICI-trial). Ann Surg. 2016;263(5):867-74.
4. Dassios T, Ali K, Makin E, Bhat R, Krokidis M, Greenough A. Prediction of Mortality in Newborn Infants With Severe Congenital Diaphragmatic Hernia Using the Chest Radiographic Thoracic Area. Pediatr Crit Care Med. 2019;20(6):534-9.
5. Snoek KG, Capolupo I, Morini F, van Rosmalen J, Greenough A, van Heijst A, et al. Score for Neonatal Acute Physiology-II Predicts Outcome in Congenital Diaphragmatic Hernia Patients. Pediatr Crit Care Med. 2016;17(6):540-6.
6. Bruns AS, Lau PE, Dhillon GS, Hagan J, Kailin JA, Mallory GB, et al. Predictive value of oxygenation index for outcomes in left-sided congenital diaphragmatic hernia. J Pediatr Surg. 2018.
7. Congenital Diaphragmatic Hernia Study G. Estimating disease severity of congenital diaphragmatic hernia in the first 5 minutes of life. J Pediatr Surg. 2001;36(1):141-5.
8. Patel MJ, Bell CS, Lally KP, Lally PA, Katakam LI, Congenital Diaphragmatic Hernia Study G. Lowest PaCO2 on the first day of life predicts mortality and morbidity among infants with congenital diaphragmatic hernia. J Perinatol. 2018.
9. Brindle ME, Cook EF, Tibboel D, Lally PA, Lally KP, Congenital Diaphragmatic Hernia Study G. A clinical prediction rule for the severity of congenital diaphragmatic hernias in newborns. Pediatrics. 2014;134(2):e413-9.
10. Bent DP, Nelson J, Kent DM, Jen HC. Population-Based Validation of a Clinical Prediction Model for Congenital Diaphragmatic Hernias. J Pediatr. 2018.
11. Bucher BT, Guth RM, Saito JM, Najaf T, Warner BW. Impact of hospital volume on in-hospital mortality of infants undergoing repair of congenital diaphragmatic hernia. Ann Surg. 2010;252(4):635-42.
12. Russo FM, Eastwood MP, Keijzer R, Al-Maary J, Toelen J, Van Mieghem T, et al. Lung size and liver herniation predict need for extracorporeal membrane oxygenation but not pulmonary hypertension in isolated congenital diaphragmatic hernia: systematic review and meta-analysis. Ultrasound Obstet Gynecol. 2017;49(6):704-13.
13. Cruz-Martinez R, Figueras F, Moreno-Alvarez O, Martinez JM, Gomez O, Hernandez-Andrade E, et al. Learning curve for lung area to head circumference ratio measurement in fetuses with congenital diaphragmatic hernia. Ultrasound Obstet Gynecol. 2010;36(1):32-6.

14. Jani JC, Peralta CF, Nicolaides KH. Lung-to-head ratio: a need to unify the technique. Ultrasound Obstet Gynecol. 2012;39(1):2-6.
15. Snoek KG, Peters NCJ, van Rosmalen J, van Heijst AFJ, Eggink AJ, Sikkel E, et al. The validity of the observed-to-expected lung-to-head ratio in congenital diaphragmatic hernia in an era of standardized neonatal treatment; a multicenter study. Prenat Diagn. 2017.
16. Russo FM, Cordier AG, De Catte L, Saada J, Benachi A, Deprest J, et al. Proposal for standardized prenatal ultrasound assessment of the fetus with congenital diaphragmatic hernia by the European reference network on rare inherited and congenital anomalies (ERNICA). Prenat Diagn. 2018;38(9):629-37.
17. Canadian Congenital Diaphragmatic Hernia C. Diagnosis and management of congenital diaphragmatic hernia: a clinical practice guideline. CMAJ. 2018;190(4):E103-E12.
18. Reiss I, Schaible T, van den Hout L, Capolupo I, Allegaert K, van Heijst A, et al. Standardized postnatal management of infants with congenital diaphragmatic hernia in Europe: the CDH EURO Consortium consensus. Neonatology. 2010;98(4):354-64.
19. Snoek KG, Greenough A, van Rosmalen J, Capolupo I, Schaible T, Ali K, et al. Congenital Diaphragmatic Hernia: 10-Year Evaluation of Survival, Extracorporeal Membrane Oxygenation, and Foetoscopic Endotracheal Occlusion in Four High-Volume Centres. Neonatology. 2018;113(1):63-8.
20. Oluyomi-Obi T, Kuret V, Puligandla P, Lodha A, Lee-Robertson H, Lee K, et al. Antenatal predictors of outcome in prenatally diagnosed congenital diaphragmatic hernia (CDH). J Pediatr Surg. 2017;52(5):881-8.
21. Basta AM, Lusk LA, Keller RL, Filly RA. Fetal Stomach Position Predicts Neonatal Outcomes in Isolated Left-Sided Congenital Diaphragmatic Hernia. Fetal Diagn Ther. 2016;39(4):248-55.
22. Romiti A, Viggiano M, Conforti A, Valfre L, Rava L, Ciofi Degli Atti M, et al. Ultrasonographic assessment of mediastinal shift angle (MSA) in isolated left congenital diaphragmatic hernia for the prediction of postnatal survival: MSA in left CDH. J Matern Fetal Neonatal Med. 2018:1-121.
23. Terui K, Nagata K, Hayakawa M, Okuyama H, Amari S, Yokoi A, et al. Novel Risk Score for Fetuses with Congenital Diaphragmatic Hernia Based on Ultrasound Findings. Eur J Pediatr Surg. 2019.
24. Le LD, Keswani SG, Biesiada J, Lim FY, Kingma PS, Haberman BE, et al. The congenital diaphragmatic hernia composite prognostic index correlates with survival in left-sided congenital diaphragmatic hernia. J Pediatr Surg. 2012;47(1):57-62.
25. Tan YW, Ali K, Andradi G, Sasidharan L, Greenough A, Davenport M. Prognostic value of the oxygenation index to predict survival and timing of surgery in infants with congenital diaphragmatic hernia. J Pediatr Surg. 2018.
26. Mank A, Carrasco Carrasco C, Thio M, Clotet J, Pauws SC, DeKoninck P, et al. Tidal volumes at birth as predictor for adverse outcome in congenital diaphragmatic hernia. Arch Dis Child Fetal Neonatal Ed. 2019.
27. Schultz CM, DiGeronimo RJ, Yoder BA, Congenital Diaphragmatic Hernia Study G. Congenital diaphragmatic hernia: a simplified postnatal predictor of outcome. J Pediatr Surg. 2007;42(3):510-6.

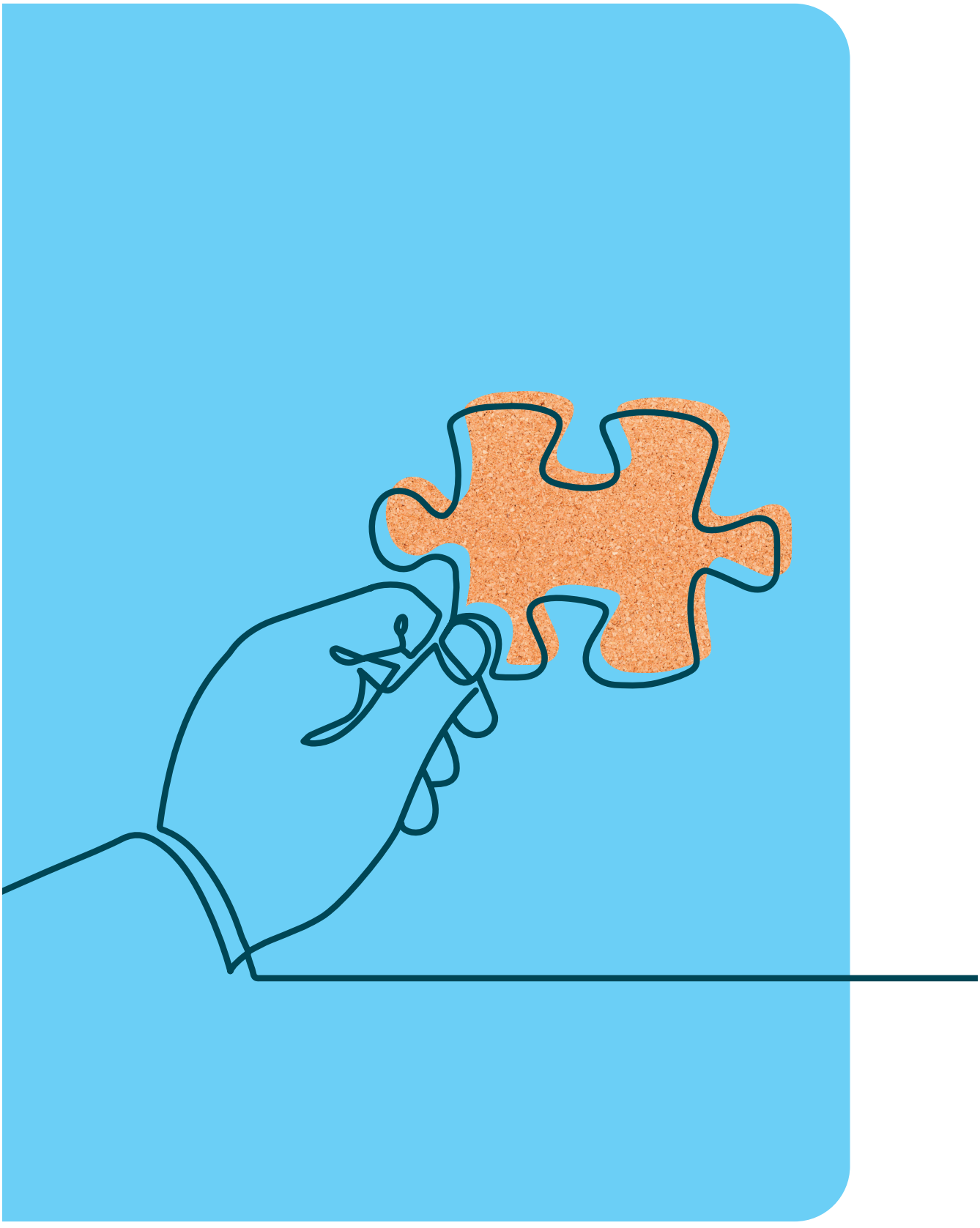
28. Sekhon MK, Fenton SJ, Yoder BA. Comparison of early postnatal prediction models for survival in congenital diaphragmatic hernia. J Perinatol. 2019.
29. Jancelewicz T, Brindle ME, Harting MT, Tolley EA, Langham MR, Jr., Lally PA, et al. Extracorporeal Membrane Oxygenation (ECMO) Risk Stratification in Newborns with Congenital Diaphragmatic Hernia (CDH). J Pediatr Surg. 2018.
30. Oh C, Youn JK, Han JW, Yang HB, Lee S, Seo JM, et al. Predicting Survival of Congenital Diaphragmatic Hernia on the First Day of Life. World J Surg. 2018.
31. Forster K, Sass S, Ehrhardt H, Mous DS, Rottier RJ, Oak P, et al. Early Identification of Bronchopulmonary Dysplasia Using Novel Biomarkers by Proteomic Screening. Am J Respir Crit Care Med. 2018;197(8):1076-80.
32. Macauley MS, Crocker PR, Paulson JC. Siglec-mediated regulation of immune cell function in disease. Nat Rev Immunol. 2014;14(10):653-66.
33. Ali SR, Fong JJ, Carlin AF, Busch TD, Linden R, Angata T, et al. Siglec-5 and Siglec-14 are polymorphic paired receptors that modulate neutrophil and amnion signaling responses to group B Streptococcus. J Exp Med. 2014;211(6):1231-42.
34. Modepalli V, Kumar A, Sharp JA, Saunders NR, Nicholas KR, Lefevre C. Gene expression profiling of postnatal lung development in the marsupial gray short-tailed opossum (Monodelphis domestica) highlights conserved developmental pathways and specific characteristics during lung organogenesis. BMC Genomics. 2018;19(1):732.
35. Higgins RD, Jobe AH, Koso-Thomas M, Bancalari E, Viscardi RM, Hartert TV, et al. Bronchopulmonary Dysplasia: Executive Summary of a Workshop. J Pediatr. 2018;197:300-8.
36. Jobe AH. Mechanisms of Lung Injury and Bronchopulmonary Dysplasia. Am J Perinatol. 2016;33(11):1076-8.
37. Berkelhamer SK, Mestan KK, Steinhorn R. An update on the diagnosis and management of bronchopulmonary dysplasia (BPD)-associated pulmonary hypertension. Semin Perinatol. 2018;42(7):432-43.
38. Sluiter I, Veenma D, van Loenhout R, Rottier R, de Klein A, Keijzer R, et al. Etiological and pathogenic factors in congenital diaphragmatic hernia. Eur J Pediatr Surg. 2012;22(5):345-54.
39. Lally KP, Lally PA, Langham MR, Hirschl R, Moya FR, Tibboel D, et al. Surfactant does not improve survival rate in preterm infants with congenital diaphragmatic hernia. J Pediatr Surg. 2004;39(6):829-33.
40. Boucherat O, Benachi A, Chailley-Heu B, Franco-Montoya ML, Elie C, Martinovic J, et al. Surfactant maturation is not delayed in human fetuses with diaphragmatic hernia. PLoS Med. 2007;4(7):e237.
41. Mous DS, Kool HM, Wijnen R, Tibboel D, Rottier RJ. Pulmonary vascular development in congenital diaphragmatic hernia. Eur Respir Rev. 2018;27(147).
42. Kool HM, Burgisser PE, Edel GG, de Kleer I, Boerema-de Munck A, de Laat I, et al. Inhibition of retinoic acid signaling induces aberrant pericyte coverage and differentiation resulting in vascular defects in congenital diaphragmatic hernia. Am J Physiol Lung Cell Mol Physiol. 2019;317(3):L317-L31.

43. Davis JM, Penney DP, Notter RH, Metlay L, Dickerson B, Shapiro DL. Lung injury in the neonatal piglet caused by hyperoxia and mechanical ventilation. J Appl Physiol (1985). 1989;67(3):1007-12.
44. Zhang Z, Wu S, Stenoien DL, Pasa-Tolic L. High-throughput proteomics. Annu Rev Anal Chem (Palo Alto Calif). 2014;7:427-54.
45. Piersigilli F, Syed M, Lam TT, Dotta A, Massoud M, Vernocchi P, et al. An omic approach to congenital diaphragmatic hernia: a pilot study of genomic, microRNA, and metabolomic profiling. J Perinatol. 2020.
46. Croitor-Sava A, Beck V, Sandaite I, Van Huffel S, Dresselaers T, Claus F, et al. High-Resolution (1) H NMR Spectroscopy Discriminates Amniotic Fluid of Fetuses with Congenital Diaphragmatic Hernia from Healthy Controls. J Proteome Res. 2015;14(11):4502-10.
47. Kononikhin AS, Starodubtseva NL, Chagovets VV, Ryndin AY, Burov AA, Popov IA, et al. Exhaled breath condensate analysis from intubated newborns by nano-HPLC coupled to high resolution MS. J Chromatogr B Analyt Technol Biomed Life Sci. 2017;1047:97-105.
48. Cartlidge PH, Mann NP, Kapila L. Preoperative stabilisation in congenital diaphragmatic hernia. Arch Dis Child. 1986;61(12):1226-8.
49. Wung JT, Sahni R, Moffitt ST, Lipsitz E, Stolar CJ. Congenital diaphragmatic hernia: survival treated with very delayed surgery, spontaneous respiration, and no chest tube. J Pediatr Surg. 1995;30(3):406-9.
50. van den Hout L, Tibboel D, Vijfhuize S, te Beest H, Hop W, Reiss I, et al. The VICI-trial: high frequency oscillation versus conventional mechanical ventilation in newborns with congenital diaphragmatic hernia: an international multicentre randomized controlled trial. BMC Pediatr. 2011;11:98.
51. Harrison MR, Keller RL, Hawgood SB, Kitterman JA, Sandberg PL, Farmer DL, et al. A randomized trial of fetal endoscopic tracheal occlusion for severe fetal congenital diaphragmatic hernia. N Engl J Med. 2003;349(20):1916-24.
52. Vermeulen E, van den Anker JN, Della Pasqua O, Hoppu K, van der Lee JH, Global Research in P. How to optimise drug study design: pharmacokinetics and pharmacodynamics studies introduced to paediatricians. J Pharm Pharmacol. 2017;69(4):439-47.
53. Benjamin DK, Smith PB, Jadhav P, Gobburu JV, Murphy MD, Hasselblad V, et al. Pediatric antihypertensive trial failures - Analysis of end points and dose range. Hypertension. 2008;51(4):834-40.
54. Steinhorn RH, Kinsella JP, Pierce C, Butrous G, Dilleen M, Oakes M, et al. Intravenous sildenafil in the treatment of neonates with persistent pulmonary hypertension. J Pediatr. 2009;155(6):841-7 e1.
55. Mous DS, Buscop-van Kempen MJ, Wijnen RMH, Tibboel D, Rottier RJ. Changes in vasoactive pathways in congenital diaphragmatic hernia associated pulmonary hypertension explain unresponsiveness to pharmacotherapy. Respir Res. 2017;18(1):187.
56. Keller RL, Tacy TA, Hendricks-Munoz K, Xu J, Moon-Grady AJ, Neuhaus J, et al. Congenital diaphragmatic hernia: endothelin-1, pulmonary hypertension, and disease severity. Am J Respir Crit Care Med. 2010;182(4):555-61.

57. Skarda DE, Yoder BA, Anstadt EE, Lally PA, Greene T, McFadden M, et al. Epoprostenol Does Not Affect Mortality in Neonates with Congenital Diaphragmatic Hernia. Eur J Pediatr Surg. 2015;25(5):454-9.
58. Lawrence KM, Hedrick HL, Monk HM, Herkert L, Waqar LN, Hanna BD, et al. Treprostinil Improves Persistent Pulmonary Hypertension Associated with Congenital Diaphragmatic Hernia. J Pediatr. 2018.
59. Van der Feen DE, Kurakula K, Tremblay E, Boucherat O, Bossers GPL, Szulcek R, et al. Multicenter Preclinical Validation of BET Inhibition for the Treatment of Pulmonary Arterial Hypertension. Am J Respir Crit Care Med. 2019;200(7):910-20.
60. Duffett M, Choong K, Foster J, Meade M, Menon K, Parker M, et al. High-Quality Randomized Controlled Trials in Pediatric Critical Care: A Survey of Barriers and Facilitators. Pediatr Crit Care Med. 2017;18(5):405-13.
61. Marshall JC, Kwong W, Kommaraju K, Burns KE. Determinants of Citation Impact in Large Clinical Trials in Critical Care: The Role of Investigator-Led Clinical Trials Groups. Crit Care Med. 2016;44(4):663-70.
62. European Union. Clinical trials: clearer rules, better protection [press release]. 2015.
63. Viele K, Berry S, Neuenschwander B, Amzal B, Chen F, Enas N, et al. Use of historical control data for assessing treatment effects in clinical trials. Pharm Stat. 2014;13(1):41-54.
64. Ristl R, Hothorn L, Ritz C, Posch M. Simultaneous inference for multiple marginal generalized estimating equation models. Stat Methods Med Res. 2020;29(6):1746-62.
65. Storme L, Boubnova J, Mur S, Pognon L, Sharma D, Aubry E, et al. Review shows that implementing a nationwide protocol for congenital diaphragmatic hernia was a key factor in reducing mortality and morbidity. Acta Paediatr. 2017.
66. Jancelewicz T, Brindle ME, Guner YS, Lally PA, Lally KP, Harting MT, et al. Toward Standardized Management of Congenital Diaphragmatic Hernia: An Analysis of Practice Guidelines. J Surg Res. 2019;243:229-35.
67. Morini F, Capolupo I, van Weteringen W, Reiss I. Ventilation modalities in infants with congenital diaphragmatic hernia. Semin Pediatr Surg. 2017;26(3):159-65.
68. Tolbert VP, Matthay KK. Neuroblastoma: clinical and biological approach to risk stratification and treatment. Cell Tissue Res. 2018;372(2):195-209.
69. Patel N, Lally PA, Kipfmueller F, Massolo AC, Luco M, Van Meurs KP, et al. Ventricular Dysfunction is a Critical Determinant of Mortality in Congenital Diaphragmatic Hernia. Am J Respir Crit Care Med. 2019.
70. Dao DT, Patel N, Harting MT, Lally KP, Lally PA, Buchmiller TL. Early Left Ventricular Dysfunction and Severe Pulmonary Hypertension Predict Adverse Outcomes in "Low-Risk" Congenital Diaphragmatic Hernia. Pediatr Crit Care Med. 2020.
71. Patel N, Kipfmueller F. Cardiac dysfunction in congenital diaphragmatic hernia: Pathophysiology, clinical assessment, and management. Semin Pediatr Surg. 2017;26(3):154-8.

72. DeKoninck P, Richter J, Van Mieghem T, Van Schoubroeck D, Allegaert K, De Catte L, et al. Cardiac assessment in fetuses with right-sided congenital diaphragmatic hernia: case-control study. Ultrasound Obstet Gynecol. 2014;43(4):432-6.
73. Hooper SB, Binder-Heschl C, Polglase GR, Gill AW, Kluckow M, Wallace EM, et al. The timing of umbilical cord clamping at birth: physiological considerations. Matern Health Neonatol Perinatol. 2016;2:4.
74. Hooper SB, Polglase GR, te Pas AB. A physiological approach to the timing of umbilical cord clamping at birth. Arch Dis Child Fetal Neonatal Ed. 2015;100(4):F355-60.
75. Kipfmueller F, Schroeder L, Berg C, Heindel K, Bartmann P, Mueller A. Continuous intravenous sildenafil as an early treatment in neonates with congenital diaphragmatic hernia. Pediatr Pulmonol. 2018.
76. Lawrence KM, Monos S, Adams S, Herkert L, Peranteau WH, Munson DA, et al. Inhaled Nitric Oxide Is Associated with Improved Oxygenation in a Subpopulation of Infants with Congenital Diaphragmatic Hernia and Pulmonary Hypertension. J Pediatr. 2020;219:167-72.
77. Loh E, Stamler JS, Hare JM, Loscalzo J, Colucci WS. Cardiovascular effects of inhaled nitric oxide in patients with left ventricular dysfunction. Circulation. 1994;90(6):2780-5.
78. Mears M, Yang M, Yoder BA. Is Milrinone Effective for Infants with Mild-to-Moderate Congenital Diaphragmatic Hernia? Am J Perinatol. 2019.
79. Acker SN, Kinsella JP, Abman SH, Gien J. Vasopressin improves hemodynamic status in infants with congenital diaphragmatic hernia. J Pediatr. 2014;165(1):53-8 e1.
80. Abolmaali N, Koch A, Gotzelt K, Hahn G, Fitze G, Vogelberg C. Lung volumes, ventricular function and pulmonary arterial flow in children operated on for left-sided congenital diaphragmatic hernia: long-term results. Eur Radiol. 2010;20(7):1580-9.
81. Moenkemeyer F, Patel N. Right ventricular diastolic function measured by tissue Doppler imaging predicts early outcome in congenital diaphragmatic hernia. Pediatr Crit Care Med. 2014;15(1):49-55.
82. Abman SH, Hansmann G, Archer SL, Ivy DD, Adatia I, Chung WK, et al. Pediatric Pulmonary Hypertension: Guidelines From the American Heart Association and American Thoracic Society. Circulation. 2015;132(21):2037-99.
83. Egan MJ, Husain N, Stines JR, Moiduddin N, Stein MA, Nelin LD, et al. Mid-term differences in right ventricular function in patients with congenital diaphragmatic hernia compared with controls. World J Pediatr. 2012;8(4):350-4.
84. Patel N, Massolo AC, Kipfmueller F. Congenital diaphragmatic hernia-associated cardiac dysfunction. Semin Perinatol. 2020;44(1):151168.
85. Tanaka T, Inamura N, Ishii R, Kayatani F, Yoneda A, Tazuke Y, et al. The evaluation of diastolic function using the diastolic wall strain (DWS) before and after radical surgery for congenital diaphragmatic hernia. Pediatr Surg Int. 2015;31(10):905-10.
86. Costerus SA, van Hoorn CE, Hendrikx D, Kortenbout J, Hunfeld M, Vlot J, et al. Towards integrative neuromonitoring of the surgical newborn: A systematic review. Eur J Anaesthesiol. 2020.

87. Soleymani S, Borzage M, Noori S, Seri I. Neonatal hemodynamics: monitoring, data acquisition and analysis. Expert Rev Med Devices. 2012;9(5):501-11.
88. Ketharanathan N, De Jonge RCJ, Klouwen I, Wildschut ED, Reiss IKM, Tibboel D, et al. Hyperoxia in pediatric severe traumatic brain injury (TBI): a comparison of patient classification by cutoff versus cumulative (area-under-the-curve) analysis. Brain Inj. 2020;34(7):958-64.



CHAPTER 9

Summary

SUMMARY

The research described in this thesis addresses several aspects of the perinatal and neonatal management of patients with CDH. Most research is conducted in cooperation with members of the CDH Euro Consortium, a clinical and scientific collaboration between tertiary medical centers in Europe with expertise in the treatment of CDH patients.

PART I Introduction

In **Chapter 1** the historical background of CDH is described. Also the importance of predicting outcome is discussed. Thereafter, prenatal parameters, such as observed to expected lung-to-head ratio (O/E LHR), and their clinical relevance are explained. Additionally, postnatal parameters and models are discussed, next to the role of biochemical biomarkers in the prediction of chronic lung disease (CLD) and pulmonary hypertension (PH) in CDH patients. The focus of the international guidelines is described, preventing ventilator induced lung injury and establishing cardiovascular stability. Subsequently, the three mayor pathways involved in regulation of the pulmonary vascular resistance are explained and the drugs that act on different steps of the pathway. Lastly, the survival, but also the morbidity and long-term follow-up are discussed briefly.

PART II Prediction

Chapter 2 presents an editorial on a new prediction tool, the preoperative chest radiographic thoracic area (CRTA), tracing the lung on an anterior-posterior chest x-ray. The role of CRTA is discussed and placed in a broader perspective of prediction tools used in CDH patients. This new tool seems to be a relatively reliable new tool, but will need additional validation. Many tools used in this group of patients struggle with the same issues; they were only tested on small groups, are difficult to apply or don't have a standardized definition or measurement method. The latter holds for PH on echocardiogram and the O/E LHR, decreasing its reliability.

In **Chapter 3** the Congenital Diaphragmatic Hernia Study Group prediction rule, an early model to predict mortality in CDH patients, is validated with a cohort of 343 CDH patients born in 4 large European centers. All centers use the same standardized CDH-EURO Consortium treatment protocol. In this group the rule shows a fair predictive performance. To further improve the rule, the pre- and postnatal parameters of 620 CDH patients are used to modify the rule. Interestingly, only liver herniation is of additional value to improve the rules ability to predict mortality in CDH patients. Also, the variable "missing Apgar score" is taken out of the model as it is not of significant value in our population.

In addition to the discovery of novel early biomarkers SIGLEC-14, BCAM, and ANGPTL3 to predict bronchopulmonary dysplasia in preterm infants, these biomarkers are tested

in preterm infants, CDH patients and adults in **Chapter 4**. The role of these biomarkers in predicting BPD in preterm infants is validated and protein concentrations correlate with the duration of mechanical ventilation, oxygen supplementation and structural lung changes. Although chronic lung disease in CDH patients cannot be predicted, it does separate surviving from non-surviving CDH patients and SIGLEC-5/14 seems to predict PH. In adults the biomarkers cannot separate patients from healthy controls.

PART III Treatment

In **Chapter 5** a novel, more individualized approach in the delivery room is described. In contrast to common practice, newborns with mild CDH, defined as isolated left-sided CDH, O/E LHR ≥50% and intra-abdominal liver position, are not routinely intubated in the delivery room, but only when they develop respiratory failure. This approach is successful in 40% of the patients, decreasing possible iatrogenic complications of prompt intubation, while it does not seem to negatively affect outcomes in the patients who develop respiratory failure.

To develop an adequate dosing regimen of intravenous sildenafil in CDH patients, **in Chapter 6** a pharmacokinetic model is developed, using samples from 23 infants with CDH. A two-compartment disposition model of sildenafil and a one-compartment disposition model of desmethyl sildenafil is observed. Only postnatal age increases sildenafil clearance and sildenafil is tolerated well in this relatively small group. The widely used loading dose of 0.4mg/kg in 3 hours, followed by 1.6mg/kg/day continues infusion, achieves appropriate sildenafil plasma levels in CDH patients.

The related trial protocol of the CoDiNOS trial is discussed in **Chapter 7.** The CoDiNOS trial is an international randomized controlled trial in which intravenous sildenafil is compared to inhaled nitric oxide for the first-step treatment of PH in neonates with CDH. We hypothesize that intravenous sildenafil is superior to inhaled nitric oxide. Primary outcome is the absence of pulmonary hypertension on echocardiography on day 14 without therapy and without treatment failure and/or death within the first 28 days of life. With a sample size of 330 patients we will be able to detect a 25% relative difference in outcome between both drugs. Secondary outcome parameters are the need for ECMO, time on intervention drug, the incidence of chronic lung disease and the external validation of the pharmacokinetic model for sildenafil, among others.

PART IV Discussion and summary

The general discussion in this thesis addresses the association of this research with the literature, as well as the implications for future research in CDH and in orphan diseases in general. The strengths and the weaknesses of the research are discussed. The main recommendations are:

- Standardization of measuring techniques and reporting for several prenatal and postnatal parameters is urgently needed, for prediction and for evaluation of therapy.

- Although the role of prediction models in the postnatal treatment of CDH patients is limited at the moment, patient selection based on prediction scores, initiating individualized care, should be further investigated as it can potentially improve outcome. It can prevent overtreatment in mild cases and might have a role in prevention and early treatment in severe cases.

- Multicentre research collaboration is essential in orphan diseases such as CDH. However, an RCT in this population is very challenging. Newly developed statistical tools are currently not implemented in clinical research. These tools can decrease the number of patients needed to answer the research question and should be further investigated and implemented.

- The treatment of the ventricular dysfunction needs to be further investigated, with and without the presence of PH as the treatment of PH can further exacerbate LV dysfunction.

NEDERLANDSE SAMENVATTING

Het onderzoek in dit proefschrift beschrijft verschillende aspecten van de perinatale en neonatale behandeling van patiënten met congenitale hernia diafragmatica (CHD). Het meeste onderzoek is verricht in samenwerking met de leden van het CDH EURO Consortium, een klinisch en wetenschappelijk samenwerkingsverband tussen tertiaire medische centra in Europa met expertise in de behandeling van CHD patiënten.

Deel I Introductie

In **Hoofdstuk 1** wordt de historische context van CHD beschreven. Ook het belang van predictie wordt bediscussieerd. Vervolgens worden prenatale parameters, zoals geobserveerde-versus-verwachtte long-hoofd-ratio (O/E LHR), en de klinische relevantie besproken. Daarna worden postnatale parameters en modellen besproken, als ook de rol van biochemische biomarkers voor de predictie van chronische longziekte en pulmonale hypertensie (PH) in CHD patiënten. De focus van de internationale richtlijnen, het voorkomen van beademings-geïnduceerde longschade en het bewerkstelligen van cardiovasculaire stabiliteit, wordt beschreven. Vervolgens wordt uitgelegd welke 3 belangrijkste routes betrokken zijn bij de regulatie van de pulmonale vaatweerstand en welke medicamenten op de verschillende stappen van de route aangrijpen. Als laatste worden kort de mortaliteit, de morbiditeit en de lange-termijn follow-up besproken.

Deel II Predictie

Hoofdstuk 2 bevat een editorial over een nieuwe postnatale predictor in CHD patiënten, het preoperatief meten van de longomtrek op een anterior-posterior genomen röntgenfoto (CRTA). De rol van CRTA wordt besproken en in het breder perspectief van predictiemodellen voor CHD patiënten geplaatst. Deze nieuwe predictor lijkt een vrij betrouwbaar nieuw instrument, maar dient nog extern gevalideerd te worden. Veel instrumenten die gebruikt worden voor deze groep patiënten kennen dezelfde problemen; ze zijn alleen getest in kleine groepen patiënten, ze zijn moeilijk toe te passen of hebben geen gestandaardiseerde definitie of meetmethode. Dit laatste geldt bijvoorbeeld voor zowel PH als de O/E LHR, wat de betrouwbaarheid van deze parameters vermindert.

In **Hoofdstuk 3** wordt de "Congenital Diaphragmatic Hernia Study Group prediction rule", of het CDHSG predictiemodel, gevalideerd in een cohort van 343 CHD patiënten die geboren zijn in 4 Europese centra. Dit model is een vroege voorspeller van mortaliteit in CHD patiënten. Alle centra gebruiken hetzelfde CDH-EURO Consortium behandelprotocol. In deze groep blijkt het model een redelijke voorspellende waarde te hebben. Om het model verder te verbeteren worden de pre- en postnatale gegevens van 620 CHD patiënten gebruikt om het model aan te passen. Interessant genoeg blijkt alleen de herniatie van de lever in de thorax van additionele waarde te zijn om het predictiemodel

te verbeteren. Daarnaast wordt de variabel " afwezige Apgar score" uit het model gehaald, omdat het in onze populatie geen significante bijdrage aan het model levert.

Als vervolg op de ontdekking van de nieuwe vroege biomarkers SIGLEC-14, BCAM, en ANGPTL3 als voorspellers van bronchopulmonale dysplasie (BPD) in premature kinderen, worden deze biomarkers getest in prematuren, CHD patiënten en volwassenen in **Hoofdstuk 4**. De rol van deze biomarkers in het voorspellen van BPD in prematuren wordt gevalideerd en plasmaconcentraties van de biomarkers correleren in deze groep ook met de duur van mechanische beademing, zuurstoftoediening en structurele longafwijkingen. Alhoewel chronisch longlijden in CHD patiënten niet kan worden voorspeld, differentieert het wel tussen de CHD patiënten die overleven en die overlijden. Daarnaast lijkt SIGLEC-5/14 een voorspeller van PH. In volwassenen kan met behulp van de biomarkers geen onderscheid worden gemaakt tussen patiënten en gezonde controles.

Deel III Behandeling

In **Hoofdstuk 5** wordt een nieuwe, meer geïndividualiseerde benadering van pasgeborenen met CHD in de verloskamers beschreven. In tegenstelling tot voorheen worden pasgeborenen met milde CHD, gedefinieerd als geïsoleerde linkszijdige CHD, O/E LHR ≥50% en intra-abdominale leverpositie, niet standaard geïntubeerd in de verloskamer, maar alleen als ze respiratoir falen ontwikkelen. Deze benadering is succesvol in 40% van de patiënten, waarmee mogelijk iatrogene complicaties van het direct intuberen worden verminderd. Daarnaast lijkt het geen negatief effect te hebben op de patiënten die respiratoir falen ontwikkelen.

Om een geschikt doseringsadvies voor intraveneus sildenafil bij CHD patiënten te ontwikkelen, wordt in **Hoofdstuk 6** een farmacokinetisch model ontwikkeld met behulp van bloedmonsters van 23 zuigelingen met CHD. De verdeling van sildenafil kan worden beschreven met een twee-compartimentenmodel en desmethylsildenafil met een een-compartimentmodel. Alleen postnatale leeftijd verhoogt de klaring van sildenafil en sildenafil wordt goed verdragen in deze relatief kleine groep. Het vaak gebruikte doseringsadvies van 0.4mg/kg in 3 uur, gevolgd door 1.6mg/kg/dag middels continue toediening, zorgt voor adequate plasmaspiegels van sildenafil in CHD patiënten.

Het hieraan gerelateerde studieprotocol van de CoDiNOS studie wordt besproken in **Hoofdstuk 7**. De CoDiNOS studie is een internationale gerandomiseerde studie waarbij intraveneus sildenafil wordt vergeleken met de inhalatie van stikstofoxide (iNO) als eerste stap van de behandeling van PH in neonaten met CHD. De hypothese is dat intraveneus sildenafil superior is ten opzichte van iNO. De primaire uitkomst is de afwezigheid van PH bij echocardiografische evaluatie op dag 14, zonder behandeling op dag 14 en zonder falen van de behandeling eerder, danwel overlijden binnen de eerste 28 dagen van het

leven. Met een steekproefomvang van 330 patiënten zullen we een relatief verschil van 25% in uitkomst tussen de twee therapieën kunnen vinden. Secundaire uitkomstmaten zijn onder andere de noodzaak tot extracorporale membraan oxygenatie, duur van behandeling met interventiemedicatie, de incidentie van chronische longziekte en de externe validatie van het in hoofdstuk 6 beschreven pharmacokinetische model van sildenafil.

Deel IV Discussie en samenvatting

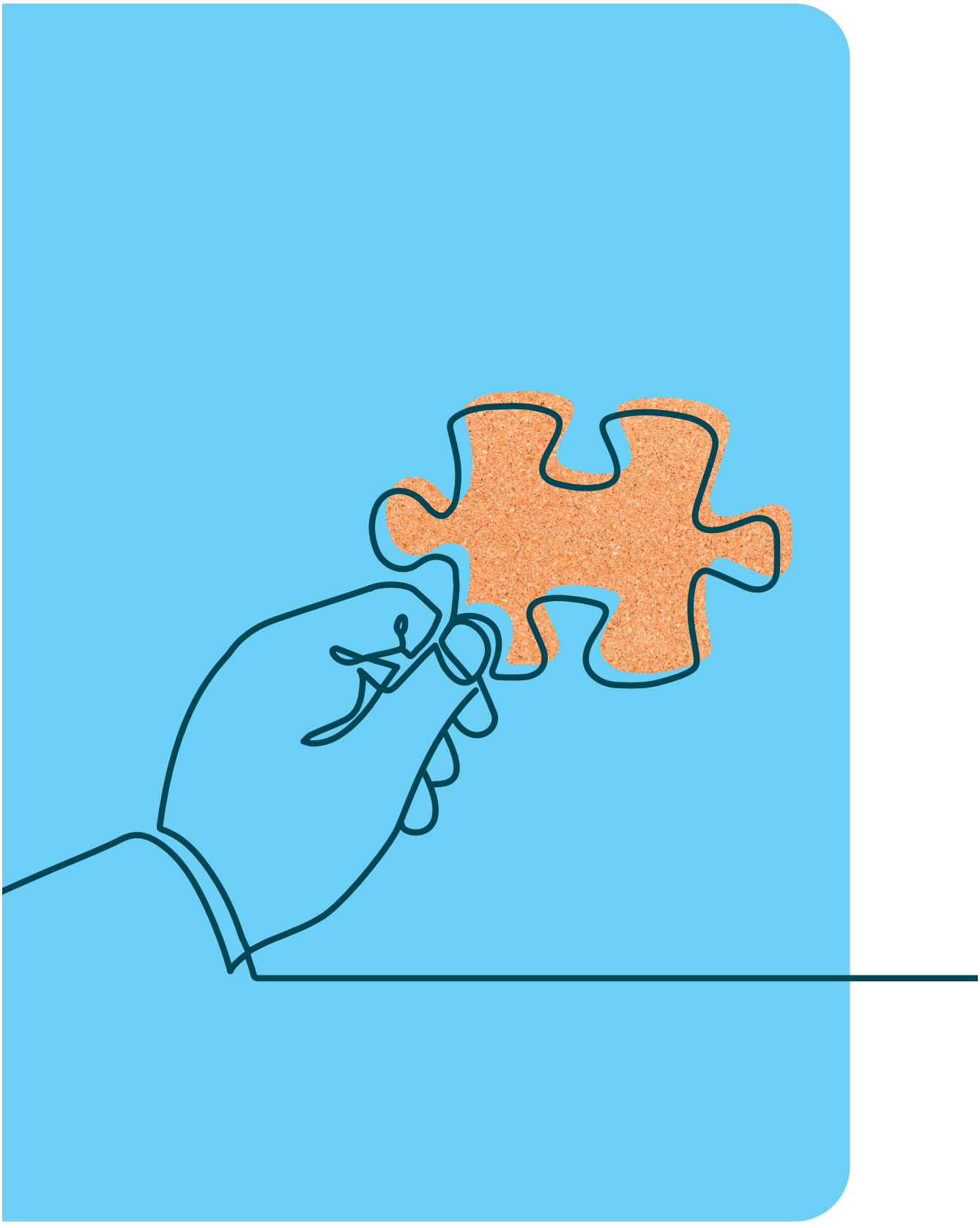
De discussie van dit proefschrift beschrijft de associatie van dit onderzoek met de beschikbare literatuur alsook de implicaties voor verder onderzoek in CHD en zeldzame ziektes in het algemeen. De kracht en zwaktes van het onderzoek worden bediscussieerd. De belangrijkste aanbevelingen zijn:

- Standaardisatie van meetmethodes en rapportering van verschillende prenatale en postnatale parameters is dringend nodig, voor predictie maar ook voor de evaluatie van behandelingen.

- Hoewel de rol van predictiemodellen in de postnatale behandeling van CHD patienten beperkt is op dit moment, zou patiënt selectie gebaseerd op predictie scores potentieel de uitkomst kunnen verbeteren en zorgen voor meer geïndividualiseerde zorg. Het kan overbehandeling voorkomen in patienten met milde CHD en het zou een rol kunnen hebben in preventie en vroege behandeling in de ernstige casussen.

- Multicenter onderzoekssamenwerking is essentieel in zeldzame ziektes zoals CHD. Maar een RCT in deze populatie blijft een grote uitdaging. Nieuw ontwikkelde statistische technieken worden op dit moment niet gebruikt in klinisch onderzoek. Deze technieken kunnen het aantal benodigde patienten om de onderzoeksvraag te beantwoorden, fors verlagen en zouden dus verder onderzocht en geïmplementeerd dienen te worden.

- De behandeling van ventriculaire disfunctie dient beter onderzocht te worden, met en zonder pulmonale hypertensie, aangezien de behandeling van pulmonale hypertensie de linker ventrikel disfunctie verder kan verslechteren.



PART V

Appendices

LIST OF ABBREVIATIONS

ALAT	alanine transaminase
ANGPTL3	angiopoietin-like protein 3
ASAT	aspartate transaminase
AUC	area under the curve
BCAM	basal cell adhesion molecule
BPD	bronchopulmonary dysplasia
CDH	congenital diaphragmatic hernia
CDHSG	Congenital Diaphragmatic Hernia Study Group
cGMP	cyclic guanosine monophosphate
CHD	congenitale hernia diafragmatica
CI	confidence interval
CL	clearance
CLD	chronic lung disease
COPD	chronic obstructive pulmonary disease
CRTA	chest radiographic thoracic area
CYP	cytochrome-P-450 enzyme
DMS	desmethylsildenafil
DV	observed concentration
EBC	exhaled breath condensate
ECMO	extracorporeal membrane oxygenation
EDTA	ethylenediamine tetraacetic acid
ELISA	enzyme-linked immunosorbent assay
ERNICA	European Reference Network for rare Inherited and Congenital (digestive and gastrointestinal) Anomalies
FDA	US Food and Drug Administration
FETO	foetal endoscopic tracheal occlusion
FiO2	inspiratory oxygen fraction
FLV	fetal lung volume
FOCE+1	first-order conditional estimation method with interaction
HsTnT	High-sensitivity troponin T
GA	gestational age
GTP	guanosine-5′-triphosphate
IIV	inter-individual variability
iNO	inhaled nitric oxide
IOV	inter occasion variability
IPF	idiopathic pulmonary fibrosis
IPRED	predicted concentration
IQR	interquartile range

IRB	institutional review board
kPa	kilopascal
LC-MS	liquid chromatography-mass spectrometry
LHR	lung-to-head ratio
LV	left ventricle
MCA	multiple congenital anomalies
mPAP	mean pulmonary artery pressure
MRI	magnetic resonance imaging
NPDE	normalized prediction distribution errors
NONMEM	Nonlinear mixed effects modelling
NT-proBNP	N-terminal pro-brain natriuretic peptide
O/E LHR	observed-to-expected lung-to-head ratio
OI	oxygenation index
PaO2	partial pressure of oxygen
PaCO2	partial pressure of carbon dioxide
PDE3/5	selective phosphodiesterase type 3/5
PEA	proximity extension assay
PH	pulmonary hypertension
PHT	pulmonary hypertension
PK	pharmacokinetic
PKPD	pharmacokinetic pharmacodynamic
PPHN	persistent pulmonary hypertension of the newborn
PPM	parts per million
RCT	randomized controlled trial
ROC	receiver operating characteristic
RV	right ventricle
SAE	serious adverse event
SIGLEC-14	sialic acid binding Ig-like lectin 14
SNAP II score	Score for Neonatal Physiology version II
sRAGE	soluble receptor for advanced glycation end products
SUSAR	suspected unexpected serious adverse event
TGF- β	transforming growth factor beta
TFLV	total fetal lung volume
US	ultrasound
V	volume of distribution
VA ECMO	veno-arterial extracorporeal membrane oxygenation
VEGFA	vascular endothelial growth factor A
VILI	ventilator induced lung injury
VIS	vasoactive-inotropic score
VPC	visual predictive check

CURRICULUM VITAE

Suzanne Cornelia Maria Cochius – den Otter was born on July 15th 1980 in Nijmegen. She grew up in Odiliapeel and graduated from the Kruisheren Kollege in Uden in 1998. She obtained her medical degree "cum laude" at Maastricht University in 2005. During her medical training she finished a clinical internship pediatrics at the Sint Vincentius Ziekenhuis in Paramaribo, Surinam. She worked as a pediatric resident (ANIOS) at the department of neonatology in the RadboudUMC –Amalia Pediatric Hospital in Nijmegen in 2005 and at the department of pediatrics in Juliana Pediatric Hospital in The Hague in 2006. In July 2007 she started her pediatric training (AIOS) at the Erasmus MC – Sophia's Pediatric Hospital in Rotterdam (Prof. Dr. M. de Hoog) and the Reinier de Graaf Hospital in Delft (Dr. N van de Lely). After she finished her training as a pediatrician, she started her fellowship pediatric intensive care at the Erasmus MC – Sophia's Pediatric Hospital and at the Royal Children's Hospital in Melbourne, Australia (Prof. Dr. W Butt) in 2013. After becoming a pediatric intensivist in 2014, she commenced her PhD project (promotors Prof. Dr. D Tibboel and Prof. Dr. K Allegaert) on congenital diaphragmatic hernia at the Intensive Care and Department of Pediatric Surgery, while also working clinically as a pediatric intensivist. Suzan is married with Timo Cochius and they live in Rotterdam, together with their three children, Tessel, Faas and Pelle.

LIST OF PUBLICATIONS

Kindt ASD*, Förster K*, **Cochius-den Otter SCM,** Flemmer AW, Hauck SM, Kamphuis J, Stócklein S, Karrasch S, Behr J, Franz A, Härtel C, Krumsiek J, Tibboel D, Hilgendorff A. Implementing disease-specific biomarkers for the early detection of Bronchopulmonary Dysplasia**.** *Submitted for publication*

Cochius – den Otter SCM, Horn – Oudshoorn EJJ, Allegaert K, DeKoninck PLJ, Peters NCJ, Cohen TE, Reiss IKM, Tibboel D. Routine Intubation in Newborns with Congenital Diaphragmatic Hernia. *Accepted for publication*. Pediatrics

Hermelijn SM, Zwartjes RR, Tiddens HAWM, **Cochius - den Otter SCM**, Reiss IKM, Wijnen RMH, Schnater JM. Associated Anomalies in Congenital Lung Abnormalities: A 20-year Experience. Neonatology 2020 Aug;25:1-7

Horn-Oudshoorn EJJ, Knol R, Te Pas AB, Hooper SB, **Cochius-den Otter SCM,** Wijnen RMH, Schaible T, Reiss IKM, DeKoninck PLJ. Perinatal stabilisation of infants born with congenital diaphragmatic hernia: a review of current concepts. Arch Dis Child Fetal Neonatal Ed. 2020 Jul;10594):449-454

Cochius-den Otter S*, Erdem Ö*, Rosmalen van J, Schaible T, Peters NCJ, Cohen-Overbeek TE, Capolupo I, Falk CJ, van Heijst AFJ, Schäffelder R, Brindle ME, Tibboel D. Validation and modification of the prediction rule for mortality in congenital diaphragmatic hernia. Pediatrics. 2020:145(4)

Cochius-den Otter SCM, Kipfmueller F, de Winter BCM, Allegaert K, Tibboel D, Mueller A, Koch BCP. Pharmacokinetic modeling of intravenous sildenafil in newborns with congenital diaphragmatic hernia. Eur J Clin Pharmacol. 2020 Feb;76(2):219-227

Cochius-den Otter S, Schaible T, Greenough A, van Heijst A, Patel N, Allegaert K, van Rosmalen J, Tibboel D, CDH Euro Consortium. The CoDiNOS trial protocol: an international randomised controlled trial of intravenous sildenafil versus inhaled nitric oxide for the treatment of pulmonary hypertension in neonates with congenital diaphragmatic hernia. BMJ Open. 2019;9(11):e032122.

Cochius-den Otter SCM, Tibboel D. Light at the Horizon? Predicting Mortality in Infants With Congenital Diaphragmatic Hernia. Pediatr Crit Care Med. 2019;20(6):575-7.

Boeschoten SA, Buysse CMP, Merkus P, van Wijngaarden JMC, Heisterkamp SGJ, de Jongste JC, van Rosmalen J, **Cochius-den Otter SCM**, Boehmer ALM, de Hoog M. Children with

severe acute asthma admitted to Dutch PICUs: A changing landscape. Pediatr Pulmonol. 2018;53(7):857-65.

Kraemer U*, **Cochius-den Otter S***, Snoek KG, Tibboel D. Pharmacodynamic considerations in the treatment of pulmonary hypertension in infants: challenges and future perspectives. Expert Opin Drug Metab Toxicol. 2016;12(1):1-19.

Cochius-den Otter SC, Joosten KF, de Jongste JC, Hop WC, de Hoog M, Buysse CM. Insulin therapy in hyperglycemic children with severe acute asthma. J Asthma. 2015;52(7):681-6.

den Otter SC, de Mol AC, Eggink AJ, van Heijst AF, de Bruijn D, Wijnen RM. Major sacrococcygeal teratoma in an extreme premature infant: a multidisciplinary approach. Fetal Diagn Ther. 2008;23(1):41-5.

*contibuted equally

PHD PORTFOLIO

Name of PhD student	Suzan Cochius – den Otter
Erasmus MC Department	Intensive Care and Department of Pediatric Surgery
PhD period	March 2014 – July 2020
Promotors	Prof. Dr. D. Tibboel
	Prof. Dr. K Allegaert

	Year	Workload (ECTS)
1. PhD training		
General courses		
BROK (Basiscursus Regelgeving en organisatie voor Klinisch Onderzoekers- Good Clinical Practice for clinical research)	2014	1.5
Renewal BROK registration	2019	1.5
Biomedical English writing course	2018	2.0
Integrity in scientific research	2018	1.0
Basic introduction course SPSS	2017	1.0
Open Clinica	2017	0.2
EndNote	2015	0.2
Symposia, workshops and meetings		
PICU research meeting, Rotterdam	2018 – present	2.0
SICK symposium, Haarzuilens	2016, 2018	1.0
Presentations		
Presentation CDH conference Houston, USA	2020	0.5
Presentation PICU research meeting, Rotterdam	2019	0.5
Presentation ECMO 25 jaar in het Erasmus MC, Rotterdam	2018	1.0
Presentation Rotterdam Leiden Pediatric Pharmacology meeting, Noordwijk	2017	0.5
Presentation CDH Meeting Liverpool, UK	2017	0.5
Presentation CDH Meeting Rotterdam	2014	0.5
(Inter)national conferences		
Euro Elso	2017, 2018	2.0
CDH conference	2014, 2017, 2020	3.0
Eupsa	2019	1.5
ISICEM, Brussels, Belgium	2016, 2018, 2019	1.5
2. Teaching		
Teach-the-teacher III	2017	0.5
Supervisor PICU nurse-practitioner in training	2015 - 2017	3.0
PICU teaching (registrars, consultants, nursing staff)	2014 – present	0.5
3. Other		
CoDiNOS study coordinator	2016 – 2020	4.0
Study side coordinator P3T Investigators	2014 - 2017	1.0
Grants		
SSWO (2 year young researcher grant); Awarded Jan Molenaarprijs for best presentation	2016	1.0

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

DANKWOORD

Na 6 jaar en met de hulp van velen is het boekje af. Het voelt niet als een afsluiting, maar een nieuwe stap. Dit onderzoek is tot stand gekomen mede dankzij de hulp en medewerking van velen. Een aantal van hen wil ik hier graag in het bijzonder bedanken.

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