Lung function in school-aged congenital diaphragmatic hernia patients; a longitudinal evaluation

Leontien C.C. Toussaint-Duyster,
Monique H.M. van der Cammen-van Zijp,
Marjolein Spoel, Harm A.W.M. Tiddens, Dick Tibboel,
Rene M.H. Wijnen, Joost van Rosmalen,
Hanneke IJsselstijn

Pediatric Pulmonology 2019; 54(8):1257-1266

ABSTRACT

Objective

Children with congenital diaphragmatic hernia (CDH) are at risk for pulmonary morbidity. Data on longitudinal evaluation of lung function in CDH are scarce. We hypothesized that CDH patients would have impaired lung function that worsens over time. We evaluated lung function and its determinants at ages 8 and 12 years.

Methods

Dynamic and static lung volumes, and diffusion capacity were measured. Extracorporeal membrane oxygenation (ECMO) treatment, the standardized European neonatal treatment protocol, patch-repair, duration of ventilation, type of initial mechanical ventilation, and nitric oxide treatment were entered as covariates in linear mixed models with standard deviation score (SDS) lung function parameters (FEV_1, $FEF_{25-75,}$ and K_{CO}) as dependent variables.

Results

Seventy-six children (27 ECMO-treated) born between 1999 and 2009 performed 113 reliable lung-function tests. Severity of airflow obstruction deteriorated significantly from age 8 to 12 years: estimated mean difference (95% confidence interval [CI]) SDS FEV_1 was -0.57 (-0.79 to -0.36) and SDS FEF_{25-75} was -0.63 (-0.89 to -0.37), both $p<0.001$. Static lung volumes were within normal range and unchanged over time: estimated mean difference (95% CI) SDS TLC -0.27 (-0.58 to 0.04), $p=0.085$. SDS K_{CO} was below normal at 8 and 12 years and remained stable: -0.06 (-0.22 to 0.35), $p=0.648$. These observations were irrespective of ECMO-treatment. FEV_1 and FEF_{25-75} were negatively associated with duration of ventilation ($p<0.001$). Baseline data were not related with TLC or $K_{CO.}$

Conclusions

CDH patients should be followed into adulthood as they are at risk for worsening airflow obstruction and decreased diffusion capacity at school age, irrespective of ECMO-treatment.

INTRODUCTION

Congenital diaphragmatic hernia (CDH) combines a developmental defect of the diaphragm with pulmonary hypoplasia, abnormal pulmonary vascular development, increased vaso-reactivity,[1] and increased susceptibility for chronic lung disease.[2] Advances in surgical and neonatal management, and the implementation of a standardized European CDH neonatal treatment protocol since November 2007[3] (referred to as "postnatal treatment protocol") (Table 1 of the supplemental file), have significantly contributed to improved survival, with reported rates from 68% to 90%.[2,4] The increase in survival may lead to an increase in morbidity for the survivors.

Pulmonary morbidity in CDH patients is common.[5,6] Lung hypoplasia with persistent airflow obstruction,[7,8] iatrogenic lung damage due to mechanical ventilation, and microstructural changes in the lung[9] are factors that may contribute to long-term pulmonary morbidity. While normal lung tissue continues to develop alveoli into adolescence,[10,11] it is unknown if and when catch-up growth of the abnormal lungs of CDH patients will occur.[12] Besides, several studies have shown that these patients' lung development is affected by inhibited pulmonary vascular growth.[13,14]

Besides these structural pulmonary abnormalities, CDH patients suffer from gastrointestinal and respiratory problems. Gastroesophageal reflux and recurrent episodes of lower respiratory tract infections often occur not only in the first years after birth, but also later in life.[15-17] All these together may lead to decreased lung function at school age.

A previous study from our group found hyperinflation of the lungs with larger functional residual capacity and decreased expiratory flows in CDH patients' first year of life, especially among those treated with extracorporeal membrane oxygenation (ECMO).[18] While a few studies found normal pulmonary function later on,[19] other studies showed airflow obstruction, high prevalence of increased airway responsiveness and decreased diffusion capacity in CDH patients.[6,8,20,21] However, many of these studies often have a cross-sectional design and evaluated lung function in CDH patients born several decades ago. As since then important advances in surgical and neonatal management have been made, including the implementation of the CDH Euro Consortium postnatal treatment protocol in November 2007,[3] more recent data on lung function need to be evaluated longitudinally over time.

Therefore, we longitudinally evaluated CDH patients' lung function at the ages of 8 and 12 years. A secondary aim was to gain insight into the clinical determinants of lung function and the effect of the use of the postnatal treatment protocol.

MATERIALS AND METHODS

Patients, procedures and study design

We included all children born with CDH between January 1999 and June 2009 who joined the standardized prospective follow-up program at the Erasmus MC-Sophia Children's Hospital according to the present standard of care for children born with major anatomical congenital anomalies. These children and their parents are followed by a multidisciplinary team, and eight standardized assessments are performed from the ages of 6 months to 17 years as published by our research group.[22,23] We analyzed data of children who had been clinically stable for at least 3 weeks prior to the assessment of the follow-up program at ages 8 and 12 years and who performed reproducible lung function tests.

We excluded data from patients diagnosed with CDH after 7 days of age, those with paraesophageal diaphragmatic defects, those with a diaphragmatic eventration, and those with an unreliable lung function test. Until November 2007, ECMO treatment was applied in cases of reversible severe respiratory failure by using the entry criteria as reported by Stolar et al.[24] After November 2007, children were treated according to the standardized CDH EURO Consortium consensus treatment protocol which included ECMO criteria[3] (Table 1 of the supplemental file). These criteria were no different from earlier criteria.

The children were seen by a dedicated team of physicians and allied health professionals. A pediatrician and a pediatric surgeon performed standardized physical examinations. Lung function was measured by a specialized technician. Perinatal and demographic characteristics were retrieved from medical records.

All data were collected during routine care and subjects were not submitted to any handling and no rules of human behavior were imposed. Therefore, institutional review board approval was waived (MEC-2016-111). Parents of all children were routinely informed about the study and provided permission to use the de-identified data for research purposes.

Measurements

Baseline data

The following baseline data were recorded: sex, age, gestational age, birth weight, ethnicity, side of hernia, type of repair, duration of mechanical ventilation, ventilation-free days in the first 28 days of life, nitric oxide (NO) treatment, type of initial mechanical ventilation, duration of intensive care unit (ICU) stay, duration of initial hospital stay, presence of chronic lung disease (CLD),[25] congenital cardiac anomalies, treatment with phosphodiesterase type 5 inhibitor (PDE5 treatment), β_2-mimetica, prophylactic

inhaled corticosteroid, atopy, number of respiratory tract infections in the previous year treated with antibiotics prescribed by a family physician, local pediatrician or pediatric pulmonologist, treatment with prophylactic antibiotics, bronchodilators, symptoms of gastroesophageal reflux (heartburn, chest pain, regurgitation, nocturnal cough, dysphagia, dysphonia), Nissen fundoplication, tube feeding, and dietetics.

Lung function measurement

Airway patency was assessed with an electronic spirometer (Masterscreen PFT; Carefusion; San Diego, CA) before and after inhalation of 400 μg salbutamol.[26] Children using inhalation medication had been instructed to stop short-acting β_2-agonists 8 hours before and long-acting β_2-agonists 24 hours before assessment. Forced expiratory volume in 1 s (FEV_1), forced vital capacity (FVC), FEV_1/FVC and forced expiratory flows between 25% and 75% of vital capacity (FEF_{25-75}) were expressed as absolute values, and as SDS based on sex-, age-, and length-related reference values.[27] Reversible airway obstruction was defined as an increase of FEV_1 > 11% after bronchodilatation (BD).[28]

Total lung capacity (TLC_{pleth}), RV/TLC_{pleth} ratio, and functional residual capacity (FRC_{pleth}) were determined by whole body plethysmography (Masterscreen Body Plethysmography; Carefusion) and expressed as absolute values and percentile scores. Diffusion capacity for carbon monoxide (DL_{CO}) and diffusion capacity corrected for alveolar volume (K_{CO}) were measured using a multigas analyzer (Masterscreen PFT; Carefusion) by the single-breath method. Percentile scores for static lung volumes and diffusion capacity obtained by the sex-, age-, and length-related reference equations of Koopman et al.[29] were transformed into SDS using an inverse normal transformation.

The fraction of exhaled NO (FeNO) was measured online using the NIOX analyzer (Aerocrine, Solna, Sweden) according to previously described guidelines and compared against the American Thoracic Society cut-off point.[30,31]

Equipment and procedures fulfilled European Respiratory Society criteria.[26]

Statistical analysis

Differences in baseline data between "participants in the follow-up program" and "non-participants in the follow-up program" and the children "treated with neonatal ECMO" and "not treated with neonatal ECMO" were evaluated using Mann-Whitney U tests for continuous variables and χ^2 tests for categorical variables.

To evaluate lung function parameters (spirometry, body plethysmography, and diffusion capacity) longitudinally and to compare these lung function parameters of patients with the norm population (SDS=0), we used linear mixed models. This method can account for within-subject correlations and allows for missing values in the dependent variable.[32] To test whether lung function parameters differ between ECMO-treated and non-ECMO-treated CDH patients, Mann-Whitney U tests were used.

To investigate whether perinatal and demographic characteristics had a significant influence on SDS lung function parameters (FEV_1 after BD, FEF_{25-75} after BD and K_{CO}), we considered the following baseline data in the linear mixed model as covariates: ECMO treatment, postnatal treatment protocol, patch repair, log transformation of duration of ventilation, type of initial mechanical ventilation, and NO treatment. Independent variables with $p>0.20$ for all outcomes were removed from the model. The two-way interaction effect between timepoint of assessment and log duration of ventilation or timepoint of assessment and ECMO treatment was added to the resulting model if the interaction effect was statistically significant ($p<0.05$). The results of the linear mixed models are reported using estimated marginal means, which are the predicted values of the dependent variable adjusted for the effect of covariates.

Multicollinearity was assessed using variance inflation factors (VIFs). VIFs < 3.0 were considered acceptable, whereas higher values were taken as a sign of multicollinearity. Analyses were performed using SPSS 24.0 (IBM, Chicago, IL), and all statistical tests used a two-sided significance level of 0.05.

RESULTS

Patients

Between January 1999 and June 2009, 167 neonates were born with CDH in the Erasmus MC-Sophia Children's Hospital. Forty-seven (27.6%) died. Forty-one (34.2%) of the 120 survivors were excluded for various reasons, mainly because of psychomotor retardation/syndrome (n=9), late diagnosis (n=10) or they were lost to follow-up/ refused to visit our follow-up (n=13) (Figure 1). Seventy-six (96.2%) (8 years only n=39; 8 and 12 years n= 37) of the 79 children who were eligible for follow-up performed a reliable lung function test (113 measurements) of whom 27 (35.5%) had received ECMO treatment (Figure 1).

No significant differences in background characteristics, except for ethnicity (p=0.001) and side of hernia (p=0.021), were found between the participants and non-participants of our follow-up program (data not shown).

Patient characteristics are presented in Tables 1 and 2. Compared to those without ECMO treatment, children who received ECMO treatment were ventilated longer, had a higher incidence of CLD, had a longer hospital stay, needed more often patch repair, were more frequently treated with PDE5 and an atopic history was reported more frequently at 8 years.

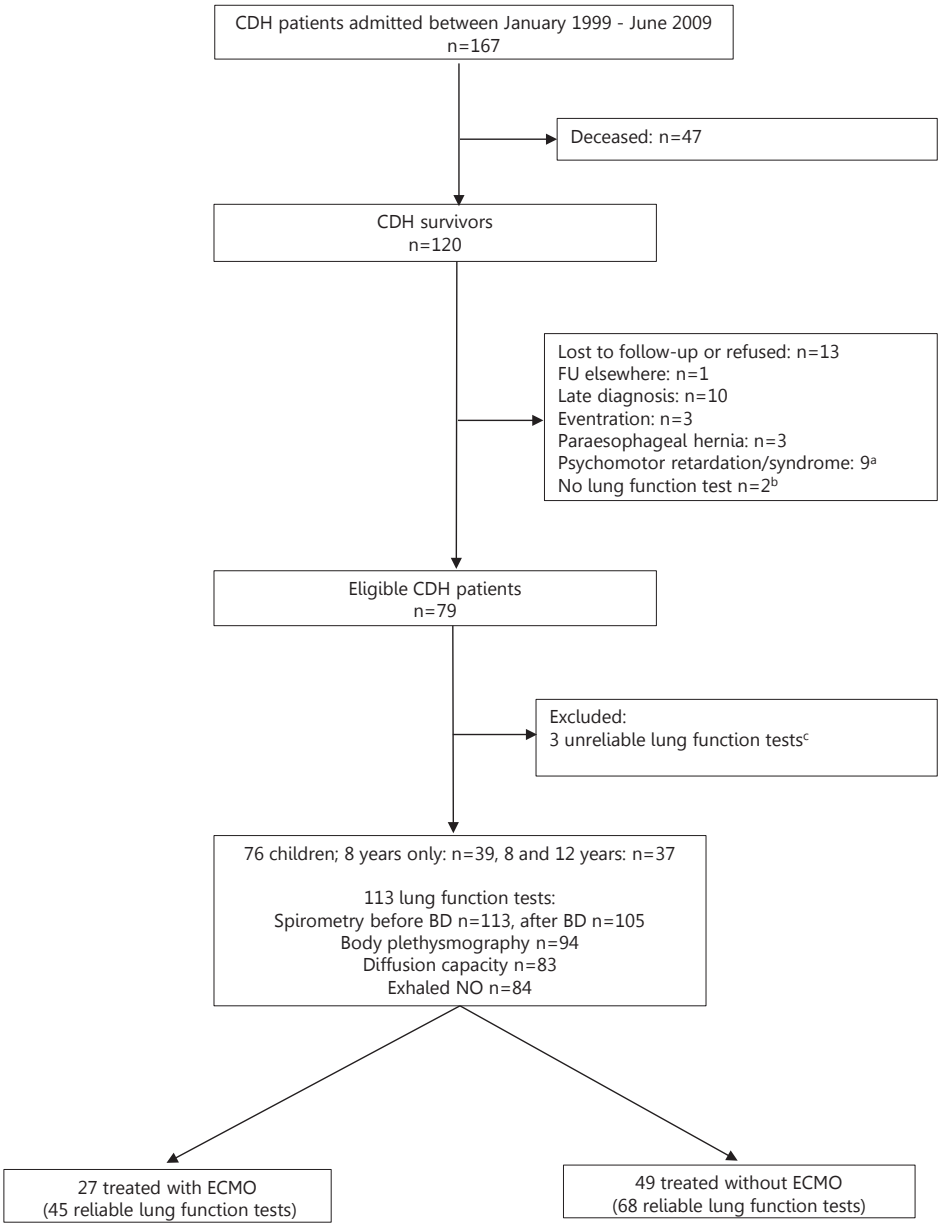

Figure 1 - Study inclusion flowchart

[a] *chromosome aberration (n=1), Cohen syndrome (n=1), Loeys-Dietz syndrome (n=1), Simpson-Golabi-Behmel syndrome (n=2), Wolf-Hirschhorn syndrome (n=1), autism (n=1), PMR (n=2)*

[b] *no lung function test performed due to a tracheacanule (n=1), organizational reasons (n=1)*

[c] *unreliable lung function test due to insufficient technique (n=3)*

BD = bronchodilitation; CDH = congenital hernia diaphragmatic; ECMO = extracorporeal membrane oxygenation; NO = nitric oxide

Table 1 - Patients characteristics

	Total n=76	ECMO n=27	non-ECMO n=49	p-value
Background				
Gestational age (weeks)	39.0 ± 1.5	39.0 ± 1.5	38.7 ± 1.6	0.225
Birth weight (kilograms)	3.1 ± 0.4	3.1 (0.4)	3.0 ± 0.6	0.204
Male (%)	41 (53.9)	18 (66.7)	23 (46.9)	0.099
Ethnicity				0.129
Dutch (%)	64 (84.2)	21 (77.8)	43 (87.8)	
Other (%)	12 (15.8)	6 (22.2)	6 (12.2)	
Left-sided hernia (%)	68 (89.5)	25 (92.6)	43 (87.8)	0.511
Patch repair (%)	54 (71.1)	23 (85.2)	31 (63.3)	0.044
Days of mechanical ventilation	15 (7-24)	28 (15-48)	10 (6-18)	<0.001
Ventilator-free days[a]	13 (0-21)	0 (0-12)	18 (10-23)	<0.001
Type of initial mechanical ventilation				0.843
CMV	33 (43.4)	12 (44.4)	21 (42.9)	
HFO	41 (53.9)	14 (51.9)	27 (55.1)	
Missing	2 (2.6)	1 (3.7)	1 (2.0)	
Nitric Oxide treatment (%)	107 (56.0)	64 (95.5)	43 (34.7)	<0.001
Days of ICU stay	24 (16-51)	52 (28-77)	19 (13-33)	<0.001
Days of initial hospital stay	39 (23-63)	77 (36-99)	29 (20-51)	<0.001
Chronic lung disease (%)				<0.001
No	45 (59.2)	8 (29.6)	37 (75.5)	
Mild	14 (18.4)	3 (11.1)	11 (22.4)	
Moderate	5 (6.6)	5 (18.5)	-	
Severe	11 (14.5)	10 (37.0)	1 (2.0)	
Missing	1 (1.3)	1 (3.7)	-	
Congenital heart disease[b]	7 (9.2)	4 (14.8)	3 (6.1)	0.210
PDE5 treatment	9 (11.8)	7 (25.9)	2 (4.1)	0.005

Data are presented as mean ± SD, median (IQR or number [percentage]), as appropriate

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

[b] Mild = requiring at least 28 days of supplemental oxygen therapy and discharge or termination of supplemental oxygen therapy by 36 weeks postmenstruation age; Moderate = requiring at least 28 days of supplemental oxygen therapy with less than 30% oxygen at 36 weeks postmenstruation age; Severe = requiring at least 28 days of supplemental oxygen therapy with 30% oxygen or greater at 36 weeks postmenstruation age

[c] Congenital heart disease: Ventricle Septum Defect and Atrium Septum Defect (n=1), Double Outlet Right Ventricle + transposition blood vessel + Open Foramen Ovale + Open Ductus Botalli (n=1), Open Ductus Botalli + Open Foramen Ovale + tricuspidalis and mitral insufficiency (n=1), Open Ductus Botalli + Atrium Septum Defect with surgery (n=3), dysplastic pulmonic valve and tricuspidalis insufficiency (n=1)

CDH = congenital diaphragmatic hernia; CMV = coventional mechanical ventilation; ECMO = extracorporeal membrane oxygenation; HFO = high frequency oscillation; PDE5 treatment = treatment with phosphodiesterase type 5 inhibitor

Lung function

SDS FEV_1, SDS FEV_1/FVC, and SDS FEF_{25-75} before and after bronchodilation were significantly below normal at the ages of 8 and 12 years (Table 3; all p≤0.01). Twenty-four children (31.6%) had reversible airflow obstruction at 8 years; nine had been treated with ECMO (one-third of all 8-year-old ECMO-treated neonates).

Hyperreactivity did not change over time: 11 children (29.7%) had reversible airflow obstruction at 12 years; six of them had been treated with ECMO (one-third of all 12-year-old ECMO-treated neonates).

Airflow obstruction had deteriorated significantly from 8 to 12 years (Table 4). This phenomenon was irrespective of ECMO treatment (Table 4, Figure 2, and Table 2 of supplemental file).

Although spirometry results differed significantly between ECMO and non-ECMO patients (Table 2) –with lower spirometry results in ECMO treated patients– ECMO had no significant effect on the deterioration of spirometry parameters (FEV_1 and FEF_{25-75}). These two groups showed a similar trend in deterioration (Figure 2).

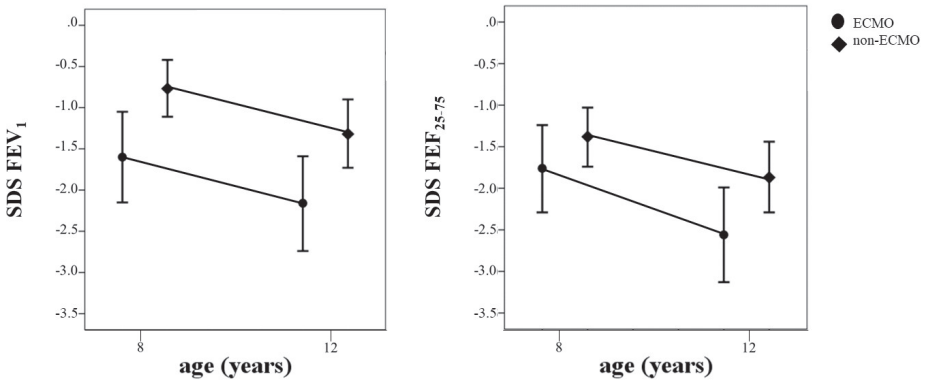

Figure 2 - FEV_1 and FEF_{25-75} before bronchodilation from 8 to 12 years
Data shown are linear mixed models estimates of mean values with 95% confidence intervals
Circles: CDH with ECMO, lozenge: CDH without ECMO
CDH = congenital diaphragmatic hernia; ECMO = extracorporeal membrane oxygenation; FEV_1 = forced expiratory volume in 1 second; FVC = forced vital capacity; FEF_{25-75} = forced expiratory flows between 25% and 75% of vital capacity; SDS = standard deviation score

Table 2 - Characteristics at follow-up assessments

	Total	ECMO	non-ECMO	p-value
At 8 years of age	n=76	n=27	n=49	
β2-mimetica	7 (9.2)	4 (14.8)	3 (6.1)	0.207
Daily	1 (1.3)	-	1 (2.0)	
Taken as needed	6 (7.9)	4 (14.8)	2 (4.1)	
Inhaled corticosteroid treatment	3 (3.9)	1 (3.7)	2 (4.1)	0.935
Atopic history	9 (11.8)	6 (22.2)	3 (6.1)	0.029
Gastroesophageal reflux[a]	13 (17.1)	4 (14.8)	9 (18.4)	0.689
Lower respiratory infections past year with antibiotics	2 (2.6)	-	2 (4.1)	0.287
Prophylactic antibiotics	1 (1.3)	-	1 (2.0)	
Therapeutic antibiotics	1 (1.3)	-	1 (2.0)	
>1 therapeutic course	1 (1.3)	-	1 (2.0)	
Nissen fundoplication	10 (13.2)	5 (18.5)	5 (10.2)	0.274
Tube feeding	-	-	-	-
Dietitian	5 (6.6)	2 (7.4)	3 (6.1)	0.829
At 12 years of age	n=37	n=18	n=19	
β2-mimetica	5 (13.5)	4 (22.3)	1 (5.3)	0.286
Daily	1 (2.7)	1 (5.6)	-	
Taken as needed	4 (10.8)	3 (16.7)	1 (5.3)	
Inhaled corticosteroid treatment	2 (5.4)	2 (11.1)	-	0.179
Atopic history	7 (18.9)	5 (27.8)	2 (10.5)	0.153
Gastroesophageal reflux[a]	4 (10.8)	1 (5.6)	3 (15.8)	0.222
Lower respiratory infections past year with antibiotics	3 (8.1)	1 (5.6)	2 (10.5)	0.512
Prophylactic antibiotics	-	-	-	
Therapeutic antibiotics	3 (8.1)	1 (5.6)	2 (10.5)	
>1 therapeutic course	-	-	-	
Nissen fundoplication	7 (18.9)	5 (27.8)	2 (10.5)	0.181
Tube feeding	-	-	-	
Dietary intervention	1 (2.7)	1 (5.6)	-	0.298

Data are presented in number (percentage)

[a]Symptoms of gastroesophageal reflux (heartburn, chest pain, regurgitation, nocturnal cough, dysphagia, dysphonia)

ECMO = extracorporeal membrane oxygenation

Table 3 - Lung function of CDH patients treated with and without ECMO

	Total	ECMO	non-ECMO	p-value[d]
8 years of age	n=76	n=27	n=49	
SDS FVC before BD	-0.44 (-0.74 - -0.14) [b]	-0.96 (-1.56 - -0.36) [a]	-0.15 (-0.46 - 0.17)	0.023
SDS FVC after BD	-0.15 (-0.46 - 0.16)	-0.61 (-1.24 - 0.02)	0.10 (-0.22 - 0.42)	0.033
SDS FEV_1 before BD	-1.06 (-1.37 - -0.76) [a]	-1.60 (-2.19 - -1.01) [a]	-0.77 (-1.10 - -0.43) [a]	0.006
SDS FEV_1 after BD	-0.51 (-0.84 - -0.19) [a]	-1.08 (-1.77 - -0.39) [a]	-0.21 (-0.52 - -0.10)	0.010
SDS FEV_1/FVC before BD	-1.04 (-1.35 - -0.73) [a]	-1.09 (-1.60 - -0.59) [a]	-1.00 (-1.41 - -0.60) [a]	0.903
SDS FEV_1/FVC after BD	-0.69 (-0.98 - -0.39) [a]	-0.95 (-1.54 - -0.35) [a]	-0.55 (-0.87 - -0.22) [a]	0.279
SDS FEF_{25-75} before BD	-1.53 (-1.82 - -1.23) [a]	-1.77 (-2.32 - -1.22) [a]	-1.38 (-1.74 - -1.04) [a]	0.332
SDS FEF_{25-75} after BD	-0.94 (-1.26 - -0.63) [a]	-1.42 (-2.03 - -0.82) [a]	-0.67 (-1.03 - -0.32) [a]	0.061
SDS RV_{pleth}	0.56 (0.30 - 0.82) [a]	0.85 (0.25 - 1.45) [b]	0.39 (0.16 - 0.63) [a]	0.049
SDS TLC_{pleth}	-0.00 (-0.30 - 0.30)	-0.16 (-0.78 - 0.47)	0.09 (-0.25 - 0.43)	0.686
SDS RV/TLC_{pleth}	0.68 (0.42 - 0.94) [a]	1.00 (0.44 - 1.56) [a]	0.49 (0.22 - 0.76) [a]	0.009
SDS FRC_{pleth}	0.13 (-0.07 - 0.34)	0.12 (-0.29 - 0.53)	0.13 (-0.11 - 0.37)	0.499
SDS DL_{CO}	-0.91 (-1.25 - -0.58) [a]	-1.39 (-1.78 - -1.00) [a]	-0.72 (-1.16 - -0.27) [a]	0.008
SDS K_{CO}	-1.29 (−1.52 - -1.05) [a]	-1.61 (-2.10- -1.12) [a]	-1.15 (-1.41- -0.88) [a]	0.130
12 years of age	n=37	n=18	n=19	
SDS FVC before BD	-0.61 (-1.01 - -0.21) [a]	-1.06 (-1.75 - -0.38) [a]	-0.37 (-0.92 - 0.17)	0.175
SDS FVC after BD	-0.46 (-0.85 - -0.06) [a]	-0.72 (-1.43 - -0.11) [c]	-0.38 (-0.91 - 0.14)	0.325
SDS FEV_1 before BD	-1.64 (-1.97 - -1.30) [a]	-2.14 (-2.64 - -1.64) [a]	-1.43 (-1.94 - -0.92) [a]	0.014
SDS FEV_1 after BD	-0.99 (-1.36 - -0.63) [a]	-1.53 (-2.18 - -0.88) [a]	-0.73 (-1.20 - -0.27) [a]	0.018
SDS FEV_1/FVC before BD	-1.63 (-2.01 - -1.25) [a]	-1.85 (-2.40 - -1.30) [a]	-1.43 (-1.98 - -0.87) [a]	0.245
SDS FEV_1/FVC after BD	-0.94 (-1.31 - -0.56) [a]	-1.51 (-2.03 - -0.98) [a]	-0.42 (-0.94 - 0.10)	0.055
SDS FEF_{25-75} before BD	-2.16 (-2.50 - -1.82) [a]	-2.56 (-3.11 - -2.02) [a]	-1.86 (-2.29 - -1.43) [a]	0.038
SDS FEF_{25-75} after BD	-1.38 (-1.74 - -1.05) [a]	-2.00 (-2.54 - -1.47) [a]	-0.99 (-1.41 - -0.56) [a]	0.007
SDS RV_{pleth}	0.63 (0.24 -1.02) [a]	0.52 (-0.20 - 1.23)	0.74 (0.27 - 1.21) [a]	0.781
SDS TLC_{pleth}	-0.27 (-0.67 - 0.12)	-0.39 (-1.12 - 0.34)	-0.31 (-0.81 - 0.19)	0.205
SDS RV/TLC_{pleth}	0.74 (0.28 - 1.20) [a]	0.63 (-0.23 -1.50)	0.90 (0.32 - 1.47) [a]	0.687
SDS FRC_{pleth}	0.17 (-0.12 - 0.45)	0.04 (-0.44 - 0.52)	0.30 (-0.13 - 0.73)	0.422
SDS DL_{CO}	-0.99 (-1.32 - -0.65) [a]	-1.14 (-1.62 - -0.65) [a]	-0.86 (-1.32 - -0.40) [a]	0.367
SDS K_{CO}	-1.22 (-1.48 - -0.96) [a]	-1.41 (-1.93 - -0.90) [a]	-1.09 (-1.37 - -0.82) [a]	0.217

Data are presented as estimated marginal mean (95% confidence interval) SDS lung function parameters Significantly below/above the population norm (SDS =0): [a] $p \leq 0.001$; [b] $p \leq 0.01$; [c] $p=0.025$; [d] Mann Whitney U tests: differences in lung function parameters between ECMO- and non-ECMO-treated CDH patients
CDH = congenital diaphragmatic hernia; ECMO = extracorporeal membrane oxygenation; SDS = standard deviation score; BD = bronchodilation; FEV_1 = forced expiratory volume in 1 second; FVC = forced vital capacity; FEF_{25-75} = forced expiratory flows between 25%-75% of vital capacity; RV_{pleth} = plethysmographic derived residual volume; TLC_{pleth} = plethysmographic derived total lung capacity; FRC_{pleth} = functional residual capacity; DLco = transfer factor for carbon monoxide; K_{CO} = diffusion capacity corrected for alveolar volume

Static lung volumes, except for RV_{pleth} and RV/TLC_{pleth} ratio, were within normal ranges at ages 8 and 12 years (Table 3). Diffusion capacity, either corrected or uncorrected for alveolar volume, was significantly below normal in both groups and ages (both $p \leq 0.001$; Table 3) and had not changed significantly over time (Table 4).

The median interquartile range (IQR) FeNO of 51 successful measurements at 8 years was 10 (9-15) ppb. The majority of children (84.4%) had FeNO less than 20 ppb.

The median (IQR) FeNO of 33 successful measurements at 12 years was 14 (9-27) ppb. More than two-thirds of children (69.7%) had FeNO less than 20 ppb (table 3 of the supplemental file).

Associations between lung function parameters and baseline data

Duration of initial hospital stay and ventilation free days were excluded as independent variables from the linear mixed model because the VIFs were more than 3.0. VIFs of the other covariates were all less than 2.1 after removing these two independent variables.

The independent variables type of repair, type of initial ventilation and NO treatment were removed from the linear mixed model, because of p-values above the cutoff of p=0.20.

FEV_1 after BD was negatively associated with log duration of ventilation (estimated coefficient [95% CI] -0.865 [-1.215 to -0.515], p<0.001) (Figure 2, Table 2 of the supplemental file). The FEV_1 after BD of children not treated with the postnatal treatment protocol was significantly higher than that of children treated with this protocol (0.767 [0.104 to 1.430], p=0.024) (Tables 2 and 4 of the supplemental file). No other significant association was found between FEV_1 and baseline data (Table 2 of the supplemental file). Besides, no significant interaction effects were found between timepoint of assessment and log duration of ventilation or timepoint of assessment and ECMO treatment for FEV_1 (data not shown).

FEF_{25-75} after BD had a negative association with log duration of ventilation (estimated coefficient [95% CI] -0.827 [-1.162 to -0.491], p<0.001) (Figure 2, Table 2 of the supplemental file). FEF_{25-75} after BD of children not treated with the postnatal treatment protocol was significantly higher than that of children treated with the protocol (0.717 [0.053 to 1.380], p=0.035) (Tables 2 and 4 of the supplemental file). No other significant associations were found between FEF_{25-75} and baseline data (Table 2 of the supplemental file). Besides, no significant interaction effects were found between timepoint of assessment and log duration of ventilation or timepoint of assessment and ECMO treatment for FEF_{25-75} (data not shown).

No significant associations were found between K_{CO} and baseline data (Table 2 of the supplemental file).

Table 4 - Estimated mean differences of lung function parameters from 8 to 12 years in CDH patient treated with and without ECMO

	Estimated mean differences (95% CI)		
Lung function parameters	**Total**	**ECMO**	**non-ECMO**
SDS FVC before BD	-0.17 (-0.45 - 0.11)	-0.11 (-0.47 - 0.26)	-0.23 (-0.68 - 0.23)
SDS FVC after BD	-0.31 (-0.62 - 0.01)	-0.11 (-0.62 - 0.40)	-0.48 (-0.92 - -0.04) [b]
SDS FEV_1 before BD	-0.57 (-0.79 - -0.36) [a]	-0.54 (-0.84 - -0.24) [a]	-0.66 (-0.99 - -0.33) [a]
SDS FEV_1 after BD	-0.48 (-0.77 - -0.19) [a]	-0.45 (-0.97 - 0.07)	-0.52 (-0.86 - -0.19) [b]
SDS FEV_1/FVC before BD	-0.59 (-0.92 - -0.27) [a]	-0.76 (-1.19 - -0.32) [a]	-0.43 (-0.93 - 0.08)
SDS FEV_1/FVC after BD	-0.25 (-0.61 - 0.10)	-0.56 (-1.08 - -0.04) [b]	0.13 (-0.32 - 0.57)
SDS FEF_{25-75} before BD	-0.63 (-0.89 - -0.37) [a]	-0.79 (-1.23 - -0.35) [a]	-0.48 (-0.81 - -0.14) [b]
SDS FEF_{25-75} after BD	-0.45 (-0.75 - -0.16) [a]	-0.58 (-1.11 - -0.05) [b]	-0.31 (-0.66 - 0.04)
SDS RV_{pleth}	0.07 (-0.37 - 0.51)	-0.33 (-1.14 - 0.47)	0.35 (-0.16 - 0.85)
SDS TLC_{pleth}	-0.27 (-0.58 - 0.04)	-0.23 (-0.76 - 0.29)	-0.40 (-0.79 - -0.01)
SDS RV/$TLC_{pleth\ ratio}$	0.06 (-0.41 - 0.53)	-0.37 (-1.13 - 0.39)	0.41 (-0.17 - 0.99)
SDS FRC_{pleth}	0.04 (-0.20 - 0.27)	-0.09 (-0.35 - 0.18)	0.17 -0.27 - 0.60)
SDS DL_{CO}	-0.08 (-0.40 - 0.24)	0.25 (-0.17 - 0.66)	-0.14 (-0.58 - 0.29)
SDS K_{CO}	0.06 (-0.22 - 0.35)	0.19 (-0.36 - 0.75)	0.05 (-0.27 - 0.37)

Significant change of estimated mean differences (based on estimated marginal means) from 8 to 12 years: [a] *$p \leq 0.001$;* [b] *$p \leq 0.01$*

CDH = congenital diaphragmatic hernia; ECMO = extracorporeal membrane oxygenation; SDS = standard deviation score; CI = confidence interval; BD = bronchodilation; FEV_1 = forced expiratory volume in 1 second; FVC = forced vital capacity; FEF_{25-75} = forced expiratory flows between 25% and 75% of vital capacity; RV_{pleth} = plethysmographic derived residual volume; TLC_{pleth} = plethysmographic derived total lung capacity; FRC_{pleth} = functional residual capacity; DLco = transfer factor for carbon monoxide; K_{CO} = diffusion capacity corrected for alveolar volume

DISCUSSION

This longitudinal follow-up study in school-aged children born with CDH revealed deterioration of severity of airflow obstruction from 8 to 12 years, though reduced diffusion capacity corrected for alveolar volume remained stable. These findings were irrespective of ECMO treatment. The static lung volumes were within normal ranges and did not change from 8 to 12 years. Longer duration of mechanical ventilation was associated with significantly more airflow obstruction.

Most studies that have evaluated pulmonary morbidity in school-aged children with CDH were cross-sectional and revealed airflow obstruction.[6,8,20,21,33] The study populations of Haliburton et al.[21] and Turchetta et al.[33] overlapped in birth years with our study population. Cross-sectional data from Haliburton et al. were obtained from retrospective chart analysis and only 33 of 118 patients (28%) had reproducible pulmonary function tests. Their mean age was 11.3 ± 3.4 years. Sixty-three percent of these children vs 57%

in our study had reduced FEV_1 and 52% vs 59% had reduced FEV_1/FVC. In the cross-sectional study of Turchetta et al., only 30 children of the 210 survivors were randomly selected to be enrolled in the study, of whom 18 participated. The mean age of this study population was 6.6 ± 2.6 years. Contrary to our study, the mean FEV_1 reached the lower range of normal. Twenty-two percent of the children had a significant increase of FEV_1 after bronchodilation vs 30% of the children in our study. Differences in study design, selection of subjects and numbers of subjects may explain differences between these studies and our results. Selection bias might have occurred in both previous cross-sectional studies.

To our knowledge, only our group has previously reported on longitudinal lung function in children and young adults who survived CDH.[6,7] In a study of ECMO-treated patients with small numbers of CDH patients, lung function had deteriorated over time at school age.[7] In a cohort born before ECMO was introduced in the Netherlands (1991), mild airflow obstruction had deteriorated from childhood to young adulthood.[6] Micro-structural pulmonary changes not only in the ipsilateral lung but also in the contralateral lung were found in non-smoking young adults in this cohort.[9] Taking into account the very short duration of mechanical ventilation in this older group[6] and the change in mortality rates over time,[2] we assume that children with CDH who survived in the past decade suffered from more severe lung hypoplasia, were ventilated longer, and have more airflow obstruction. As we show in this study deterioration of airflow obstruction between 8 and 12 years, and in a previous study of our research group deterioration of airflow obstruction from childhood to adulthood,[6] we recommend further research on long-term pulmonary morbidity including assessment of exercise tolerance and lung morphology. Lung perfusion scans revealed that pulmonary vascular development in CDH patients remains abnormal.[14] These patients' lung development is affected by inhibited pulmonary vascular growth. Pulmonary vascular catch-up growth does not always occur, especially not in the ipsilateral lung.[10,13,14] Lung hypoplasia with limited catch-up growth and vascular maldevelopment will probably contribute to the development of CLD and affect lung function later in life.

We assume that ECMO-treated CDH patients have more severe lung hypoplasia, pulmonary hypertension and vascular maldevelopment than non-ECMO-treated CDH patients. Therefore, they may need longer mechanical ventilation, which is associated with risk of CLD. This may be reflected by more severe airflow obstruction at school age.

The ECMO-treated CDH patients in this study indeed had longer duration of mechanical ventilation and more often CLD. They also had significant lower spirometry parameters than non-ECMO-treated CDH patients. However, the change of airflow obstruction over time was not affected by ECMO treatment.

This suggests that CLD could be responsible for the lower spirometry parameters at school age, but not for the deterioration of these parameters. Therefore, we recommend

that future research should focus on long-term longitudinal assessment of pulmonary morbidity and imaging of lung morphology into adulthood.

Interestingly, the airflow obstruction in the children treated after introduction of the postnatal treatment protocol was more severe than that in the children born earlier. This result supports the aforementioned assumption that with the decreased mortality from 33% to 12% after the introduction of the postnatal treatment protocol, there might be an increase of the presence of more severe lung hypoplasia in the survivors. However, ECMO treatment was more frequent before the introduction of the protocol, and was usually applied in children with more severe lung hypoplasia. Still, ECMO-treated children who participated in the UK ECMO trial had a slightly better lung function than those who were conventionally ventilated,[34] as they might have been spared prolonged ventilation and consequent barotrauma. Our study included only two CDH patients treated with ECMO after the implementation of the postnatal treatment protocol. More patients treated according to the postnatal treatment protocol should be studied to demonstrate possible significant effects of this protocol on lung function.

Significant reversibility of airflow obstruction was observed in almost one-third of our participants. Our data do not allow for speculations about the reason for this phenomenon, but it emphasizes the importance of long-term follow-up of all children with CDH. Supportive care by pediatric pulmonologists including prescription of bronchodilators may be useful to reduce pulmonary morbidity and improve exercise tolerance.[35]

The strengths of our study are the relatively large cohort and the longitudinal design. Another strength of our study is the absence of significant differences in baseline data reflecting severity of illness between the participants and non-participants of our follow-up program. Selection bias is therefore unlikely.

Several limitations need to be addressed, however. First, data of only a relatively small number of children assessed at 12 years of age (n=37) were included, although mixed models account for data missing at random. Secondly, although we had a large number of reliable lung function tests, some lung function assessment data were missing (flowchart), mainly concerning children with no previous experience in lung function testing. Thirdly, we found a significant effect of the use of the postnatal treatment protocol on lung function. Children treated after the introduction of the postnatal treatment protocol had more severe airflow obstruction than the children treated before. A larger sample size of 8- and 12-year-olds is needed to unravel the relation between the introduction of the postnatal treatment protocol, severity of lung hypoplasia, microstructural pulmonary changes of the lungs and airflow obstruction. Finally, patient-reported outcomes such as atopy and lower respiratory tract infections (RTIs) treated with antibiotics and/or gastroesophageal reflux (Table 2), were not considered as covariates in the linear mixed model, because only a small number of patients reported those complaints. To minimize the risk of recall bias we recorded only the number of RTIs treated with antibiotics in the

last year. Digital exchange of information of prescribed antibiotics between family physician, pharmacy and hospital could contribute to reliable monitoring of the individual use of antibiotics over several years.

In conclusion, airflow obstruction deteriorates over time, though reduced diffusion capacity remains stable. These observations are irrespective of ECMO treatment. Good pulmonary care into adulthood should be provided to minimize respiratory dysfunction on the long term. Early risk stratification may be important to offer timely intervention.

ACKNOWLEDGEMENTS

The authors thank all members of the surgical long-term follow-up team and the staff of the lung function department for their contributions. Ko Hagoort provided editorial advice.

REFERENCES

1. Sluiter I, Reiss I, Kraemer U, Krijger R, Tibboel D, Rottier RJ. Vascular abnormalities in human newborns with pulmonary hypertension. Expert Rev Respir Med. 2011;5:245-256.
2. van den Hout L, Schaible T, Cohen-Overbeek TE, et al. Actual outcome in infants with congenital diaphragmatic hernia: the role of a standardized postnatal treatment protocol. Fetal Diagn Ther. 2011;29:55-63.
3. Reiss I, Schaible T, van den Hout L, et al. Standardized postnatal management of infants with congenital diaphragmatic hernia in Europe: the CDH EURO Consortium consensus. Neonatology. 2010;98:354-364.
4. Ackerman KG, Pober BR. Congenital diaphragmatic hernia and pulmonary hypoplasia: new insights from developmental biology and genetics. Am J Med Genet C Semin Med Genet. 2007;145C:105-108.
5. Majaesic CM, Jones R, Dinu IA, Montgomery MD, Sauve RS, Robertson CM. Clinical correlations and pulmonary function at 8 years of age after severe neonatal respiratory failure. Pediatr Pulmonol. 2007;42:829-837.
6. Spoel M, van der Cammen-van Zijp MH, Hop WC, Tibboel D, de Jongste JC, IJsselstijn H. Lung function in young adults with congenital diaphragmatic hernia; a longitudinal evaluation. Pediatr Pulmonol. 2013;48:130-137.
7. Spoel M, Laas R, Gischler SJ, et al. Diagnosis-related deterioration of lung function after extracorporeal membrane oxygenation. Eur Respir J. 2012;40:1531-1537.
8. Peetsold MG, Heij HA, Nagelkerke AF, et al. Pulmonary function and exercise capacity in survivors of congenital diaphragmatic hernia. Eur Respir J. 2009;34:1140-1147.
9. Spoel M, Marshall H, IJsselstijn H, et al. Pulmonary ventilation and micro-structural findings in congenital diaphragmatic hernia. Pediatr Pulmonol. 2016;51:517-524.
10. Hislop AA. Airway and blood vessel interaction during lung development. J Anat. 2002;201:325-334.
11. Narayanan M, Owers-Bradley J, Beardsmore CS, et al. Alveolarization continues during childhood and adolescence: new evidence from helium-3 magnetic resonance. Am J Respir Crit Care Med. 2012;185:186-191.
12. Beals DA, Schloo BL, Vacanti JP, Reid LM, Wilson JM. Pulmonary growth and remodeling in infants with high-risk congenital diaphragmatic hernia. J Pediatr Surg. 1992;27:997-1001.
13. Sluiter I, Veenma D, van Loenhout R, et al. Etiological and pathogenic factors in congenital diaphragmatic hernia. Eur J Pediatr Surg. 2012;22:345-354.
14. Hayward MJ, Kharasch V, Sheils C, et al. Predicting inadequate long-term lung development in children with congenital diaphragmatic hernia: an analysis of longitudinal changes in ventilation and perfusion. J Pediatr Surg. 2007;42:112-116.
15. Kawahara H, Okuyama H, Nose K, et al. Physiological and clinical characteristics of gastroesophageal reflux after congenital diaphragmatic hernia repair. J Pediatr Surg. 2010;45:2346-2350.
16. Peetsold MG, Kneepkens CMF, Heij HA, IJsselstijn H, Tibboel D, Gemke RJBJ. Congenital Diaphragmatic Hernia: Long-term Risk of Gastroesophageal Reflux Disease. J Pediatr Gastr Nutr. 2010;51:448-453.
17. Chiu PP, IJsselstijn H. Morbidity and long-term follow-up in CDH patients. Eur J Pediatr Surg. 2012;22:384-392.

18. Spoel M, van den Hout L, Gischler SJ, et al. Prospective longitudinal evaluation of lung function during the first year of life after repair of congenital diaphragmatic hernia. Pediatr Crit Care Med. 2012;13:e133-139.
19. Koumbourlis AC, Wung JT, Stolar CJ. Lung function in infants after repair of congenital diaphragmatic hernia. J Pediatr Surg. 2006;41:1716-1721.
20. IJsselstijn H, Tibboel D, Hop WJ, Molenaar JC, de Jongste JC. Long-term pulmonary sequelae in children with congenital diaphragmatic hernia. Am J Respir Crit Care Med. 1997;155:174-180.
21. Haliburton B, Mouzaki M, Chiang M, et al. Pulmonary function and nutritional morbidity in children and adolescents with congenital diaphragmatic hernia. J Pediatr Surg. 2017;52:252-256.
22. Gischler SJ, van der Cammen-van Zijp MHM, Mazer P, et al. A prospective comparative evaluation of persistent respiratory morbidity in esophageal atresia and congenital diaphragmatic hernia survivors. J Pediatr Surg. 2009;44:1683-1690.
23. Mazer P, Gischler SJ, van der Cammen-van Zijp MHM, et al. Early developmental assessment of children with major non-cardiac congenital anomalies predicts development at the age of 5 years. Dev Med Child Neurol. 2010;52:1154-1159.
24. Stolar CJ, Snedecor SM, Bartlett RH. Extracorporeal membrane oxygenation and neonatal respiratory failure: experience from the extracorporeal life support organization. J Pediatr Surg. 1991;26:563-571.
25. Jobe AH, Bancalari EH. Controversies about the definition of bronchopulmonary dysplasia at 50 years. Acta Paediatr. 2017;106:692-693.
26. Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. Eur Respir J. 2005;26:319-338.
27. Quanjer PH, Stanojevic S, Cole TJ, et al. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. Eur Respir J. 2012;40:1324-1343.
28. Casan P, Roca J, Sanchis J. Spirometric Response to a Bronchodilator - Reference Values for Healthy-Children and Adolescents. B Eur Physiopath Res. 1983;19:567-569.
29. Koopman M, Zanen P, Kruitwagen CL, van der Ent CK, Arets HG. Reference values for paediatric pulmonary function testing: The Utrecht dataset. Resp Med. 2011;10f:15-23.
30. Baraldi E, de Jongste JC, Force T. Measurement of exhaled nitric oxide in children. Eur Respir J. 2002;20:223-237.
31. Dweik RA, Boggs PB, Erzurum SC, et al. An Official ATS Clinical Practice Guideline: Interpretation of Exhaled Nitric Oxide Levels (FENO) for Clinical Applications. Am J Resp Crit Care. 2011;184:602-615.
32. Fitzmaurice GM, Laird NM, Ware JH. Applied Longitudinal Analysis. 1st edition: Hoboken, John Wiley & Sons; 2004.
33. Turchetta A, Fintini D, Cafiero G, et al. Physical activity, fitness, and dyspnea perception in children with congenital diaphragmatic hernia. Pediatr Pulmonol. 2011;46:1000-1006.
34. Beardsmore C, Dundas I, Poole K, Enock K, Stocks J. Respiratory function in survivors of the United Kingdom Extracorporeal Membrane Oxygenation Trial. Am J Respir Crit Care Med. 2000;161:1129-1135.
35. GOLD Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease 2018 report. Available from: www.goldcopd.org.

SUPPLEMENTAL FILE

Table 1 - Recommendations of the standardized postnatal protocol

Prenatal management and delivery
Following prenatal diagnosis, the absolute and O/E LHR and the position of the liver should be evaluated
Planned vaginal delivery or caesarean section after a gestational age of 37 weeks in a high-volume tertiary center should be pursued
In case of preterm labor prior to 34 weeks of gestation, antenatal steroids should be given
Delivery room management and treatment in a very early phase
After delivery, the infant should be intubated immediately without bag and mask ventilation
The goal of treatment in the delivery room is achieving acceptable preductal saturations levels between 80 and 95%
Ventilation in the delivery room may be done by conventional ventilator or ventilation bag with a peak pressure as low as possible, preferably below 25 cm H_2O
An oro- or nasogastric tube with continuous or intermittent suction should be placed
Arterial blood pressure has to be maintained at a normal level for gestational age. In case of hypotension and/ or poor perfusion, 10- 20 ml/kg NaCl 0.9% should be administered 2 times
Sedatives and analgesics should be given
No routine use of surfactant in either term or preterm infants with CDH
Ventilation management in the Intensive Care Unit
Adapt treatment to reach a preductal saturation between 85 and 95% and a postductal saturation above 70%
In individual cases, preductal saturation above 80% might be acceptable, as long as organs are well perfused
The target $PaCO_2$ range should be between 45 and 60 mmHg
Pressure-controlled ventilation initial settings are a PIP of 20-25 cm H_2O and a PEEP of 2-5 cm H_2O; ventilator rate of 40- 60/min
HFOV: initial setting mean airway pressure 13-17 cm H_2O, frequency 10 Hz, ΔP 30-50 cm H_2O depending on chest wall vibration
After stabilization, the FiO_2 should be decreased if preductal saturation is above 95%
Further management in the Intensive Care Unit
Infants should be sedated and be monitored using validated analgesia and sedation scoring systems
Neuromuscular blocking agents should be avoided if possible
If symptoms of poor perfusion and/or blood pressure below the normal level for gestational age occur and are associated with preductal saturation below 80%, echocardiographic assessment should be performed
In case of hypovolemia, isotonic fluid therapy 10-20 ml/kg NaCl 0.9% up to 3 times during the first 2 hours may be given and inotropics should be considered
Pulmonary hypertension
Perform echocardiography within the first 24 h after birth
Blood pressure support should be given to maintain arterial blood pressure levels at normal levels for gestational age
iNO should be considered if there is evidence of extrapulmonary right-to-left shunting and the oxygenation index is above 20 and/or the saturation difference is more than 10%
In case of suprasystemic pulmonary artery pressure and right-to-left shunting through the foramen ovale, i.v. prostaglandin E1 has to be considered

Table 1 - Recommendations of the standardized postnatal protocol *(continued)*

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

Reiss, I., et al., Standardized postnatal management of infants with congenital diaphragmatic hernia in Europe: the CDH EURO Consortium consensus. Neonatology, 2010. 98(4): p. 354-64.

Table 2 - Possible determinants of lung function parameters

Dependent variable Predictor	**estimated coefficient (95% CI)**	**p-value**
FEV_1 after BD		
Time of assessment	0.466 (0.172 - 0.760)	0.003
ECMO	-0.271 (-0.368 - 0.911)	0.400
Before postnatal protocol	0.767 (0.104 - 1.430)	0.024
Log duration of initial ventilation	-0.865 (-1.215 - -0.515)	<0.001
FEF_{25-75} after BD		
Time of assessment	0.460 (0.168 - 0.751)	0.003
ECMO	-0.266 (-0.339 - 0.870)	0.383
Before postnatal protocol	0.717 (0.053 - 1.380)	0.035
Log duration of initial ventilation	-0.827 (-1.162 - -0.491)	<0.001
K_{CO}		
Time of assessment	-0.109 (-0.411 - 0.192)	0.462
ECMO	-0.266 (-0.237 - 0.769)	0.295
Before postnatal protocol	0.019 (-0.507- 0.546)	0.942
Log duration of initial ventilation	-0.180 (-0.487 - 0.126)	0.244

Linear mixed model: data are presented as estimated coefficient (95% confidence intervals) SDS lung function parameters

FEV_1 = forced expiratory volume in 1 second; FEF_{25-75} = forced expiratory flows between 25% and 75% of vital capacity; K_{CO} = diffusion capacity corrected for alveolar volume; ECMO = extracorporeal membrane oxygenation; SDS = standard deviation score

Table 3 - FeNO of ECMO and non-ECMO treated CDH patients

	Total	**ECMO**	**non-ECMO**
8 years	n=51	n=20	n=31
FeNO, median (IQR)	10 (9-15)	12 (8-20)[a]	10 (9-13)[a]
<20 ppb, n(%)	43 (84.4)	15 (75.0)	28 (90.3)
20-35 ppb, n(%)	6 (11.8)	4 (20.0)	2 (6.5)
>35 ppb, n(%)	2 (3.9)	1 (5.0)	1 (3.2)
12 years	n=30	n=14	n=16
FeNO, median (IQR)	14 (9-27)	14 (7-46)[b]	14 (11-20)[b]
<20 ppb, n(%)	23 (76.7)	11 (64.7)	12 (75.0)
20-35 ppb, n(%)	3 (10.0)	1 (5.9)	2 (12.5)
>35 ppb, n(%)	7 (23.3)	5 (29.4)	2 (12.5)

Mann-Whitney test: no significance difference between ECMO and non-ECMO treated CDH patients: [a]p=0.699; [b]p=0.914

CDH = congenital diaphragmatic hernia, ECMO = extracorporeal membrane oxygenation, n = number of patients, IQR = interquartile range, FeNO = fraction of exhaled nitric levels, ppb = parts per billion

Table 4 - Lung function of CDH patients treated with and without the postnatal treatment protocol

	8 years	12 years
Treated without protocol, n=94	n=57	n=37
FVC before BD	-0.29 (-1.30 - 0.57)	-0.69 (-1.71 - 0.74)
FVC after BD	0.15 (-1.01 - 0.83)	-0.28 (-1.12 - 1.00)
FEV_1 before BD	-0.95 (-1.79 - 0.06)	-1.50 (-2.42 - -0.64)
FEV_1 after BD	-0.39 (-1.26 - 0.57)	-0.62 (-1.83 - 0.09)
FEV_1/FVC before BD	-0.74 (-2.09 - -0.08)	-1.83 (-2.43 - -0.65)
FEV_1/FVC after BD	-0.65 (-1.20 - 0.36)	-1.27 (-1.90 - -0.18)
FEF_{25-75} before BD	-1.29 (-2.40 - -0.56)	-2.03 (-3.03 - -1.17)
FEF_{25-75} after BD	-0.81 (-1.79 - -0.02)	-1.27 (-2.28 - -0.48)
RV_{pleth}	0.76 (0.07 - 1.21)	-1.18 (-1.88 - -0.50)
TLC_{pleth}	0.08 (-0.61 - 0.90)	-1.28 (-1.88 - -0.84)
RV/TLC_{pleth}	0.71 (0.19 - 1.24)	0.92 (0.15 - 1.55)
FRC_{pleth}	0.15 (-0.23 - 0.84)	0.28 (-0.81 - 1.08)
DL_{CO}	-0.84 (-1.41 - 0.27)	0.47 (-0.55 - 0.99)
Treated with protocol, n=19[a]	n=19	n=0
FVC before BD	-0.51 (-0.88 - 0.11)	-
FVC after BD	0.26 (-0.56 - 0.22)	-
FEV_1 before BD	-1.44 (-2.23 - -0.26)	-
FEV_1 after BD	-0.64 (-1.28 - 0.10)	-
FEV_1/FVC before BD	-1.72 (-2.47 - -0.65)	-
FEV_1/FVC after BD	-0.95 (-1.66 - 0.02)	-
FEF_{25-75} before BD	-2.23 (-2.71 - -1.04)	-
FEF_{25-75} after BD	-1.15 (-2.13 - -0.18)	-
RV_{pleth}	0.36 (-0.08 - 0.98)	-
TLC_{pleth}	-0.28 (-0.90 - 0.18)	-
RV/TLC_{pleth}	0.58 (0.14 - 1.35)	-
FRC_{pleth}	-0.10 (-0.55 - 0.23)	-
DL_{CO}	-0.99 (-2.05 - -0.41)	-
K_{CO}	-1.18 (-2.05 - -0.52)	-

Data are presented as median (interquartile range) SDS lung function parameters

[a] 2/19 CDH patients received neonatal ECMO treatment

BD = bronchodilation; FVC = forced vital capacity; FEV1 = forced expiratory volume in 1 second; FEF25-75 = forced expiratory flows between 25%-75% of vital capacity; RVpleth = plethysmographic derived residual volume; TLCpleth = plethysmographic derived total lung capacity; FRCpleth = functional residual capacity; DLco = transfer factor for carbon monoxide; KCO = diffusion capacity corrected for alveolar volume; n = number of patients