THE VULNERABLE BRAIN

NEURODEVELOPMENT AFTER NEONATAL CRITICAL ILLNESS

RAISA SCHILLER

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Neurodevelopment after neonatal critical illness

Raisa Schiller

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The Vulnerable Brain
Neurodevelopment after neonatal critical illness

Het kwetsbare brein
Neurocognitieve ontwikkeling na zeer ernstige ziekte als pasgeborene

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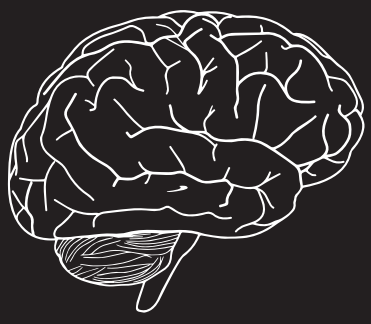
The vulnerable brain in critically ill infants

CHAPTER 1



GENERAL INTRODUCTION

A young girl comes to our outpatient clinic for her follow-up visit at 8 years of age. As a neonate, she was treated with extracorporeal membrane oxygenation for severe meconium aspiration syndrome. Upon discharge, her cranial ultrasound was normal and routine visits in the first years of life showed favourable outcomes. In her records we see that on her routine IQ assessment at 5 years of age, she scored well-above average. She is a bright young girl, plays sports and is healthy. However, when asked how she is doing, she starts to cry. She says she keeps forgetting the plans she has made with friends or the homework she has to turn in the next day. She is in tears because she fears people will think she is not smart. Her parents are worried, they also question what will happen when she has to make the transition from primary school to high school. They do not understand what is wrong with her…

The number of critically ill neonates surviving after neonatal intensive care admission is increasing worldwide.[1,2] The girl described here is, unfortunately, not unique. It is therefore of utmost importance that our focus broadens from prevention of mortality to long-term outcome in critically ill neonates.

A clearly delimited group of survivors of neonatal critical illness are children treated with neonatal extracorporeal membrane oxygenation (ECMO) or congenital diaphragmatic hernia (CDH) treated without ECMO. Since the first neonatal ECMO treatment applied in 1975, nearly 40,000 neonates have been treated with ECMO worldwide.[3] The annual number of neonatal ECMO runs has decreased over the years and there has been a shift from respiratory to cardiac runs. Nonetheless, the most frequent underlying diagnoses for neonatal ECMO remain meconium aspiration syndrome (MAS) and congenital diaphragmatic hernia (CDH). The survival rate following MAS is over 90%. CDH is a rare congenital anatomical malformation associated with significant mortality and morbidity due to pulmonary hypoplasia and pulmonary hypertension. In the most severe cases of CDH, patients require treatment with ECMO and mortality rates are 49%.[3] Over the past decade, standardized treatment protocols for CDH patients have led to less need for ECMO and to lower mortality rates.[4]

The assessment of long-term outcome in these children is therefore increasingly important. Of particular concern is the neuropsychological outcome following neonatal critical illness. The brain is rapidly developing during the first months of life and therefore particularly vulnerable in these children.[5] Given the importance of neuropsychological functioning both for academic performance and daily life activities, it is imperative to correctly identify and treat survivors of neonatal ECMO and/or CDH at risk of such long-term impairments.

IDENTIFICATION OF PATIENTS AT RISK

Neuropsychological assessment

Within the last decade, a number of studies have evaluated long-term neuropsychological outcome following neonatal ECMO and/or CDH. Fortunately, a significant number of children survive without overt neurological abnormalities, such as haemorrhage or periventricular leukomalacia.[6,7] Moreover, general cognitive outcome seems generally comparable to that of healthy children at various stages of development.[8-12] Strikingly, however, the incidence of school problems is significantly higher in these children compared to the general population.[11,13] This is highly suggestive of an alternative explanation related to specific neuropsychological deficits rather than general intellectual functioning.

A limited number of follow-up studies have assessed specific neuropsychological functions in neonatal ECMO and/or CDH survivors. Sustained attention has been evaluated in 8-year-old survivors of CDH, both in patients treated with and without ECMO. Attention deficits were found in 68% of children compared to the general population, with no influence of treatment type.[13] In 8-year-old neonatal ECMO survivors following CDH as well as other diagnoses, sustained attention deficits were found as well, while visual-motor integration was normal.[11] In the UK ECMO Trial, verbal and visual memory were assessed in 7-year-old survivors of severe respiratory failure randomized to receive either neonatal ECMO or conventional management.[10] Both groups had significantly worse verbal and visual memory compared to the norm.[10] Taken together, these studies suggest both memory and attention deficits following neonatal critical illness, while intelligence and visual-motor integration are normal.[10,11,13] However, neuropsychological assessment including all major cognitive domains in the same cohort is lacking. As such, the domains most affected following neonatal ECMO and/or CDH remain largely speculative. To improve identification of patients at risk, clear delineation of the neuropsychological profile following neonatal ECMO and/or CDH is needed.

Neuroimaging

It is crucial to identify patients at risk of school problems as early as possible. Illness and treatment characteristics, such as underlying diagnosis or the duration of mechanical ventilation, may be useful to predict neuropsychological impairments as early as in infancy. However, as of yet, results have not been conclusive. Severity of illness rather than independent clinical characteristics may increase a child's risk of long-term impairments[11,14], but quantifying severity of illness is difficult and clinically useful risk factors remain unknown. Therefore, it is important to investigate alternative ways to improve early identification. The use of advanced neuroimaging techniques to parcellate specific neurobiological correlates of impaired outcome may be useful in this respect. Studies

utilizing sophisticated neuroimaging methods to study survivors of neonatal ECMO and/or CDH are scarce.[14,15] Van den Bosch et al. showed cortical thickness and global brain volumes in 8-to-15 year-old neonatal ECMO survivors to be similar to healthy controls, despite verbal memory problems in survivors.[15] These results suggest that the underlying brain injury in ECMO survivors may be more specific and/or subtle. In school-age children who experienced neonatal hypoxia, specific alterations in bilateral hippocampal volume were found compared to healthy controls, which were associated with memory deficits in patients.[9] In preterm infants, hippocampal volume measured at term-equivalent age correlated with memory outcomes both at two years and seven years of age.[16,17] These findings indicate a potential predictive value of MRI. The neurobiological alterations associated with long-term neuropsychological deficits are therefore of interest, but remain unknown in survivors of neonatal ECMO and/or CDH.

TREATMENT OF PATIENTS AT RISK

Given the increased risk of neuropsychological impairments and school failure following neonatal ECMO and/or CDH, it is essential to find ways to prevent or diminish impaired outcome. However, few such intervention strategies are available. Cognitive training programs are based on the idea that repetitive mental exercise of one cognitive task results in improved functioning that may generalize to other tasks with similar underlying skills. A widely evaluated cognitive training for children with working-memory problems is Cogmed Working-Memory Training (CWMT).[18] In children born preterm or with ADHD, studies have demonstrated near- and, although to a lesser extent, far-transfer effects after CWMT, i.e. improvements on trained and untrained cognitive functions.[19,20] As working-memory is one of the fundamental building blocks for higher cognitive functioning and highly associated with academic performance, CWMT may be beneficial for survivors of neonatal ECMO and/or CDH.[21] However, its effectiveness remains unstudied in these children.

AIMS AND OUTLINE OF THIS THESIS

Growing up after neonatal ECMO and/or CDH has long-term neurodevelopmental consequences.[10,11,13-15] Therefore, long-term follow-up is of great importance in these children. Neuropsychological follow-up after neonatal critical illness should have two main objectives: 1) (early) identification of patients at risk; 2) improving neuropsychological outcome in patients at risk. This thesis addresses these objectives (Figure 1).

Identification. The specific neuropsychological profile and its underlying neurobiology remain largely unknown – knowledge that is essential in order to improve (early) identification of patients at risk. First, the specific neuropsychological profile following neonatal ECMO and/or CDH is delineated, from infancy to school-age (chapters 2 & 3) and into adolescence (chapter 4). Secondly, the neurobiology following neonatal ECMO is compared to healthy controls using advanced neuroimaging techniques (chapter 5), and the associations between brain alterations and long-term neuropsychological deficits are investigated in survivors of neonatal ECMO and/or CDH (chapter 6). Lastly, the pathophysiology underlying the brain alterations and associated long-term neuropsychological deficits across survivors of common causes of neonatal critical illness is explored by reviewing the literature (chapters 9 & 10).

Treatment. In addition to reliable and early identification of patients at risk, there is a need for treatment modalities or intervention strategies to improve neuropsychological outcome in these children. Therefore, the effects of a cognitive training program on neuropsychological outcome (chapter 7) and brain connectivity (chapter 8) in school-age survivors of neonatal ECMO and/or CDH are studied.

Finally, the results of the studies are placed in a broader perspective and aims for future research are described (chapter 11).

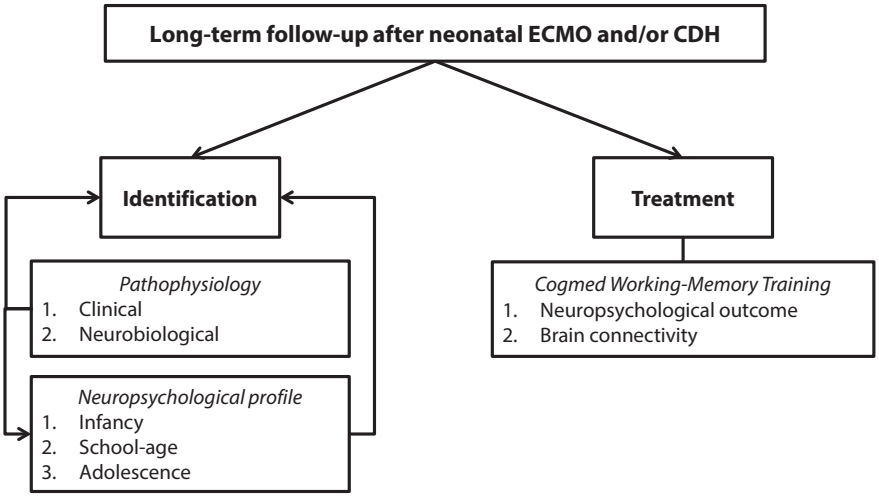


Figure 1. A schematic overview of the contents of this thesis

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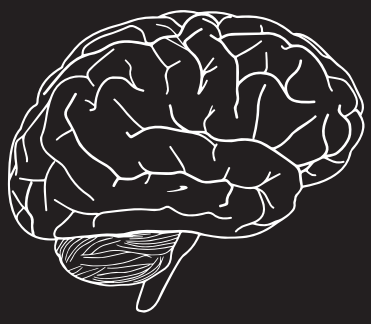
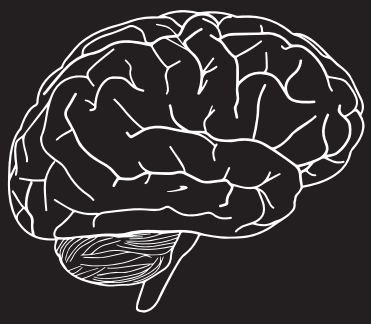


COGNITION AND THE BRAIN AFTER NEONATAL CRITICAL ILLNESS

CHAPTER 2



NEUROPSYCHOLOGICAL FOLLOW-UP AFTER NEONATAL ECMO

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ABSTRACT

Objective To assess the longitudinal development of intelligence and its relation to school performance in a nationwide cohort of neonatal ECMO survivors as well as evaluate predictors of outcome at eight years.

Methods Repeated measurements of intelligence in neonatal ECMO survivors were collected at two, five and eight years (n = 178) with validated, standardized instruments. Selective attention (n = 148) and type of education were evaluated in the eight-year-olds.

Results Intelligence was found to remain stable and average across development (mean IQ(SD) at 2 years = 102(18); at 5 years = 100(17); at 8 years = 99(17)), $p = .15$. Children attending regular education without the need for help (n = 101, mean z-score(SD) = -1.50(1.93)) performed significantly better on the selective attention task compared to those children in need of extra help (n = 65, mean z-score(SD) = -2.54(3.18)) or those attending special education (n = 13, mean z-score(SD) = -4.14(3.63)), $p = .03$. However, only children attending special education had below average intelligence (mean IQ(SD) = 76(15)), compared to average intelligence for those attending regular education, both with (mean IQ(SD) = 95(15)) and without help (mean IQ(SD) = 105(16)). Children with congenital diaphragmatic hernia scored significantly lower on both IQ (CDH mean IQ(SD) = 93(20); MAS mean IQ(SD) = 100(15); other diagnoses mean IQ(SD) = 100(19), $p = .04$) and selective attention (CDH, mean z-score(SD) = -3.48(3.46)); MAS mean z-score(SD) = -1.60(2.13); other diagnoses mean z-score(SD) = -1.65(2.39), $p = .002$) compared to other diagnoses.

Conclusion Intelligence testing alone does not identify those at risk for academic problems for the majority of neonatal ECMO survivors. We propose internationally standardized follow-up protocols that focus on long-term problem-oriented neuropsychological assessment.

INTRODUCTION

Extracorporeal membrane oxygenation (ECMO) has been used in over 28,000 neonates with severe respiratory failure who are unresponsive to conventional medical management.[1] Survival rates have remained stable over the years with 5-10% surviving with severe neurological complications.[1] The remaining 90% of survivors are at risk for subtler long-term neurodevelopmental problems.[2-4] Despite increasing awareness of these problems, the current standardized (international) follow-up protocols are inadequate for the detection of neuropsychological deficits in neonatal ECMO survivors.[1,5] As the ELSO recommendations have not been reviewed since 1997, an evidence-based update is mandatory.[6]

In many follow-up programs, intelligence remains the primary outcome measure.[5,6] Previous studies have shown intelligence to be comparable to that of healthy children at various stages of development.[2,3,7-9] IQ testing can give valuable insight into the overall cognitive functioning of an individual, but is not suited to detect subtle neuropsychological impairments.[10] Extensive neuropsychological testing in neonatal ECMO survivors has demonstrated deficits especially in the attention and (working) memory domains in 8- and 17-year-olds[2,4] with an increased need for extra help in school.[2,4,8] Since IQ is generally within the average range, the school problems are likely due to specific neuropsychological impairments. However, this remains largely speculative and IQ has not been studied longitudinally.

In this study, we aimed to investigate the relationship between school problems and cognitive outcome in neonatal ECMO survivors. To do so, we first assessed the longitudinal development of intelligence at two, five and eight years of age in a nationwide cohort of neonatal ECMO survivors. We then evaluated type of education attendance in relation to intelligence and to selective attention at eight years of age. Finally, we studied whether school performance and cognitive outcome at eight years of age were influenced by clinical characteristics. We hypothesized that intelligence is normal across the three ages and unrelated to the school problems observed in neonatal ECMO survivors. Based on this, we propose standardized, problem-oriented follow-up aimed at specific neuropsychological domains that can be internationally implemented.

METHODS

Population

Patients born between January 1996 and December 2006 treated with ECMO within the first 28 days of life and participating in the structured prospective post-ECMO follow-up program were eligible for the current study (n = 278). Children were either part of the

follow-up program that was initiated in 2001 at the Erasmus MC-Sophia Children's Hospital in Rotterdam (n = 143) or at the Radboud University Medical Centre in Nijmegen initiated in 1998 (n = 135). ECMO support was given according to the criteria described by Stolar et al[11] which did not change over time. Entry and exclusion criteria for follow-up were previously described.[2,7] The post-ECMO follow-up program is the standard of care in the Netherlands[2,7,12], therefore Institutional Review Board approval was waived. Only those children of whom at least the mental developmental index at two and IQ at eight years of age were evaluated were included (Rotterdam, n = 96, Nijmegen, n = 82) (Figure 1). Demographic and medical characteristics of the patients are reported in Table 1.

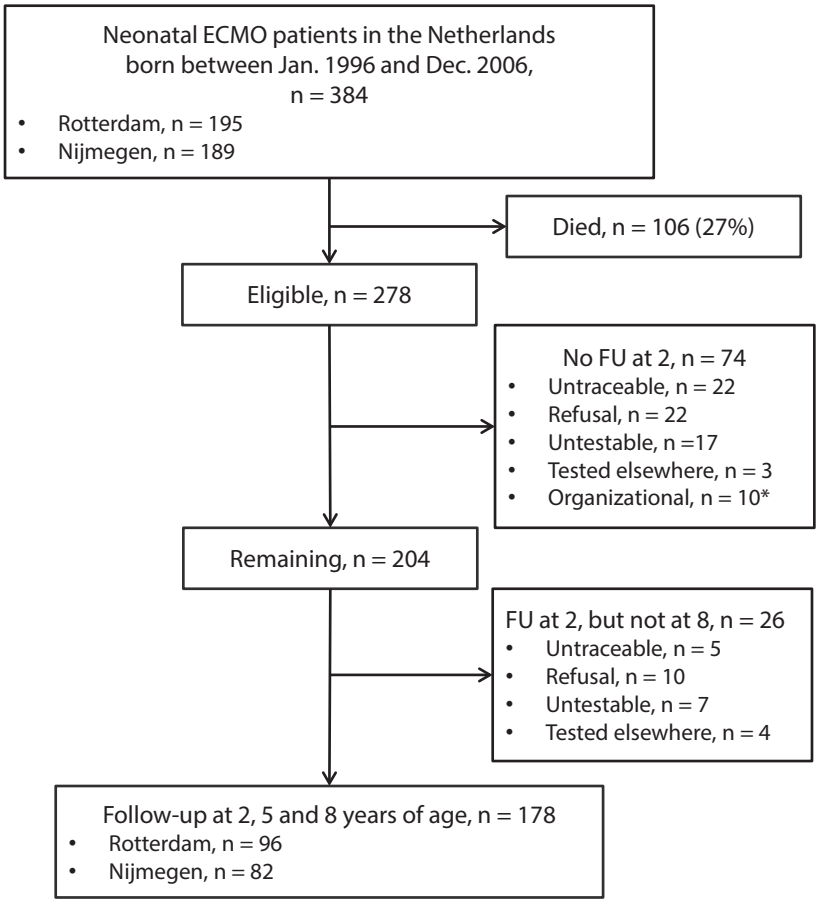

Figure 1. Inclusion flowchart of the neonatal ECMO survivors

Table 1. Patient characteristics

	All (n = 178)	MAS (n = 97)	CDH (n = 36)	Other (n = 45)
a) Demographic				
Gender				
Male	96 (54)	46 (47)	24 (67)	26 (58)
Female	82 (46)	51 (53)	12 (33)	19 (42)
Ethnicity				
Dutch	143 (81)	75 (78)	31 (86)	37 (82)
Non-Dutch	34 (19)	21 (22)	5 (14)	8 (18)
Unknown	1	1	0	0
MEL				
Low	45 (27)	21 (23)	11 (34)	13 (30)
Moderate	65 (39)	38 (42)	10 (30)	17 (40)
High	56 (34)	31 (35)	12 (36)	13 (30)
Unknown	12	7	3	2
Type of education at 8				
Regular	100 (56)	58 (60)	18 (51)	24 (53)
Regular with help	65 (37)	36 (37)	15 (43)	14 (31)
Special education	12 (7)	3 (3)	2 (6)	7 (16)
Unknown	1	0	1	0
b) Clinical				
Birthweight (grms)	3461 (552)	3512 (551)	3316 (436)	3465 (624)
Gestational age (wks)	40 (2)	41 (2)	39 (1)	39 (2)
Type of ECMO				
VA	155 (87)	77 (79)	36 (100)	42 (93)
VV	21 (12)	18 (19)	0 (0)	3 (7)
VV conversion to VA	2 (1)	2 (2)	0 (0)	0 (0)
Unknown	1	0	0	0
Age start ECMO (dys)	1 (0-23)	1 (0-10)	1 (0-11)	2 (0-23)
Hours on ECMO	140 (24-369)	135 (24-288)	177 (63-369)	138 (53-288)
Mechanical vent. (dys)	14 (3-68)	13 (6-32)	28 (7-68)	13 (3-40)
O2 post-ECMO				
1 day – 1 week	87 (53)	51 (56)	9 (30)	27 (63)
>1 week - <1 month	64 (39)	35 (39)	14 (47)	15 (35)
>1 month	13 (8)	5 (5)	7 (23)	1 (2)
Unknown	14	6	6	2
CLD presence				
Yes	39 (23)	18 (20)	16 (50)	5 (11)
No	129 (77)	74 (80)	16 (50)	39 (89)
Unknown	10	5	4	1

Table 1. Patient characteristics (continued)

	All (n = 178)	MAS (n = 97)	CDH (n = 36)	Other (n = 45)
Abnormal CUS				
Yes	17 (10)	6 (6)	2 (6)	9 (20)
No	159 (90)	91 (94)	33 (94)	35 (80)
Unknown	2	0	1	1

N (%) is reported for all demographic variables. Non-Dutch refers to children with at least one non-native Dutch parent. The mean (SD) is reported for birthweight and gestational age. The median (range) is reported for age start ECMO in days, total amount of hours on ECMO and time on mechanical ventilation in days. N (%) was reported for extra oxygen need post-ECMO, type of ECMO and CLD presence. Other diagnoses were sepsis (n = 10), persistent pulmonary hypertension of the newborn (PPHN; n = 30), pneumonia (n = 2), congenital cystic adenomatoid malformation of the lung (n = 1), pneumothorax (n = 1) and infant respiratory distress syndrome (n = 1). Abbreviations: MAS, meconium aspiration syndrome; CDH, congenital diaphragmatic hernia; MEL; maternal educational level; grms, grams; wks, weeks; dys, days; ECMO, extracorporeal membrane oxygenation; O2 post-ECMO, extra oxygen supply post-extubation; VA, venoarterial; VV, venovenous; CLD, chronic lung disease; CUS, cranial ultrasound.

Neuropsychological assessment

Intelligence

Intelligence was measured at two, five and eight years of age. For two-year-olds, the Bailey Developmental Scales (BOS 2-30) (n = 100) or, from December 2003, the Bailey Scales of Infant Development – Second Edition – Dutch version (BSID-II-NL) (n = 78) were used to assess mental outcome. These standardized instruments both assess verbal and non-verbal development of 2-to-30 month-old children and are substantially related to each other.[13]

The Revised Amsterdam Intelligence Test (RAKIT) short-form was used at five years.[14] For the eight-year-olds, the RAKIT (n = 102) or the Wechsler Intelligence Scale for children (WISC-III-NL; n = 76) was used.[15] Both tests assess verbal and non-verbal intelligence, have been shown to have good reliability and validity[14,15], and have been used interchangeably by our group before.[16]

For all four tests, a normalized population mean of 100 with a standard deviation of 15 is used.[13-15] The outcome on all four tests is referred to as intelligence or IQ.

Selective attention

Selective attention was measured in the eight-year-old children (n = 148) with the Dot Cancellation paper-and-pencil test. The main outcome measure was working-speed, which was converted into z-scores (individual score minus the population score divided by the population standard deviation). Good validity, sensitivity, reliability and Dutch normative data have been reported.[17]

Procedures and study design

Children underwent neuropsychological evaluation by a psychologist at two, five and eight years of age. Parents filled in questionnaires on ethnicity (Dutch/≥1 non-native Dutch parent) and maternal educational level (MEL; high/moderate/low). MEL refers to the highest type of education completed by the mother.[18] Various medical characteristics were recorded prospectively: birth weight, gestational age, diagnosis, age at the start of ECMO, ECMO duration/type (venoarterial (VA)/venovenous (VV)/VV conversion to VA), duration of mechanical ventilation, extra oxygen supply post extubation, chronic lung disease (CLD; yes/no)[19] and abnormal cranial ultrasound (CUS; yes (i.e. parenchymal or intracranial bleedings)/no).

Data analysis

Clinical characteristics of participants and non-participants of the follow-up program were compared using independent-samples T-tests for the normally distributed data and Mann-Whitney U tests for the non-normally distributed data.

The developmental trajectory of intelligence was evaluated using repeated-measures ANOVA. Normality tests were performed. Mauchly's test was used to assess and correct for sphericity.

Type of education attendance at eight years of age and its relations to intelligence at two, five and eight and selective attention at eight were analyzed using Kruskal-Wallis H tests. For post-hoc analyses, the three education categories were transformed into two dummy variables: 1) regular education with help versus regular education and special education and 2) special education versus regular education with and without help. Independent samples T-tests were then conducted to evaluate which groups differed.

Next, the effect of diagnosis (meconium aspiration syndrome (MAS), congenital diaphragmatic hernia (CDH), other diagnoses) on intelligence at all ages, and selective attention at eight years were evaluated using Kruskall-Wallis H tests, as previous research has shown CDH patients to perform worse compared to children with other diagnoses.[2]

Finally, associations between IQ at two years, IQ at five years and outcome at eight years of age were evaluated using multivariate linear regression analyses, adjusted for MEL and parents' ethnicity.[20-22] Parents' ethnicity was used because a child's verbal skills, and thus neurodevelopmental outcome, may be affected by a parent who was born outside of the Netherlands and does not speak Dutch as their first language.[21] The influence of medical characteristics on IQ and selective attention at eight years of age was tested in two separate models. Diagnosis, type of ECMO, duration of mechanical ventilation and CLD were added into the multivariate linear regression analyses. The assumptions for multivariate linear regression analysis were checked with normal probability plots of the residuals and the Durbin-Watson test. Multicollinearity was evaluated using the criterion that variance inflation factors could not exceed 2.5.[23]

Analyses were performed with SPSS 22.0 (IBM, Chicago, IL, USA). For all analyses, a *p*-value of < .05 was considered statistically significant.

RESULTS

Participants had a significantly higher birthweight than non-participants (mean birthweight (SD) = 3461 (552) and 3294 (556) grams respectively; $p = .02$). No other clinical differences were found between participants and non-participants.

Developmental trajectory of intelligence

Intelligence fell within the normal range at two, five and eight years of age (Figure 2).[13-15] Mauchly's test indicated that the assumption of sphericity had been violated ($p < .001$), therefore Greenhouse-Geisser corrected tests are reported ($\varepsilon = .01$). Intelligence was found to remain stable from two, to five, to eight years of age ($p = .15$, n = 152). At eight years old, six children (3%) had low IQ scores (<70), 39 children (22%) had below average IQ scores (≤85), 103 children (58%) had average IQ scores (85-115) and 30 children (17%) had an above average IQ (≥115).

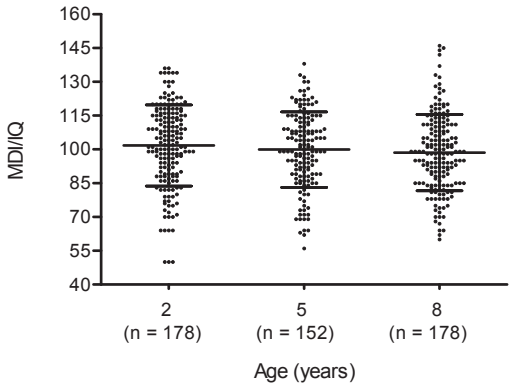

Figure 2. Longitudinal assessment of intelligence following neonatal ECMO
Mean (SD) of MDI/IQ was reported at two, five and eight years of age. The population mean IQ (SD) = 100 (15). Abbreviations: MDI, mental developmental index; IQ, intelligence quotient.

Outcome and type of education

Intelligence

Sixty-five (37%) of the ECMO survivors needed extra help at school at eight years versus 20% of children in the general population.[24] Twelve children (7%) in our cohort attended special education at eight years of age (Table 1a), compared to 4.4% in the general population.[24] To get a better understanding of the relatively high proportions of children receiving extra help and attending special education, we analyzed its relationship with intelligence. Children attending special education had significantly lower intelligence

from two years onwards, whereas those attending regular education, irrespective of their need for extra help, had comparable intelligence to the general population (Table 2).

Selective attention

The ECMO survivors who attended regular education without help performed significantly better on the selective attention task compared to those needing extra help or attending special education ($p = .02$). Selective attention did not significantly differ between the ECMO survivors attending special education and those needing extra help ($p = .75$) (Table 2).

Table 2. Outcome based on type of education attendance at eight years of age

	MDI 2 yrs (n = 178)	IQ 5 yrs (n = 152)	IQ 8 yrs (n = 178)	Selective attention 8 yrs (n = 148)
Regular education	105 (16)	106 (14)	104 (16)	-1.50 (1.93)
Regular education with help	100 (19)	95 (17)	95 (15)	-2.54 (3.18)
Special education	83 (19)*	81 (15)*	77 (15)*	-2.91 (2.21)

Mean (SD) of MDI/IQ and mean z-score (SD) of selective attention as measured by working speed are reported based type of education. Mean z-score (SD) = 0 (1). IQ population mean (SD) = 100 (15).
*Significantly different IQ score than the general population at $p < .001$.
Abbreviations: MDI, mental developmental index; yrs, years.

Diagnosis

Intelligence did not differ at two and five years of age between MAS, CDH or other diagnoses. At eight years of age, CDH patients had a significantly lower IQ than those with other diagnoses, ($p = .04$). Furthermore, significant differences were found on selective attention between the three diagnostic groups ($p = .007$), with the CDH group scoring lowest on the selective attention task (Table 3).

Table 3. Neuropsychological outcome based on diagnosis

	MDI 2 yrs	IQ 5 yrs	IQ 8 yrs	Selective attention 8 yrs
MAS	104 (18)	101 (14)	100 (15)	-1.60 (2.13)
n	*97*	*81*	*97*	*86*
CDH	98 (18)	98 (21)	93 (20)*	-3.48 (3.46)*
n	*36*	*32*	*36*	*30*
Other	99 (18)	99 (18)	100 (19)	-1.39 (1.88)
n	*45*	*39*	*45*	*32*

Mean (SD) of MDI/IQ and mean z-score (SD) of selective attention as measured by working speed are reported based on diagnosis. The IQ population mean = 100 (15). Mean z-score (SD) = 0 (1). *Significantly different compared to other diagnostic groups at $p < .05$.
Other diagnoses include persistent pulmonary hypertension of the newborn, sepsis, cardinal respiratory insufficiency, persistent fetal circulation and respiratory syncytial infection.
Abbreviations: MDI, mental developmental index; MAS, meconium aspiration syndrome; CDH, congenital diaphragmatic hernia; yrs, years.

Predictors of outcome at eight years

Low MEL increased the likelihood of having a lower IQ at eight years of age. Also, children with higher IQ scores at five years of age were more likely to have higher IQ at eight. Having a lower IQ score at five years of age increased the likelihood of a poorer score on the selective attention task at eight years of age (Table 4).

None of the medical characteristics were significantly related to outcome at eight years of age (Table 4).

Table 4. Predictors of outcome at eight years

	IQ 8 yrs	Selective attention 8 yrs
Demographic predictors		
Low MEL	***B* = -4.72, *p* = .03** **(CI -8.90 – -0.53)**	*B* = 0.04, *p* = .94 (CI -1.00 – 1.08)
Dutch ethnicity	*B* = -1.80, *p* = .45 (CI -6.51 – 2.92)	*B* = -1.10, *p* = .09 (CI -2.27 – 0.15)
MDI at two	*B* = 0.07, *p* = .22 (CI -0.04 – 0.18)	*B* = -0.01, *p* = .54 (CI -0.04 – 0.02)
IQ at five	***B* = 0.75, *p* < .001** **(CI 0.63 – 0.88)**	***B* = -0.07, *p* < .001** **(CI -0.10 – -0.04)**
Medical predictors		
CDH	*B* = -5.02, *p* = .25 (CI -13.65 – 3.62)	*B* = 1.31, *p* = .07 (CI -0.08 – 2.70)
MAS	*B* = -0.43, *p* = .89 (CI -6.70 – 5.83)	*B* = -0.01, *p* = .98 (CI -1.04 – 1.02)
VA	*B* = 2.82, *p* = .49 (CI -5.21 – 10.84)	*B* = -0.10, *p* = .87 (CI -1.27 – 1.08)
Mech. vent. (days)	*B* = -.19, *p* = .19 (CI -0.46 – 0.09)	*B* = 0.04, *p* = .06 (CI -0.00 – 0.09)

Multivariate regression analyses to assess the influence of demographic and medical characteristics on outcome at eight years of age. Selective attention is measured by working-speed given in seconds; a higher score represents slower working-speed and vice versa. MEL (high MEL versus low and moderate MEL; low MEL versus high and moderate MEL) and diagnosis (CDH versus rest; MAS versus rest) are dummy variables (yes = 1, no = 0). Ethnicity is Dutch (1) or non-Dutch (0).
A ***P*-value** of > .05 was considered statistically significant.
Abbreviations: yrs, years; MEL, maternal educational level; MDI, mental developmental index; CDH, congenital hernia diaphragmatic; MAS, meconium aspiration syndrome; VA ECMO, venoarterial extracorporeal membrane oxygenation; CLD, chronic lung disease.

DISCUSSION

This is the first longitudinal assessment of IQ in a large group of children treated with neonatal ECMO. We showed that intelligence falls within the average range at two, five and eight years of age and remains stable. This is in line with previous cross-sectional

studies.[2,3,7,8,21] Strikingly, a large group of children attending regular education needed extra help in school, despite average intelligence. We found that these school difficulties were related to selective attention problems. As current follow-up protocols focus mainly on IQ, language and visuomotor integration – domains that have been shown to remain intact following neonatal ECMO[6,7,25-27] – those ECMO survivors at risk for school problems will not be identified. Our results underline the importance of standardized, evidence-based and problem-oriented neuropsychological follow-up following neonatal ECMO.

Despite the fact that the ECMO survivors included in this study did not have severe neurological morbidity, twice as many children in our cohort needed extra help at regular education compared to the general population[24], as previously found by our group.[2] However, all had average intelligence, which did not differ from those who did not need help in school. Also, a relatively high number of ECMO survivors attended special education compared to the general population.[24] These children had below average intelligence. Interestingly, both the ECMO survivors needing extra help and the ones attending special education performed significantly worse on the selective attention task compared to the ECMO survivors not needing help in school. Our findings therefore allow us to identify two groups of neonatal ECMO survivors who (without overt neurological deficits) are at risk of long-term school problems: those with lower IQ and related neuropsychological impairments and those with average IQ but with isolated neuropsychological deficits. For children attending special education, poor selective attention is more in accordance with – and may be partly explained by – their below average intelligence. The combination of which may lead to the need for special education. However, for those children needing extra help, isolated neuropsychological deficits may cause the need for educational support.

For identification of those at risk of school problems, especially of the ECMO survivors with average intelligence, problem-oriented neuropsychological assessment that goes beyond testing global cognitive functioning with the use of an IQ test[10] is essential. Attention and (working) memory have been shown to be overlapping constructs that share much of the same pathways in the brain.[29] The attention problems observed in our cohort could therefore be accompanied by (working)memory deficits. Indeed, earlier studies have shown neuropsychological problems to lie mainly in the attention and memory domains in these children.[2-4,28] It is therefore highly recommended that, besides intelligence, both attention and memory are focused on following neonatal ECMO. Using the current guidelines, neonatal ECMO survivors at risk for school problems will not be identified.[6,7,25,27,30] We therefore propose a problem-oriented revision of follow-up protocols.

Since neuropsychological impairments in neonatal ECMO survivors have shown to emerge in childhood and to persist even into adolescence[2-4], neuropsychological

follow-up that extends beyond the age of five is crucial.[6] Neonatal follow-up of premature infants has shown early developmental assessments of high-risk infants to often be imprecise – especially for those with milder impairments that at a later age do affect their school performance.[31] It is likely that this is similar to neonatal ECMO follow-up. Moreover, neuropsychological testing beyond early childhood is important as these types of deficits at a later age may not only continue to hamper academic performance, but also affect the ability to participate in society and thus lead a fulfilling life.[4] However, as we have shown intelligence at five years of age to be highly predictive of IQ at eight, elaborate IQ assessment both at five and at eight may be redundant. To make most efficient use of time and resources, assessment of a full IQ can be considered at five years of age so that specified neuropsychological assessment can be conducted at eight years of age. At eight years, IQ can be screened with the use of a few subtests and, only if needed, a full IQ test can be administered. Such a problem-oriented approach will make risk stratification and early identification of those neonatal ECMO survivors at risk for school problems more feasible.

Finally, within follow-up of neonatal ECMO survivors, certain risk factors of impaired neurodevelopment should be taken into account. IQ at eight years old and selective attention were lower in CDH patients compared to children with other underlying diagnoses. These findings confirm earlier work demonstrating CDH patients to have generally worse outcomes.[2] Our proposal of problem-oriented and evidence-based neuropsychological follow-up therefore seems even more critical for this particular patient group. None of the other clinical characteristics studied were found to predict outcome at eight years of age, this is in line with previous findings.[2,4] Low MEL significantly increased the likelihood of lower IQ at eight years of age. Although this result is not specific to neonatal ECMO survivors[31,32], it is important to take into account during neonatal ECMO follow-up.

In this nationwide study we are the first to longitudinally assess intelligence in a large group of neonatal ECMO survivors. We have identified two specific groups of neonatal ECMO survivors who may be at risk for school problems: those with neuropsychological impairments despite having average intelligence and those with below average intelligence and neuropsychological impairments. As sources for educational support are available for all schools in the Netherlands, the number of children needing educational support or special education reported in this study are likely to be accurate. Furthermore, due to the high level of compliance, selection bias is highly unlikely. Nonetheless, our study has some limitations. First, a Dutch test measuring selective attention was used which limits cross-sectional comparisons. On the other hand, we were able to compare our data to Dutch normative data. Second, 11% of children (n = 31) did not complete the neuropsychological assessment at two and/or eight years of age due to cognitive or behavioral impairments (n = 14, seen elsewhere with severe cognitive impairment (i.e.

IQ < 70); n = 11, too tired or uncooperative at time of assessment; n = 6, tested elsewhere but had average IQ scores), which may have resulted in a bias. However, the percentage of dropouts due to severe cognitive impairment was relatively low in comparison to the total number of participants, making significant bias unlikely. Third, treatment technologies, especially the use of centrifugal pumps, a smaller priming volume and new membranes with subsequently other adherence of commonly used drugs, are constantly changing and this may affect long-term outcomes. The current findings may thus not be generalizable to patients treated in recent years. Future studies should compare outcome between patients treated in different time periods to see what the effects of technology changes are in the long-term. Finally, at the time of data collection, our neuropsychological follow-up consisted only of intelligence and attention tests. Therefore, other cognitive functions that might be susceptible to impairment following neonatal ECMO, such as memory and executive functioning[4], were not evaluated. Future studies should include these cognitive functions when assessing long-term neurodevelopment in neonatal ECMO survivors.

CONCLUSION

Neonatal ECMO survivors have an overall average and stable IQ from two, to five, to eight years of age. Despite this, a large group is at risk for school problems. In the majority of ECMO survivors at risk, these school problems were related to isolated selective attention deficits. IQ alone is therefore not a reliable predictor of school performance or even eventual participation in society. As current neonatal ECMO follow-up protocols mainly focus on IQ and language and visuomotor integration, those children at risk will not be identified. Our findings emphasize the need for evidence-based, problem-oriented neuropsychological follow-up with a focus on attention and memory functioning following neonatal ECMO. Taken into account should be risk factors such as low MEL and/or a CDH diagnosis. As neuropsychological impairments have been shown to emerge in childhood and persist into adolescence, follow-up should extend beyond five years of age.

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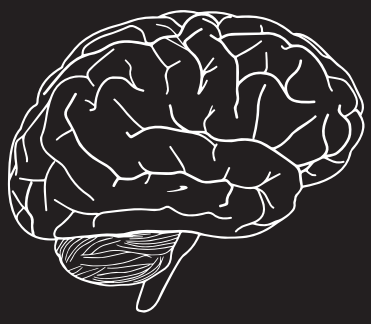
CHAPTER 3



RISK FACTORS OF IMPAIRED NEUROPSYCHOLOGIC OUTCOME IN SCHOOL-AGED SURVIVORS OF NEONATAL CRITICAL ILLNESS

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ABSTRACT

Objective Until now, long-term outcome studies have focused on general cognitive functioning and its risk factors following neonatal extracorporeal membrane oxygenation (ECMO) and/or congenital diaphragmatic hernia (CDH). However, it is currently unknown which neuropsychological domains are most affected in these patients, and which clinical variables can be used to predict specific neuropsychological problems. This study aimed to identify affected neuropsychological domains and its clinical determinants in survivors of neonatal ECMO and/or CDH.

Design Prospective follow-up study.

Setting Tertiary university hospital.

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

Interventions None.

Measurements and Main Results Intelligence, attention, memory, executive functioning and visuospatial processing were evaluated using validated tests and compared with Dutch reference data. Assessed risk factors of outcome were illness severity indicators, number of anesthetic procedures in the first year of life and growth at one year. Patients had average intelligence (mean IQ±SD: 95±16), but significantly poorer sustained attention (mean z-score±SD: —2.73±2.57), verbal (immediate: —1.09±1.27; delayed: —1.14±1.86) and visuospatial memory (immediate: —1.48±1.02; delayed: —1.57±1.01; recognition: —1.07±3.10) than the norm. ECMO-treated CDH patients had significantly lower mean IQ (84±12) than other neonatal ECMO patients (94±10) and CDH patients not treated with ECMO (100±20). Maximum vasoactive-inotropic score was negatively associated with delayed verbal (B = —0.02, 95%CI: —0.03 to —0.002, p =.026) and visuospatial memory (B = —0.01, 95%CI: —0.02 to —0.001, p = .024).

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

INTRODUCTION

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

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

In this study, neuropsychological outcome was evaluated in school-aged CDH survivors treated with or without neonatal ECMO and in neonatal ECMO-treated survivors following other diagnoses. We hypothesized that children would mainly have attention and memory deficits, despite a generally average IQ. We expected markers of increased severity of illness and hypoperfusion as well as poor growth in the first year of life to have negatively affected neuropsychological outcome at school-age.

MATERIAL AND METHODS

Population

We included CDH and neonatal ECMO patients born between January 2006 and March 2009. Participants were routinely seen at eight years of age as part of a structured prospective longitudinal follow-up program that includes regular physical and neurodevelopmental assessments until 18 years.(12) ECMO treatment was applied in case of severe respiratory

failure using the criteria described by Stolar et al.(13). Since November 2007, CDH patients were treated according to the standardized CDH-EURO Consortium treatment protocol.(14) In case of persistent poor tissue perfusion and/or hypotension (arterial blood pressure below normal levels for gestational age and not improving after fluid boluses), treatment with dobutamine and/or dopamine was initiated, followed by norepinephrine, epinephrine or milrinone in case of persistent hypotension. Exclusion criteria were: genetic syndromes known to affect neurodevelopment, severe neurologic or developmental impairments preventing standardized assessments, late CDH diagnosis (>7 days of life), or a paraesophageal hernia. We used a protocol with extended neuropsychological assessments, implemented in January 2014 (Supplemental File 1).(1,2) Included children were divided into three groups: ECMO patients following other diagnoses than CDH ("ECMO-other"), CDH patients treated with ECMO ("CDH-ECMO") and CDH patients not treated with ECMO ("CDH-non-ECMO"). This post-ECMO/CDH follow-up program is standard of care, therefore Institutional Review Board approval was waived (MEC-2017-185).(2,15)

Data collection

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

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

Neuropsychological evaluation was performed by an experienced pediatric psychologist. Socioeconomic status was assessed from maternal education level.(21)

Neurodevelopmental outcome tests

Validated neuropsychological tests were administered in their Dutch versions to assess skills in six domains (Supplemental File 1):

1 IQ:
   a. Two-subtest short-form (Block Design and Vocabulary) of the Wechsler Intelligence Scale for Children (WISC-III-NL)(22).
2 Attention:
   a. Processing speed: Trail Making Test section A (TMTA)(23,24).
   b. Selective attention and cognitive flexibility: Stroop color-word test (STROOP)(23,24) and Trail Making Test section B (TMTB)(23,24).

   c. Sustained attention: Dot Cancellation Test (DCT)(25).
3 Verbal memory:
   a. Working-memory: subtest Digit Span of the WISC-III-NL(22).
   b. Immediate and delayed recall: Rey Auditory Verbal Learning Test (RAVLT)(26).
4 Visuospatial memory:
   a. Working-memory: subtest Spatial Span of the Wechsler Nonverbal Scale of Ability (WNV)(27).
   b. Immediate and delayed recall: Rey Complex Figure Test (RCFT)(28).
5 Executive functioning:
   a. Key Search and Modified Six Elements of the Behavioral Assessment of the Dysexecutive Syndrome (BADS-C-NL)(29).
6 Visuospatial processing:
   a. Copy of the Rey Complex Figure Test (RCFT Copy)(28).

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

Statistical analysis

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

Univariable analyses were performed to assess the influence of clinical characteristics on the following neuropsychological outcomes of interest: intelligence, sustained attention, verbal memory immediate recall, verbal memory delayed recall, visuospatial memory immediate recall and visuospatial memory delayed recall. The independent variables included: maximum VIS, ECMO, type of ECMO (venoarterial or venovenous), sepsis, ventilator-free days in the first 28 days of life, duration of initial hospital stay, growth z-scores at 1 year (paired t-test showed largest growth deflection from 6 months to 1 year) and number of anesthetic procedures during the first year of life. Next, multivariable linear regression analyses were used to identify which independent variables remained significant predictors in a multivariable model. The assumptions for linear regression analysis were checked with normal probability plots of the residuals. Multicollinearity was evaluated in the multivariable models using the criterion that variance inflation factors should not exceed 2.5(30).

Analyses were performed with SPSS 21.0 (IBM, Armonk, NY, USA), a two-sided *p*-value < .05 was considered statistically significant.

RESULTS

Patient characteristics

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

Table 1. Patient characteristics

Characteristics	ECMO-other[1] (n = 25)	CDH-ECMO (n = 10)	CDH-non-ECMO (n = 30)	*p*-value
Gestational age (weeks)	40.9 (40.0-41.1)	39.2 (36.7-40.7)	38.5 (38.0-39.3)	**<0.001**
Birth weight (kilograms)	3.5 (0.5)	3.1 (0.8)	2.9 (0.4)	**0.001**
Male	14 (56%)	5 (50%)	18 (60%)	0.84[2]
Ethnicity				0.60[2]
Dutch	19 (76%)	9 (90%)	26 (87%)	
Other	6 (24%)	1 (10%)	4 (13%)	
Maternal Education Level				0.59[2]
Low	6 (24%)	3 (33%)	8 (29%)	
Moderate	12 (48%)	3 (33%)	16 (57%)	
High	7 (28%)	3 (33%)	4 (14%)	
Unknown	0	1	2	
Inborn	4 (16%)	4 (40%)	18 (60%)	**0.003[2]**
ECMO-related				
Highest oxygenation index prior to ECMO	33 (28-40)	38 (26-54)		0.72
Age start ECMO (days)	2 (1-4)	1 (1-2)		0.30

Table 1. Patient characteristics (continued)

Characteristics	ECMO-other[1] (n = 25)	CDH-ECMO (n = 10)	CDH-non-ECMO (n = 30)	*p*-value
Duration of ECMO (hours)	92 (54-100)	172 (131-212)		**<0.001**
ECMO mode				**<0.001[2]**
VA	7 (28%)	10 (100%)		
VV converted to VA	1 (4%)			
VV	17 (68%)			
CDH-related				
Left sided hernia		8 (80%)	25 (83%)	1.00[2]
Age at surgery (days)		4 (3-6)	3 (2-4)	0.32
Patch repair		9 (90%)	17 (57%)	0.07[2]
Surgical technique				
Laparotomy		10 (100%)	20 (67%)	**0.04[2]**
Thoracoscopy			10 (33%)	
Hospital Admission-related				
PELOD-2 score[3]	7 (7-9)	9 (8-9)	6 (5-7)	**<0.001**
Maximum VIS[4]	40 (35-70)	107 (91-142)	40 (5-76)	**<0.001**
Dobutamine treatment	24 (96%)	10 (100%)	19 (63%)	**0.04[2]**
Dopamine treatment	22 (88%)	7 (70%)	13 (43%)	**0.01[2]**
Norepinephrine treatment	13 (52%)	10 (100%)	15 (50%)	**0.02[2]**
Epinephrine treatment	2 (8%)	-	2 (7%)	1.00[2]
Milrinone treatment	-	2 (20%)	-	**0.03[2]**
Vasopressin treatment	-	-	-	-
CPR during initial hospital stay	2 (8%)	1 (10%)	0 (0%)	0.18[2]
Sepsis during initial hospital stay[5]	3 (12%)	4 (40%)	1 (3%)	**0.01[2]**
Days of mechanical ventilation	10 (7-11)	40 (16-51)	10 (5-18)	**0.001**
Ventilator-free days in the first 28 days of life	18 (17-21)	0 (0-12)	19 (10-23)	**0.001**
Days of initial PICU stay	13 (10-16)	70 (24-101)	21 (12-35)	**<0.001**
Days of initial hospital stay	24 (21-29)	91 (48-156)	36 (20-53)	**0.004**
Pulmonary hypertension				
Yes	13 (57%)	8 (80%)	12 (48%)	0.25[2]
No	10 (43%)	2 (20%)	13 (52%)	
Missing	2	0	5	
Inhaled nitric oxide treatment	22 (88%)	10 (100%)	10 (33%)	**<0.001[2]**
Sildenafil treatment	2 (8%)	6 (60%)	1 (3%)	**<0.001[2]**
Chronic lung disease[6]				
Yes	2 (10%)	8 (80%)	8 (28%)	**<0.001[2]**
No	19 (90%)	2 (20%)	21 (72%)	
Missing	4		1	
Follow-up				

Table 1. Patient characteristics (continued)

Characteristics	ECMO-other[1] (n = 25)	CDH-ECMO (n = 10)	CDH-non-ECMO (n = 30)	*p*-value
Number of anesthetic procedures first year of life	2 (2-3)	4 (3-4)	1 (1-2)	**<0.001**
Weight-for-height z-score at 1 year	-0.38 (0.86)	-1.80 (0.76)	-0.88 (0.97)	**0.001**
Height-corrected-for-target height z-score at 1 year	-0.27 (0.96)	-0.36 (0.69)	-0.43 (0.78)	**0.02**
Head circumference-for-age z-score at 1 year	-0.47 (1.16)	-0.50 (1.31)	-0.39 (1.15)	0.96

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

P-value = significant difference between the groups.

[1] ECMO treatment was given in case of meconium aspiration syndrome (n = 18), persistent pulmonary hypertension of the newborn (n = 2), congenital heart disease (monoventricular heart with transposition of the great vessels and total anomalous pulmonary venous return) (n = 1), sepsis (n = 1), respiratory insufficiency due to respiratory syncytial virus (n = 1), infant respiratory distress syndrome with bilateral pneumothorax (n = 1), pulmonary hypoplasia due to bilateral hydrothorax (n = 1).

[2] Fisher's exact test was used.

[3] PELOD-2 score in the first 24 hours of PICU stay or up to ECMO cannulation if ECMO was initiated in the first 24 hours of PICU stay was calculated.[16]

[4] The maximum VIS recorded during PICU stay up until ECMO cannulation for the ECMO-treated patients or up until hernia repair for the CDH non-ECMO patients.[17] VIS was maximal at the median age of 1 (1-1) day in the ECMO patients, at the median age of 1 (0-1) day in the CDH ECMO patients, and at the median age of 0 (0-1) days in the CDH non-ECMO patients.

[5] Sepsis during initial hospital stay (clinical suspicion of sepsis with positive blood culture). ECMO-other: 2 patients had sepsis during ECMO; 1 patients after ECMO; maximum VIS during sepsis: 10 (5-15). CDH-ECMO: 4 patients had sepsis after ECMO; maximum VIS during sepsis: 2.5 (0-32.5). CDH non-ECMO: 1 patient had sepsis after hernia repair; maximum VIS during sepsis: 0.

[6] Chronic lung disease defined as oxygen dependency at 28 days of life.(18)

Abbreviations: CDH = congenital diaphragmatic hernia; CPR = cardiopulmonary resuscitation; ECMO = extracorporeal membrane oxygenation; PELOD-2 = Pediatric Logistic Organ Dysfunction-2; PICU=pediatric intensive care unit; VA = venoarterial; VIS = vasoactive-inotropic score; VV = venovenous.

Neuropsychological outcome

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

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

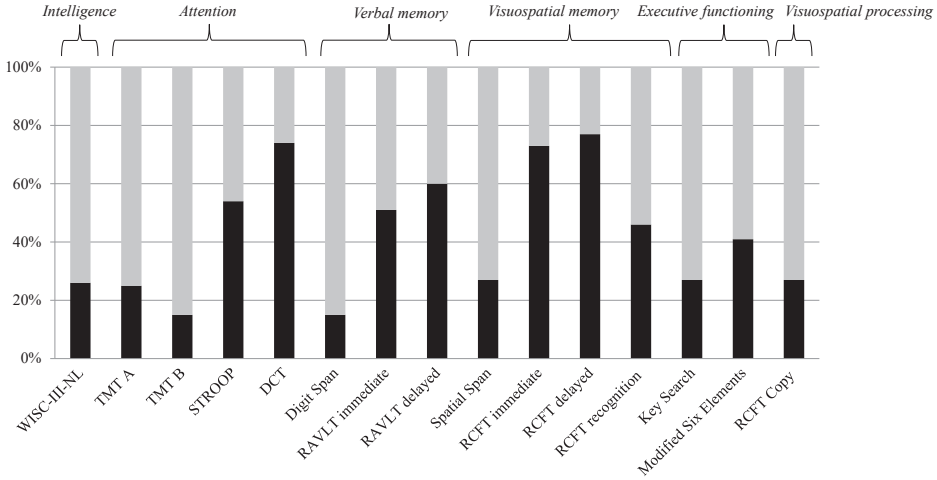

Figure 1. Presence of neuropsychological impairments in the study population
Percentage of patients with a z-score ≤ -1 (impaired; as shown by the dark colored bars) and > -1 (normal; as shown by the grey colored bars) on each of the neuropsychological tests.
Abbreviations: WISC-III-NL = Wechsler Intelligence Scale for Children, Dutch version; TMT = Trail Making Test; Stroop = Stroop Color Word Test; DCT = Dot Cancellation Test; RAVLT = Rey Auditory Verbal Learning Test; RCFT = Rey Complex Figure Test.
Please refer to the Supplemental File for a description of the tests.

Predictors of neuropsychological outcome

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

In the multivariable analyses, a higher maximum VIS remained associated with worse verbal and visuospatial memory delayed recall, such that an increase in maximum VIS of 20 points would result in a decrease of the verbal and visuospatial memory delayed recall z-scores of 0.4 and 0.2, respectively. Patients with impaired verbal memory had a significantly higher maximum VIS (72±44) than patients with normal verbal memory (40±32), $p = .003$. Patients with impaired visuospatial memory had a higher maximum VIS (64±45) than patients with normal visuospatial memory (44±33), although this was not significant ($p = .118$). The VIS remained a significant predictor of verbal and visuospatial memory delayed recall when group (ECMO-other, CDH-ECMO, CDH-non-ECMO) was added to the model (data not shown).

Growth or other illness severity indicators were no longer associated with neuropsychological outcome in the multivariable analyses (Supplemental Table 1).

Table 2. Overview of neuropsychological outcome

Neuropsychological test	All (n = 65)	ECMO-other (n = 25)[1]	CDH-ECMO (n = 10)	CDH-non-ECMO (n = 30)	*p*-value
Intelligence					
WISC-III-NL	95 (16)	94 (10)	84 (12)	100 (20)	**.029**
Attention					
TMT A	-0.33 (0.86)	-0.25 (1.05)	-0.45 (0.53)	-0.36 (0.79)	.963
TMT B	-0.18 (0.98)	0.08 (1.03)	-0.28 (1.08)	-0.37 (0.88)	.267
STROOP	-0.61 (1.01)	-1.00 (0.76)	-0.29 (1.11)	-0.39 (1.10)	.081
DCT	-2.73 (2.57)	-2.88 (2.09)	-3.88 (2.91)	-2.25 (2.76)	.173
Verbal memory					
WISC-III-NL Digit span	0.06 (1.09)	-0.08 (1.15)	-0.34 (0.94)	0.31 (1.07)	.706
RAVLT immediate	-1.09 (1.27)	-1.26 (1.24)	-1.55 (1.03)	-0.79 (1.33)	.664
RAVLT delayed	-1.14 (1.86)	-1.38 (1.46)	-1.87 (1.16)	-0.70 (2.24)	.117
Visuospatial memory					
WNV Spatial Span	-0.31 (0.99)	-0.39 (0.79)	-0.85 (0.76)	-0.06 (1.14)	.613
RCFT immediate	-1.48 (1.02)	-1.52 (1.02)	-1.86 (0.69)	-1.31 (1.09)	.417
RCFT delayed	-1.57 (1.01)	-1.56 (1.01)	-1.89 (0.77)	-1.47 (1.09)	.689
RCFT recognition	-1.07 (3.10)	-1.09 (1.51)	-0.62 (0.95)	-0.47 (1.22)	.117
Executive functioning					
Key Search	-0.12 (0.94)	-0.26 (0.98)	-0.04 (1.09)	-0.05 (0.89)	.694
Modified Six Elements	-0.60 (0.87)	-0.95 (0.90)	-0.46 (0.47)	-0.46 (0.92)	.194
Visual spatial processing					
RCFT copy	-0.26 (1.02)	-0.36 (1.00)	-0.69 (1.02)	-0.02 (1.01)	.107

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

DISCUSSION

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

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

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

Variables	Intelligence	Sustained attention	Verbal memory immediate	Verbal memory delayed	Visuospatial memory immediate	Visuospatial memory delayed
Univariable analyses with medical predictors						
CDH-non-ECMO[1]	**B = 9.84, p = .031 (CI 0.96 – 18.72)**	**B = 1.73, p = .016 (CI 0.34 – 3.12)**	B = 0.70, p = .068 (CI -0.05 – 1.46)	B = 0.86, p = .145 (CI -0.31 – 2.02)	B = 0.31, p = .257 (CI -0.23 – 0.86)	B = 0.30, p = .302 (CI -0.28 – 0.88)
CDH-ECMO[2]	**B = -14.11, p = .016 (CI -25.48 – -2.74)**	B = -1.84, p = .060 (CI -0.08 – 3.76)	B = -0.73, p = .144 (CI -1.73 – 0.26)	B = -0.97, p = .204 (CI -2.48 – 0.54)	B = -0.45, p = .205 (CI -1.16 – 0.26)	B = -0.37, p = .328 (CI -1.12 – 0.38)
ECMO-other[3]	B = -1.59, p = .745 (CI -11.37 – 8.19)	B = -0.77, p = .312 (CI -2.30 – 0.75)	B = -0.29, p = .475 (CI -1.11 – 0.53)	B = -0.31, p = .622 (CI -1.55 – 0.94)	B = -0.48, p = .868 (CI -0.63 – 0.53)	B = -0.09, p = .772 (CI -0.70 – 0.53)
VA-ECMO[4]	**B = -12.59, p = .002 (CI -19.97 – -5.20)**	B = -0.42, p = .670 (CI -2.46 – 1.61)	B = -0.38, p = .457 (CI -1.41 – 0.66)	B = -0.25, p = .677 (CI -1.47 – 0.97)	B = -0.23, p = .530 (CI -0.97 – 0.51)	B = -0.26, p = .499 (CI -1.02 – 0.51)
Sepsis[5]	B = -12.36, p = .064 (CI -25.46 – 0.74)	B = -0.86, p = .451 (CI -1.42 – 3.15)	B = -0.52, p = .363 (CI -1.65 – 0.62)	B = -0.93, p = .281 (CI -2.63 – 0.78)	B = -0.23, p = .565 (CI -1.04 – 0.57)	B = -0.25, p = .556 (CI -1.10 – 0.60)
VIS[6]	B = -0.08, p = .105 (CI -0.17 – 0.02)	B = -0.01, p = .217 (CI 0.01 – -0.03)	**B = -0.01, p = .014 (CI -0.02 – -0.002)**	**B = -0.02, p = .004 (CI -0.03 – -0.01)**	**B = -0.01, p = .028 (CI -0.01 – -0.001)**	**B = -0.01, p = .006 (CI -0.01 – -0.002)**
Ventilator-free days[7]	**B = 0.70, p = .006 (CI 0.22 – 1.19)**	B = 0.07, p = .098 (CI 0.16 – -0.01)	**B = 0.04, p = .035 (CI 0.00 – 0.09)**	**B = 0.08, p = .025 (CI 0.01 – 0.14)**	**B = 0.03, p = .040 (CI 0.00 – 0.06)**	**B = 0.03, p = .043 (CI 0.01 – 0.07)**
Initial hospital stay (days)	**B = -0.16, p = .004 (CI -0.27 – -0.06)**	B = -0.01, p = .133 (CI 0.00 – -0.03)	**B = -0.01, p = .045 (CI -0.02 – 0.000)**	B = -0.01, p = .133 (CI -0.03 – 0.004)	B = -0.00, p = .281 (CI -0.01 – 0.003)	B = -0.00, p = .347 (CI -0.01 – 0.004)
Anesthetics[8]	**B = -2.92, p = .027 (CI -5.49 – -0.35)**	**B = -0.44, p = .036 (CI -0.03 – -0.85)**	B = -0.15, p = .179 (CI -0.37 – 0.72)	B = -0.23, p = .175 (CI -0.57 – 0.11)	B = -0.04, p = .615 (CI -0.20 – 0.12)	B = -0.04, p = .626 (CI -0.21 – 0.13)
Univariable analyses with growth predictors						
Weight-for-height z-score at 1 year	**B = 6.41, p = .002 (CI 2.48 – 10.33)**	B = 0.42, p = .275 (CI -0.35 – 1.19)	B = 0.35, p = .053 (CI -0.01 – 0.69)	B = 0.29, p = .323 (CI -0.30 – 0.89)	B = 0.23, p = .087 (CI -0.03 – 0.49)	B = 0.22, p = .112 (CI -0.05 – 0.50)
Height-corrected-for-target height z-score at 1 year	B = 0.54, p = .835 (CI -4.70 – 5.79)	B = 0.21, p = .639 (CI 0.68 – 1.10)	B = 0.28, p = .203 (CI -0.16 – 0.71)	B = 0.38, p = .287 (CI -0.33 – 1.09)	B = -0.01, p = .936 (CI -0.34 – 0.31)	B = -0.05, p = .766 (CI -0.39 – 0.29)

Table 3. Patient characteristics and neuropsychological outcome at eight years of age (continued)

Variables	Intelligence	Sustained attention	Verbal memory immediate	Verbal memory delayed	Visuospatial memory immediate	Visuospatial memory delayed
Head circumference-for-age z-score at 1 year	*B* = 3.98, *p* = .060 (CI -0.18 – 8.13)	*B* = 0.27, *p* = .413 (CI -0.39 – 0.94)	*B* = 0.21, *p* = .237 (CI -0.14 – 0.55)	*B* = 0.23, *p* = .445 (CI -0.37 – 0.83)	*B* = 0.13, *p* = .351 (CI -0.14 – 0.40)	*B* = 0.11, *p* = .423 (CI -0.17 – 0.39)
Multivariable analyses						
VIS				***B* = -0.02, *p* = .026 (CI -0.03 – -0.002)**		***B* = -0.01, *p* = .024 (CI -0.02 – -0.001)**

Maternal education level, ethnicity and gender were adjusted for in all models. Growth parameters were z-scores. Variables found to be significant predictors in the univariable analyses were added into the multivariable model. Only those variables found to be significant in the multivariable analyses are reported. Results indicate significant associations at *p-value* < .05.

[1] CDH -non-ECMO patients were compared to patients treated with ECMO.

[2] CDH-ECMO patients were compared to patients treated with ECMO following other diagnoses and to CDH patients not treated with ECMO.

[3] ECMO patients following other diagnosis were compared to CDH patients treated with and without ECMO.

[4] Patients treated with VA-ECMO were compared to patients treated with VV-ECMO.

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

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

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

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

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

the need for and timing of ECMO treatment in neonates with severe respiratory failure, which should be investigated in future studies. The VIS has been validated to predict clinical outcomes in infants who require cardiac surgery(17,34), representing another group of critically ill children requiring circulatory support by vasopressor drugs. Future studies are needed to further validate the usefulness of this score for long-term outcome in neonatal critical illness survivors, and to study the direct association between maximum VIS and brain areas susceptible to ischemic-reperfusion injury such as the hippocampus.

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

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

Our study has some limitations. First, the relatively small sample sizes of the three diagnostic groups is a frequent problem in follow-up studies including patients with rare diagnoses, limiting the interpretability of our regression analyses. Multicenter collaborations with standardized management and structured follow-up are important to increase sample sizes and get a better understanding of the pathophysiology underlying long-term outcome. Second, Magenetic Resonance Imaging data were not available and we therefore could not examine whether maximum VIS was associated with brain structures susceptible to cerebral hypoperfusion. Standardized neuroimaging studies

both at neonatal and school-age will aid in understanding pathophysiologic concepts of early brain development and long-term outcome, and are therefore important in future studies. Third, there are likely multiple factors involved in the development of long-term neuropsychological impairments following ECMO and/or CDH, such as exposure to inflammation(7), anesthetics(39), and stress(40) in early life, and/or a complex interplay amongst these factors. As of now, techniques to reliably measure these mechanisms and their interactions are lacking. Future studies are needed to develop specific brain monitoring techniques that can be used during PICU stay for early identification of patients at risk of long-term impairments.

CONCLUSION

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

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SUPPLEMENTARY MATERIAL

Supplemental methods. Description of data collection.

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

Supplemental File 1. Descriptions of the neurodevelopmental tests.

Intelligence

Wechsler Intelligence Scale for Children (WISC-III-NL)

The RAKIT or the Wechsler Intelligence Scale for children was used. Both tests assess verbal and non-verbal intelligence, have been shown to have good reliability and validity(1, 2), and have been used interchangeably by our group before.(3) For both tests, a normalized population mean of 100 with a standard deviation of 15 is used.(1, 2)

Attention

Dot Cancellation Test

This paper-and-pencil test measures sustained selective attention and concentration in terms of speed and accuracy. It consists of a paper on which figures made of three, four or five dots are displayed in 33 rows. The child is instructed to cross off all figures with four dots, as precise and as fast as they can.(4)

Stroop Color Word Test (STROOP)

The Stroop consists of three trials: in the first trial (Stroop 1) the subject must read color names, in the second trial (Stroop 2) name printed colors, and in the third trial (Stroop 3) name printed colors not denoted by the color name. The test can be administered to children and adults in the age range 8-65 years. Selective attention is measured with this test.(5, 6)

Trail Making Test (TMT)

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

Verbal memory

WISC-III-NL – subtest Digit Span

The Digit Span consists of random number sequences that increase in length and that the examiner reads aloud at the rate of 1 number per second. The child has to reproduce these numbers in the same order. Next, the sequences must be recalled backwards (3-5-7 becomes 7-5-3). The first part of the test measures short-term auditory memory and short-term retention capacity. The second part measures auditory working memory. A

difference of 4 or more points between forward and backward Digit Span in favor of forward is indicative of a working-memory problem.(7)

Rey Auditory Verbal Learning Test (RAVLT)

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

Visuospatial memory

Wechsler Nonverbal Scale of Ability (WNV) – subtest Spatial Span

The Spatial Span requires the child to touch a group of blocks arranged on a board in a nonsystematic manner in the same and reverse order as demonstrated by the examiner. The first part of the test measures short-term visuospatial memory and short-term retention capacity. The second part measures visuospatial working-memory.(10)

Rey Complex Figure Test (RCFT)

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

Executive functioning

Key Search

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

Modified Six Elements

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

Visual spatial processing

Rey Complex Figure Test (RCFT)

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

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Supplemental Table 1. Patient characteristics and neuropsychological outcome at eight years of age

Variables	Intelligence	Sustained attention	Verbal memory immediate	Verbal memory delayed	Visuospatial memory immediate	Visuospatial memory delayed
Univariable analyses with medical predictors						
CDH-non-ECMO[1]	**B = 9.84, p = .031 (CI 0.96 – 18.72)**	**B = 1.73, p = .016 (CI 0.34 – 3.12)**	B = 0.70, p = .068 (CI -0.05 – 1.46)	B = 0.86, p = .145 (CI -0.31 – 2.02)	B = 0.31, p = .257 (CI -0.23 – 0.86)	B = 0.30, p = .302 (CI -0.28 – 0.88)
CDH-ECMO[2]	**B = -14.11, p = .016 (CI -25.48 – -2.74)**	B = -1.84, p = .060 (CI -0.08 – 3.76)	B = -0.73, p = .144 (CI -1.73 – 0.26)	B = -0.97, p = .204 (CI -2.48 – 0.54)	B = -0.45, p = .205 (CI -1.16 – 0.26)	B = -0.37, p = .328 (CI -1.12 – 0.38)
ECMO-other[3]	B = -1.59, p = .745 (CI -11.37 – 8.19)	B = -0.77, p = .312 (CI -2.30 – 0.75)	B = -0.29, p = .475 (CI -1.11 – 0.53)	B = -0.31, p = .622 (CI -1.55 – 0.94)	B = -0.48, p = .868 (CI -0.63 – 0.53)	B = -0.09, p = .772 (CI -0.70 – 0.53)
VA-ECMO[4]	**B = -12.59, p = .002 (CI -19.97 – -5.20)**	B = -0.42, p = .670 (CI -2.46 – 1.61)	B = -0.38, p = .457 (CI -1.41 – 0.66)	B = -0.25, p = .677 (CI -1.47 – 0.97)	B = -0.23, p = .530 (CI -0.97 – 0.51)	B = -0.26, p = .499 (CI -1.02 – 0.51)
Sepsis[5]	B = -12.36, p = .064 (CI -25.46 – 0.74)	B = -0.86, p = .451 (CI -1.42 – 3.15)	B = -0.52, p = .363 (CI -1.65 – 0.62)	B = -0.93, p = .281 (CI -2.63 – 0.78)	B = -0.23, p = .565 (CI -1.04 – 0.57)	B = -0.25, p = .556 (CI -1.10 – 0.60)
VIS[6]	B = -0.08, p = .105 (CI -0.17 – 0.02)	B = -0.01, p = .217 (CI 0.01 – -0.03)	**B = -0.01, p = .014 (CI -0.02 – -0.002)**	**B = -0.02, p = .004 (CI -0.03 – -0.01)**	**B = -0.01, p = .028 (CI -0.01 – -0.001)**	**B = -0.01, p = .006 (CI -0.01 – -0.002)**
Ventilator-free days[7]	**B = 0.70, p = .006 (CI 0.22 – 1.19)**	B = 0.07, p = .098 (CI 0.16 – -0.01)	**B = 0.04, p = .035 (CI 0.00 – 0.09)**	**B = 0.08, p = .025 (CI 0.01 – 0.14)**	**B = 0.03, p = .040 (CI 0.00 – 0.06)**	**B = 0.03, p = .043 (CI 0.01 – 0.07)**
Initial hospital stay (days)	**B = -0.16, p = .004 (CI -0.27 – -0.06)**	B = -0.01, p = .133 (CI 0.00 – -0.03)	**B = -0.01, p = .045 (CI -0.02 – 0.000)**	B = -0.01, p = .133 (CI -0.03 – 0.004)	B = -0.00, p = .281 (CI -0.01 – 0.003)	B = -0.00, p = .347 (CI -0.01 – 0.004)
Anesthetics[8]	**B = -2.92, p = .027 (CI -5.49 – -0.35)**	**B = -0.44, p = .036 (CI -0.03 – -0.85)**	B = -0.15, p = .179 (CI -0.37 – 0.72)	B = -0.23, p = .175 (CI -0.57 – 0.11)	B = -0.04, p = .615 (CI -0.20 – 0.12)	B = -0.04, p = .626 (CI -0.21 – 0.13)
Univariable analyses with growth predictors						
Weight-for-height z-score at 1 year	**B = 6.41, p = .002 (CI 2.48 – 10.33)**	B = 0.42, p = .275 (CI -0.35 – 1.19)	B = 0.35, p = .053 (CI -0.01 – 0.69)	B = 0.29, p = .323 (CI -0.30 – 0.89)	B = 0.23, p = .087 (CI -0.03 – 0.49)	B = 0.22, p = .112 (CI -0.05 – 0.50)
Height-corrected-for-target height z-score at 1 year	B = 0.54, p = .835 (CI -4.70 – 5.79)	B = 0.21, p = .639 (CI 0.68 – 1.10)	B = 0.28, p = .203 (CI -0.16 – 0.71)	B = 0.38, p = .287 (CI -0.33 – 1.09)	B = -0.01, p = .936 (CI -0.34 – 0.31)	B = -0.05, p = .766 (CI -0.39 – 0.29)
Head circumference-for-age z-score at 1 year	B = 3.98, p = .060 (CI -0.18 – 8.13)	B = 0.27, p = .413 (CI -0.39 – 0.94)	B = 0.21, p = .237 (CI -0.14 – 0.55)	B = 0.23, p = .445 (CI -0.37 – 0.83)	B = 0.13, p = .351 (CI -0.14 – 0.40)	B = 0.11, p = .423 (CI -0.17 – 0.39)
Multivariable analyses						
CDH-non-ECMO[1]	B = 7.33, p = .213 (CI -4.42 – 19.07)	B = 1.29, p = .110 (CI -2.90 – 0.31)				

Supplemental Table 1. Patient characteristics and neuropsychological outcome at eight years of age (continued)

Variables	Intelligence	Sustained attention	Verbal memory immediate	Verbal memory delayed	Visuospatial memory immediate	Visuospatial memory delayed
CDH-ECMO[2]	*B* = -1.66, *p* = .851 (CI -19.51 – 16.18)					
VA-ECMO[3]	*B* = -8.03, *p* = .131 (CI -18.83 – 2.76)					
VIS[6]			*B* = -0.01, *p* = .055 (CI -0.02 – -0.000)	***B* = -0.02, *p* = .026 (CI -0.03 – -0.002)**	*B* = -0.01, *p* = .084 (CI -0.01 – -0.001)	***B* = -0.01, *p* = .024 (CI -0.02 – -0.001)**
Ventilator-free days[7]	*B* = -0.28, *p* = .571 (CI -1.29 – 0.72)		*B* = 0.02, *p* = .580 (CI -0.06 – 0.10)	*B* = 0.02, *p* = .555 (CI -0.06 – 0.10)	*B* = 0.01, *p* = .587 (CI -0.03 – 0.05)	*B* = 0.00, *p* = .813 (CI -0.03 – 0.04)
Initial hospital stay (days)	*B* = -0.14, *p* = .225 (CI -0.36 – 0.09)		*B* = 0.00, *p* = .784 (CI -0.01 – 0.02)			
Anesthetics[8]	*B* = 0.40, *p* = .831 (CI -3.36 – 4.15)	*B* = 0.25, *p* = .285 (CI -0.22 – 0.72)				
Weight-for-height z-score at 1 year	*B* = 3.85, *p* = .180 (CI -1.88 – 9.58)					

Maternal education level, ethnicity and gender were adjusted for in all models. Growth parameters were z-scores. Variables found to be significant predictors in the univariable analyses were added into the multivariable model. Only those variables found to be significant in the multivariable analyses are reported. **Results** indicate significant associations at *p-value* < .05.

[1] CDH -non-ECMO patients were compared to patients treated with ECMO.

[2] CDH-ECMO patients were compared to patients treated with ECMO following other diagnoses and to CDH patients not treated with ECMO.

[3] ECMO patients following other diagnosis were compared to CDH patients treated with and without ECMO.

[4] Patients treated with VA-ECMO were compared to patients treated with VV-ECMO.

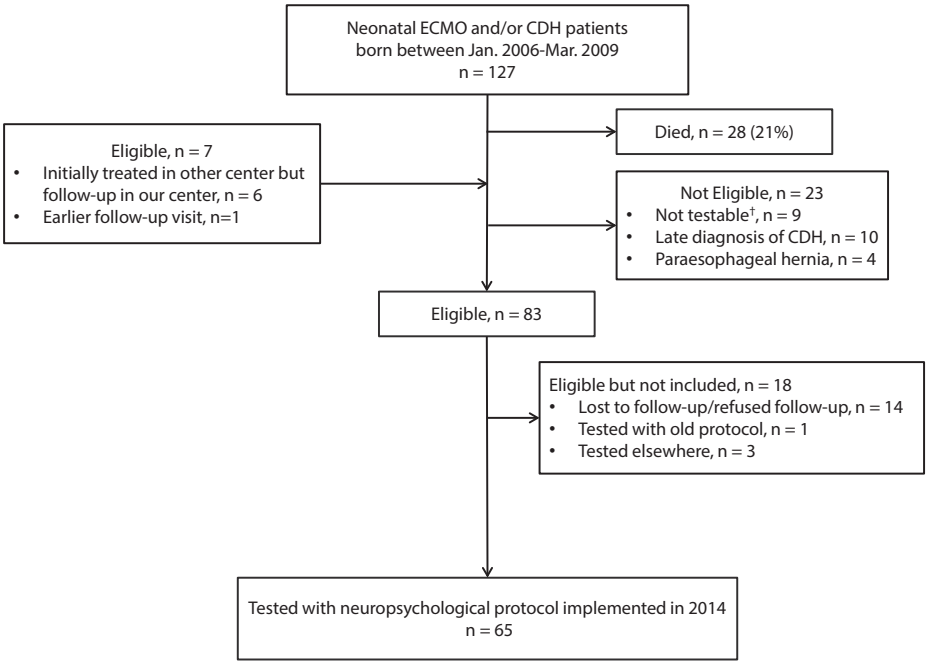
[5] Sepsis during initial hospital stay (clinical suspicion of sepsis with positive blood culture).

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

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

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

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



Supplemental Figure 1. Flowchart of the study population

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

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

CHAPTER 4

GROWING UP AFTER CRITICAL ILLNESS: VERBAL, VISUAL-SPATIAL AND WORKING MEMORY PROBLEMS IN NEONATAL ECMO SURVIVORS

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ABSTRACT

Objectives To assess neuropsychological outcome in 17- and 18-year–old neonatal extracorporeal membrane oxygenation survivors.

Design A prospective longitudinal follow-up study.

Setting Follow-up program at the Erasmus MC-Sophia Children's Hospital in Rotterdam, The Netherlands.

Patients Thirty adolescents 17 or 18 years old, treated between 1991 and 1997, underwent neuropsychological assessment.

Interventions None.

Measurements and Main Results Attention, memory, executive functioning, visual-spatial functions, social-emotional functioning, and behavior were assessed with validated instruments, and data were compared with reference data. Included predictors for analysis of adverse outcome were diagnosis, age at start extracorporeal membrane oxygenation, convulsions, and use of anti-epileptics. Adolescents' performance (expressed as mean [SD] z-score) was significantly lower than the norm on short-term and long-term verbal memory (z-score = -1.40 [1.58], p = .016; z-score = -1.54 [1.67], p = .010, respectively), visual-spatial memory (z-score = -1.65 [1.37], p = .008; z-score = -1.70 [1.23], p = .008, respectively), and working memory (32% vs 9% in the norm population). Parents reported more problems for their children regarding organization of materials (z-score = −0.60 [0.90]; p = .03) and behavior evaluation (z-score = −0.53 [0.88]; p = .05) on a questionnaire. Patients reported more withdrawn/depressed behavior (z-score = −0.47 [0.54]; p = .02), somatic complaints (z-score = −0.43 [0.48]; p = .03), and social problems (z-score = −0.41 [0.46]; p = .04). Patients reported more positive feelings of self-esteem and an average health status.

Conclusions Adolescents treated with neonatal extracorporeal membrane oxygenation are at risk of verbal, visual-spatial, and working-memory problems. Future research should focus on 1) the longitudinal assessment of specific neuropsychological skills in adolescence and adulthood; 2) identifying risk factors of neuropsychological dysfunction; 3) evaluating to what extent "severity of illness" is responsible for acquired brain injury; and 4) effects of timely cognitive rehabilitation.

INTRODUCTION

Neonatal extracorporeal membrane oxygenation (ECMO) stabilizes and supports critically ill newborns with acute and potentially reversible, respiratory failure.(1) Worldwide, approximately 28,000 neonates have been treated with ECMO for this reason, with 75% surviving to discharge or transfer.(2) The most common underlying conditions were meconium aspiration syndrome (MAS), congenital diaphragmatic hernia (CDH), and persistent pulmonary hypertension of the newborn.(2) The best results were obtained in MAS patients (94% survival) and the worst in CDH patients (51%).(2)

Survivors are at risk of serious complications, such as intra-cranial hemorrhage and infarctions.(3) Internationally, routine neuroimaging during ECMO treatment showed abnormalities in 10–59% of infants, depending on case mix and case selection.(3) In the Netherlands, the prevalence of brain injury during ECMO treatment was 17.3% nationwide.(4) These findings alone are sufficient reasons for early identification of survivors at risk of adverse neurodevelopmental outcome and close monitoring in the long-term. Moreover, critical illness, ECMO-treatment itself, and post-ECMO treatment factors may have consequences for outcomes in general.

Various studies have reported neuropsychological outcome of neonatal ECMO survivors until school age, showing that intellectual outcome did not differ from that of norm populations but that these children had deficits in attention, memory, and visual-spatial functions and more frequently had special educational needs.(5–9) Neonatal ECMO survivors might be at risk of neuropsychological problems at older age because of the poor development of executive functioning, such as working-memory and planning. Executive functioning is needed to develop academic, behavioral, and social functioning and prepare for effective participation in society (e.g., finding a job). As these neuropsychological functions start to develop in early childhood but continue into young adulthood, these children may be at risk of "growing into deficits".(10) The aim of this study was to evaluate the neuropsychological outcome at adolescent age.

MATERIALS AND METHODS

Population

Data were obtained within the framework of a post-ECMO follow-up program – initiated in 2001 in our hospital – in which children's lung function, growth, and development are regularly assessed until 18 years old.(11,12) This study concerned 17- to 18-year–old adolescents who between February 1991 and June 1997 had been treated with neonatal venoarterial ECMO at the ICU of the Erasmus MC-Sophia Children's Hospital in Rotterdam. In all cases, the cannula had been placed by the same surgical team in the right

cervical region. ECMO support was given in case of severe respiratory failure and an estimated mortality risk of higher than 80% using the entry criteria reported by Stolar et al(13). These criteria were: an oxygenation index (OI) greater than 40 beyond 4 hours or an alveolar arterial oxygen gradient greater than 600 during 6–8 hours, with an Fio2 of 100%, and signs of barotraumas or acute deterioration. During the study period, these entry criteria did not change in our institution.

The follow-up program is the standard of care for ECMO-treated neonates in the Netherlands.(11,12) The Erasmus MC Medical Ethical Review Board stated that "the Medical Research in Human Subjects Act (in Dutch: "WMO") does not apply to this study because subjects are not being submitted to any handling nor are there rules of human behavior being imposed". All participants and their parents provided permission to use the data.

Design

During the routine follow-up visit, structured questionnaires were used: parents of the adolescents provided information on socioeconomic status (SES; based on maternal education)(14) and ethnicity (at least one parent of Dutch/non-Dutch origin). Adolescents were asked about their academic achievement.

Overall intelligence was taken as the intelligence quotient (IQ) score at age 8 or 12 years. If IQ had been assessed at both ages, we used the IQ score assessed at 12 years. The follow-up program provides for a formal neuropsychological assessment by a pediatric psychologist. As part of the neuropsychological assessment, the parents filled out questionnaires in the waiting room of the hospital and the adolescents in the consultation room. In the first 10 years of the follow-up program, these assessments were limited and geared to individual needs, but from January 2012, a standard assessment battery was used for the neuropsychological tests (Supplemental Digital Content 1).

The following clinical data were retrieved: underlying disease (CDH, MAS, and other diagnoses); gestational age; birth weight; age at start ECMO; time on ECMO; highest OI and mean airway pressure prior to ECMO; duration of ventilation; oxygen dependency after extubation (< 1wk, 1wk to 1 mo, and > 1 mo); the presence of chronic lung disease (CLD) (15); abnormal cranial ultrasound (CUS: no and yes); use of morphine or other sedatives (< 1 wk, 1 wk to 1 mo, and > 1 mo); use of muscle relaxants (no, ICU: 1 d to 1wk, and ICU: > 1wk); the presence of convulsions (no, clinical but not tested, and confirmed on an electroencephalograph); use of antiepileptics (no, prophylactically, therapeutically < 1 mo, and therapeutically > 1 mo); and diagnosis of epilepsy at later age (no, yes, and dubious).

Instruments

Validated neuropsychological tests and questionnaires were administered in their Dutch versions to assess skills in different domains (see brief descriptions of the tests in the Supplemental Digital Content 1).(16–25)

Neuropsychological tests:

1. Intelligence: Wechsler Intelligence Scale for Children.
2. Attention: Trail Making Test and Stroop Color-Word test.
3. Memory: subtests Rebus Learning and Auditory Comprehension of the Kaufman Intelligence Test; subtest digit span of the Wechsler Adult Intelligence Scale; the Rey Auditory Verbal Learning Test (RAVLT); and the Rey Complex Figure Test (RCFT).
4. Executive functioning: Tower test.
5. Visual-spatial processing: RCFT copy.

Questionnaires:

1. Executive functioning: Behavior Rating Inventory of Executive Functioning (BRIEF; filled out by parents).
2. Social-emotional functioning: self-esteem: Self Perception Profile for Children (SPPC); health status: Pediatric Quality of Life Inventory (PedsQL).
3. Behavior: Youth Self-Report (YSR).

Data analysis

Differences in medical and background characteristics (Table 2) between participants and nonparticipants were assessed with either the Mann-Whitney U test (continuous variables) or the chi square test (categorical variables).

To enable comparison of the results of the different neuropsychological assessments, all outcomes were converted into z-scores (individual score minus the mean population score divided by the population SD). These z-scores were compared with the z-score of IQ using paired sample t-tests. In this way, we analyzed whether the outcomes of the neuropsychological tests were concordant with IQ. Outcomes in z-scores on the neuropsychological tests and questionnaires were then compared with the z-scores of the general population (mean z-score = 0; SD = 1) using paired samples t-tests. The assumption of normality was assessed. The difference between the digit-span forward and backward was assessed using the chi-square test.

The influence of each medical variable on the neuropsychological domains on which the adolescents showed impaired performance compared with the general population was assessed using univariate linear regression analyses. Those medical variables that were found to be significant predictors of impaired neuropsychological outcome were then added together – with SES as a covariate – in a multivariate linear regression model.

To check the assumptions for linear regression analysis and to examine the applicability of the model, normal probability plots of the residuals and multicollinearity was evaluated.(26)

All analyses were done using SPSS Statistics for Windows, Version 22.0 (IBM, Armonk, NY). A *p* value of .05 was used.

RESULTS

Patients

Between February 1991 and June 1997, 72 neonates had been treated with ECMO. Eighteen (25%) had died prior to original hospital discharge (nine CDH; three MAS; six other diagnoses). Twenty of the 54 survivors (37%) did not attend follow-up at 17 years old: 11 refused, four had follow-up elsewhere, two only underwent medical examination because they had recently been tested elsewhere (MAS with developmental delay and other diagnosis with intellectual disability), two had emigrated, and one was not seen because of organizational reasons. For 17 of these 20 patients, data on outcome or state of health were available. Seven had average intelligence (one with epilepsy and an infarct, one with attention deficit hyperactivity disorder, and one with attention deficit disorder), six had developmental delay, and four had intellectual disability (one with epilepsy and one with attention deficit hyperactivity disorder). Most medical and background characteristics did not statistically differ between participants and nonparticipants; the exceptions are abnormal CUS, use of muscle relaxants, and the presence of convulsions (Table 1).

Neuropsychological assessment and questionnaires

Of the 34 adolescents (63%) attending follow-up, three had abnormal CUS as neonates (occlusion of the middle and anterior cerebral artery, subependymal hemorrhage grade 1, and watershed stroke).

Of all 34 participants, three could not complete the assessment battery because of intellectual disability and/or behavioral problems and only filled in questionnaires on self-esteem, health status, and behavior (CDH with intellectual disability, MAS with intellectual disability and possible autism, and CDH with intellectual disability [including a chromosome X duplication] and autism). For organizational reasons, one other patient (CDH with average intelligence) was not tested and only filled in the questionnaires. The other 30 adolescents had been assessed at the age of 17 or 18 years, but not all had performed all tests because of the fact that the follow-up assessments were first geared to individual needs and standardized from January 2012 (Table 2). The assumption of normality was met for the neuropsychological data and questionnaires.

Table 1. Medical and background variables

	Participants (n=36)	Non-participants (n=18)	*p* value
Diagnosis, No. (%)			0.34
MAS	22 (61)	9 (50)	
CDH	5 (14)	1 (6)	
Other	9 (25)	8 (44)	
Gestational age in weeks, median (range)	40 (34-43)	40 (35-42)	0.94
Birth weight in grams, median (range)	3295 (2160-4980)	3220 (2300-4360)	0.99
Age start ECMO in hours, median (range)	28 (8-600)	25 (8-120)	0.26
Time on ECMO in hours, median (range)	122 (47-309)	127 (72-510)	0.65
Highest oxygenation index prior to ECMO, median (range)	54 (27-130)	55 (32-95)	0.81
Highest mean airway pressure prior to ECMO (cm H_2O), median (range)	21 (14-28)	20 (16-26)	0.63
Duration of ventilation in days, median (range)	10 (4-37)	10 (5-34)	0.95
Oxygen dependency post extubation, No. (%)			**0.04**
1 day- 1 week	18 (50)	7 (39)	
>1 week- 1 month	6 (17)	6 (33)	
>1 month	9 (25)	-	
Missing	3 (8)	5 (28)	
CLD, No. (%)			0.16
yes	10 (28)	1 (5)	
Missing	3 (8)	4 (22)	
Abnormal cranial ultrasound, No. (%)			**0.04**
yes	5 (14)	7 (39)	
Missing	-	1 (6)	
Use of morphine or other sedatives, No. (%)			0.77
<1 week	11 (31)	6 (33)	
1 week- 1 month	21 (58)	8 (44)	
>1 month	4 (11)	3 (17)	
Missing	-	1 (6)	
Use of muscle relaxants, No. (%)			0.09
No	3 (8)	-	
Intensive Care Unit 1 day- 1 week	20 (56)	14 (78)	
Intensive Care Unit >1 week	13 (36)	2 (11)	
Missing	-	2 (11)	
Presence of convulsions, No. (%)			**0.007**
No	25 (69)	10 (56)	
Clinical, but not tested	10 (28)	2 (11)	
Confirmed on electroencephalograph	1 (3)	6 (33)	
Use of anti- epileptics, No. (%)			0.26
No	14 (39)	4 (22)	

Table 1. Medical and background variables (continued)

	Participants (n=36)	Non-participants (n=18)	*p* value
Prophylactically	11 (31)	6 (33)	
Therapeutically <1 month	5 (14)	1 (6)	
Therapeutically >1 month	6 (17)	7 (39)	
Diagnosis of epilepsy at later age, No. (%)			-
No	27 (75)	-	
Yes	3 (8)	-	
Dubious	3 (8)	-	
Missing	3 (8)	18 (100)	
Male gender, No. (%)	17 (47)	11 (61)	0.40
Medical and background variables (continued)			
Ethnicity, No. (%)			0.25
Dutch	24 (67)	8 (44)	
Missing	-	9 (50)	
SES, No. (%)			0.95
Low	19 (53)	5 (28)	
Moderate	12 (33)	4 (22)	
High	3 (8)	1 (6)	
Missing	2 (6)	8 (44)	

Results are presented as n (%) or median (range). MAS = meconium aspiration syndrome; CDH = congenital diaphragmatic hernia; other = persistent pulmonary hypertension in the newborn (n=7), sepsis (n=5), asphyxia (n=3), pulmonary hypoplasia due to kidney failure (n=1), respiratory syncytial virus (n=1); ECMO = extracorporeal membrane oxygenation; CLD = chronic lung disease as defined by Jobe and Bancalari(15). SES = socio-economic status based on maternal education(40).
P value: difference between participants and non-participants; the Mann-Whitney test was used for continuous variables; the chi-square test was used for categorical variables

Academic achievement

Of the 30 adolescents assessed at age 17 or 18 years, five were enrolled in special education (15%), eight in secondary education (24%; two in preparatory vocational secondary education and training [in Dutch: VMBO], five in Senior General Secondary Education [in Dutch: HAVO], and one in University Preparatory Education [in Dutch: VWO]). Twenty adolescents were enrolled in senior secondary vocational education and training (60%; in Dutch: MBO), and one (3%) was enrolled in first cycle higher education (in Dutch: HBO).

In the Dutch population, 6% of adolescents who are 17 and 18 years old attend special education, 27% secondary education, 49% vocational education, and 18% first cycle higher education(27). The percentages differed significantly from the reference norm (chi-square test, 63.8; $p < .001$).

Neuropsychological outcome

Intelligence

Based on outcomes of intelligence tests at 8 years (n = 3) or 12 years (n = 27), the mean IQ(SD) for the entire group was calculated to be 90.3(19.4). The mean IQ z-score was -0.65(1.29), which is significantly below the norm of the Dutch population (p = .03). Six children had scored below 70 at age 8 or 12 years. When those six were removed from the analyses, the IQ of the remainder of the group was 97.5(13.8).

Attention

Selective and divided attention did not differ from what was expected based on their IQ (Table 2).

Memory

The adolescents scored significantly lower (Table 2) on short-term and long-term verbal (RAVLT immediate and delayed recall) and visual-spatial memory (RCFT immediate and delayed recall) than what was expected based on their IQ (Table 2). One adolescent had visual problems that could primarily affect the RCFT results but was able to copy the figure (RCFT copy) without major problems, indicating specific visual-spatial memory problems to explain the low scores on the RCFT immediate and delayed recall.

The adolescents scored significantly higher (Table 2) than expected based on their IQ on auditory short-term memory (digit span). However, the proportion of adolescents scoring lower on the backward span than on the forward span (indicative of a working-memory problem [28]) was significantly higher than the norm population (25) (32% vs 9%; $p < .01$).

Executive Functioning

The adolescents scored significantly higher than expected based on their IQ on the Tower test (Table 2), which measures planning.

Visual-spatial processing

Results did not differ from what was expected based on their IQ (Table 2).

Questionnaires

Executive Functioning

Figure 1 graphically shows that the parents of the entire group on average evaluated executive functioning of their children to be less positive than that of the normal population (BRIEF organization of materials: mean [SD] = -0.60 [0.90], p = .03; BRIEF behavior evaluation: mean [SD] = -0.53 [0.88], p = .05).

Table 2. Overview of neuropsychological outcome

Neuropsychological test	n	IQ mean (SD)	Mean (SD)	*p* value
Attention				
TMT A	22	-0.69 (1.36)	-0.71 (1.75)	0.95
TMT B			-0.90 (1.28)	0.39
TMT B/A			-0.50 (0.93)	0.34
Stroop 1	22	-0.69 (1.36)	-0.94 (1.59)	0.18
Stroop 2			-0.79 (1.56)	0.74
Stroop 3			-0.90 (1.33)	0.45
Stroop			-0.41 (1.08)	0.34
Memory				
KAIT rebus learning	29	-0.59 (1.28)	-0.83 (1.11)	0.38
KAIT rebus learning delayed			-0.74 (1.56)	0.82
KAIT auditory comprehension			-0.34 (0.87)	0.22
KAIT auditory comprehension delayed			-0.70 (1.24)	0.63
WAIS Digit span	22	-0.69 (1.36)	-0.12 (1.12)	**0.014**
RAVLT immediate	28	-0.63 (1.33)	-1.40 (1.58)	**0.02**
RAVLT delayed			-1.54 (1.67)	**0.010**
RAVLT recognition	14	-0.66 (1.24)	-0.69 (1.53)	0.76
RCFT immediate	9	-0.47 (1.21)	-1.65 (1.37)	**0.008**
RCFT delayed			-1.70 (1.23)	**0.008**
RCFT recognition			-1.07 (0.69)	0.20
Executive functioning				
Tower	29	-0.64 (1.32)	-0.06 (0.96)	**0.03**
Visual spatial processing				
RCFT copy	9	-0.47 (1.21)	-0.97 (1.48)	0.29

n= number of adolescents assessed with that specific neuropsychological test. IQ mean (SD) = average z-score IQ of the adolescent group assessed with that specific test. Mean (SD) = average z-score of the neuropsychological test. *P* value = outcome of the paired samples t-test between IQ and neuropsychological outcome. ***P* value** = significant outcome of the paired sample t-test. TMT = Trail Making Test; Stroop = Stroop Color Word Test; KAIT = Kaufman Adult Intelligence Test; WAIS = Wechsler Adolescent Intelligence Scale; RAVLT = Rey Auditory Verbal Learning Test; RCFT = Rey Complex Figure Test; Tower = Tower test.

Self-Esteem

The adolescents on average evaluated their behavior more positive than the reference population (SPPC behavior: mean [SD] = 0.67 [1.00], $p < .01$).

Health Status

The adolescents' health status on average did not significantly differ from that of the normal population. The adolescents rated their physical, emotional, social, school, and psychosocial functioning similarly to healthy peers (PedsQL physical functioning: mean

[SD] = 0.25 [1.06]; PedsQL emotional functioning: mean [SD] = 0.49 [0.92]; PedsQL social functioning: mean [SD] = 0.06 [1.08]; PedsQL school functioning: mean [SD] = -0.01 (1.14); PedsQL psychosocial functioning: mean [SD] = 0.24 [0.95]).

Behavior Problems

The adolescents on average reported more internalizing behavior problems than found in the normal population (YSR withdrawn/depressed: mean [SD] = -0.47 [0.54], p = .02; YSR somatic complaints: mean [SD] = -0.43 (0.48), p = .03; YSR social problems: mean [SD] = -0.41 [0.46], p = .04) (Figure 1).

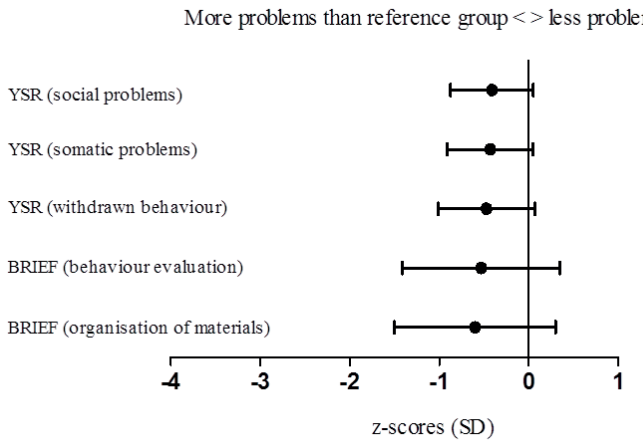

Figure 1. Proxy- and self-reported outcome
YSR = Youth Self Report.
BRIEF = Behavior Rating Inventory of Executive Functioning.
Z-score = Individual norm score minus the population norm score divided by the standard deviation of the population.

Factors influencing neuropsychological outcome

Regression analyses were performed to assess whether any medical characteristics (Table 1) could predict the four neuropsychological outcome variables that were significantly lower than in the normal population (RAVLT, both immediate and delayed recall; RCFT, both immediate and delayed recall).

First, univariate regression analyses with all medical variables identified four medical variables with an individual significant influence on one or more of the outcome variables: diagnosis, age at start ECMO, the presence of convulsions, and use of antiepileptics (Table 3). Next, multiple regression analyses were done with these four medical variables while correcting for SES. In the analyses, multicollinearity was evaluated. The multivariate regression model showed none of the medical variables to be significant predictors once added all together.

Table 3. Predictors of neuropsychological outcome

Univariate Regression Analyses	RAVLT Immediate Recall	RAVLT Delayed Recall	RCFT Immediate Recall	RCFT Delayed Recall
Diagnosis	NS	NS	$\beta = -0.79$; $p = 0.01$ (CI, −3.66 to −0.69)	$\beta = -0.69$; $p = 0.04$ (CI, −3.30 to −0.11)
Age start ECMO	NS	$\beta = 0.40$; $p = 0.04$ (CI, 0.001 to 0.029)	NS	NS
Presence of convulsions	$\beta = 0.43$; $p = 0.03$ (CI, 0.20 to 2.66)	NS	NS	NS
Use of antiepileptics	$\beta = -0.46$; $p = 0.30$ (CI, −3.54 to −0.21)	$\beta = -0.45$; $p = 0.03$ (CI, −3.67 to −0.20)	NS	NS

RAVLT = Rey Auditory Verbal Learning Test, RCFT = Rey Complex Figure Test, NS = not significant, ECMO = extracorporeal membrane oxygenation.

DISCUSSION

In this first worldwide study of neuropsychological outcome of neonatal ECMO survivors at adolescent age, we found that short-term and long-term verbal deficits and visual-spatial memory problems were present in a large proportion of these adolescents, even after adjustment for IQ. Impaired visual acuity could have caused the poor visual-spatial memory results (RCFT immediate and delayed recall) in our study. One adolescent indeed had visual problems but still was able to copy the figure (RCFT copy) without major problems. Therefore, we believe that our results indicate specific visual-spatial memory problems in this population. Poorer processing skills combined with poor spatial abilities and lower scores on visual memory testing have been reported in 7-year-old neonatal ECMO survivors.(8) Our findings suggest that these problems may persist into later life and could possibly affect academic performance and participation in society.

In this study, the subjects performed significantly better than expected based on their IQ on auditory short-term memory (digit span). The digit span is comprised of a forward span and a backward span component. The forward component requires more from short-term auditory memory(29), whereas the backward component requires more from working-memory(28) and may evoke more visuospatial image processes.(30) Almost one-third of adolescents in this study scored significantly lower on the backward component than on the forward component compared to 9% in the norm population(25), suggesting deficits in working-memory. Deficits in working-memory have been identified in children with acquired brain injury as well.(31)

Concerning the adolescents' executive functioning skills, in this study, parents reported more problems compared with the reference norm on the scales organization of materials and behavior evaluation in the adolescents. In practice, this could mean, for example, that they have difficulty in organizing homework assignments or do not evaluate their work after they have finished. We did not assess these specific executive

functions, only planning. Further research should make clear whether the problems reported by the parents could be because of other poor executive functioning skills or (working)memory problems.

The key brain regions supporting working-memory are the dorsolateral and ventrolateral prefrontal and parietal cortex.(32) These regions are also involved in organization skills and behavior evaluation (functions that parents reported to be suboptimal in their children). It would, therefore, be worthwhile to specifically study these brain regions in neonatal ECMO survivors with the use of neuroimaging. Another brain region, the hippocampus, is highly and selectively susceptible to injury caused by hypoxia-ischemia (including ECMO treatment)(33–36) and has been shown to lead to memory dysfunctions in later childhood.(33) This type of injury might be a partial explanation for the verbal and visual-spatial memory problems found in this study. Neuroimaging studies should, therefore, also focus on the more subcortical located hippocampus.

Regarding social-emotional functioning, the adolescents reported more withdrawn behavior and somatic and social problems compared with the norm population. On the other hand, they had a significantly more positive feeling of self-competence. This is not an unusual finding. In a study with adolescent, preterm born children, the vast majority were content with their activities and participation in society although most of them were neither in school nor employed at age 19.(37) In the same vein, we have previously found that self-reported emotional functioning of children with congenital anomalies treated with or without neonatal ECMO was not affected at the age of 8 years.(7)

We were unable to identify significant predictors of the memory problems. Still, we believe that "severity of illness" is a predictor for neuropsychological outcome of neonatal ECMO survivors, rather than ECMO treatment itself. This notion is supported by findings in children with CDH who were not treated with ECMO.(7) Outcome after neonatal ECMO treatment is determined by many different factors: pretreatment related (e.g., congenital anomalies or loss of cerebral autoregulation), treatment related (such as intracranial hemorrhage), and posttreatment related (e.g., CLD and prolonged hospitalization).(9) However, a specific severity of illness scoring system for ECMO treated patients is not available.(38) We believe that the lack of such a scoring system – together with the small sample size – may explain why we did not find significant predictors of outcome at older age.

This study has some limitations. First, only 63% of participants in the follow-up program had been assessed at adolescent age. We started our follow-up program in 2001, and therefore, a large proportion of the survivors was invited a long time after their treatment. This resulted in a small sample. In other studies with participants born from 1996 onward, we had participation rates above 85%.(11,12) Bias may have occurred because significantly more nonparticipants than participants had abnormal CUS during ECMO (Table 1). Second, the fact that the average IQ was significantly below the norm

is a potential source of bias. In previous studies in (pre)school-aged ECMO survivors, intelligence was found to be equal to the norm.(7,8,11) We assume that the generally low SES in this study contributes to the low IQ in this group, as low SES is associated with poorer cognitive development.(39) To avoid confounding the neuropsychological outcome, we corrected for both IQ and SES in the statistical analyses. Further to this issue, the use of IQ obtained at 8 and 12 years old, rather than at adolescent age, could be considered a limitation. However, previous evidence that intelligence is stable from infancy into adolescence (40) was confirmed for the 14 adolescents who were evaluated at both 8 and 12 years in this study. Third, we addressed selective and divided attention but not sustained attention. In a previous study, we found sustained attention problems in 8-year-old children treated with neonatal ECMO.(7) Because poor sustained attention can interfere with memory, it is not clear whether the observed memory problems are primary or secondary dysfunctions. Fourth, we did not correct for multiple testing (n = 20) in the analyses of the neuropsychological tests. If we had done so, none of the results would have been significant. We decided to present the uncorrected results because this first study on neuropsychological outcome in neonatal ECMO survivors at adolescent age yields further directions for improvement of care and future follow-up studies. Fifth, we did not have a control group of adolescents who had similar severity of illness in the neonatal period but who did not undergo ECMO treatment. With two ECMO centers in the Netherlands, covering a relatively small geographical area, the large majority of neonates with similar severity of illness who are not born prematurely (i.e., born after 34 weeks of gestation with birth weight > 2,000 gm) are treated with ECMO. Therefore, it will be difficult to obtain controls with similar severity of illness who survived without ECMO. As an alternative, patients from countries with less access to ECMO treatment could serve as controls. However, variety in treatment protocols and neuropsychological assessments may bias the results of such a study. For similar reasons, we consider the use of a historic control group of infants treated before the ECMO era not appropriate.

Despite the limitations, this study is of value as it is the first reporting on neuropsychological outcome following neonatal ECMO at 17–18 years and has important implications for patient care. Considering that we found neuropsychological deficits persisting into adolescence – thereby extending the evidence of "growing into deficit" in these patients – it is of utmost importance that we report outcome at this age in order to understand at which neuropsychological processes interventions should be aimed.

CONCLUSION

This study showed verbal, visual-spatial, and working-memory problems in adolescents of 17–18 years old treated with neonatal ECMO. Furthermore, parents reported some

aspects of executive functioning as impaired in their children. Positively, health status and sense of self-competence did not seem affected. Considering the findings of this study in light of the outcome at school-age, future research should focus on 1) the longitudinal neuropsychological outcome, specifically of (working) memory, 2) developing a standardized scoring system to quantify "severity of illness", 3) evaluating to what extent "severity of illness" is responsible for acquired brain injury, and 4) evaluation of effects of cognitive rehabilitation (which is currently performed in our department). To achieve these goals, it would be necessary to set up multicenter follow-up programs with internationally standardized assessment instruments and neuroimaging.

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SUPPLEMENTARY MATERIAL

Supplemental Digital Content 1. Descriptions of the neuropsychological tests.

Intelligence

Wechsler Intelligence Scale for Children (WISC-III-NL)

Intelligence test for children between the ages 6 and 16. The test generates a full scale IQ (FSIQ), Total Verbal IQ (TVIQ) and Total Performance IQ (TPIQ). The IQ scores are derived from five verbal and five performance subtests. There are also three supplemental tests (1, 2).

Attention

Trail Making Test (TMT)

This paper and pencil test consists of two parts. In the first part (part A), the subject must draw lines to consecutively connect numbered circles on a sheet. In the second part (part B), the subject must consecutively but alternately connect numbered and lettered circles on another worksheet. The goal of the test is to finish each part as quickly as possible. The test can be administered to children and adults in the age range 6-89 years. This test measures visual conceptual and visuomotor tracking as well as divided attention (3, 4).

Stroop Color Word Test (Stroop)

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

Memory

Kaufman Intelligence Test (KAIT) – subtest Rebus Learning

The subject learns a word or concept associated with a rebus (a picture that stands for a word) and then has to read aloud phrases and sentences that are composed of these rebuses. This is repeated after 30 minutes. This test measures short- and long-term visual/verbal associative memory. Adolescents and adults in the age range 14-85 years can take the test (5, 6).

Kaufman Intelligence Test (KAIT) – subtest Auditory Comprehension

The subject has to listen to a recording of a news story, then answer literal and inferential questions about the story. This test measures short- and long-term (after 45 minutes)

verbal memory and verbal logic reasoning. Adolescents and adults in the age range 14-85 years can take the test (5, 6).

Wechsler Adult Intelligence Scale – Third Edition (WAIS-III) – subtest Digit Span

The Digit Span consists of random number sequences that increase in length and that the examiner reads aloud at the rate of 1 number per second. The subject has to reproduce these numbers in the same order. Next, the sequences must be recalled backwards (3-5-7 becomes 7-5-3). The first part of the test measures short-term auditory memory and short-term retention capacity. The second part measures auditory working memory. A difference of 4 or more points between forward and backward Digit Span in favor of forward is indicative of a working-memory problem. The test is applicable to adolescents and adults in the age range 16-85 years (7).

Rey Auditory Verbal Learning Test (RAVLT)

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

Memory and visual-spatial functioning

Rey Complex Figure Test (RCFT)

The RCFT consists of three trials. First the subject has to copy a complex figure. Then after 3 and after 30 minutes the figure must be drawn from memory. Next, different figures are shown and the subject has to indicate whether these figures were in the original figure. This test measures visual integration, short- and long-term visual-spatial memory, and visual-spatial recognition. It can be completed by children and adults in the age range 6-89 years (4, 10).

Executive functioning

Tower Test (Tower)

The subject must plan ahead to rearrange five colored rings in varying sizes from the initial position on three upright sticks to a new predetermined position in as few moves as possible. When two or more rings are at the same stick, the smaller ones must always be on top of the larger ones. Only one ring can be moved at the same time. This test measures planning ability and can be administered to children and adults in the age range 8-89 years (11, 12).

Behavior Rating Inventory of Executive Functioning questionnaire (BRIEF)

This questionnaire is filled out by parents of children and adolescents between the ages of 6 and 18 years. Different areas of executive functioning are addressed in 75 questions that form 8 subscales: Inhibition, Cognitive Flexibility/Shifting, Emotional Control, Initiate, Working-memory, Plan/organize, Organization of materials, Monitor. The 8 subscales make up two indices: Behavior Regulation Index (ability to adjust thinking and regulate emotions and behavior) and Metacognition Index (ability to independently carry out tasks and solve problems based on the judgment of own behavior). A total executive functioning score can be derived as well (13, 14).

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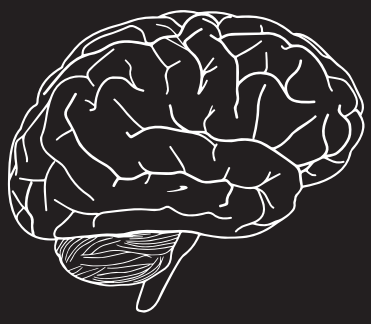
CHAPTER 5



NEONATAL CRITICAL ILLNESS AND DEVELOPMENT: WHITE MATTER AND HIPPOCAMPUS ALTERATIONS IN SCHOOL-AGE NEONATAL ECMO SURVIVORS

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ABSTRACT

Aim To examine the neurobiology of long-term neuropsychological deficits following neonatal extracorporeal membrane oxygenation (ECMO).

Method This cross-sectional study assessed white matter integrity and hippocampal volume of ECMO survivors (8-15yrs) and healthy controls (8-17yrs) using Diffusion Tensor Imaging and structural MRI, respectively. Neuropsychological outcome was evaluated in patients. Included clinical predictors of white matter integrity: age start ECMO, ECMO duration, highest oxygenation index before ECMO, highest mean airway pressure and mechanical ventilation duration.

Results ECMO survivors (n = 23) had lower global fractional anisotropy than healthy children (n = 54) (patients = .368; comparison group = .381; $p = .018$), but similar global mean diffusivity ($p = .41$). ECMO survivors had lower fractional anisotropy in the left cingulum bundle (patients = .345; controls = .399; $p < .001$) and higher mean diffusivity in a region of the left parahippocampal cingulum (patients = .916; comparison group = .871; $p < .001$). Higher global mean diffusivity predicted worse verbal memory in ECMO survivors (n = 17) ($\beta = -.74$, $p = .008$). ECMO survivors (n = 23) had smaller bilateral hippocampal volume than healthy children (n = 43) (left: $p < .001$; right: $p < .001$) and this was related to worse verbal memory (left: $\beta = .65$, $p = .018$; right: $\beta = .71$, $p = .006$).

Interpretation Neonatal ECMO survivors are at risk for long-term brain alterations, which may partly explain long-term neuropsychological impairments. Neuroimaging may contribute to better risk stratification of long-term impairments.

INTRODUCTION

Extracorporeal membrane oxygenation (ECMO) has been used in over 28,000 neonates with severe respiratory failure, 74% of whom survived to discharge or transfer.[1] Previous studies found 10-59% of ECMO patients to have abnormalities on routine neuroimaging during treatment.[2] Furthermore, neurological complications such as hypoxia and cerebrovascular injury have been reported.[3,4]

Neurodevelopmental follow-up of these children shows long-term (subtle) neuropsychological impairments that emerge in childhood and persist into adolescence.[5-7] Despite normal intelligence, these attention and memory deficits result in patients being at risk for school failure.[5-7] These results suggest a 'growing into deficit' phenomenon where subtle brain injuries acquired at a young age become functionally evident over time when demands on cognitive functioning increases.[8] This 'growing into deficit' is nested within different developmental processes that occur in the brain (i.e. myelination) during childhood and adolescence. Thus, it is important to study the underlying neurobiology of long-term neuropsychological impairments within a developmental framework.

Studies utilizing sophisticated neuroimaging methods to study neonatal ECMO survivors are scarce.[9,10] Structural MRI in school-age children who experienced neonatal hypoxia have shown bilateral lower hippocampal volume compared to healthy controls.[9] Furthermore, the lower hippocampal volume was associated with the extent of memory deficits. Our group previously found cortical thickness and global brain volumes in neonatal ECMO survivors (8-15 years) to be similar to healthy controls, despite verbal memory problems in the patients.[10] These results suggest that the underlying brain injury in ECMO survivors is specific and/or subtle and therefore may not always be identifiable using high-resolution structural MRI. Alternative advanced imaging techniques may be better suited to identify subtle brain alterations in ECMO survivors.

Diffusion Tensor Imaging (DTI) is an imaging technique that can quantify microstructural characteristics of white matter. White matter has been shown to be especially vulnerable in the neonatal period, a time when it is undergoing rapid development.[11] Neonatal ECMO survivors may therefore be at an increased risk for white matter abnormalities. Moreover, white matter integrity has been associated with neuropsychological outcome.[11] Since white matter is important for high-speed transmission of neuronal signals between distant brain regions, aberrations in white matter development could affect the orchestration of specific cognitive functions. Thus, the long-term neuropsychological impairments observed in both 8-year-old and 17-year-old ECMO survivors could be due to underlying white matter alterations.[5-7]

This study aimed to assess whether 8-to-15 year-old ECMO survivors have white matter alterations and whether these are partially responsible for the long-term neuropsychological deficits observed in these patients. We hypothesized to find white matter

alterations in ECMO survivors, specifically in tracts associated with (working) memory and attention. Identification of neurobiological correlates of long-term neuropsychological impairments following neonatal ECMO may contribute to better risk stratification of these impairments in neonatal ECMO survivors.

METHOD

ECMO survivors

Children born between January 1997 and December 2003 treated with venoarterial neonatal ECMO in the Erasmus MC in Rotterdam, the Netherlands were included. ECMO support was given according to the entry criteria described by Stolar et al[12]. For each patient, cannulas were placed in the right cervical region by the same surgical team. Further recruitment details and in- and exclusion criteria are described elsewhere.[10] Of 60 eligible patients, six families were not traceable, 17 declined participation and one child had dental braces, leaving 36 participants. Of these, 23 had reliable DTI and structural data (8-15 years) (Supplementary Figure 1). Background characteristics retrieved from the medical records are presented in Table 1.

Comparison group

Healthy children (8-18 years) were recruited through two different approaches. First, participating families were asked if their child had a friend who would be interested in participating. Second, we sent invitation letters to the parents of children attending a primary school in Rotterdam. In- and exclusion criteria are described elsewhere.[10] Eleven of 75 controls were excluded because of either preterm birth or because they were greater than six months younger/older than the youngest/oldest patient, leaving 64 eligible controls. Of these, 54 had reliable DTI data and 43 had reliable structural data (Supplementary Figure 1).

Study procedure

The study was performed in compliance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) and approved by the Institutional Review Board at the Erasmus MC (MEC-2010-299). Informed consent was obtained prior to participation from the parents of each child and from children 12 years and older. All children had been administered subtests of the Dutch edition of the NEPSY-II-NL neuropsychological test battery (Pearson, Amsterdam).[13] Only performance of the ECMO survivors was analyzed as the relationship between cognitive functioning and brain alterations is largely unknown following ECMO. Children between 8 and 12 years performed nine subtests, older children performed only six due to the age limit of three tests.[13]

Table 1. Clinical characteristics of the ECMO survivors

	Participants (n = 23)
IQ, mean (SD)	100 (19)
Gestational age (weeks), median (IQR)	40 (2)
Birth weight (grams), median (IQR)	3530 (810)
Diagnosis, n (%)	
MAS	15 (66)
CDH	4 (17)
Other	4 (17)
Age start ECMO (hours), median (IQR)	20 (24)
ECMO duration (hours), median (IQR)	124 (100)
Highest oxygenation index prior to ECMO, median (IQR)	46 (27)
Highest mean airway pressure, median (IQR)	20 (5)
Mechanical ventilation (days), median (IQR)	11 (9)
O2 post-ECMO, n (%)	
1 day – 1 week	11 (48)
>1 week - <1 month	11 (48)
>1 month	1 (4)
BPD presence, n (%)	
Yes	2 (9)
No	21 (91)
Nitric Oxide pre-ECMO, n (%)	
Yes	16 (69)
No	5 (22)
Unknown	2 (9)
Inotropic use, n (%)	
Yes	21 (92)
No	1 (4)
Unknown	1 (4)
Morphine use, n (%)	
<1 week	4 (18)
1 week – 1 month	15 (65)
>1 month	3 (13)
Unknown	1 (4)
Muscle relaxant use, n (%)	
No	2 (8)
Perioperative only, n (%)	3 (13)
1 day – week	12 (53)
>1 week	5 (22)
Unknown	1 (4)
Corticosteroids use, n (%)	
Yes	2 (8)
No	21 (91)

Other diagnoses consist of persistent pulmonary hypertension of the newborn (PPHN) (n = 1), pneumonia (n = 2), and sepsis (n = 1). abbreviations: IQ, intelligence quotient; IQR, interquartile range; MAS, meconium aspiration syndrome; CDH, congenital diaphragmatic hernia; ECMO, extracorporeal membrane oxygenation; O2 post-ECMO, extra oxygenation need post-ECMO; BPD, bronchopulmonary dysplasia.

Neuroimaging

Participants first underwent a mock scanning session to become familiarized with the MR-environment.[14] MRI data were acquired on a 3 Tesla GE MR-750 system using an 8-channel head coil (General Electric, Milwaukee, WI). A full description of these methods is provided in Supplementary File 1. Briefly for the DTI data, after data processing, the voxel-wise scalar maps fractional anisotropy (FA) and mean diffusivity (MD) were computed. FA is the degree of directionality of diffusion and ranges from 0 to 1, where a higher FA generally represents a greater coherence of white matter fibers. MD is the rate of diffusion of hydrogen averaged in all directions. Lower MD is suggestive of increased integrity in axonal membranes, packing, or myelin. Fully automated probabilistic fiber tractography was performed using the FSL plugin "AutoPtx"[15] to create subject-specific, probabilistic representations of multiple white matter fiber bundles. Raw image quality was assessed using automated software[16] and visual inspection, leaving 77 datasets (ECMO survivors = 23, comparison group = 54).

Statistical analysis

Age at MRI, gestational age and gender differences between groups were assessed using independent samples t-tests and a chi-squared test, respectively. As previous research showed congenital diaphragmatic hernia (CDH) patients tend to have lower IQ, we compared IQ between patients with meconium aspiration syndrome (MAS), CDH or other diagnoses using ANOVA.[6]

Global white matter integrity of patients and controls was analyzed first. The association fibers and limbic system fibers (uncinate, inferior fronto-occipital fasciculus, superior longitudinal fasciculus, inferior longitudinal fasciculus, cingulum bundle and parahippocampal part of cingulum) were used to compute a measure of global FA and global MD. These fibers have been associated with neuropsychological functioning and in particular with attention and (working) memory – domains that have been shown to be affected in neonatal ECMO survivors.[6,17] The global measures were computed by multiplying the FA/MD values of each tract with the average volume of each tract, and then dividing this by the sum of the volumes of all tracts (Formula 1). This ensured that tracts of different size were appropriately weighted in their contribution to the global mean.

Formula 1:

$$\text{Global FA} = \frac{\sum_{i=1}^{6} FAtract_i Voltract_i}{\sum_{i=1}^{6} Voltract_i}$$

The same formula was used to compute global MD. Assumptions of normality were met for FA and MD.

Differences between groups in global FA, MD and the diffusion metrics within individual white matter tracts were analyzed using ANCOVA, adjusted for age at MRI and gender.[17] The white matter tracts were assessed in the left and right hemispheres separately. Global FA/MD was added as a covariate to identify differences in the individual tracts only. The False Discovery Rate (FDR) correction[19] was used once to account for multiple testing in all analyses of FA/MD differences between groups. Results were considered statistically significant at the FDR-corrected $p < .05$.

Multivariable linear regression models were used to evaluate the effect of five clinical characteristics (age start ECMO, ECMO duration, highest oxygenation index prior to ECMO, highest mean airway pressure and mechanical ventilation duration) on global FA, global MD, and the altered white matter tracts. Age at MRI was adjusted for. For all clinical characteristics, log transformations were applied to satisfy parametric statistics assumptions.

In the ECMO survivors, white matter microstructure and verbal memory were assessed to get a better understanding of the poor verbal memory performance in the ECMO survivors previously shown by our group.[10] Separate multivariable linear regression models were used to evaluate the effect of global FA, global MD and the altered tracts on verbal memory, adjusted for age at MRI. The associations between white matter microstructure on the other eight NEPSY-II-NL subtests in the ECMO survivors was explored using multivariable linear regression analyses, as the relationship between cognition and white matter microstructure remains largely unknown in this group. FDR correction[19] was used to account for multiple testing for every set of analyses with the same NEPSY subtest. Results were considered statistically significant at the FDR-corrected $p < .05$.

Next, hippocampal volumes were compared between groups using ANCOVA. This was done separately for the right and left hippocampus, adjusted for age, gender and global brain volume. In ECMO survivors, multivariable linear regression analyses were performed to analyze the relationship between hippocampal volume and verbal memory, adjusted for age, gender and global brain volume.

SPSS Statistics Version 22.0 (Armonk, NY: IBM Corp.) and the Stats package in R Statistical Software version 3.1.3 (R Core Team, 2014) were used for statistical analyses and the FDR correction, respectively. For all regression analyses, the regression coefficient beta (β) and p-values are reported. Multicollinearity was not found in the models (variance inflation factors < 2.5[18]). Effect sizes were calculated using partial eta squared (h_p^2). Results were considered statistically significant at $p < .05$.

RESULTS

Study population

The ECMO group consisted of 23 children (11 boys, 12 girls) with a mean age (SD) of 11.9 (2.6) years. All ECMO survivors attended regular education, but 35% (n = 8) needed extra help in school. The comparison group consisted of 54 children (22 boys, 32 girls) with a mean age (SD) of 11.4 (2.5) years. All healthy children attended regular education. Age at MRI, gestational age and gender did not differ between groups (p = .34; p = .57; p = .57, respectively).

None of the ECMO survivors (n = 23) had abnormal neonatal cranial ultrasounds or suffered cardiac arrests. Other clinical characteristics are reported in Table 1. Consistent with previous results, mean IQ (SD) did not differ between the diagnostic groups in the ECMO survivors (MAS: 100 (23); CDH: 95 (8); other diagnoses: 107 (15)), p = .70.[6]

DTI results

ECMO survivors had significantly lower global FA (n = 23, global FA = .368) compared to healthy children (n = 54, global FA = .381), p = .02, h_p^2 = .07. Further analyses were performed on the individual tracts. Significantly lower FA was found in the left cingulum bundle (CB) of ECMO survivors, p < .001, h_p^2 = .13 (Table 2, Figure 1A). Because the CB is a large bundle in which functional differences between the anterior and posterior parts have been demonstrated[20], we additionally analyzed the left anterior and posterior cingulate bundles (CBa, CBp) separately. We found significantly lower FA in patients in the left CBa and CBp (Table 2).

Global MD did not differ between ECMO survivors (global MD = .803) and healthy children (global MD = .794), p = .41, h_p^2 = .01. Because DTI has not been used in neonatal ECMO survivors before, further analyses on MD in the individual tracts were conducted. Higher MD was found in the left parahippocampal cingulum (PHC) in ECMO survivors, p < .001, h_p^2 = .12 (Table 2, Figure 1B).

White matter microstructure and clinical characteristics in ECMO survivors

None of the clinical characteristics significantly predicted global FA or MD. Higher oxygenation indices before ECMO predicted higher FA in the right CB, β = .50, p = .03. Longer duration of mechanical ventilation, β = .36, p = .03, and younger age at the start of ECMO predicted higher MD in the left PHC, β = -.62, p = .002.

White matter microstructure and neuropsychological outcome in ECMO survivors

Global MD significantly predicted cued and free recall verbal memory, β = -.74, p = .01 (n = 17) (Figure 2). Global FA (p = .20), FA in the left CB (p = .97), and MD in the left PHC (p =

Table 2. FA and MD group differences in white matter tracts

Tract	Hemisphere	Mean FA Controls	Mean FA Patients	$p_{uncor.}$	p	η_p^2	Mean MD Controls	Mean MD Patients	$p_{uncor.}$	p	η_p^2
UNC	Left	.347 (.03)	.334 (.03)	.69	.83	.00	.819 (.03)	.826 (.03)	.92	.93	.00
	Right	.361 (.03)	.346 (.02)	.46	.71	.01	.813 (.03)	.817(.02)	.57	.80	.01
IFO	Left	.415 (.03)	.401 (.02)	.81	.93	.00	.808 (.03)	.815 (.03)	.93	.93	.00
	Right	.422 (.03)	.407 (.03)	.66	.83	.00	.808 (.03)	.813 (.02)	.42	.71	.01
SLF	Left	.367 (.03)	.357 (.03)	.32	.71	.01	.769 (.03)	.779 (.03)	.40	.71	.01
	Right	.367 (.03)	.361 (.02)	.07	.28	.04	.769 (.03)	.775 (.03)	.48	.71	.01
ILF	Left	.401 (.02)	.388 (.02)	.40	.71	.01	.822 (.03)	.829 (.03)	.68	.83	.00
	Right	.407 (.02)	.397 (.02)	.89	.93	.00	.833 (.03)	.836 (.03)	.22	.60	.02
CB	**Left**	**.399 (.05)**	**.345 (.04)**	.002	**.02**	**.13**	.761 (.04)	.782 (.04)	.06	.28	.05
	Anterior	**.376 (.05)**	**.321 (.04)**	**.00**		**.13**					
	Posterior	**.429 (.06)**	**.381 (.04)**	**.02**		**.07**					
	Right	.368 (.05)	.323 (.04)	.01	.10	.08	.762 (.03)	.778 (.03)	.12	.35	.03
PHC	Left	.271 (.03)	.249 (.03)	.11	.35	.04	**.871 (.05)**	**.916 (.06)**	.002	**.02**	**.12**
	Right	.275 (.03)	.259 (.03)	.39	.71	.01	.897 (.05)	.932 (.06)	.03	.20	.06

Results of ANCOVA's showing differences between patients (n = 23) and controls (n = 57) on all association and limbic system fiber tracts. Additional analyses on the anterior and posterior parts of the cingulum were done only for FA in the left cingulum bundle as differences between patients and controls were found in this specific tract. Mean weighted average FA (SD) is given for each tract per group. The partial eta squared (η_p^2) is given as an effect size. The size of the effect is interpreted according to Cohen's guidelines[23] which states 0.01 to be a small effect size, 0.06 to be a medium effect size, and 0.14 to be large. Age, gender and global FA or MD were added as covariates in the ANCOVA's. **FDR-corrected *p*-values** <.05 were considered statistically significant. $P_{uncor.}$ gives the uncorrected *p*-value. Abbreviations: FA, fractional anisotropy; MD, mean diffusivity; UNC, uncinate fasciculus; ILF, inferior longitudinal fasciculus; IFO, inferior fronto-occipital fasciculus; SLF, superior longitudinal fasciculus; CB, cingulum bundle; PHC, parahippocampal cingulum bundle.

.21) were not related to verbal memory. None of the other subtests of the NEPSY-II were related to the white matter alterations (Supplementary Table 1).

Hippocampal volume

ECMO survivors had significantly smaller mean hippocampal volume (SD) than healthy children, in the left, $p < .001$, $h_p^2 = .41$ (patients = 3597 (407), n = 23; comparison group = 4245 (398), n = 43), and right hemispheres, $p < .001$, $h_p^2 = .22$ (patients = 3646 (515); comparison group = 4111 (490)).

Left, $\beta = .65$, $p = .02$, and right, $\beta = .71$, $p = .01$, hippocampal volumes were associated with verbal memory performance in ECMO survivors (n = 17).

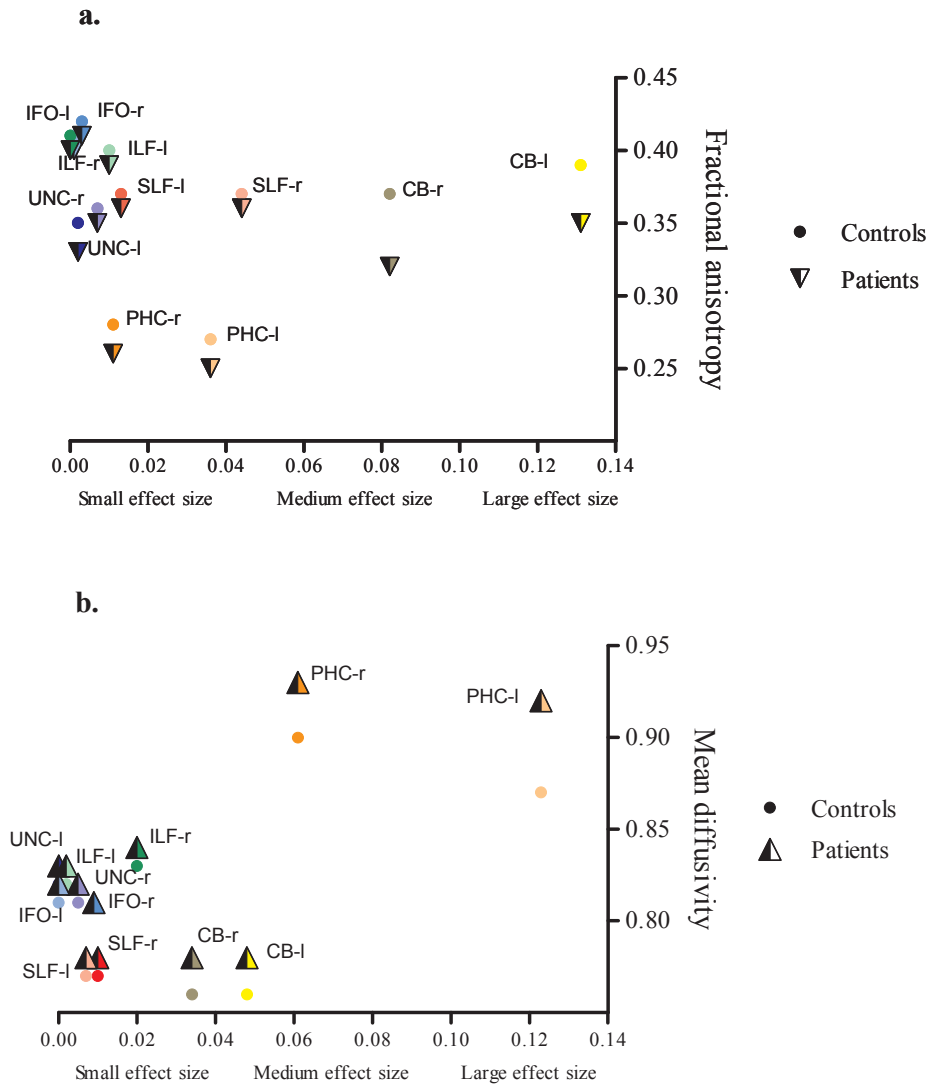

Figure 1. FA/MD differences between ECMO patients and healthy controls

Figures 1a and 1b show FA and MD (10^{-3} mm^2/s) for the individual white matter tracts of patients and controls. Effect sizes (partial eta squared) are used to show the magnitude of the difference between groups. Effect sizes of 0.01 are considered to be small, 0.06 to be medium, and 0.14 to be large(23). In Figure 1a, FA in the left CB differs significantly between patients and controls. In Figure 1b, MD in the left parahippocampal cingulum differs significantly between patients and controls. Abbreviations: -l, left; -r, right; FA, fractional anisotropy; UNC, uncinate fasciculus; ILF, inferior longitudinal fasciculus; IFO, inferior fronto-occipital fasciculus; SLF, superior longitudinal fasciculus; CB, cingulum bundle; PHC, parahippocampal cingulum bundle.

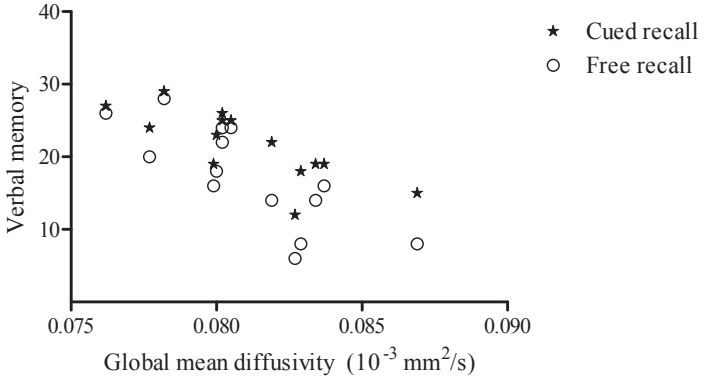

Figure 2. Verbal memory and white matter integrity
Scatterplot showing the relationship between global Mean Diffusivity (MD) and cued and free recall verbal memory in neonatal ECMO patients. Verbal memory minimum and maximum scores: 0-34.

DISCUSSION

We found global white matter alterations and smaller bilateral hippocampal volume in school-age neonatal ECMO survivors compared to healthy children, as well as specific white matter alterations in the CB and PHC. Lower verbal memory performance in ECMO survivors was related to higher global MD and smaller bilateral hippocampal volume. These results suggest long-term brain alterations that persist through childhood and into adolescence.

Global FA was significantly lower in ECMO survivors. FA has been suggested to be a measure of coherence of hydrogen diffusion within microstructures (i.e. axons, microtubules) and to be lower in disorganized white matter tracts.[21] Lower global FA may indicate long-term injury to neural tracts. This is in line with earlier results showing brain alterations following neonatal hypoxia and ECMO treatment.[9] Interestingly, global MD did not differ between ECMO survivors and healthy children. This may be because the white matter alterations are specific (i.e. disorganization of axon fibers), leaving other structures (i.e. cellular structures) relatively intact.[11] However, this remains speculative as FA and MD are summary parameters that cannot give detailed histological information.

We recently demonstrated verbal memory deficits in both 17-year-old ECMO survivors and in the ECMO survivors that are part of this study.[5,10] In the latter group, we found that higher global MD predicted lower verbal memory. It is unclear why MD rather than FA predicted verbal memory, as only global FA differed between ECMO survivors and healthy children. Nonetheless, our findings are in line with a previous neuroimaging study showing lower white matter volume and memory deficits in 11-to-13 year-old neonatal ECMO survivors.[9]

Lower FA was found in the entire left CB in neonatal ECMO survivors, indicating aberrations along the entire tract. Even though global MD did not differ, ECMO survivors had significantly higher MD in the left PHC. The limbic system fibers (i.e. CB and PHC) have been found to develop rapidly in the first six months of life, causing FA to increase and MD to decrease.[11] Because our participants were critically ill in the first weeks of life, the development of these fibers may be at risk.

We found white matter alterations only in the left hemisphere. Left side predominance of brain injury following neonatal ECMO has been previously reported using cranial ultrasound.[4] Of note, before correcting for multiple comparisons, right hemisphere differences in the CB and PHC were also found. Furthermore, unlike the small effect sizes found in the majority of the tract comparisons, differences in the right CB and PHC showed medium effect sizes.[22] The lack of right hemisphere differences may thus be due to a small sample size rather than being indicative of an increased left hemisphere vulnerability.

CB or PHC alterations in the ECMO survivors were not associated with verbal memory. The CB is a large bundle involved in various cognitive functions, including working- and visuospatial memory and attention.[23] The CB alterations may thus be specific to other neuropsychological deficits that have been observed following neonatal ECMO.[5-7] Furthermore, the verbal memory task used focused on episodic memory, whereas the parahippocampal region seems involved in semantic memory.[24] As episodic memory has been associated with the hippocampus, a structure vulnerable to hypoxic injuries, hippocampal rather than CB or PHC alterations may partly explain the verbal memory deficits observed.[9] Indeed, we found smaller bilateral hippocampal volume in ECMO survivors compared to controls. Moreover, smaller hippocampal volume was associated with worse verbal memory in ECMO survivors.

Other than verbal memory, no relationships were found between performance on the NEPSY-II and white matter integrity. This suggests specificity between the verbal memory deficit and hippocampal volume in ECMO survivors. Nonetheless, ECMO survivors have been shown to be at risk for working-memory, visuospatial memory and attention impairments, as well as school failure.[5] Subtle neuropsychological deficits have been shown to be difficult to detect with the NEPSY-II[25], which could explain the lack of findings with some of the subtests. In future studies it is therefore critical to use tests specifically designed to measure these types of deficits. Such outcomes should be combined with neuroimaging to improve our understanding of brain alterations and their clinical impact following neonatal ECMO.

No clinical predictors of global white matter were found, but longer duration of mechanical ventilation and younger age at the start of ECMO negatively influenced MD in the left PHC. These findings provide some support that severity of illness negatively influences outcome.[6,9] However, we found that a higher OI prior to ECMO was associated

with higher FA in the right CB. As higher FA is generally associated with better organization of white matter, these analyses should be replicated by future studies before any firm conclusions can be drawn.

Our study has some limitations. First, the small size of the patient group restricts our findings to those with moderate to large effect sizes and limits the interpretability of the multivariable regression analyses. Second, more elaborate neuropsychological assessment offering finer details of specific neuropsychological domains is better suited to assess white matter alterations in ECMO survivors. Third, while we do have age, gender and NEPSY outcomes of the healthy controls, we do not have IQ scores of this group. However, because all children performed within normal ranges on the NEPSY and attended regular education, they are likely to have average intelligence.[13] Fourth, all patients had been treated with VA ECMO. However, while the application of VV ECMO has increased, VA ECMO remains the most frequently used modality for neonatal respiratory or cardiac failure.[1] Lastly, we have limited clinical information of the patients due to lack of a digital patient management system (introduced in our unit in 2003) at the time of treatment.

Despite the limitations, this study is the first to use DTI to study white matter microstructure in school-age neonatal ECMO survivors and show long-term global and specific white matter alterations in neonatal ECMO survivors. Global MD alterations and lower hippocampal volume were associated with worse verbal memory performance in patients. These results help define the underlying neurobiology involved in the long-term neuropsychological deficits following ECMO. Furthermore, severity of illness may have partly influenced white matter development. The use of advanced neuroimaging techniques such as DTI may contribute to better risk stratification and earlier identification of long-term neurodevelopmental impairments in critically ill infants.

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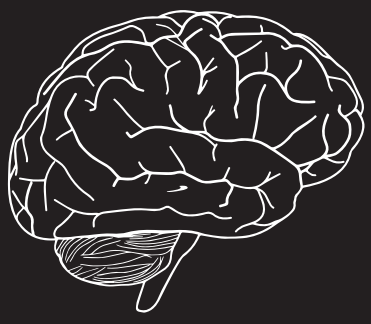
CHAPTER 6



NEUROBIOLOGIC CORRELATES OF ATTENTION AND MEMORY DEFICITS FOLLOWING CRITICAL ILLNESS IN EARLY LIFE

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ABSTRACT

Objective Survivors of critical illness in early life are at risk of long-term memory and attention impairments. However, their neurobiological substrates remain largely unknown.

Design A prospective follow-up study.

Setting Erasmus MC-Sophia Children's Hospital, Rotterdam, the Netherlands.

Patients Thirty-eight school-age (8-12 years) survivors of neonatal extracorporeal membrane oxygenation (ECMO) and/or congenital diaphragmatic hernia (CDH) with an IQ ≥ 80 and a below average score (z-score ≤ -1.5) on one or more memory tests.

Interventions None.

Measurements and Main Results Intelligence, attention, memory, executive functioning and visuospatial processing were assessed and compared with reference data. White matter microstructure and hippocampal volume were assessed using Diffusion Tensor Imaging and structural MRI, respectively. Global fractional anisotropy (FA) was positively associated with selective attention ($\beta = 0.53$, $p = .030$) and sustained attention ($\beta = 0.48$, $p = .018$). Mean diffusivity (MD) in the left parahippocampal region of the cingulum (PHC) was negatively associated with visuospatial memory, both immediate ($\beta = -0.48$, $p = .030$) and delayed recall ($\beta = -0.47$, $p = .030$). MD in the PHC was negatively associated with verbal memory delayed recall (left: $\beta = -0.52$, $p = .021$; right: $\beta = -0.52$, $p = .021$). Hippocampal volume was positively associated with verbal memory delayed recall (left: $\beta = 0.44$, $p = .037$; right: $\beta = 0.67$, $p = .012$). ECMO treatment or ECMO type did not influence the structure-function relationships.

Conclusions Our findings indicate specific neurobiological correlates of attention and memory deficits in school-age survivors of neonatal ECMO and/or CDH. A better understanding of the neurobiology following critical illness, both in early and in adult life, may lead to earlier identification of patients at risk for impaired neuropsychological outcome with the use of neurobiological markers.

INTRODUCTION

The number of children admitted to neonatal intensive care units has increased over the last decade and medical improvements have led to higher survival rates.(1) The long-term neurodevelopment following critical illness in early life is therefore, now more than ever, of major concern. Previous studies have shown that growing up after critical illness in early life, either due to prematurity, specific forms of cardiac anomalies or major congenital anomalies, is associated with neuropsychological deficits and school problems. Attention and memory deficits have been reported in pre-adolescent and adolescent survivors, irrespective of underlying cause or birthweight.(2-7) However, the neurobiological substrates of these impairments remain largely unknown.

A clearly delimited group of critical illness survivors are children treated with neonatal extracorporeal membrane oxygenation (ECMO) or congenital diaphragmatic hernia (CDH) treated without ECMO. Recently, we showed altered global white matter microstructure and specific alterations in limbic system regions in 11-year-old ECMO survivors.(8) Hippocampal volume was positively associated with verbal memory.(8) However, as only a limited cognitive assessment was available, specific aspects of neuropsychological outcome in relation to brain alterations have yet to be explored. This is of interest as, despite a generally average IQ, not only verbal memory but visuospatial memory and attention deficits have been shown following ECMO and CDH treated without ECMO.(2,5,7)

The identification of impaired neurodevelopment currently relies solely on neuropsychological assessment. Understanding the neurobiological correlates of impaired outcome may lead to earlier identification of children at risk with the use of advanced neuroimaging techniques. In this study, we aimed to find neurobiological substrates of neuropsychological deficits in school-age (8-12 years) survivors of critical illness in early life by combining elaborate neuropsychological assessment with structural MRI and diffusion tensor imaging (DTI). We hypothesized that previously demonstrated brain alterations in neonatal ECMO survivors (i.e. in global white matter microstructure, white matter microstructure in the cingulum bundle and parahippocampal region of the cingulum, and hippocampal volume(8)) would be specifically associated with memory and attention deficits. We expect our findings to aid in earlier identification of patients at risk of long-term cognitive deficits with the use of neurobiological markers.

MATERIALS AND METHODS

Population

Participants of an ongoing trial on working-memory training (NTR4571) at the Erasmus MC-Sophia Children's Hospital with usable neuroimaging data at baseline were included. Inclusion criteria for the trial were: school-age (8-12 years) children treated with ECMO or treated for CDH without ECMO in the first weeks of life at the Erasmus MC-Sophia Children's Hospital in Rotterdam or the Radboud University Medical Center in Nijmegen (the Netherlands), IQ ≥ 80 and memory impairment (*z*-score ≤ -1.5 on at least one (working) memory test(9)). Children who met the inclusion criteria underwent neuroimaging. ECMO treatment had been applied in case of reversible severe respiratory failure using the entry criteria by Stolar et al(10): oxygenation index > 25 with 3-hour intervals, persistent low pH (< 7.15) for 3-6 hours, and non-responding to changes in therapy. Entry criteria for ECMO did not change over time. Exclusion criteria were: psychopharmaceutic drugs (e.g. methylphenidate) and/or genetic syndromes known to affect neuropsychological functioning.

This study was performed in compliance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) and approved by the Institutional Review Board (MEC-2014-001). All parents and children ≥ 12 years signed an informed consent prior to their inclusion in the study. The neuropsychological and neuroimaging data collected at baseline are presented in this study.

Neuropsychological assessment

Validated neuropsychological tests, with Dutch validated reference values, were used to assess cognitive skills across four domains (brief test descriptions in Supplemental Digital Content 1):

1. Intelligence:
    a. Two-subtest short-form (Block Design and Vocabulary)(11) of the Wechsler Intelligence Scale for Children (WISC-III-NL)(12).
2. Attention:
    a. Sustained attention: Dot Cancellation Test (DCT)(13).
    b. Selective attention: Trail Making Test B (TMTB)(9), STROOP color-word test (STROOP)(9).
    c. Processing speed: Trail Making Test A (TMTA)(9).
3. Memory:
    a. Working-memory: subtest Digit Span of the WISC-III-NL(12), subtest Spatial Span of the Wechsler Nonverbal Scale of Ability (WNV)(14).
    b. Verbal memory: Rey Auditory Verbal Learning Test (RAVLT)(15).
    c. Visuospatial memory: Rey Complex Figure Test (RCFT)(16).

4. Executive functioning:
    a. Subtests Key Search and Modified Six Elements of the Behavioral Assessment of the Dysexecutive Syndrome(17).

Neuroimaging

All children first underwent a mock scanning session to become familiarized with the MR-environment.(18) MRI data were acquired on a 3 Tesla GE MR-750 system using an 8-channel head coil (General Electric, Milwaukee, WI). A full description of the methods is provided in Supplemental Digital Content 2. After DTI data processing, the voxel-wise scalar maps fractional anisotropy (FA) and mean diffusivity (MD) were computed. FA indicates the degree of directionality of water diffusion and ranges from 0 to 1. MD is the rate of diffusion of water (hydrogen) averaged in all directions. The FSL plugin 'AutoPtx' for fully automated probabilistic fiber tractography was used to create subject-specific, probabilistic representations of multiple white matter bundles.(19) Automated(20) and visual inspection of the data left 33 DTI datasets (87%) and 35 structural MRI datasets (92%) with usable image quality. All scans were reviewed by a board-certified neuroradiologist (M.S.), blinded for medical history and outcome. No serious clinically relevant abnormalities were reported. In one patient with a hemorrhagic infarct in the posterior middle cerebral artery as shown on neonatal cranial ultrasound, abnormalities (ulegyria) in this region were still visible on the long-term MRI scan. Sensitivity analyses were performed without this child's data. As the results did not change, the child was not excluded from the analyses.

Statistical analysis

Neuropsychological test scores were converted to z-scores (individual score minus the population mean divided by the population SD). Scores were inverted where appropriate so that higher scores always equated with better performance. Described in more detail in our previous study(8), global white matter microstructure was calculated using a weighted (by tract volume) average score of FA/MD of the association and limbic system fibers (uncinate, inferior fronto-occipital fasciculus, superior longitudinal fasciculus, inferior longitudinal fasciculus, cingulum bundle and parahippocampal part of cingulum (PHC)):

Formula 1:

$$\text{Global FA} = \frac{\Sigma_{i=1}^{6} FAtract_i Voltract_i}{\Sigma_{i=1}^{6} Voltract_i}$$

Where *i* denotes the tract, and *Vol* the volume of the tract. The same formula was used to calculate global MD.

Our primary aim was to examine whether previously reported brain alterations in ECMO survivors (global white matter structure, FA in the cingulum bundle, MD in the PHC and hippocampal volume(8)) were associated with specific neuropsychological deficits in ECMO survivors and survivors of CDH treated without ECMO. Linear regression analyses were used to assess the associations between the brain regions of interest and neuropsychological outcome. Brain structures were analyzed in the left and right hemispheres separately. If a significant association existed between an individual white matter tract and neuropsychological outcome, global FA/MD was added to identify whether associations were specific to an individual white matter tract above and beyond a global effect. The False Discovery Rate (FDR) correction(21) was applied once for each set of analyses between the same neuropsychological task and white matter microstructure outcomes (i.e. once for every six tests). The same method was used for the analyses between neuropsychological outcome and hippocampal volume. In post-hoc analyses, we adjusted for IQ to assess whether the structure-function relationships were specific to that neuropsychological domain or driven by general intellectual functioning.

Next, we assessed whether ECMO treatment (yes/no) or type of ECMO treatment (veno-arterial ECMO [VA-ECMO]/veno-venous ECMO [VV-ECMO]) influenced the associations between the brain regions of interest and neuropsychological outcome using linear regression analyses.(5, 22)

Finally, we determined if neuropsychological outcome was significantly different from the general population (mean z-score = 0; SD = 1) using one-sample t-tests, to inform us about the potential meaning of a significant structure-function relationship.

SPSS Statistics Version 22.0 (Armonk, NY: IBM Corp.) was used for the statistical analyses. Normality tests were performed for all data and assumptions of ANCOVA were checked and met before analyses were conducted. No multicollinearity was found (variance inflation factors < 2.5 (23)). In all regression analyses, we adjusted for age at the time of assessment and gender.(24) Total brain volume was included in the analyses with hippocampal volume.(25) The standardized regression coefficient beta (β), uncorrected (p_{uncor}) and FDR-corrected p-values were reported. Effect sizes were calculated using partial eta squared (h_p^2) and interpreted according to Cohen's guidelines (0.01 = small, 0.06 = medium, 0.14 = large)(26). Results were considered statistically significant at FDR-corrected $p < .05$.

RESULTS

Study population

Thirty-eight children participated (15 girls, 23 boys) with a mean age (SD) of 9.7 (1.5) years. Twenty-six children (68%) had been treated with ECMO (MAS = 18; CDH = 2;

Other = 6) and 12 CDH patients (32%) had not required ECMO. Seventeen ECMO patients (65%) had undergone VA-cannulation and nine (35%) VV-ECMO. Patient characteristics retrieved from medical records are reported in Table 1.

Table 1. Patient characteristics

Patient characteristics	All (n = 38)	ECMO (n = 26)	CDH non-ECMO (n = 12)
Age at assessment (years)	9.7 ± 1.5	9.6 ± 1.7	10.0 ± 1.2
Gestational age (weeks)	40.0 ± 0.3	41 ± 1.4	39 ± 2.3
Birth weight (grams)	3410 (3205-3790)	3595 (3296-3822)	3133 (2414-3423)
Male	23 (61%)	14 (54%)	9 (75%)
Dutch ethnicity	32 (84%)	20 (77%)	12 (100%)
Days of mechanical ventilation	10 (8-16)	11 (9-17)	9 (6-16)
Neonatal brain abnormalities[1]	3 (9%)	3 (13%)	0
Inborn	7 (18%)	1 (4%)	6 (50%)
Diagnosis			
MAS		18 (69%)	
CDH		2 (8%)	12 (100%)
Other[2]		6 (23%)	
Age start ECMO (days)		2 (1-3)	
Duration of ECMO (hours)		114 (89-181)	
Type of ECMO			
VA		16 (61%)	
VV		9 (35%)	
VV to VA conversion		1 (4%)	

Data are expressed as mean ± SD, median (IQR) or number (percentage), as appropriate.

[1] Abnormalities seen on cranial ultrasound or MRI in neonatal period: hemorrhagic infarct in the posterior middle cerebral artery (n= 1), bilateral thalamic lesions (n=2).

[2] Other diagnoses are PPHN (n=3), respiratory insufficiency due to respiratory syncytial virus (n=2), mono-ventricular heart with transposition of the great vessels (n=1).

Abbreviations: ECMO, extracorporeal membrane oxygenation; CDH, congenital diaphragmatic hernia; MAS, meconium aspiration syndrome; PPHN, persistent pulmonary hypertension of the newborn.

White matter microstructure and neuropsychological outcome

Lower global FA was associated with worse selective attention, $\beta = 0.45$, $p = .036$, $h_p^2 = 0.24$, and sustained attention, $\beta = 0.48$, $p = .018$, $h_p^2 = 0.23$ (Table 2). Lower FA in the right cingulum bundle was associated with worse sustained attention, $\beta = 0.46$, $p = .018$, $h_p^2 = 0.24$, whereas the association with FA in the left cingulum bundle disappeared after correcting for multiple testing, $\beta = 0.40$, $p = .046$, $h_p^2 = 0.26$. These findings were no longer significant when global FA was added (Table 2).

Table 2. White matter microstructure and neuropsychological outcome

Neuropsychological test, n = 33	Global FA	FA CB left	FA CB right	Global MD	MD PHC left	MD PHC right
Intelligence						
WISC-III-NL	β = 0.16, $p_{uncor.}$= .426, p = .680	β = 0.08, $p_{uncor.}$ = .680, p = .680	β = 0.26, $p_{uncor.}$= .158, p = .474	β = -0.13, $p_{uncor.}$= .564, p = .680	β = 0.09, $p_{uncor.}$= .642, p = .680	β = 0.27, $p_{uncor.}$= .146, p = .474
Attention						
TMT A	β = 0.20, $p_{uncor.}$= .324, p = .648	β = 0.15, $p_{uncor.}$= .468, p = .702	β = 0.23, $p_{uncor.}$= .227, p = .648	β = 0.12, $p_{uncor.}$= .587, p = .704	β = -0.00, $p_{uncor.}$= .983, p = .983	β = 0.32, $p_{uncor.}$= .083, p = .498
TMT B	**β = 0.53, $p_{uncor.}$= .005, p = .030**	β = 0.22, $p_{uncor.}$= .277, p = .397	β = 0.36, $p_{uncor.}$= .045, p = .135	β = -0.35, $p_{uncor.}$= .103, p = .206	β = -0.19, $p_{uncor.}$= .331, p = .397	β = 0.13, $p_{uncor.}$= .489, p = .489
Stroop interference	β = -0.14, $p_{uncor.}$= .450, p = .675	β = -0.32, $p_{uncor.}$= .064, p = .384	β = -0.01, $p_{uncor.}$= .966, p = .969	β = 0.24, $p_{uncor.}$= .223, p = .460	β = 0.01, $p_{uncor.}$= .969, p = .969	β = 0.20, $p_{uncor.}$= .230, p = .460
DCT	**β = 0.48, $p_{uncor.}$= .006, p = .018**	β = 0.40, $p_{uncor.}$= .023, p = .046*	**β = 0.46, $p_{uncor.}$= .004, p = .018***	β = -0.35, $p_{uncor.}$= .069, p = .104	β = -0.29, $p_{uncor.}$= .110, p = .132	β = -0.08, $p_{uncor.}$= .620, p = .620
Verbal memory						
Digit span	β = 0.08, $p_{uncor.}$= .685, p = .685	β = 0.14, $p_{uncor.}$= .484, p = .581	β = 0.26, $p_{uncor.}$= .158, p = .358	β = 0.25, $p_{uncor.}$= .257, p = .386	β = 0.32, $p_{uncor.}$= .108, p = .358	β = 0.25, $p_{uncor.}$= .179, p = .358
RAVLT immediate	β = 0.32, $p_{uncor.}$= .111, p = .333	β = 0.22, $p_{uncor.}$= .274, p = .411	β = 0.09, $p_{uncor.}$= .631, p = .631	β = -0.21, $p_{uncor.}$= .350, p = .420	β = -0.41, $p_{uncor.}$= .036, p = .216	β = -0.25, $p_{uncor.}$= .192, p = .384
RAVLT delayed	β = 0.21, $p_{uncor.}$= .313, p = .470	β = 0.04, $p_{uncor.}$= .851, p = .851	β = -0.04, $p_{uncor.}$= .831, p = .851	β = -0.24, $p_{uncor.}$= .284, p = .670	**β = -0.52, $p_{uncor.}$= .007, p = .021**	**β = -0.52, $p_{uncor.}$= .004, p = .021**
RAVLT recognition	β = 0.15, $p_{uncor.}$= .464, p = .873	β = 0.16, $p_{uncor.}$= .437, p = .873	β = 0.03, $p_{uncor.}$= .878, p = .893	β = 0.03, $p_{uncor.}$= .893, p = .893	β = -0.12, $p_{uncor.}$= .582, p = .873	β = -0.24, $p_{uncor.}$= .209, p = .873

Table 2. White matter microstructure and neuropsychological outcome (continued)

Neuropsychological test, n = 33	Global FA	FA CB left	FA CB right	Global MD	MD PHC left	MD PHC right
Visuospatial memory						
Spatial Span	β = 0.02, $p_{uncor.}$ = .913, p = .945	β = 0.04, $p_{uncor.}$= .848, p = .945	β = -0.10, $p_{uncor.}$= .945, p = .945	β = -0.02, $p_{uncor.}$= .913, p = .945	β = 0.13, $p_{uncor.}$= .519, p = .945	β = 0.25, $p_{uncor.}$= .164, p = .945
RCFT immediate	β = 0.18, $p_{uncor.}$= .320, p = .480	β = -0.05, $p_{uncor.}$= .778, p = .934	β = -0.01, $p_{uncor.}$= .963, p = .963	β = -0.30, $p_{uncor.}$= .131, p = .393	**β = -0.48, $p_{uncor.}$= .005, p = .030**	β = -0.20, $p_{uncor.}$= .243, p = .480
RCFT delayed	β = 0.23, $p_{uncor.}$= .203, p = .406	β = -0.02, $p_{uncor.}$= .932, p = .932	β = -0.04, $p_{uncor.}$= .806, p = .932	β = -0.29, $p_{uncor.}$= .130, p = .390	**β = -0.47, $p_{uncor.}$= .005, p = .030**	β = -0.16, $p_{uncor.}$= .324, p = .486
RCFT recognition	β = 0.36, $p_{uncor.}$= .056, p = .112	β = 0.18, $p_{uncor.}$= .346, p = .519	β = 0.05, $p_{uncor.}$= .765, p = .820	β = -0.44, $p_{uncor.}$= .028, p = .084	β = -0.42, $p_{uncor.}$= .022, p = .084*	β = 0.04, $p_{uncor.}$= .820, p = .820
Executive functioning						
Key Search	β = -0.04, $p_{uncor.}$= .808, p = .808	β = -0.26, $p_{uncor.}$= .138, p = .549	β = -0.12, $p_{uncor.}$= .461, p = .692	β = 0.25, $p_{uncor.}$= .183, p = .549	β = 0.06, $p_{uncor.}$= .752, p = .808	β = 0.15, $p_{uncor.}$= .357, p = .692
Modified six elements	β = 0.20, $p_{uncor.}$= .333, p = .635	β = 0.15, $p_{uncor.}$= .451, p = .635	β = 0.13, $p_{uncor.}$= .529, p = .635	β = -0.09, $p_{uncor.}$= .686, p = .686	β = -0.26, $p_{uncor.}$= .200, p = .600	β = -0.26, $p_{uncor.}$= .200, p = .600
Visuospatial processing						
RCFT copy	β = 0.24, $p_{uncor.}$ = .248, p = .743	β = 0.13, $p_{uncor.}$= .522, p = .743	β = 0.11, $p_{uncor.}$= .549, p = .743	β = -0.29, $p_{uncor.}$= .188, p = .743	β = 0.01, $p_{uncor.}$= .953, p = .953	β = 0.10, $p_{uncor.}$= .619, p = .743

Results of linear regression analyses assessing associations between our brain regions of interest(8) and neuropsychological outcome, adjusted for gender and age at assessment. *puncor.* gives the uncorrected *p*-value. ***P*** < .05 is considered statistically significant after correcting for multiple testing using the false discovery rate correction. *No longer significant when global measure of microstructure was added to the model.

Abbreviations: FA, fractional anisotropy; CB, cingulum bundle; MD, mean diffusivity; PHC, parahippocampal region of the cingulum; WISC-III-NL, Wechsler Intelligence Scale for Children Dutch version; TMT, Trail Making Test; DCT, Dot Cancellation Test; RAVLT, Rey Auditory Verbal Learning Test; RCFT, Rey Complex Figure Test.

Table 3. Hippocampal volume and neuropsychological outcome

Neuropsychological test (n= 38)	Hippocampal volume, left	Hippocampal volume, right
Intelligence		
WISC-III-NL	β = 0.12, $p_{uncor.}$= .583, p = .868	β = 0.04, $p_{uncor.}$= .868, p = .868
Attention		
TMT A	β = -0.04, $p_{uncor.}$= .862, p = .862	β = -0.08, $p_{uncor.}$= .799, p = .862
TMT B	β = -0.20, $p_{uncor.}$= .364, p = .470	β = -0.19, $p_{uncor.}$= .470, p = .470
Stroop interference	β = 0.10, $p_{uncor.}$= .645, p = .900	β = 0.03, $p_{uncor.}$= .900, p = .900
DCT	β = 0.07, $p_{uncor.}$= .716, p = .950	β = -0.02, $p_{uncor.}$= .950, p = .950
Verbal Memory		
Digit span	β = -0.10, $p_{uncor.}$= .624, p = .624	β = -0.42, $p_{uncor.}$= .073, p = .146
RAVLT immediate	β = 0.40, $p_{uncor.}$= .057, p = .114	β = 0.36, $p_{uncor.}$= .148, p = .148
RAVLT delayed	**β = 0.44, $p_{uncor.}$= .037, p = .037**	**β = 0.67, $p_{uncor.}$= .006, p = .012**
RAVLT recognition	β = 0.27, $p_{uncor.}$= .238, p = .238	β = 0.40, $p_{uncor.}$= .144, p = .238
Visuospatial memory		
Spatial Span	β = 0.04, $p_{uncor.}$= .842, p = .920	β = -0.03, $p_{uncor.}$= .920, p = .920
RCFT immediate	β = 0.30, $p_{uncor.}$= .133, p = .133	β = 0.39, $p_{uncor.}$= .103, p = .133
RCFT delayed	β = 0.22, $p_{uncor.}$= .264, p = .290	β = 0.24, $p_{uncor.}$= .290, p = .290
RCFT recognition	β = -0.05, $p_{uncor.}$= .825, p = .825	β = -0.08, $p_{uncor.}$= .748, p = .825
Executive Functioning		
Key Search	β = 0.17, $p_{uncor.}$= .358, p = .716	β = 0.04, $p_{uncor.}$= .859, p = .859
Modified six elements	β = 0.22, $p_{uncor.}$= .328, p = .328	β = 0.49, $p_{uncor.}$= .074, p = .074
Visual spatial processing		
RCFT copy	β = -0.00, $p_{uncor.}$= .987, p = .987	β = -0.04, $p_{uncor.}$= .889, p = .987

Results of linear regression analyses assessing associations between neuropsychological outcome and hippocampal volume in the left and right hemispheres. *Puncor.* gives the uncorrected *p*-value. ***P*** < .05 is considered statistically significant after correcting for multiple testing using the false discovery rate correction. Abbreviations: WISC-III-NL, Wechsler Intelligence Scale for Children Dutch version; TMT, Trail Making Test; DCT, Dot Cancellation Test; RAVLT, Rey Auditory Verbal Learning Test; RCFT, Rey Complex Figure Test.

No associations were found between global MD and any of the neuropsychological tests (Table 2). Higher MD in the left PHC was associated with worse visuospatial memory immediate recall, β = -0.48, p = .030, h_p^2 = 0.24, and delayed recall, β = -0.47, p = .030, h_p^2 = 0.24. MD in both the left and right PHC were also negatively associated with verbal memory delayed recall (left: β = -0.52, p = .021, h_p^2 = 0.23; right: β = -0.52, p = .021, h_p^2 = 0.26) (Table 2, Figure 1).

IQ did not affect the associations between white matter microstructure and neuropsychological outcome.

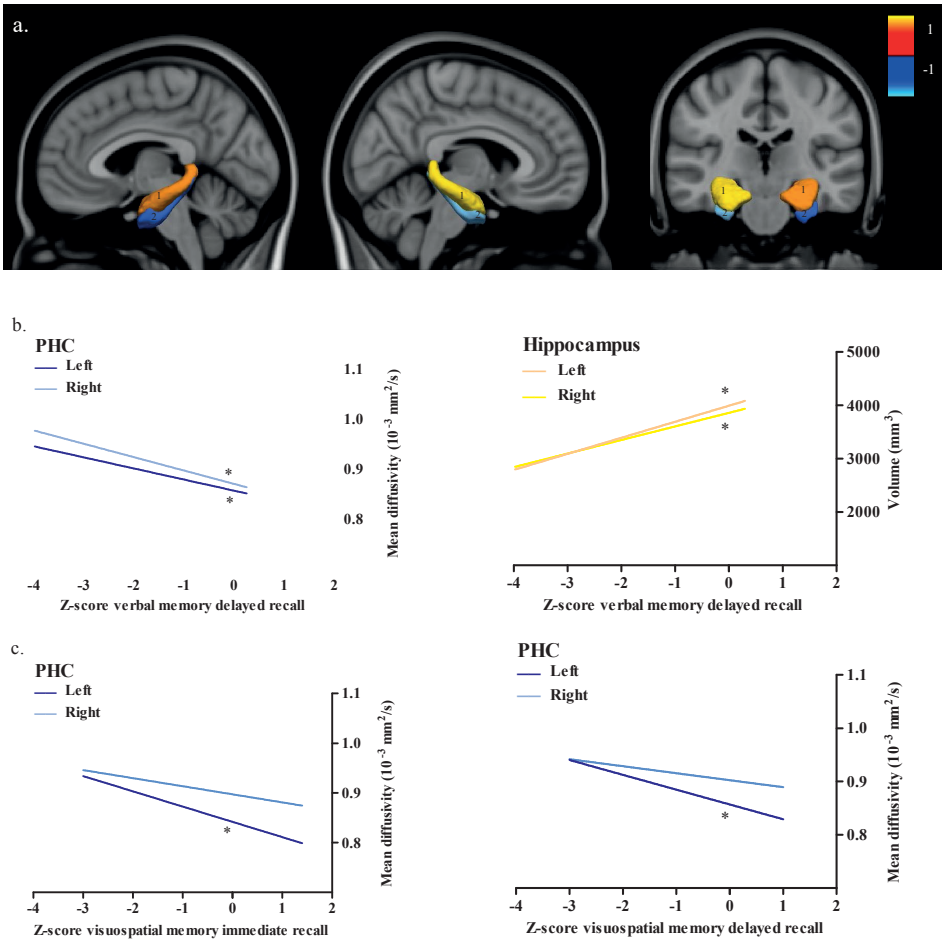

Figure 1. Associatons between brain alterations and neuropsychological outcome
Yellow and orange colors indicate a positive association and blue colors indicate a negative association between brain regions and neuropsychological outcome. a) Parasaggital and coronal views of the left and right hemispheres showing the left and right hippocampal volumes(1) and the left and right parahippocampal regions of the cingulum(2). b) Showing the associations between verbal memory delayed recall and mean diffusivity in the parahippocampal region of the cingulum and the hippocampal volume in both hemispheres. c) Showing the associations between visuospatial memory immediate and delayed recall and mean diffusivity in the parahippocampal region of the cingulum in both hemispheres. *Indicates a significant association at $p < .05$. Abbreviations: PHC, parahippocampal region of the cingulum.

Hippocampal volume and neuropsychological outcome

Smaller hippocampal volumes in the left and right hemispheres were associated with lower scores on verbal memory delayed recall (left: $\beta = 0.44$, $p = .037$, $h_p^2 = 0.16$; right: $\beta = 0.67$, $p = .012$, $h_p^2 = 0.14$) (Table 3, Figure 1). IQ did not affect these associations.

Treatment characteristics and neurodevelopmental outcome

The significant associations found between neuropsychological outcome and the brain were the same in ECMO and non-ECMO patients, and VA-ECMO and VV-ECMO patients (Supplemental Digital Content 3).

Neuropsychological functioning compared to the norm population

Participants had an average IQ but scored significantly lower on all verbal and visuospatial memory tasks (immediate recall, delayed recall and recognition). Participants also had significantly lower sustained attention than the reference population (Table 4).

Table 4. Overview of neuropsychological outcome

Neuropsychological test	All (n = 38)	*P* value[1]
Intelligence		
WISC-III-NL[2]	100 (12)	.968
Attention		
TMT A	-0.33 (0.95)	.057
TMT B	-0.03 (1.18)	.954
Stroop interference	-0.31 (1.00)	.063
DCT	**-1.13 (2.01)**	**.001**
Memory		
WISC-III-NL Digit span	0.21 (1.02)	.219
WNV Spatial Span	0.17 (1.02)	.336
RAVLT immediate	**-1.58 (1.10)**	**.000**
RAVLT delayed	**-1.90 (1.19)**	**.000**
RAVLT recognition	**-1.19 (1.59)**	**.000**
RCFT immediate	**-1.65 (0.97)**	**.000**
RCFT delayed	**-1.65 (1.07)**	**.000**
RCFT recognition	**-0.63 (1.45)**	**.007**
Executive functioning		
Key Search	0.01 (1.20)	.669
Modified six elements	-0.08 (0.99)	.652
Visual spatial processing		
RCFT copy	0.14 (0.68)	.205

Data are expressed as mean z-score (SD). ***P* < .05** is considered statistically significant.
[1]P value of the one-sample t-test assessing the difference between the average z-score of the participants and the general population z-score ($\mu = 0$).
[2]IQ was based on a short-form of the WISC-III-NL using two subtests, Vocabulary and Block Design.(11) Abbreviations: WISC-III-NL, Wechsler Intelligence Scale for Children Dutch version; TMT, Trail Making Test; DCT, Dot Cancellation Test; RAVLT, Rey Auditory Verbal Learning Test; RCFT, Rey Complex Figure Test.

DISCUSSION

The aim of this study was to identify neurobiological substrates of long-term impaired neuropsychological outcome following critical illness in early life. In CDH survivors treated with and without ECMO and in survivors of ECMO treatment following other diagnoses, specific associations were found between attention and memory deficits and global white matter microstructure and regions of the limbic system. These results – that were irrespective of ECMO or conservative ventilator management – provide more insight in the underlying neurobiology of long-term outcome following critical illness in early life. Furthermore, our findings were irrespective of general IQ. This supports a 'growing into deficit' phenomenon in these patients, where subtle brain injuries acquired at a young age become functionally evident when higher cognitive functioning is required at a later age.

The PHC and hippocampus are part of the limbic system, one of the main brain networks involved in memory. The PHC is bidirectionally connected to the hippocampus and forms a larger memory circuit together with cortical structures.(27) While the hippocampus is viewed as the main hub for memory, its connections are highly important for intact functioning of various memory types.(27,28) In line with this, our findings showed higher MD, suggestive of decreased integrity in axonal membranes, packing, or myelin(29), in the PHC to be associated with lower visuospatial and verbal memory. Further, smaller hippocampal volume was associated with impaired verbal memory. Interestingly, studies on other types of critical illness in early life have demonstrated similar structure-function relationships. Hippocampal alterations and related verbal memory impairments were previously found by our group in a different cohort of neonatal ECMO survivors, but also by others in survivors of neonatal hypoxia and in patients with severe forms of congenital heart disease.(3,8,30) In preterm born children, infant hippocampal volume was negatively associated with school-age verbal memory, and white matter volume in the PHC with non-verbal memory.(31,32)

The present study further showed that lower global FA was related to poorer attention. Lower FA can be interpreted as reduced coherence of white matter fibers.(29) Global white matter abnormalities have been associated with lower attention in preterm born children as well.(33) Although we also found a significant association between lower FA in the cingulum bundle and worse sustained attention, this association disappeared when global FA was added. These findings support the notion of more widespread white matter network alterations underlying attention impairments.(34)

In our study, the majority of significant structure-function relationships were found in the left and right hemispheres, although associations with FA in the left cingulum bundle disappeared after multiple testing correction. The associations with visuospatial memory were found only in the left PHC. Predominantly left hemispheric alterations

have been suggested to be due to right internal jugular vein cannulation in neonatal ECMO patients.(35) However, our results did not show an effect of ECMO. CDH survivors treated without ECMO showed structure-function relationships similar to ECMO-treated patients. Also, no differences were found between VA- and VV-ECMO treated patients. However, these findings should be interpreted with caution due to the small sample size. Furthermore, previous findings have shown more brain abnormalities in VA-ECMO-treated patients compared to VV-treated patients, but associations with neuropsychological outcome were not studied.(22) Future studies with larger sample sizes, also including near-ECMO patients, are needed to further delineate the effects of treatment or diagnosis on neurodevelopment following critical illness.

Although further research is needed, our results suggest similar neuropsychological deficits and structure-function relationships in CDH survivors treated with and without ECMO and ECMO survivors following other diagnoses. This may suggest potentially similar neurodevelopmental mechanisms following various types of critical illness in early life.(3,30-32) The brain matures in a nonlinear fashion from childhood into adulthood, indicating that the timing of microstructural changes differs per brain region.(24,36) Consequently, the timing of injuries is likely to have specific effects on brain development.(37) As the limbic system undergoes rapid development throughout the third trimester and neonatal period, these structures may be particularly vulnerable in critically ill newborns, born prematurely and at term.(38,39) Furthermore, the hippocampus is sensitive to both internal and external influences. Hypoxic-ischemic injury and (chronic) stress have been associated with hippocampal alterations in term and preterm infants.(40,41) White matter limbic system fibers, such as the PHC and cingulum bundle, may be more vulnerable to these types of injuries because of their connections with the hippocampus and their periventricular location.(37) Interestingly, similar long-term cognitive impairments were reported in adult patients after treatment at intensive care units (ICU).(42) Although newborns are likely to be at higher risk of limbic system alterations due to its rapid development during this time, hippocampal vulnerability has been shown in the adult brain as well(43), but needs further exploration in adult ICU survivors.

While this study contributes to the limited research on the neurobiology of neuropsychological outcome following critical illness, there are some limitations. First, our comparisons between ECMO and non-ECMO, and VA- and VV-ECMO treated patients should be interpreted with caution as the small sample size limits the interpretability of the regression analyses. Second, we did not have a (healthy) control group to compare our neuroimaging data with. Due to major differences in scanner hardware and software, we could not compare our data to data obtained elsewhere. We did however have normative data of the neuropsychological tests. Since we were primarily interested in finding potential neurobiological substrates of impaired outcome following critical illness, we were still able to adequately address these questions. Third, our sample includes only

children with a below average score on one or more memory tests and an IQ of 80 or above. However, since previous studies have shown that the majority of ECMO and/or CDH survivors have average intelligence but an increased likelihood of attention and memory impairments(2,5,7), we believe that this sample is representative of critical illness survivors and suits the aim of this study. Nonetheless, future studies should explore how below average IQ affects the structure-function relationships.

CONCLUSION

We showed that regions of the limbic system and global white matter microstructure were specifically related to impaired neuropsychological outcome in school-age survivors of CDH, treated with and without ECMO, and in ECMO survivors following other diagnoses. Our findings may lead to earlier identification of those at risk of neurodevelopmental impairment with the use of neurobiological markers, such as low hippocampal volume. Also, a better understanding of the neurobiology will contribute to a more critical appraisal of potential intervention modalities. As similar neurodevelopmental outcomes have been found in survivors of various causes of critical illness, future research should assess neurodevelopment longitudinally across different patient groups, using both neuroimaging and neuropsychological assessment, and compare outcomes to age-matched healthy controls.

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SUPPLEMENTARY MATERIAL

Supplemental Digital Content 1. Descriptions of the neuropsychological tests.

Intelligence

Wechsler Intelligence Scale for Children (WISC-III-NL)

A short-form with two subtests, Block Design and Vocabulary, of the WISC-III-NL were used to assess general intelligence.(1) The WISC-III-NL has been shown to have good reliability and validity.(2) A normalized population mean of 100 with a standard deviation of 15 is used.(2)

Attention

Dot Cancellation Test

This paper-and-pencil test measures sustained attention and concentration in terms of speed. It consists of a paper on which figures made of three, four or five dots are displayed in 33 rows. The child is instructed to mark all figures with four dots, as precise and as fast as they can.(3)

Stroop Color Word Test (Stroop)

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

Trail Making Test (TMT)

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

Memory

WISC-III-NL – subtest Digit Span

The Digit Span consists of random number sequences that increase in length and that the examiner reads aloud at the rate of 1 number per second. The child has to reproduce these numbers in the same order. Next, the sequences must be recalled backwards (3-

5-7 becomes 7-5-3). The first part of the test measures short-term auditory memory and short-term retention capacity. The second part measures auditory working memory.(2)

Wechsler Nonverbal Scale of Ability (WNV) – subtest Spatial Span

The Spatial Span requires the child to touch a group of blocks arranged on a board in a nonsystematic manner in the same and reverse order as demonstrated by the examiner. The first part of the test measures short-term visuospatial memory and short-term retention capacity. The second part measures visuospatial working-memory.(6)

Rey Auditory Verbal Learning Test (RAVLT)

The RAVLT consists of five presentations with recall of a 15-word list, a sixth recall trial after 30 minutes, and a seventh recognition trial. This test measures memory span, short- and long term verbal memory, verbal recognition, and learning curve. It can be administered to children and adults in the age range 6-89 years.(7, 8)

Memory and visual-spatial functioning

Rey Complex Figure Test (RCFT)

The RCFT consists of three trials. First the child has to copy a complex figure (Copy). Then after 3 and after 30 minutes the figure must be drawn from memory (Recall). Next, different figures are shown and the child has to indicate whether these figures were in the original figure (Recognition). This test measures visual integration, short- and long-term visual-spatial memory, and visual-spatial recognition. It can be completed by children and adults in the age range 6-89 years.(9, 10)

Executive functioning

Key Search

A test of strategy formation. The child is asked to demonstrate how they would search a field for a set of lost keys and their strategy is scored according to its efficiency and functionality.(11)

Modified Six Elements

The child is asked to work on six different tasks for which they have five minutes. The child needs to make sure that by the end of the five minutes, all six of the tasks have been done and the child has done as much as possible of each task. This is a test of planning, task scheduling and performance monitoring.(11)

We used Dutch versions of all tests.

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Supplemental Digital Content 2. Neuroimaging methods.

Image Acquisition

Prior to neuroimaging, all participants underwent a 30-minute mock scanning session to become familiarized with the MR-environment[1]. Magnetic resonance imaging data were acquired on a 3 Tesla GE MR-750W system using an 8 channel receive-only head coil (General Electric, Milwaukee, WI). In order to support the participant's head and minimize head motion, cushions were placed on both sides of the child's head inside of the head coil. Participants were able to watch a movie during the scans. MRI-compatible headphones were used to reduce the scanner noise and allow participants to listen to the movie's audio track. Communication with the MR operator was also enabled through the headphones before and after scans. The DTI data were acquired using a single-shot, echo-planar imaging sequence with the following parameters: TR = 12,500 ms, TE = 72 ms, flip angle = 90, matrix = 120 x 120, FOV = 240 mm x 240 mm, slice thickness = 2 mm, number of slices = 65, ASSET acceleration factor = 2. In total, 35 volumes with diffusion weighting (b = 900 s/mm^2) and 3 volumes without diffusion weighting (b = 0 s/mm^2) were acquired. The high-resolution structural T1-weighted images were acquired using an inversion recovery fast spoiled gradient recalled BRAVO sequence with the following parameters: TR=8.77ms, TE = 3.4ms, inversion time=600ms, flip angel=10, matrix

220x220, FOV=220mm x220mm, ARCimaging acceleration factor of 2, slice thickness = 1mm, and a final $1mm^3$ isotropic resolution.

MR-Image Preprocessing

Data were processed using the Functional MRI of the Brain's Software Library (FMRIB, FSL) (2) and the Camino Diffusion MRI Toolkit within Python (version 2.7) and the Neuroimaging in Python Pipelines and Interfaces package (Nipype, version 0.92)[3,4]. First, motion and eddy-current induced artifacts[5] were addressed using the FSL "eddy_correct" tool[6]. In order to account for the rotations applied to the diffusion data after adjusting for these artifact, the resulting transformation matrices were used to rotate the "B-matrix" gradient direction table[7,8]. The FSL Brain Extraction Tool was used to remove non-brain tissue[9]. In order to minimize the limitations observed with respect to the ordinary least squares fit method[10], the diffusion tensor was fit using the RESTORE method implemented in Camino[11]. Voxel-wise scalar maps (i.e. FA, MD) were then computed. FA is the degree of directionality of diffusion and ranges from 0 to 1, where a higher FA generally represents a greater coherence of white matter fibers. MD is the rate of diffusion of hydrogen averaged in all directions. Lower MD is suggestive of increased integrity in axonal membranes, packing, or myelin. White matter continues to mature throughout childhood, even into young adulthood, causing FA to increase and MD to decrease. Abnormal brain development typically leads to lower FA and higher MD in white matter tracts[12].

Probabilistic Fiber Tractography

Fully automated probabilistic fiber tractography was performed using the FSL plugin, "AutoPtx"[13]. Subject-specific, probabilistic representations of multiple white matter fiber bundles are created with this method using a combination of FSL tools from the Diffusion Toolkit (FDT). The Bayesian Estimation of Diffusion Parameters Obtained using Sampling Techniques (BEDPOSTx), accounting for two fiber orientations at each voxel, was used to esatimate diffusion parameters at each voxel[14,15]. Next, for each subject, the FA map was aligned to the FMRIB-58 FA template image with the FSL nonlinear registration tool (FNIRT). The inverse of this nonlinear warp field was computed, and applied to a series of predefined seed, target, exclusion, and termination masks provided by the AutoPtx plugin (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/AutoPtx). The FSL module "ProbtrackX" was then applied to conduct probabilistic fiber tracking using these supplied tract-specific masks (i.e., seed, target, etc.) in the native diffusion image space of each subject[14]. The connectivity distributions resulting from fiber tractography were normalized to a scale from 0 to 1 using the total number of successful seed-to-target attempts, and were subsequently thresholded to remove low-probability voxels likely related to noise. For each tract, the number of samples used for probabilistic tracking, and the probability thresholds applied to the resulting distributions (ILF: 0.005, SLF: 0.001, IFO: 0.01, UNC:

0.01, CB: 0.01, PHC: 0.02), were selected based on previously established values[13]. After thresholding the path distributions, weighted average DTI scalar measures were computed within each tract using the normalized path distributions as the weights. The methods used were based on those described by Muetzel et al[16].

Structural Image Acquisition and Analysis

The Freesurfer image analysis suite version 5.3.0 (http://surfer.nmr.mgh.harvard.edu/) for cortical reconstruction and volumetric segmentation was used. Freesurfer computes structural morphometric measures using a fully-automated approach. Technical procedures have been described extensively[19].

Image Quality Assurance

Raw DTI image quality was assessed with both a visual inspection and with automated software[16]. For the visual inspection, maps of the sum of squares error (SSE) of the tensor fit were inspected for structured signal that is consistent with motion and other artifacts in the diffusion-weighted images (e.g., attenuated slices in diffusion-weighted images). Furthermore, probabilistic tractography data were inspected visually to ensure images were properly aligned to the template and paths were reconstructed accurately[16]. Datasets determined to be of poor quality were excluded (n = 7, ~8%).

In addition to this visual inspection, slice-wise signal intensity was examined for attenuation resulting from motion, cardiac pulsation and other artifacts using the automated DTIprep quality control tool (http://www.nitrc.org/projects/dtiprep/). Four (~5%) additional datasets were excluded based on the DTIprep results, leaving 77 DTI datasets (patients = 23, controls = 54) for analysis.

FreeSurfer reconstructions were evaluated for accuracy through detailed visual inspections. Each image was visually inspected and subjects with poor quality data were excluded. In subjects with small errors in the gray/white segmentation, control points, and white matter edits were added to identify and correct misclassified white matter regions. When the segmentation improved, the corrected images were used.

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Supplemental Digital Content 3. Influence of ECMO treatment and type of ECMO on structure-function relationships

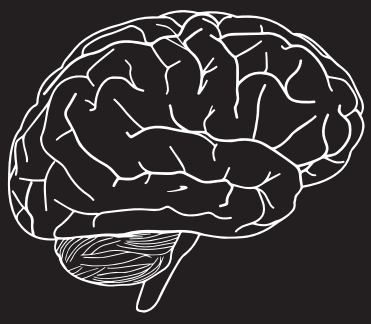
	DCT	RAVLT delayed recall	RCFT immediate recall	RCFT delayed recall
Global FA	***F*(1,29)=6.74, *p*=.015**			
ECMO treatment	*F*(1,29)=0.23, *p*=.635			
ECMO treatment*GlobalFA	*F*(1,29)=0.23, *p*=.638			
MD left PHC		***F*(1,29)=7.47, *p*=.011**	***F*(1,29)=13.05, *p*<.001**	***F*(1,29)=15.92, *p*<.001**
ECMO treatment		*F*(1,29)=0.00, *p*=.987	*F*(1,29)=0.24, *p*=.629	*F*(1,29)=1.57, *p*=.220
ECMO treatment*MDleftPHC		*F*(1,29)=0.00, *p*=.963	*F*(1,29)=0.27, *p*=.611	*F*(1,29)=1.68, *p*=.205
MD right PHC		***F*(1,29)=9.01, *p*=.005**		
ECMO treatment		*F*(1,29)=0.21, *p*=.647		
ECMO treatment*MDrightPHC		*F*(1,29)=0.29, *p*=.592		
Left hippocampal volume		*F*(1,31)=2.79, *p*=.105		
ECMO treatment		*F*(1,31)=0.05, *p*=.820		
ECMO treatment*LeftHippocampus		*F*(1,31)=0.06, *p*=.829		
Right hippocampal volume		***F*(1,31)=4.39, *p*=.044**		
ECMO treatment		*F*(1,31)=0.45, *p*=.507		
ECMO treatment*RightHippocampus		*F*(1,31)=0.27, *p*=.498		
Global FA	***F*(1,18)=8.05, *p*=.011**			
ECMO type	*F*(1,18)=0.03, *p*=.861			
ECMO type*GlobalFA	*F*(1,18)=0.04, *p*=.837			
MD left PHC		*F*(1,18)=3.60, *p*=.074	***F*(1,18)=8.05, *p*=.011**	*F*(1,18)=4.54, *p*=.047
ECMO type		*F*(1,18)=0.16, *p*=.696	*F*(1,18)=0.15, *p*=.704	*F*(1,18)=0.11, *p*=.738
ECMO type*MDleftPHC		*F*(1,18)=0.17, *p*=.685	*F*(1,18)=0.15, *p*=.700	*F*(1,18)=0.11, *p*=.741
MD right PHC		***F*(1,18)=10.23, *p*=.005**		
ECMO type		*F*(1,18)=0.34, *p*=.565		

Supplemental Digital Content 3. Influence of ECMO treatment and type of ECMO on structure-function relationships (continued)

	DCT	RAVLT delayed recall	RCFT immediate recall	RCFT delayed recall
ECMO type*MDrightPHC		$F(1,18)=0.31$, $p=.582$		
Left hippocampal volume		**$F(1,20)=5.24$, $p=.033$**		
ECMO type		$F(1,20)=0.61$, $p=.443$		
ECMO type*LeftHippocampus		$F(1,20)=0.61$, $p=.445$		
Right hippocampal volume		**$F(1,20)=9.35$, $p=.006$**		
ECMO type		$F(1,20)=0.19$, $p=.665$		
ECMO type*RightHippocampus		$F(1,20)=0.27$, $p=.608$		

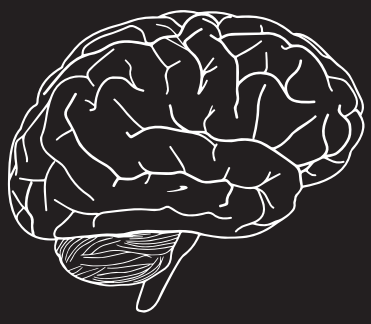
Results of linear regression analsyes assessing differences between patients treated with ECMO and without ECMO in the significant structure-function relationships. The same analyses were conducted to assess differences between patients treated with venoarterial and venovenous ECMO (ECMOtype). ***P*** < .05 is considered statistically significant.

Abbreviations: FA, fractional anisotropy; MD, mean diffusivity; PHC, parahippocampal region of the cingulum; DCT, Dot Cancellation Test; RAVLT, Rey Auditory Verbal Learning Test; RCFT, Rey Complex Figure Test.



CAN WE TRAIN THE DAMAGED BRAIN AFTER NEONATAL CRITICAL ILLNESS?

CHAPTER 7



WORKING-MEMORY TRAINING FOLLOWING NEONATAL CRITICAL ILLNESS: A RANDOMIZED CONTROLLED TRIAL

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ABSTRACT

Objective To test the immediate and long-term effectiveness of Cogmed Working-Memory Training (CWMT) following ECMO and/or CDH.

Design A nationwide randomized controlled trial assessing neuropsychological outcome immediately and one year post-CWMT, conducted between October 2014-June 2017. Researchers involved in the follow-up assessments were blinded to group allocation.

Setting Erasmus MC-Sophia Children's Hospital, Rotterdam and Radboud University Medical Center, Nijmegen, the Netherlands.

Patients Eligible participants were neonatal ECMO and/or CDH survivors (8-12 years) with an IQ ≥ 80 and a *z*-score ≤ -1.5 on at least one (working)memory test at first assessment.

Interventions CWMT, comprising 25 sessions of 45 minutes for five consecutive weeks at home.

Measurements and Main Results Participants were randomized to CWMT (n = 19) or no intervention (n = 24) (two dropped out after T0). Verbal working-memory (estimated coefficient = 0.87; p = .002) and visuospatial working-memory (estimated coefficient = 0.96, p = .003) had significantly improved in the CWMT group at T1, but were similar between groups at T2 (verbal, p = .902; visuospatial, p = .416). Improvements were found at T2 on long-term visuospatial memory following CWMT (estimated coefficient = 0.95, p = .003). Greater improvements in this domain at T2 following CWMT were associated with better self-rated school functioning (r = .541, p = .031) and parent-rated attention (r = .672, p = .006).

Conclusions Working-memory improvements after CWMT disappeared one year post-training in neonatal ECMO and/or CDH survivors. Gains in visuospatial memory persisted one year post-intervention. CWMT may be beneficial for survivors with visuospatial memory deficits.

Trial Registration NTR4571: http://www.trialregister.nl/trialreg/admin/rctview.asp?TC=4571.

INTRODUCTION

Growing up after neonatal critical illness has long-term neurodevelopmental consequences.(1-7) Specifically, children treated with neonatal extracorporeal membrane oxygenation (ECMO) and/or with congenital diaphragmatic hernia (CDH) are at risk of specific (working)memory and attention deficits at school-age, despite average intelligence.(1,3,8) These deficits become more evident as children mature, suggesting they 'grow into deficit'(9). This mechanism – where subtle brain injuries acquired in early life become evident only later in life when higher cognitive functioning is required – has recently been described by our group across survivors of neonatal critical illness.(10) As more educational problems occur following neonatal critical illness than in the general population(4,11), it is imperative to find intervention strategies to prevent or diminish impaired outcome.

Working-memory, one of the fundamental building blocks for higher cognitive functioning, is highly associated with academic performance(12) and may be at risk of impairment following neonatal ECMO.(1,13) Training programs to improve cognitive functioning have received increasing attention over the years, and are based on the idea that repetitive mental exercise of one cognitive task results in improved functioning that may generalize to other tasks with similar underlying skills. A widely evaluated cognitive training for children with working-memory problems is Cogmed Working-Memory Training (CWMT).(14) Near-transfer effects, i.e. improvements on trained and untrained working-memory tasks, as well as far-transfer effects to non-trained cognitive functions have been found immediately after CWMT.(15,16) However, whether effects persist beyond six months post intervention remains largely unknown.(17,18)

In this single-blind RCT, the immediate and long-term effectiveness of CWMT on (working)memory in school-age (8-12 years) survivors of neonatal ECMO and/or CDH are studied. We hypothesized that CWMT improved (working)memory and attention immediately after training. Furthermore, we hypothesized that these improvements persisted 12 months post-training.

MATERIALS AND METHODS

Design and setting

This RCT, conducted between October 2014 and June 2017, compared CWMT to no training in school-age neonatal ECMO and/or CDH survivors (NTR4571). Children born between February 2002 and December 2007 who were treated in either of the two referral centers for neonatal ECMO and CDH treatment in the Netherlands (Erasmus MC, Rotterdam or the Radboudumc, Nijmegen) were recruited. As we have previously shown

similar long-term cognitive outcome in CDH patients irrespective of ECMO treatment, CDH patients treated without ECMO were also recruited.(2,8) ECMO had been applied using the entry criteria described by Stolar et al.(19), which did not change over time. The study took place at the Erasmus MC-Sophia Children's Hospital. Ethical approval was granted by our institution's Review Board (MEC-2014-001).

Eligibility and recruitment

Eligible participants were: neonatal ECMO and/or CDH survivors between 8-12 years at first assessment, IQ ≥ 80, and a *z*-score ≤ -1.5 on at least one (working)memory test.(20) Children were recruited in two ways: 1) children who underwent neuropsychological assessment as part of the structured follow-up program in Rotterdam(21,22) and met the inclusion criteria were referred to our study or, 2) potentially eligible children received information by mail about the trial and were invited to contact our center. Written informed consent from all parents and children ≥ 12 years old was obtained. Exclusion criteria were: usage of psychopharmaceutic drugs (e.g. methylphenidate) and/or genetic syndromes that affect neuropsychological functioning. All children had sufficient knowledge of the Dutch language to perform the assessments.

Eligible children were randomized into either the CWMT group or the control group by an independent researcher uninvolved with the neuropsychological assessments. Randomization was performed by drawing from sealed, opaque envelopes containing a paper with either 'intervention' or 'no intervention'. The psychologists who conducted the neuropsychological assessments were blinded to group allocation.

Intervention

The CWMT^{RM} version for 7-17-year-old children was used. Children trained at home for 45 minutes a day, five days a week, for five consecutive weeks, after which the training was completed as per manufacturer's instructions.(14) Task level adapted automatically to ensure the child was continuously performing at its' maximum ability. As part of the program, children were supervised by a certified CWMT coach, who provided weekly support to the family by phone and e-mail, and closely monitored the child's performance via online access.

Children in the control group did not receive any training.

Outcome measures

After baseline assessment (T0), neuropsychological assessments were repeated in all participants one week (T1) and one year (T2) post-intervention (Figure 1). The primary outcome measure was verbal working-memory(23), assessed using the WISC-III-NL Digit Span(24), at T1. For all secondary outcome measures, please refer to Supplemental Digital Content (SDC) 1 and 2.

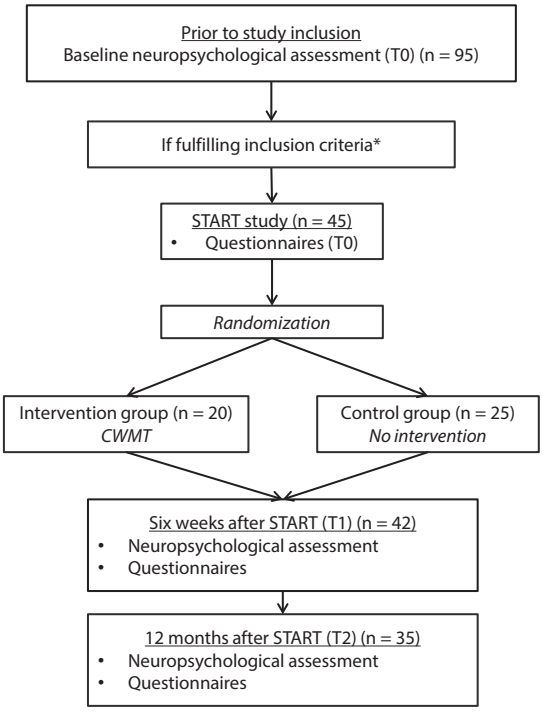

Figure 1. Trial outline
For short descriptions of the tests and questionnaires used, please refer to Supplemental Digital Content 2. *IQ > 80 and a *z*-score ≤ -1.5(20) on one or more memory tests. Abbreviations: CWMT, Cogmed Working-Memory Training.

Sample size calculation

The power calculation was based on the expected difference between the CWMT group and control group on verbal working-memory, the primary outcome measure. Based on previous findings on the effect of CWMT on verbal working-memory in children with working-memory problems(23,25,26), we expected a difference of 0.8 SD between groups (considered a large effect according to Cohen's guidelines(27)). We assumed that baseline scores would show a correlation of 50% with scores at T1. We calculated that a sample size of 25 children per group would be needed (power of 90%, alpha of .05).(28)

Statistical analysis

Clinical and demographic characteristics and neuropsychological outcome at baseline were compared between groups using independent samples t-tests and ANCOVA (normally distributed variables), Mann-Whitney U tests or Fisher's exact tests (non-normally distributed continuous or categorical variables).

All analyses were based on the intention-to-treat principle. Outcome scores were converted to z-scores (individual score minus population mean divided by population SD). Scores were inverted where appropriate so that a higher score always equated with better performance. To assess outcome after CWMT at T1 and T2, we estimated linear mixed models. This method accounts for within-subject correlations and allows

for missing values in the dependent variable. Based on the Akaike information criterion, a random intercept was included in the mixed models to account for the within-subject correlations. *P*-values for the fixed effects were calculated using t-tests with the Satterthwaite approximation method. Performance at baseline was constrained to be equal. Neuropsychological outcome was the dependent variable, and group and time-point as well as the group*time-point interaction term were independent variables. For analyses with the secondary neuropsychological outcome measures (all but verbal working-memory at T1), the False Discovery Rate (FDR)-correction(29) was used to correct for multiple testing. It was applied once for each set of tests in the same neuropsychological domain (e.g. once for the analyses done with tests measuring attention). Additionally, linear mixed models were estimated with the self- and proxy-rated outcomes as dependent variables.

Finally, if any sustained improvements were found on the neuropsychological outcome measures following CWMT at T2, we assessed whether these were associated with subjective improvements scored by parents, teachers or children on EF, working-memory, attention, self-esteem or school functioning. We conducted Pearson correlation analyses between the change-score from T0 to T2 on neuropsychological outcome and these self- and proxy-reported outcomes at T2 in the CWMT group. In secondary analyses, no correction for multiple testing was applied.

Statistical analyses were performed using SPSS 21.0 (IBM Corporation, Armonk, NY) and R Statistical Software version 3.1.3 (R Core Team, 2014)(lme4 and lmerTest packages). Results of the linear mixed models were summarized using the estimated marginal means, which are the predicted values of the dependent variable adjusted for the effects of the independent variables. These can be interpreted as z-scores. For all analyses, a two-sided (FDR-corrected) p-value $< .05$ was considered statistically significant.

RESULTS

Of 217 invited children, 54 declined to participate and 68 did not respond. Fifty assessed children were excluded because they did not meet the inclusion criteria and two dropped out after randomization, leaving 43 participants. Of these, 19 were assigned to the CWMT group and 24 to the control group (Figure 2). Age, ethnicity, gender, IQ, education type, or clinical characteristics, such as ECMO treatment, were similar between groups (Table 1). See Figure 3 for baseline neuropsychological outcome.

All children in the CWMT group completed 25 sessions, except one who completed 20 sessions. Sensitivity analyses were performed without this child's data. As the results did not change, the child was not excluded from the analyses.

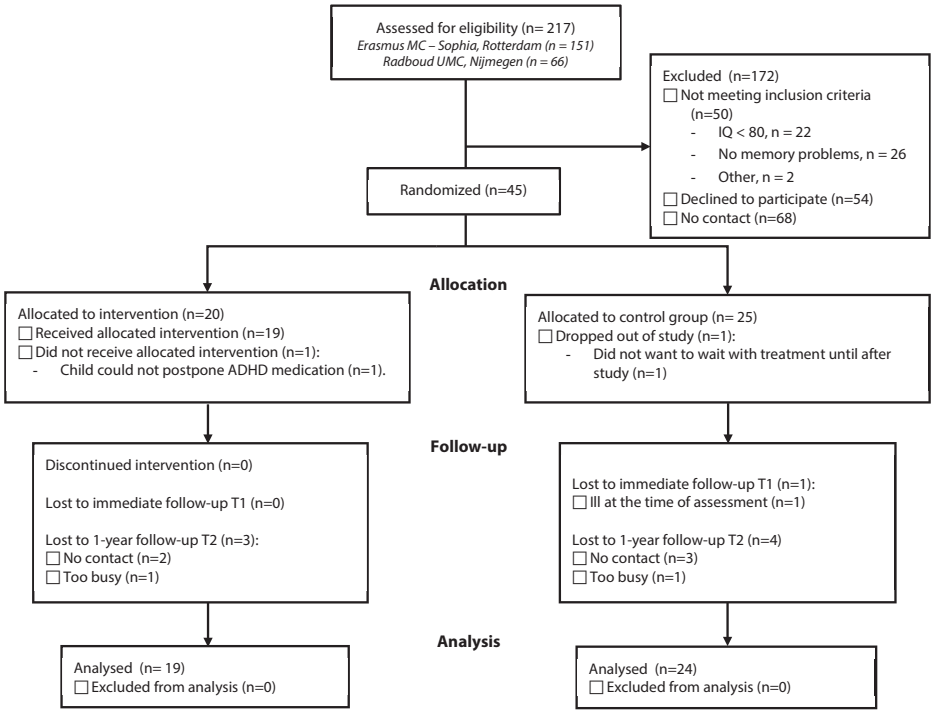

Figure 2. CONSORT flow diagram
T1 refers to the first follow-up assessment immediately after the intervention, T2 refers to the assessment one year after the intervention. Abbreviations: ADHD, Attention Deficit Hyperactivity Disorder.

Primary outcome measure

The CWMT group improved significantly on verbal working-memory at T1 compared to controls (estimated coefficient = 0.87; p = .002) (SDC3, Figure 4).

Secondary outcome measures

Working-memory

Verbal working-memory was similar between groups at T2 (estimated coefficient = -0.04, p = .902) (SDC3, Figure 4A). Additional analyses were performed to evaluate the Digit Span Forward (DSF), i.e. short-term memory, and Digit Span Backward (DSB), i.e. working-memory, separately(24). Performance on the DSF and DSB improved significantly at T1 in the CWMT group compared to the control group (forward: estimated coefficient = 0.93, p = .028; backward: estimated coefficient = 1.13, p = .033), whereas no group differences were found at T2 (forward: estimated coefficient = -0.08, p = .860; backward: estimated coefficient = 0.38, p = .497).

The CWMT group improved significantly on visual working-memory compared to the control group at T1 (estimated coefficient = 0.96, p = .003). However, this difference

Table 1. Study population characteristics

Characteristics	All (n = 43)	Controls (n = 24)	CWMT (n = 19)	*P*-value
a) Demographic				
Age (years)	10 ± 2	10 ± 2	10 ± 1	.275
Gender				.812
Male	24 (56%)	13 (54%)	11 (58%)	
Ethnicity				.127
Dutch	37 (86%)	19 (79%)	18 (95%)	
Maternal education level[a]				.407
Low	7 (16%)	3 (13%)	4 (21%)	
Moderate	13 (30%)	7 (29%)	6 (32%)	
High	23 (54%)	14 (58%)	9 (47%)	
Type of education child				.953
Regular	27 (63%)	14 (58%)	13 (68%)	
Regular with help	13 (30%)	9 (38%)	4 (21%)	
Special education	3 (7%)	1 (4%)	2 (11%)	
IQ	100 ± 12	98 ± 12	101 ± 12	.359
b) Clinical				
Birthweight (grams)	3596 ± 479	3474 ± 338	3772 ± 605	.765
Gestational age (weeks)	40 ± 1	40 ± 2	41 ±1	.492
Mechanical vent. (days)	11 (9-17)	12 (9-17)	10 (9-17)	.677
CLD presence	6 (15%)	3 (13%)	3 (19%)	.423
Abnormal CUS				.969
Yes	3 (9%)	2 (9%)	1 (9%)	
No	29 (91%)	19 (91%)	10 (91%)	
Unknown[b]	11	3	8	
CDH-non-ECMO	12 (28%)	6 (50%)	6 (50%)	.646
ECMO treatment[c]	31 (72%)	18 (75%)	13 (68%)	.643
Type of ECMO				.357
VA	21 (66%)	10 (56%)	11 (84%)	
VV	9 (31%)	8 (44%)	1 (8%)	
VV conversion to VA	1 (3%)	0 (0%)	1 (8%)	
Age start ECMO (days)	2 (1-3)	2 (1-4)	1 (1-2)	.077
Hours on ECMO	110 (90-182)	119 (87-196)	104 (90-182)	.824

N (%), mean ± SD or median (interquartile range) is reported where appropriate for the group as a whole ('All' in column 1), the control group (Controls in column 2) and the CWMT group (CWMT in column 3) separately. Dutch refers to children with two native Dutch parents. [a]Based on the highest level of education completed by the mother(41).

[b]In CDH-non-ECMO patients, cranial ultrasounds were not routinely performed in our centers.

[c]Diagnoses underlying ECMO treatment were congenital diaphragmatic hernia (n=2), meconium aspiration syndrome (n=22), persistent pulmonary hypertension of the newborn (n = 4), infant respiratory distress syndrome (n = 2), and cardiac anomaly (n=1).

Abbreviations: CWMT, Cogmed Working-Memory Training; IQ, Intelligence Quotient; CLD, chronic lung disease; CUS, cranial ultrasound; CDH, congenital diaphragmatic hernia; ECMO, extracorporeal membrane oxygenation; VA, venoarterial; VV, venovenous

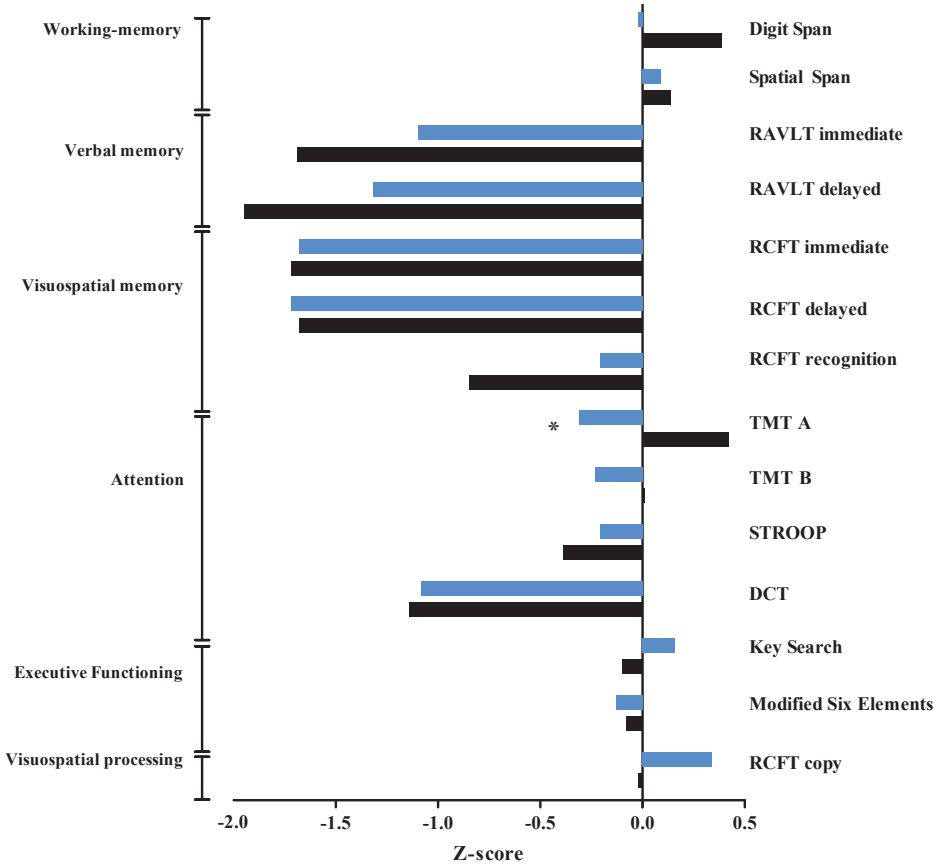

Figure 3. Neuropsychological outcome at baseline for the CWMT group and the control group
Mean z-score is given per group. Scores of the CWMT group are presented in blue, scores of the control group are presented in black. Independent samples T-test was used to identify differences between the groups. *Significant difference between the groups. Abbreviations: CWMT, Cogmed Working-Memory Training; RAVLT, Rey Auditory Learning Test; RCFT, Rey Complex Figure Test; DCT, Dot Cancellation Test; TMT, Trail Making Test; STROOP, Stroop Color Word Test.

disappeared at T2 (estimated coefficient = 0.29, p = .416) (SDC3, Figure 4A). An improvement in Spatial Span Forward was found in the CWMT group at T1 compared to controls (estimated coefficient = 1.12, p < .001), but not at T2 (estimated coefficient = -0.15, p = .613). Spatial Span Backward did not differ between the CWMT group and controls (T1: estimated coefficient = 0.43, p = .146; T2: estimated coefficient = 0.61, p = .056).

Memory

The CWMT group improved on short-term visuospatial memory at T1 and T2 compared to the control group, but this difference did not reach significance. Long-term visuospa-

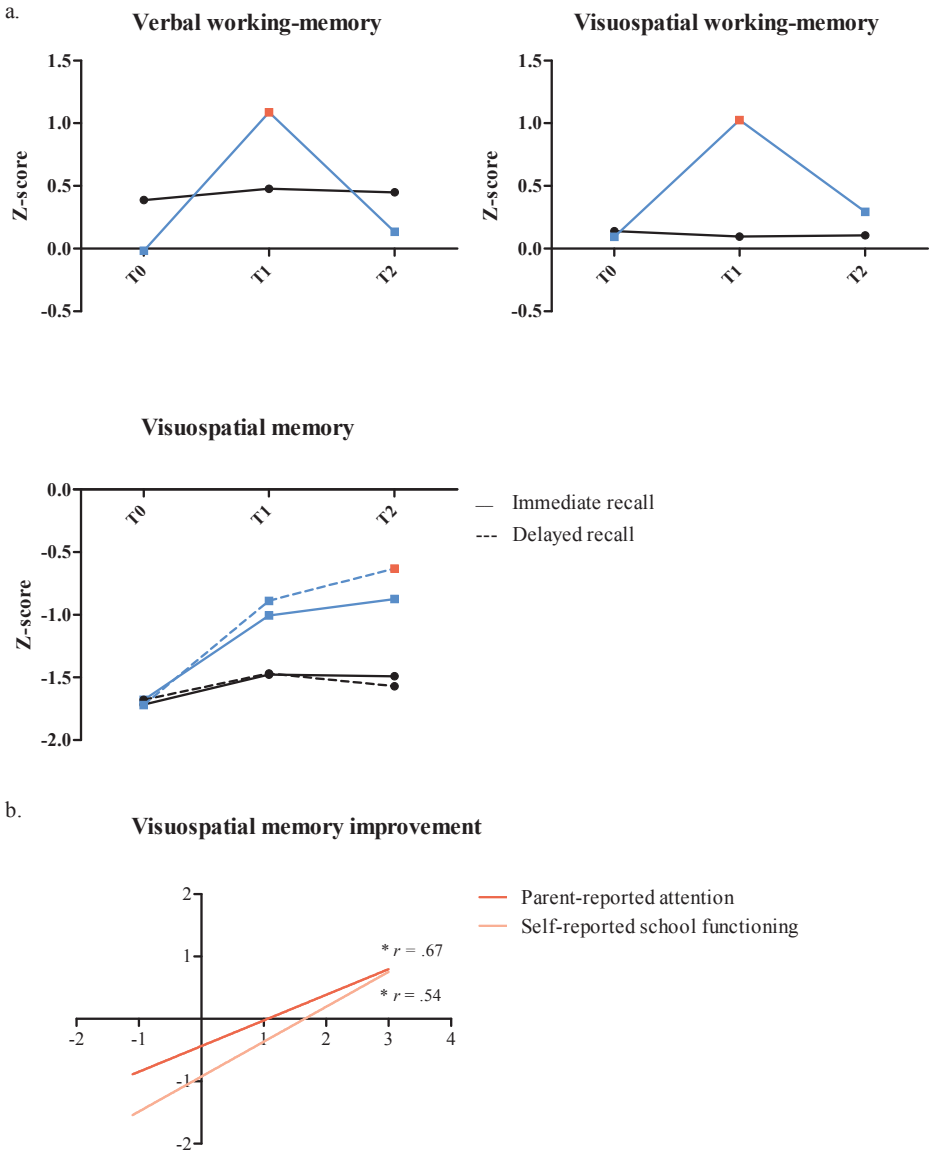

Figure 4. Neuropsychological outcome immediately and one year after CWMT in ECMO and/or CDH survivors

Blue lines represent the CWMT group, black lines represent the control group. Panel A shows verbal working-memory, visuospatial working-memory, and visuospatial memory at baseline (T0), immediately after (T1) and one year after CWMT (T2). A red dot represents a significant group by time effect, showing a significant improvement in the CWMT group compared to the control group at that time-point. Panel B shows the significant correlations between the change in z-scores from T0 to T2 in long-term visuospatial memory and z-scores on the self- and parent-reported outcomes on school functioning and attention in the CWMT group at T2. Abbreviations: CWMT, Cogmed Working Memory Training; ECMO, extracorporeal membrane oxygenation; CDH, congenital diaphragmatic hernia.

tial memory improved significantly in the CWMT compared to the control group at T2 (estimated coefficient = 0.95, p = .003) (SDC3, Figure 4A).

Verbal memory did not change (SDC3).

Other neuropsychological outcomes

Attention, processing speed, EF, and visuospatial processing were similar between groups at T1 and T2 (SDC3).

Proxy- and self-reported outcomes

Parents, but not teachers, of the CWMT group scored EF at T2 higher than the control group (estimated coefficient = 0.57, p = .034). Parent- and teacher-rated working-memory did not differ between groups (Figure 4B, SDC4).

Parents and teachers scored the child's behavior within the average range in both groups at all time-points (SDC4). Parents, but not teachers, of the CWMT group reported fewer problems with attention and hyperactivity at T2 compared to controls (estimated coefficient = 0.58, p = .042)(SDC4).

Children in the CWMT group reported better quality of life at T2 than the control group (estimated coefficient = 0.92, p = .034). Parents did not report changes in (psychosocial) quality of life following CWMT (SDC4).

Children in the CWMT group reported better school functioning at T2 than controls, but this difference did not reach significance. Proxy-reported school functioning was similar in both groups (SDC4).

Neuropsychological improvement and subjective outcome following CWMT

Larger gains in long-term visuospatial memory from T0 to T2 were associated with higher scores on school functioning scored by children in the CWMT group at T2 (r = .541, p = .031), and better parent-reported attention and hyperactivity at T2 (r = .672, p = .006) (Figure 3B). No other associations were found between visuospatial memory improvement and the subjective outcomes (not shown).

DISCUSSION

This nationwide single-blind randomized controlled trial confirmed our hypothesis by showing that school-age neonatal ECMO and/or CDH survivors who completed CWMT significantly improved on working-memory immediately post-intervention. However, this improvement did not persist one year post-intervention. We found positive far-transfer effects of CWMT to long-term visuospatial memory, persisting one year post-intervention. These children reported better school functioning and their parents

reported fewer problems with inattention and hyperactivity. As over half of our cohort had visuospatial memory deficits at baseline, these improvements following CWMT are highly relevant for this particular population.

Our findings of improved verbal and visuospatial working-memory immediately after CWMT are in line with the effects demonstrated in other groups.(30-33) The ability to memorize digits for a short period of time and manipulate them are directly trained in CWMT.(31) However, after one year, working-memory performance had returned to baseline. This suggests that active training of working-memory is needed to maintain improved functioning in these domains. A period of retraining after CWMT completion may lead to more sustained effects, but this remains speculative. Although studies with follow-up assessments more than six months post-intervention are scarce, gains in working-memory performance have been found to persist seven months(30) and one year post-training(25). The inconsistency in results may be due to differences in population and the type of neuropsychological deficits that exists between populations. For example, working-memory was within the average range in our population at baseline, in contrast to the children with working-memory deficits studied in the two other long-term studies.(25,30)

Short- and long-term verbal and visuospatial memory are at major risk of impairment following neonatal ECMO and/or CDH.(1,3) In this school-age cohort, more than half of the children had such memory deficits at baseline. However, short- and long-term verbal memory did not change following CWMT. CWMT consists of mostly visual and visuospatial training tasks, and as such may not target verbal (working)memory enough to result in far-transfer effects.(31) In line with this, children in the CWMT group did show sustained improvement on long-term visuospatial memory one year after the intervention, resulting in average performance at this time. Visuospatial memory is important for everyday life and gains in this domain are therefore of great significance.

Greater sustained improvements in the CWMT group in long-term visuospatial memory were associated with better self-reported school functioning and less proxy-reported problems with attention at T2. These findings suggest that the improvements on visuospatial memory extend to daily life. However, these results should be interpreted with caution due to the small sample size in combination with the number of analyses. The generalizability of cognitive improvements to everyday life and school performance has received considerable attention over the last few years. Studies reported both improved attention in daily life following CWMT(34) and no benefits to educational performance(35). In our study, teachers did not report any improvements following CWMT. However, they did not report any problems at baseline either. Future studies that include objective measures of academic performance such as reading or mathematical ability are needed in both preschool and school-age neonatal ECMO and/

or CDH survivors following CWMT to get a better impression of its impact on school functioning and daily life.

Attention and (working)memory share similar pathways in the brain.(36) In addition to (working)memory, attention may therefore also improve through CWMT. Sustained attention deficits have been previously found following neonatal ECMO and/or CDH(1,3), and were confirmed in this cohort. Although we found faster processing speed following CWMT at T2, significance disappeared after multiple testing correction. Selective and sustained attention did not improve post-CWMT. Neuroimaging studies in children with ADHD or childhood cancer, found improvements in attention immediately post-CWMT to be associated with fronto-parietal networks.(32,37-39) However, attention deficits following neonatal ECMO and/or CDH were found to be associated with global white matter microstructure and cingulum bundle alterations.(3,5) CWMT therefore may not target the networks responsible for attention deficits in this population. Our group is currently studying the effectiveness of CWMT following neonatal ECMO and/or CDH using advanced neuroimaging techniques. Such findings could enhance our understanding of how CWMT affects the brain in these survivors.

This is the first study investigating the effectiveness of CWMT following neonatal ECMO and/or CDH, demonstrating high feasibility of such a training in this group. However, our study has some limitations. First, we used a non-active control group for ethical considerations against subjecting children to an intensive training without potential benefits, which limits our ability to attribute our findings to the specific characteristics of the CWMT training. The self- and proxy-rated outcomes should therefore be interpreted with caution. Nonetheless, various studies have found improved outcome following CWMT when compared to a non-adaptive training program which also included weekly phone calls from a certified Cogmed training coach.(25,31,34,40) Second, our sample size was smaller than anticipated. We did not extend our inclusion time because we did not want our control group to wait longer than needed to complete CWMT if it was proven to be beneficial. Finally, our primary outcome measure was based on initial reports of neuropsychological outcome in the study population that showed working-memory problems(2,7,11) and on previous studies on CWMT(23,25,26). However, ongoing research testing all major neuropsychological domains demonstrated primarily short- and long-term memory problems in these children.(8) Given these new insights, a different primary outcome measure than working-memory would have been more appropriate for this population.

CONCLUSION

We found improved working-memory immediately after CWMT in school-age neonatal ECMO and/or CDH survivors, but this did not sustain until one year post-training. Sustained far-transfer effects on long-term visuospatial memory were found following CWMT. Given the high risk of visuospatial memory deficits in these children and the importance of memory in daily life, CWMT shows clinical utility for children with visuospatial memory deficits. Future studies with advanced neuroimaging techniques and objective measures of academic performance are needed to further delineate the effectiveness of CWMT in neonatal ECMO and/or CDH survivors.

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SUPPLEMENTARY MATERIAL

Supplemental Digital Content 1. Outcome measures

Domain	Test	Respondent	T0	T1	T2
Neuropsychological assessment					
Working-memory	Subtest Digit Span of the WISC-III-NL;	Child;	*	*	*
	Subtest Spatial Span of the WNV	Child	*	*	*
Verbal memory	RAVLT Immediate and Delayed recall	Child	*	*	*
Visuospatial Memory	RCFT Immediate and Delayed recall and Recognition	Child	*	*	*
Sustained attention	DCT	Child	*	*	*
Selective attention	TMT section B;	Child	*	*	*
	STROOP colour-word test	Child	*	*	*
Processing speed	TMT section A	Child	*	*	*
Executive functioning	Subtests Key Search and Modified Six Elements of the BADS-C-NL	Child	*	*	*
Visuospatial processing	RCFT Copy	Child	*	*	*
Questionnaires					
Executive functioning	BRIEF total score	Parents + Teacher	*	*	*
Working-memory	Subscale of BRIEF		*	*	*
Behaviour	SDQ	Parents + Teacher	*	*	*
Attention and hyperactivity	Subscale of SDQ		*	*	*
Quality of life	PedsQL	Child + Parents	*	*	*
School functioning	Subscale of PedsQL		*	*	*
Psychosocial quality of life	CHQ	Parents	*	*	*
Self-esteem	Subscale of CHQ		*	*	*

Overview of outcome measures assessed at the different time points of the study. T0 is the baseline assessment, T1 is six weeks after baseline, and T2 is 12 months after baseline. The primary outcome measure was working-memory assessed by Digit Span.

Abbreviations: WISC-III-NL, Wechsler Intelligence Scale for Children; WNV, Wechsler Non Verbal Scale of Ability; RAVLT, Rey Auditory Learning Test; RCFT, Rey Complex Figure Test; DCT, Dot Cancellation Test; TMT, Trail Making Test; BADS-C-NL, Behavioural Assessment of the Dysexecutive Syndrome; BRIEF, Behaviour Rating Inventory of Executive Functioning; SDQ, Strengths and Difficulties Questionnaire; PedsQL, Paediatric Quality of Life Inventory; CHQ, Child Health Questionnaire.

Supplemental Digital Content 2. Descriptions of the outcome measures.

Intelligence

Wechsler Intelligence Scale for Children (WISC-III-NL)

A short-form with two subtests, Block Design and Vocabulary, of the WISC-III-NL were used to assess general intelligence.[1] The WISC-III-NL has been shown to have good reliability and validity.[2] A normalized population mean of 100 with a standard deviation of 15 is used.[2]

Primary outcome measures

Verbal working-memory

WISC-III-NL – subtest Digit Span

The Digit Span consists of random number sequences that increase in length and that the examiner reads aloud at the rate of 1 number per second. The child has to reproduce these numbers in the same order. Next, the sequences must be recalled backwards (3-5-7 becomes 7-5-3). The first part of the test measures short-term auditory memory and short-term retention capacity. The second part measures auditory working memory.[2]

Secondary outcome measures

Visuospatial working-memory

Wechsler Nonverbal Scale of Ability (WNV) – subtest Spatial Span

The Spatial Span requires the child to touch a group of blocks arranged on a board in a non-systematic manner in the same and reverse order as demonstrated by the examiner. The first part of the test measures short-term visuospatial memory and short-term retention capacity. The second part measures visuospatial working-memory.[3]

Verbal memory

Rey Auditory Verbal Learning Test (RAVLT)

The RAVLT consists of five presentations with recall of a 15-word list, a sixth recall trial after 30 minutes, and a seventh recognition trial. This test measures memory span, short- and long term verbal memory, verbal recognition, and learning curve. It can be administered to children and adults in the age range 6-89 years.[4,5]

Visuospatial memory and visuospatial processing

Rey Complex Figure Test (RCFT)

The RCFT consists of three trials. First the child has to copy a complex figure (Copy). Then after 3 and after 30 minutes the figure must be drawn from memory (Recall). Next, different figures are shown and the child has to indicate whether these figures were in the original figure (Recognition). This test measures visual integration, short- and long-term

visual-spatial memory, and visual-spatial recognition. It can be completed by children and adults in the age range 6-89 years.[6,7]

Attention

Dot Cancellation Test

This paper-and-pencil test measures sustained attention and concentration in terms of speed. It consists of a paper on which figures made of three, four or five dots are displayed in 33 rows. The child is instructed to mark all figures with four dots, as precisely and as fast as they can.[8]

Stroop Colour Word Test (Stroop)

The Stroop consists of three trials: in the first trial (Stroop 1) the subject must read colour names, in the second trial (Stroop 2) name printed colours, and in the third trial (Stroop 3) name printed colours not denoted by the colour name. The test can be administered to children and adults in the age range 8-65 years. Selective attention is measured with this test, using the difference score between Stroop 2 and Stroop 3.[9,10]

Trail Making Test (TMT)

This paper and pencil test consists of two parts. In the first part (part A), the subject must draw lines to consecutively connect numbered circles on a sheet. In the second part (part B), the subject must consecutively but alternately connect numbered and lettered circles on another worksheet. The aim of the test is to finish each part as quickly as possible. The test can be administered to children and adults in the age range 6-89 years. This test measures visual conceptual and visuomotor tracking as well as divided attention.[9,10]

Executive functioning

Key Search of the Behavioural Assessment of the Dysexecutive Syndrome (BADS-C-NL)

A test of strategy formation. The child is asked to demonstrate how they would search a field for a set of lost keys and their strategy is scored according to its efficiency and functionality.[11]

Modified Six Elements of the BADS-C-NL

The child is asked to work on six different tasks for which they have five minutes. The child needs to make sure that by the end of the five minutes, all six of the tasks have been done and the child has done as much as possible of each task. This is a test of planning, task scheduling and performance monitoring.[11]

Questionnaires

Behaviour Rating Inventory of Executive Functioning (BRIEF)

To evaluate the perception of the parents and teachers of the child's executive functioning, including working-memory, the BRIEF was used. This questionnaire can be used for children between the ages 5 and 18 years. The Dutch version of the BRIEF has been validated and includes Dutch norm scores.[12]

Child Health Questionnaire (CHQ)

The Dutch version of the CHQ (CHQ-PF50) measures physical and psychosocial functioning of the child and is filled out by the parents. The CHQ comprises 14 subscales. In addition to the overall score on psychosocial functioning, the domain score on self-esteem is used. The Dutch version of the CHQ is validated and includes Dutch norm scores.[13]

Paediatric Quality of Life Inventory (PedsQL)

The PedsQL measures health-related quality of life and is filled out by the parents as well as the child. The PedsQL measures various domains, such as social functioning, physical functioning, psychosocial functioning, emotional functioning and school functioning. The overall score to measure quality of life and school functioning are used. The PedsQL includes Dutch norms for children between 4 and 16 years.[14]

Strength and Difficulties Questionnaire (SDQ)

The child's behaviour can be evaluated by the parents and teacher using the SDQ. This questionnaire can be used for children between 4 and 16 years. The child's psychosocial development is evaluated using questions about both positive and negative behaviours. The follow-up version of the SDQ was used at the post-intervention assessments to obtain ratings of the behaviour of the child over the last month. The SDQ consist of five subscales and a total score. The total score as well as the score on the subscale hyperactivity/inattention is used. The Dutch version of this test has been validated and includes Dutch norm scores.[15]

We used Dutch versions of all tests and questionnaires.

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Supplemental Digital Content 3. Neuropsychological outcome immediately and one year after CWMT in neonatal ECMO and/or CDH survivors

Measures	Variable	Estimated coefficient	95% CI	P-value
Working-memory				
Digit Span	*CWMT*Time-point:*			
	T0	Reference	Reference	-
	T1	**0.87**	**0.21 to 1.42**	**.002**
	T2	-0.04	-0.63 to 0.55	.902
Spatial Span	*CWMT*Time-point:*			
	T0	Reference	Reference	-
	T1	**0.96**	**0.47 to 1.45**	**.003**
	T2	0.29	-0.24 to 0.82	.416
Verbal memory				
RAVLT immediate	*CWMT*Time-point:*			
	T0	Reference	Reference	-
	T1	0.02	-0.67 to 0.70	.960
	T2	0.22	-0.51 to 0.96	.731
RAVLT delayed	*CWMT*Time-point:*			
	T0	Reference	Reference	-
	T1	0.52	-0.04 to 1.08	.268
	T2	0.45	-0.15 to 1.04	.280
Visuospatial memory				
RCFT Immediate	*CWMT*Time-point:*			
	T0	Reference	Reference	-
	T1	0.37	-0.17 to 0.91	.232
	T2	0.60	0.02 to 1.17	.094
RCFT Delayed	*CWMT*Time-point:*			
	T0	Reference	Reference	-
	T1	0.53	0.01 to 1.06	.094
	T2	**0.95**	**0.38 to 1.51**	**.006**
RCFT recognition	*CWMT*Time-point:*			
	T0	Reference	Reference	-
	T1	0.46	-0.23 to 1.15	.232
	T2	-0.09	-0.83 to 0.65	.808
Sustained attention				
DCT	*CWMT*Time-point:*			
	T0	Reference	Reference	-
	T1	0.02	-0.76 to 0.80	.961
	T2	-0.03	-0.87 to 0.80	.961
Selective attention				

Supplemental Digital Content 3. Neuropsychological outcome immediately and one year after CWMT in neonatal ECMO and/or CDH survivors (continued)

Measures	Variable	Estimated coefficient	95% CI	P-value
TMT section B	*CWMT*Time-point:*			
	T0	Reference	Reference	-
	T1	0.31	-0.29 to 0.91	.950
	T2	0.11	-0.53 to 0.76	.950
STROOP	*CWMT*Time-point:*			
	T0	Reference	Reference	-
	T1	-0.06	-0.64 to 0.51	.950
	T2	0.02	-0.62 to 0.66	.950
Processing speed				
TMT section A	*CWMT*Time-point:*			
	T0	Reference	Reference	-
	T1	0.21	-0.36 to 0.77	.475
	T2	0.66	0.05 to 1.27	.068
Executive functioning				
BADS-C-NL Key Search	*CWMT*Time-point:*			
	T0	Reference	Reference	-
	T1	-0.55	-1.21 to 0.10	.151
	T2	-0.21	-0.91 to 0.49	.557
BADS-C-NL Modified Six Elements	*CWMT*Time-point:*			
	T0	Reference	Reference	-
	T1	0.49	-0.12 to 1.09	.151
	T2	0.72	0.02 to 1.42	.151
Visuospatial processing				
RCFT copy	*CWMT*Time-point:*			
	T0	Reference	Reference	-
	T1	-0.10	-0.54 to 0.34	.664
	T2	0.15	-0.33 to 0.62	.664

Results of linear mixed model analyses showing the effect of CWMT on neuropsychological outcome at T1 and T2. All estimated coefficients are reported as z-scores. The control group was used as the reference group and the baseline assessment T0 as the reference time-point. FDR-correction(26) was applied to correct for multiple testing. FDR-correction was applied once for each set of tests in the same neuropsychological domain (i.e. once for the tests measuring attention). An **FDR-corrected *p*-value** <.05 is considered to be statistically significant.

Abbreviations: CWMT, Cogmed Working Memory Training; ECMO, extracorporeal membrane oxygenation; CDH, congenital diaphragmatic hernia; T1, six weeks after baseline; T2, 12 months after baseline; RAVLT, Rey Auditory Verbal Learning Test; RCFT, Rey Complex Figure Test; DCT, Dot Cancellation Test; TMT, Trail Making Test; BADS-C-NL, Behavioural Assessment of the Dysexecutive Syndrome.

Supplemental Digital Content 4. Proxy- and self-reported outcomes after CWMT in ECMO and/or CDH survivors

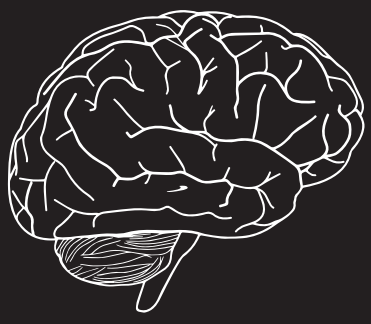
	Variable	Estimated coefficient	95% CI	P-value
Self-report				
School functioning* (PedsQL)	*CWMT*Time-point:*			
	T0	Reference	Reference	-
	T1	0.11	-0.69 to 0.90	.787
	T2	0.71	-0.15 to 1.56	.104
Quality of life (PedsQL)	*CWMT*Time-point:*			
	T0	Reference	Reference	-
	T1	0.46	-0.42 to 1.33	.304
	T2	0.75	-0.16 to 1.71	.101
Proxy-report - Parent				
School functioning* (PedsQL)	*CWMT*Time-point:*			
	T0	Reference	Reference	-
	T1	0.24	-0.25 to 0.72	.335
	T2	0.10	-0.42 to 0.62	.704
Quality of Life (PedsQL)	*CWMT*Time-point:*			
	T0	Reference	Reference	-
	T1	0.22	-0.25 to 0.68	.355
	T2	0.40	-0.10 to 0.89	.113
Working-memory (BRIEF)	*CWMT*Time-point:*			
	T0	Reference	Reference	-
	T1	-0.21	-0.78 to 0.37	.478
	T2	0.51	-0.11 to 1.13	.108
Executive Functioning (BRIEF)	*CWMT*Time-point:*			
	T0	Reference	Reference	-
	T1	-0.09	-0.58 to 0.40	.706
	T2	**0.57**	**0.04 to 1.09**	**.034**
Psychosocial Quality of Life (CHQ)	*CWMT*Time-point:*			
	T0	Reference	Reference	-
	T1	-0.16	-0.69 to 0.38	.565
	T2	-0.15	-0.74 to 0.43	.608
Self-esteem (CHQ)	*CWMT*Time-point:*			
	T0	Reference	Reference	-
	T1	0.01	-1.05 to 1.08	.979
	T2	0.13	-1.03 to 1.29	.821
Hyperactivity/attention (SDQ)	*CWMT*Time-point:*			
	T0	Reference	Reference	-
	T1	0.33	-0.18 to 0.84	.197
	T2	**0.58**	**0.02 to 1.13**	**.042**

Supplemental Digital Content 4. Proxy- and self-reported outcomes after CWMT in ECMO and/or CDH survivors (continued)

	Variable	Estimated coefficient	95% CI	P-value
Behaviour (SDQ)	*CWMT*Time-point:*			
	T0	Reference	Reference	-
	T1	0.30	-0.16 to 0.76	.193
	T2	0.38	-0.11 to 0.88	.128
Proxy-report - Teacher				
Working-memory (BRIEF)	*CWMT*Time-point:*			
	T0	Reference	Reference	-
	T1	0.07	-0.80 to 0.94	.873
	T2	0.23	-0.70 to 1.15	.627
Executive Functioning (BRIEF)	*CWMT*Time-point:*			
	T0	Reference	Reference	-
	T1	0.06	-0.53 to 0.66	.836
	T2	0.03	-0.50 to 0.67	.913
Hyperactivity/attention (SDQ)	*CWMT*Time-point:*			
	T0	Reference	Reference	-
	T1	0.05	-0.54 to 0.63	.878
	T2	0.12	-0.50 to 0.73	.704
Behaviour (SDQ)	*CWMT*Time-point:*			
	T0	Reference	Reference	-
	T1	0.21	-0.27 to 0.69	.392
	T2	0.16	-0.34 to 0.67	.516

Results of linear mixed model analyses showing the effect of CWMT on proxy- and self-reported outcomes at T1, as well as at T2. The control group was used as the reference group and the baseline assessment T0 as the reference time-point. All estimated coefficients are reported as z-scores. ***P*-value** <.05 is considered to be statistically significant. Abbreviations: T1, six weeks after baseline; T2, 12 months after baseline; CWMT, Cogmed Working Memory Training; BRIEF, Behaviour Rating Inventory of Executive Functioning; PedsQL, Paediatric Quality of Life Inventory; SDQ, Strengths and Difficulties Questionnaire; CHQ, Child Health Questionnaire.

CHAPTER 8



WHITE MATTER MICROSTRUCTURE CHANGES FOLLOWING WORKING-MEMORY TRAINING IN SURVIVORS OF NEONATAL CRITICAL ILLNESS: A RANDOMIZED CONTROLLED TRIAL

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ABSTRACT

Objective To test the effect of Cogmed Working-Memory Training (CWMT) on white matter microstructure following neonatal ECMO and/or CDH.

Design A nationwide randomized controlled trial assessing white matter microstructure immediately post-CWMT (T1) and its association with neuropsychological outcome immediately and one year (T2) post-CWMT, conducted between October 2014-June 2017. Researchers involved in the follow-up assessments were blinded to group allocation.

Setting Erasmus MC-Sophia Children's Hospital, Rotterdam and Radboud University Medical Center, Nijmegen, the Netherlands.

Patients Eligible participants were neonatal ECMO and/or CDH survivors (8-12 years) with an IQ ≥ 80 and a *z*-score ≤ -1.5 on at least one (working)memory test at first assessment.

Interventions CWMT, comprising 25 sessions of 45 minutes for five consecutive weeks at home.

Measurements and Main Results Participants were randomized to CWMT (n = 14) or non-training (n = 20). Global fractional anisotropy (FA) increased significantly in the CWMT group compared to the non-training group (estimated coefficient = .007, p = .015). Increased FA (estimated coefficient = .009, p = .033) and decreased mean diffusivity (estimated coefficient = -.010, p = .018) were found in the left superior longitudinal fasciculus in the CWMT group compared to the non-training group at T1. Children in the CWMT who improved with at least 1 SD on verbal working-memory from T0 to T1 had significantly higher FA in the left SLF at T1 (n = 6; FA left SLF at T1 = .408±.01) compared to children that did not show this improvement after CWMT (n = 6; FA left SLF at T1 = .384±.02), $F(1,12) = 6.22$, p = .041, h_p^2 = .47. No other structure-function relationships were found after CWMT.

Conclusions White matter microstructure is affected by CWMT in school-age survivors of neonatal ECMO and/or CDH. Our findings demonstrate that white matter microstructure and associated cognitive outcomes are malleable in these children.

Trial Registration NTR4571: http://www.trialregister.nl/trialreg/admin/rctview.asp?TC=4571.

INTRODUCTION

Survivors of neonatal extracorporeal membrane oxygenation (ECMO) and/or congenital diaphragmatic hernia (CDH) are at increased risk of specific attention and memory deficits that begin during the school-age years and extend into adolescence.[1,2] These deficits have been found to be specifically associated with global white matter microstructure alterations and with specific alterations in limbic regions of the brain, namely the hippocampus, cingulum and parahippocampal part of the cingulum.[3-5] Using a single-blind randomized controlled trial, we recently found that school-age survivors of neonatal ECMO and/or CDH trained with Cogmed working-memory training (CWMT[6]) showed significant improvements in verbal and visuospatial working-memory immediately post-intervention compared to untrained controls (chapter 7). However, this improvement was not maintained at the one year post-intervention assessment. Interestingly however, improvements in visuospatial memory delayed recall were found to persist one year post-intervention compared to controls (chapter 7). Given the fact that more than 50% of these survivors have visuospatial memory deficits later in life[1] and considering the importance of memory for both academic performance and participation in society, this is a promising finding.

Several studies in adults have shown plasticity in white matter microstructure immediately following working-memory training.[7,8] In particular, the superior longitudinal fasciculus (SLF) has shown plastic changes following working-memory training, likely because it connects parietal and frontal cortical regions, which have been shown to be important for working-memory.[9] Given the increased plasticity of a child's brain compared to that of an adult[10], white matter microstructure changes may be more widespread following CWMT in children compared to adults. However, this has remained unstudied until now.

The aim of this study was to investigate the neurobiological plasticity following CWMT using diffusion tensor imaging (DTI) in school-age survivors of neonatal ECMO and/or CDH that were part of a nationwide single-blind randomized controlled trial. We hypothesized that white matter microstructure, and in particular the SLF, would change following CWMT. Furthermore, we assessed whether if there were changes in white matter microstructure, these were associated with the cognitive improvements observed following CWMT (chapter 7).

METHODS

Design and population

This nationwide randomized controlled trial, conducted between October 2014 and June 2017, compared CWMT to no training in school-age neonatal ECMO and/or CDH survivors (NTR4571). Inclusion criteria for the trial were: school-age children (8-12 years) treated with ECMO or treated for CDH without ECMO in the first weeks of life at the Erasmus MC-Sophia Children's Hospital in Rotterdam or the Radboud University Medical Center in Nijmegen (the Netherlands), IQ ≥ 80 and memory impairment (*z*-score ≤ -1.5 on one or more memory tests[11]). Exclusion criteria were: usage of psychopharmaceutic drugs (e.g. methylphenidate) and/or genetic syndromes that are known to affect neuropsychological functioning. Eligible children were randomized into either the CWMT group or the non-training group by an independent researcher not involved with the assessment of the children (please refer to Figure 1 for the trial outline). The MRI exam and neuropsychological assessments were performed by researchers blinded to group allocation.

Ethical approval was granted by the Erasmus MC Medical Ethical Review Board (MEC-2014-001). All families received an application package with written informed consent for the parents and children ≥ 12 years of age that was discussed with the family and filled out before participating in the trial. The complete trial methods are described elsewhere (chapter 7).

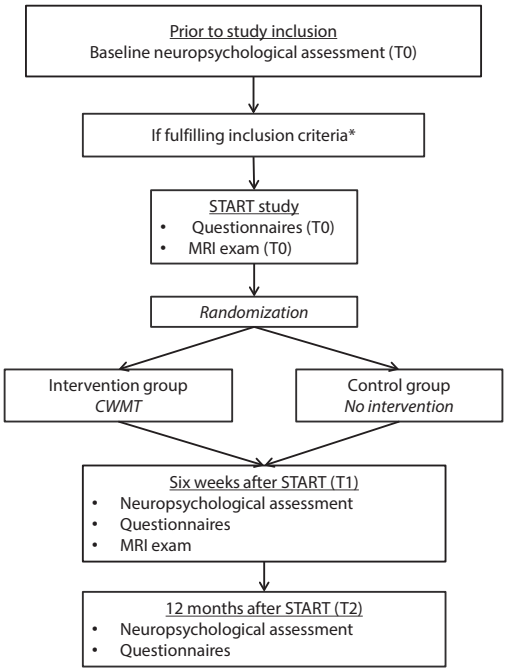

Figure 1. Trial outline
For short descriptions of the tests and questionnaires used, please refer to Supplemental File 1. For description of the MR methods please refer to Supplemental File 2. *IQ > 80 and a *z*-score ≤ -1.5(20) on one or more memory tests. Abbreviations: CWMT, Cogmed Working-Memory Training.

Intervention

Children in the CWMT group completed the CWMT^{RM} version for children from the ages 7 to 17 years. Children trained at home for 45 minutes a day, five days a week, for five consecutive weeks, as recommended in the manufacturer's instructions.[6] The level of the tasks adapted automatically to ensure that the child was continuously performing at the maximum of his or her ability. As part of the program, each child was supervised by a certified CWMT coach, who provided support to the family and feedback on the training results once a week over the phone and by e-mail. The CWMT coach was able to closely monitor the child's performance via online access.

Children in the non-training group did not receive any training.

Outcome measures

After the standardized, neuropsychological assessment at baseline to determine eligibility, eligible participants underwent an MRI exam (T0). After six weeks, the MRI exam was repeated in all participants (T1). At the same time, the neuropsychological assessment was repeated, and again after one year following the baseline measurement (T2, neuropsychological assessment only). The neuropsychological outcomes are described elsewhere (chapter 7; please refer to Supplemental File 1 for brief descriptions of all cognitive tests).

All children first underwent a mock scanning session to become familiarized with the MRI-scanner environment.[12] MRI data were acquired on a 3 Tesla GE MR-750 system using an 8-channel head coil (General Electric, Milwaukee, WI). A full description of the sequences and scanning protocol is provided in Supplemental File 2. After DTI image preprocessing, voxel-wise scalar maps of fractional anisotropy (FA) and mean diffusivity (MD) were computed. FA provides a rotationally invariant measure of diffusion, with 0 being completely isotropic (equal in all directions) and 1 being completely anisotropic (diffusion along only one axis). MD is the rate of diffusion of water (hydrogen) averaged in all directions. The FSL plugin 'AutoPtx' for fully automated probabilistic fiber tractography was used to create subject-specific, probabilistic representations of multiple white matter bundles.[13] Automated[14] and visual inspection of the neuroimaging data resulted in 61 DTI datasets (87%) with usable image quality. All scans were reviewed by a certified neuroradiologist (M.S.), blinded for medical history and outcome.

Statistical analysis

Clinical and demographic characteristics and neuropsychological outcome at baseline were compared between groups using independent samples t-tests and ANOVA (normally distributed variables), Mann-Whitney U or Fisher's exact tests (non-normally distributed continuous or categorical variables, respectively).

First, we assessed whether white matter microstructure changed as a result of CWMT. Our primary aim was to assess changes in the SLF as it is involved in working-memory and has previously been shown to be affected by CWMT.[9] Linear mixed models were estimated to assess whether white matter microstructure changed in the CWMT group compared to the non-training group at T1. This method accounts for within-subject correlations and allows for missing values in the dependent variable. Outcome at baseline was constrained to be equal. The brain diffusion measures were included as dependent variables, and group and time-point as well as the group by time-point interaction term were included as independent variables. Results of the linear mixed models were summarized using the estimated marginal means (the predicted values of the dependent variable adjusted for the effects of the independent variables) of the group by time-point interaction.

Second, we analyzed changes in global FA and global MD following CWMT using linear mixed models. Described by our group in more detail elsewhere[5], global white matter microstructure was calculated using a weighted (by tract volume) average score of FA/MD of the association and limbic system white matter tracts (uncinate, inferior fronto-occipital fasciculus, SLF, inferior longitudinal fasciculus, cingulum bundle and parahippocampal part of cingulum) (Equation 1), known to be involved with cognitive functioning in children[14,15]:

Equation 1:

$$\text{Global FA} = \frac{\Sigma_{i=1}^{n} FAtract_i Voltract_i}{\Sigma_{i=1}^{n} Voltract_i}$$

where *i* denotes the tract, *Vol* denotes the volume of the tract, and *n* is the number of tracts. The same formula was used for global MD, replacing FA for each tract with the MD measure.

If global FA or global MD changed significantly following CWMT, additional analyses were performed with the individual white matter tracts. The same setup of linear mixed models were used to now assess whether white matter microstructure in the individual tracts (independent variables) changed in the CWMT group compared to the non-training group at T1. Brain structures were analyzed in the left and right hemispheres separately as laterality differences have been shown in the organization of working-memory and specific cognitive functions.[16] In all linear mixed models, we adjusted for age at T1 and gender.[17] For the additional analyses on group differences in the individual white matter tracts, the False Discovery Rate (FDR)[18] correction was applied to account for multiple testing. These results were considered statistically significant at the FDR-corrected $p < .05$.

Our previous findings showed that children trained with CWMT improved significantly on verbal working-memory, visuospatial working-memory and visuospatial memory

delayed recall compared to non-trained children (chapter 7). For our second aim, we therefore assessed whether changes in white matter microstructure following CWMT (if any) were associated with the cognitive improvements in the CWMT group using univariate linear regression models. The dependent variable was the brain parameter at T1 in the CWMT group and the independent variable was the cognitive outcome measure, dichotomized to: improved (>1 SD improvement from T0 to T1) versus not improved (<1 SD improvement from T0 to T1). In these analyses, we adjusted for FA/MD at baseline, age at T1 and gender. Neuropsychological test scores were converted to z-scores (individual score minus the population mean divided by the population SD). Scores were inverted where appropriate so that a higher score always equated with better performance.

Statistical analyses were performed using SPSS 21.0 (IBM Corporation, Armonk, NY) and the Stats, lme4 and lmerTest packages in R Statistical Software version 3.1.3 (R Core Team, 2014). The estimated marginal means are reported by group and time-point for the linear mixed model analyses. Effect sizes were calculated in the linear regression models using partial eta squared (h_p^2) and interpreted according to Cohen's guidelines (0.01 = small, 0.06 = medium, 0.14 = large).[19]

RESULTS

Of 34 eligible children with useable DTI data, 14 were in the CWMT group (13 with an MRI at both T0 and T1) and 20 in the non-training group (18 with an MRI at both T0 and T1) (please refer to Supplemental Figure 1 for the CONSORT flow diagram). Demographic and clinical characteristics did not differ between the CWMT group and the non-training group (Table 1). There were no differences in global white matter microstructure or white matter microstructure on any of the tracts at baseline between the two groups (data not shown).

White matter microstructure following CWMT

Using a linear mixed model analysis, we found significant group by time interactions with FA in the left SLF significantly higher in the CWMT group compared to the non-training group at T1 (estimated coefficient = .009, p = .033), and lower MD in the left SLF in the CWMT group compared to the non-training group at T1 (estimated coefficient = -.010, p = .018)(Table 2).

There was a significant group by time interaction with higher global FA in the CWMT group compared to the non-training group at T1 (estimated coefficient = .007, p = .015). Additional linear mixed model analyses in the individual tracts showed a significant group by time interaction with higher FA in the right uncinate fasciculus in the CWMT group compared to the non-training group at T1 (estimated coefficient = .013, p = .029).

Table 1. Study population characteristics

Characteristics	All (n = 34)	Control (n = 19)	CWMT (n = 15)	*P*-value
a) Demographic				
Age (years)	10 ± 2	10 ± 2	10 ± 1	.821
Gender				.728
Male	21 (62%)	10 (67%)	11 (62%)	
Handedness				
Right	28 (82%)	16 (84%)	12 (80%)	.749
Ethnicity				.355
Dutch	29 (85%)	15 (79%)	14 (93%)	
Maternal education level[a]				.242
Low	5 (15%)	2 (11%)	3 (20%)	
Moderate	11 (32%)	5 (26%)	6 (40%)	
High	18 (53%)	12 (63%)	6 (40%)	
Type of education child				.228
Regular	23 (68%)	13 (68%)	10 (67%)	
Regular with help	9 (26%)	6 (32%)	3 (20%)	
Special education	2 (6%)	0 (0%)	2 (13%)	
IQ	101 ± 12	100 ± 12	102 ± 12	.576
b) Clinical				
Birthweight (kilograms)	3.5 (3.2-2.8)	3.5 (3.3-3.8)	3.4 (3.0-4.0)	.650
Gestational age (weeks)	40 (39-41)	40 (40-41)	40 (40-42)	.483
Mechanical vent. (days)	10 (8-16)	11 (8-16)	10 (9-16)	.762
Chronic lung disease[b]	3 (9%)	2 (11%)	1 (8%)	.787
Abnormal CUS[c]	3 (9%)	2 (12%)	1 (9%)	.823
CDH-non-ECMO	11 (32%)	5 (26%)	6 (40%)	.646
ECMO treatment[d]	23 (68%)	14 (74%)	9 (60%)	.475
Type of ECMO				.176
VA	15 (65%)	8 (57%)	7 (78%)	
VV	7 (30%)	6 (43%)	1 (11%)	
VV conversion to VA	1 (4%)	0 (0%)	1 (22%)	
Age start ECMO (days)	1 (1-2)	2 (1-4)	1 (1-2)	.557
Hours on ECMO	109 (85-180)	114 (84-185)	104 (84-161)	.831

N (%), mean ± SD or median (interquartile range) is reported where appropriate. Dutch refers to children with two native Dutch parents. [a]Based on the highest level of education completed by the mother(38). [b]Chronic lung disease defined as oxygen dependency at 28 days of life[29]. [c]Abnormal CUS: hemorrhagic infarct posterior cerebral artery (n=1), focal densities thalami (n=2). [d]Diagnoses underlying ECMO treatment in the neonatal period were congenital diaphragmatic hernia (n=2), meconium aspiration syndrome (n=17), persistent pulmonary hypertension of the newborn (n = 2), infant respiratory distress syndrome (n = 1), and cardiac anomaly (n=1). Abbreviations: CWMT, Cogmed Working Memory Training; IQ, Intelligence Quotient; CUS, cranial ultrasound; CDH, congenital diaphragmatic hernia; ECMO, extracorporeal membrane oxygenation; VA, venoarterial; VV, venovenous.

Table 2. FA and MD group differences in white matter tracts immediately after CWMT

Tract	Hemisphere	Mean FA T1 Non-training	Mean FA T1 CWMT	*p*	p_{FDR}	Mean MD T1 Non-training	Mean MD T1 CWMT	*p*
SLF	**Left**	**.390 (.01)**	**.396 (.01)**	**.033**		**.781 (.01)**	**.770 (.01)**	**.018**
	Right	.387 (.01)	.392 (.01)	.250		.783 (.01)	.762 (.01)	.183
Global	-	**.401 (.01)**	**.406 (.01)**	**.015**		.811 (.01)	.800 (.01)	.055
UNC	Left	.368 (.01)	.381 (.01)	.119	.298			
	Right	.378 (.01)	.392 (.01)	.029	.173			
IFO	Left	.442 (.01)	.451 (.01)	.296	.360			
	Right	.438 (.01)	.447 (.01)	.052	.173			
ILF	Left	.423 (.01)	.424 (.01)	.201	.337			
	Right	.427 (.01)	.429 (.01)	.202	.337			
CB	Left	.382 (.01)	.396 (.01)	.037	.173			
	Right	.351 (.01)	.347 (.01)	.324	.360			
PHC	Left	.276 (.01)	.286 (.01)	.296	.360			
	Right	.295 (.01)	.293 (.01)	.910	.910			

Group*time-point interaction terms of the linear mixed model analyses showing differences in white matter microstructure between children in the CWMT group (n = 14) and non-training group (n = 20) directly after the intervention. Mean weighted average FA (SD) is given for each tract per group. Results are significant at ***p-value*** < .05. The FDR-correction[18] was applied once for the set of additional analyses on the individual white matter tracts (i.e. once for 10 tests). $\boldsymbol{P_{FDR}}$ < .05 was considered statistically significant. Abbreviations: FA, fractional anisotropy; MD, mean diffusivity; UNC, uncinate fasciculus; ILF, inferior longitudinal fasciculus; IFO, inferior fronto-occipital fasciculus; SLF, superior longitudinal fasciculus; CB, cingulum bundle; PHC, parahippocampal cingulum bundle.

However, this finding did not survive the correction for multiple testing (p_{FDR} = .173). Furthermore, a significant group by time interaction was found with higher FA in the left cingulum bundle in the CWMT group compared to the non-training group at T1 (estimated coefficient = .019, p = .037), but this finding did not remain significant after correcting for multiple testing either (p_{FDR} = .173)(Table 2).

Global MD did not significantly change following CWMT (estimated coefficient = -.007, p =.055)(Table 2).

White matter microstructure and neuropsychological improvement following CWMT

Children in the CWMT group who improved with at least 1 SD on verbal working-memory from T0 to T1 had significantly higher FA in the left SLF at T1 (n = 6; FA left SLF at T1 = .408±.01) compared to children that did not show this improvement after CWMT (n = 6; FA left SLF at T1 = .384±.02), $F(1,12) = 6.22$, p = .041, h_p^2 = .47 (Figure 2). This association was not found in the non-training group where two children improved with at least 1 SD from T0 to T1 (p = .175), indicating that the association between the increase in FA in the left SLF and improvement in verbal working-memory is related to CWMT.

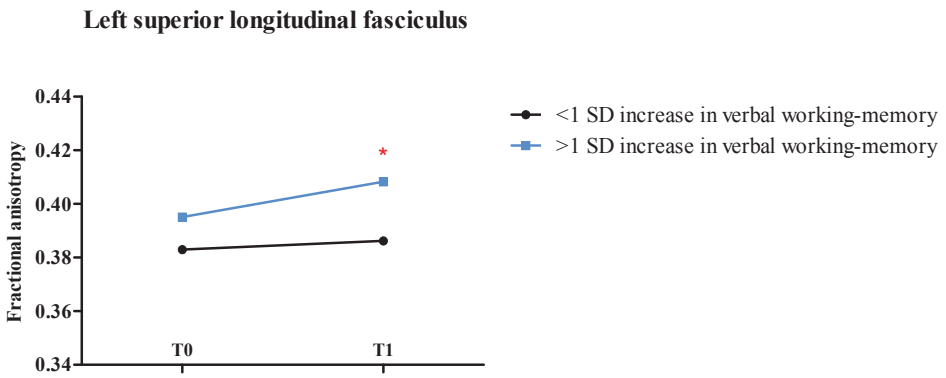

Figure 2. Associations between cognitive improvement and changes in white matter microstructure following CWMT

Results from univariate linear regression analyses showing a significant association between an improvement of >1 SD on verbal working-memory and an increase in fractional anisotropy in the SLF after CWMT. Children who improved with at least 1 SD on verbal working-memory (n=6) after CWMT, had significantly higher FA in the left SLF at T1 than children that did not show this improvement (n=6). *Indicates a significant association at $p < .05$. Abbreviations: CWMT, Cogmed Working-Memory Training.

Significant improvements in visuospatial working-memory or visuospatial memory delayed recall found after CWMT were not associated with the training-induced changes in white matter microstructure in the CWMT group.

DISCUSSION

In this single-blind randomized controlled trial, we found significant white matter microstructural changes in school-age survivors of neonatal ECMO and/or CDH who were trained with CWMT compared to non-trained survivors. We found both global and specific changes in white matter microstructure immediately post-intervention in the CWMT group compared to the non-training group. Specific changes in FA in the left SLF were associated with better verbal working-memory after CWMT. These findings demonstrate that neurobiological plasticity exists in survivors of neonatal critical illness despite significant alterations found in white matter microstructure in these children compared to healthy controls.[3,5]

Our findings of specific changes in the SLF, a white matter tract connecting the frontal and parietal cortices[9], following CWMT confirm previous findings of changes in connectivity in frontopatietal regions following working-memory training in healthy school-age children (8-11 years) as well as in childhood cancer survivors (12 years).[20,21] Although most studies have focused on brain *activity* using fMRI and/or were performed in adults, the frontoparietal network has been consistently shown to be affected by working-

memory training.[22-24] Furthermore, we found that the training-induced changes in the left SLF were significantly associated with improvements in verbal working-memory. Predominantly left hemispheric alterations have been previously found following neonatal ECMO[5], which have been suggested to be due to right internal jugular vein cannulation in neonatal ECMO patients.[25] Because of this, there may be more room for improvement in the left hemisphere, which could explain the training-induced changes in the left hemisphere only. However, we have previously shown that, although more significant alterations were found in the left hemisphere, right-hemispheric alterations were found in these children compared to healthy children as well[3], making this clarification unlikely. The majority of children in our cohort was right handed (80%) and right-handedness is generally associated with left-hemispheric dominance for language.[26] This therefore may also explain the association between verbal working-memory improvements and the left-sided changes in white matter microstructure. These results confirm previous findings that have shown that working-memory functioning is lateralized, i.e. verbal working-memory corresponds with the left hemisphere and visuospatial working-memory with the right hemisphere.[16,27] However, children in the CWMT group improved significantly on visuospatial working-memory (chapter 7), yet we did not find any changes in the right SLF. In previous neuroimaging studies following CWMT in children as well as adults, both right-lateralized and left-lateralized changes in the frontal and parietal cortices have been demonstrated.[9,21] These contrasting findings may be due to the type of image acquisition and analysis employed, such as region-of-interest versus whole-brain analyses, making it difficult to draw definitive conclusions.

In addition to changes in the left SLF, we found that global FA increased in the CWMT compared to the non-training group from T0 to T1. In the majority of the white matter tracts assessed, FA increased following CWMT. This global change may be due to the relatively high plasticity of the child's brain.[10] Increased FA may indicate enhanced orchestration in communication between neural circuits, which, in turn, may lead to enhanced cognitive functioning.[8] However, these global changes were not associated with any cognitive improvements following CWMT. In the same cohort, we previously showed an association between lower global FA and sustained attention deficits, a domain consistently found to be impaired following neonatal ECMO and/or CDH.[3] However, we did not find any direct relationships between the changes in global FA and sustained attention following CMWT. Future research is needed to better understand the clinical relevance of changes in global white matter microstructure following CWMT in children.

We have previously shown improvements in long-term visuospatial memory both immediately and one year after CWMT in this cohort (chapter 7). These cognitive improvements may be due to changes in brain activity or connectivity that were not detectable using DTI.[9] However, in the same cohort, we previously showed that impaired delayed

visuospatial memory was specifically associated with increased MD in the parahippocampal part of the cingulum.[3] A previous study using DTI in adults following CWMT did find training-induced changes in the parahippocampal cingulum.[9] In our cohort, the improvements in delayed visuospatial memory were most apparent one year post-intervention (chapter 7). Potential changes in the parahippocampal and temporal brain regions therefore may not have been detectable immediately following CWMT. In line with this, a recent study in a population-based cohort of school-age children (6-10 years) has shown downstream effects of behavior on the brain instead of the commonly assumed direction of brain shaping behavior.[28] Such a downstream mechanism may explain why we see cognitive improvements immediately following CWMT without corresponding changes in the brain. Unfortunately, this remains speculative as no MRI was made after one year. Future studies conducting multimodal neuroimaging both immediately and longitudinally over the course of the year post-intervention are needed to better understand neuroplasticity following working-memory training.

This is the first study to assess and demonstrate neuroplasticity following CWMT in neonatal ECMO and/or CDH survivors. Despite this, our study has some limitations. First, our small sample size limits the interpretability of our findings, in particular the analyses on associations between brain changes and cognitive improvement in the CWMT group. However, since the association between increased FA in the SLF and improved verbal working-memory had a large effect size (0.47)[19], coupled with prior literature supporting this link[9, 23], we regard this to be a reliable finding. Second, we used a non-active control group for ethical considerations against subjecting children to an intensive training without potential benefits, which limits our ability to attribute our findings to the specific characteristics of the CWMT training. However, neuroplasticity has been demonstrated following working-memory training even when compared to a non-adaptive training program.[9,20] Third, we were not able to conduct an MRI exam one year post-intervention, limiting our understanding of neuroplasticity following CWMT. Future studies that combine neuroimaging and neuropsychological assessment at multiple time points following CWMT are needed. Lastly, the indices extracted using DTI are not specific to any white matter property and therefore specific biological changes may be missed. In the future, studies using more sensitive neuroimaging techniques, such as MRI with higher magnetic field strength (e.g. 7 Tesla), will further increase our understanding of neuroplasticity following cognitive training.

CONCLUSION

Our findings demonstrate both global and specific changes in white matter microstructure immediately after CWMT in school-age survivors of neonatal ECMO and/or CDH

compared to non-trained survivors. Specific changes in FA in the left SLF were associated with better verbal working-memory after CWMT. These results suggest that white matter microstructure and associated cognitive outcomes are malleable in these children. Future studies on the effectiveness of cognitive interventions need to include follow-up assessments both immediately and one year after the training to further increase our understanding of neuroplasticity following neonatal critical illness.

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SUPPLEMENTARY MATERIAL

Supplemental File 1. Descriptions of the neuropsychological tests.

Intelligence

Wechsler Intelligence Scale for Children (WISC-III-NL)

A short-form with two subtests, Block Design and Vocabulary, of the WISC-III-NL were used to assess general intelligence.[1] The WISC-III-NL has been shown to have good reliability and validity.[2] A normalized population mean of 100 with a standard deviation of 15 is used.[2]

Verbal working-memory

WISC-III-NL – subtest Digit Span

The Digit Span consists of random number sequences that increase in length and that the examiner reads aloud at the rate of 1 number per second. The child has to reproduce these numbers in the same order. Next, the sequences must be recalled backwards (3-5-7 becomes 7-5-3). The first part of the test measures short-term auditory memory and short-term retention capacity. The second part measures auditory working memory.[2]

Visuospatial working-memory

Wechsler Nonverbal Scale of Ability (WNV) – subtest Spatial Span

The Spatial Span requires the child to touch a group of blocks arranged on a board in a non-systematic manner in the same and reverse order as demonstrated by the examiner. The first part of the test measures short-term visuospatial memory and short-term retention capacity. The second part measures visuospatial working-memory.[3]

Verbal memory

Rey Auditory Verbal Learning Test (RAVLT)

The RAVLT consists of five presentations with recall of a 15-word list, a sixth recall trial after 30 minutes, and a seventh recognition trial. This test measures memory span, short- and long term verbal memory, verbal recognition, and learning curve. It can be administered to children and adults in the age range 6-89 years.[4,5]

Visuospatial memory and visuospatial processing

Rey Complex Figure Test (RCFT)

The RCFT consists of three trials. First the child has to copy a complex figure (Copy). Then after 3 and after 30 minutes the figure must be drawn from memory (Recall). Next, different figures are shown and the child has to indicate whether these figures were in the original figure (Recognition). This test measures visual integration, short- and long-term

visual-spatial memory, and visual-spatial recognition. It can be completed by children and adults in the age range 6-89 years.[6,7]

Attention

Dot Cancellation Test

This paper-and-pencil test measures sustained attention and concentration in terms of speed. It consists of a paper on which figures made of three, four or five dots are displayed in 33 rows. The child is instructed to mark all figures with four dots, as precisely and as fast as they can.[8]

Stroop Colour Word Test (Stroop)

The Stroop consists of three trials: in the first trial (Stroop 1) the subject must read colour names, in the second trial (Stroop 2) name printed colours, and in the third trial (Stroop 3) name printed colours not denoted by the colour name. The test can be administered to children and adults in the age range 8-65 years. Selective attention is measured with this test, using the difference score between Stroop 2 and Stroop 3.[9,10]

Trail Making Test (TMT)

This paper and pencil test consists of two parts. In the first part (part A), the subject must draw lines to consecutively connect numbered circles on a sheet. In the second part (part B), the subject must consecutively but alternately connect numbered and lettered circles on another worksheet. The aim of the test is to finish each part as quickly as possible. The test can be administered to children and adults in the age range 6-89 years. This test measures visual conceptual and visuomotor tracking as well as divided attention.[9,10]

Executive functioning

Key Search of the Behavioural Assessment of the Dysexecutive Syndrome (BADS-C-NL)

A test of strategy formation. The child is asked to demonstrate how they would search a field for a set of lost keys and their strategy is scored according to its efficiency and functionality.[11]

Modified Six Elements of the BADS-C-NL

The child is asked to work on six different tasks for which they have five minutes. The child needs to make sure that by the end of the five minutes, all six of the tasks have been done and the child has done as much as possible of each task. This is a test of planning, task scheduling and performance monitoring.[11]

We used Dutch versions of all tests.

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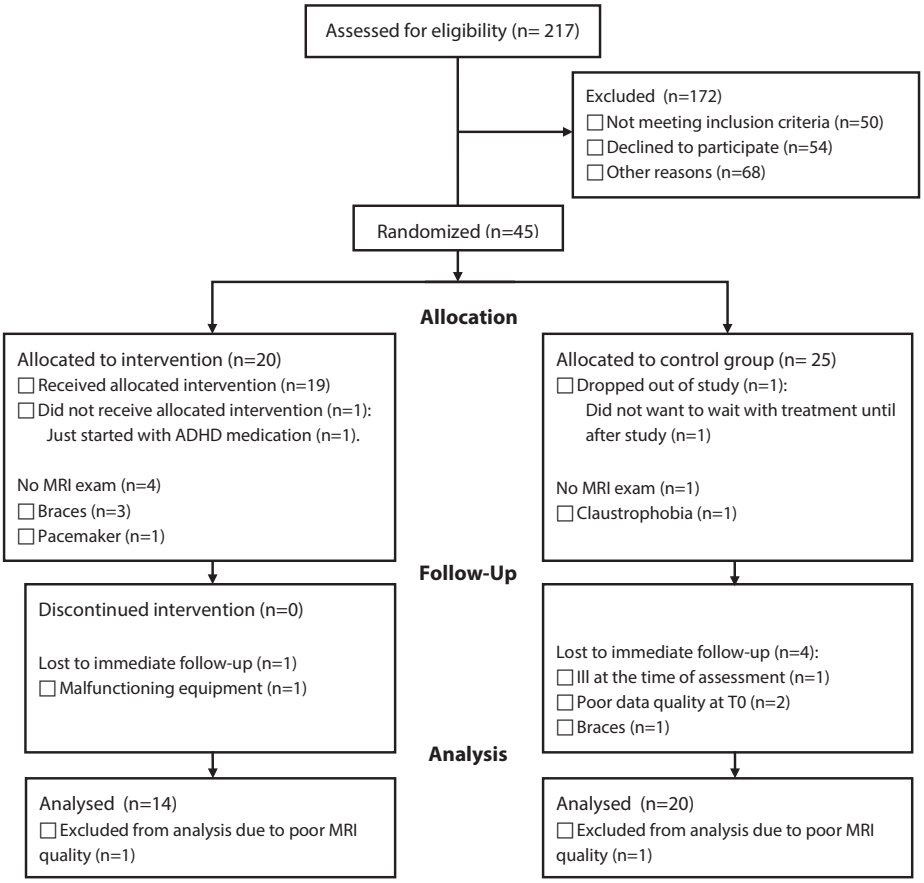

Supplemental Figure 1. CONSORT flow diagram
Abbreviations: ADHD, Attention Deficit Hyperactivity Disorder.

Supplemental File 2. Neuroimaging methods.

Image Acquisition

Prior to neuroimaging, all participants underwent a 30-minute mock scanning session to become familiarized with the MR-environment[1]. Magnetic resonance imaging data were acquired on a 3 Tesla GE MR-750W system using an 8 channel receive-only head coil (General Electric, Milwaukee, WI). In order to support the participant's head and minimize head motion, cushions were placed on both sides of the child's head inside of the head coil. Participants were able to watch a movie during the scans. MRI-compatible headphones were used to reduce the scanner noise and allow participants to listen to the movie's audio track. Communication with the MR operator was also enabled through the headphones before and after scans. The DTI data were acquired using a single-shot, echo-planar imaging sequence with the following parameters: TR = 12,500 ms, TE = 72 ms, flip angle = 90, matrix = 120 x 120, FOV = 240 mm x 240 mm, slice thickness = 2 mm, number of slices = 65, ASSET acceleration factor = 2. In total, 35 volumes with diffusion weighting (b = 900 s/mm^2) and 3 volumes without diffusion weighting (b = 0 s/mm^2) were acquired.

MR-Image Preprocessing

Data were processed using the Functional MRI of the Brain's Software Library (FMRIB, FSL) (2) and the Camino Diffusion MRI Toolkit within Python (version 2.7) and the Neuroimaging in Python Pipelines and Interfaces package (Nipype, version 0.92)[3,4]. First, motion and eddy-current induced artifacts[5] were addressed using the FSL "eddy_correct" tool[6]. In order to account for the rotations applied to the diffusion data after adjusting for these artifact, the resulting transformation matrices were used to rotate the "B-matrix" gradient direction table[7,8]. The FSL Brain Extraction Tool was used to remove non-brain tissue[9]. In order to minimize the limitations observed with respect to the ordinary least squares fit method[10], the diffusion tensor was fit using the RESTORE method implemented in Camino[11]. Voxel-wise scalar maps (i.e. FA, MD) were then computed. FA is the degree of directionality of diffusion and ranges from 0 to 1, where a higher FA generally represents a greater coherence of white matter fibers. MD is the rate of diffusion of hydrogen averaged in all directions. Lower MD is suggestive of increased integrity in axonal membranes, packing, or myelin. White matter continues to mature throughout childhood, even into young adulthood, causing FA to increase and MD to decrease. Abnormal brain development typically leads to lower FA and higher MD in white matter tracts[12].

Probabilistic Fiber Tractography

Fully automated probabilistic fiber tractography was performed using the FSL plugin, "AutoPtx"[13]. Subject-specific, probabilistic representations of multiple white matter fiber

bundles are created with this method using a combination of FSL tools from the Diffusion Toolkit (FDT). The Bayesian Estimation of Diffusion Parameters Obtained using Sampling Techniques (BEDPOSTx), accounting for two fiber orientations at each voxel, was used to esatimate diffusion parameters at each voxel[14,15]. Next, for each subject, the FA map was aligned to the FMRIB-58 FA template image with the FSL nonlinear registration tool (FNIRT). The inverse of this nonlinear warp field was computed, and applied to a series of predefined seed, target, exclusion, and termination masks provided by the AutoPtx plugin (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/AutoPtx). The FSL module "ProbtrackX" was then applied to conduct probabilistic fiber tracking using these supplied tract-specific masks (i.e., seed, target, etc.) in the native diffusion image space of each subject[14]. The connectivity distributions resulting from fiber tractography were normalized to a scale from 0 to 1 using the total number of successful seed-to-target attempts, and were subsequently thresholded to remove low-probability voxels likely related to noise. For each tract, the number of samples used for probabilistic tracking, and the probability thresholds applied to the resulting distributions (ILF: 0.005, SLF: 0.001, IFO: 0.01, UNC: 0.01, CB: 0.01, PHC: 0.02), were selected based on previously established values[13]. After thresholding the path distributions, weighted average DTI scalar measures were computed within each tract using the normalized path distributions as the weights. The methods used were based on those described by Muetzel et al[16].

Image Quality Assurance

Raw DTI image quality was assessed with both a visual inspection and with automated software[16]. For the visual inspection, maps of the sum of squares error (SSE) of the tensor fit were inspected for structured signal that is consistent with motion and other artifacts in the diffusion-weighted images (e.g., attenuated slices in diffusion-weighted images). Furthermore, probabilistic tractography data were inspected visually to ensure images were properly aligned to the template and paths were reconstructed accurately[16]. Datasets determined to be of poor quality were excluded (n = 7, ~8%).

In addition to this visual inspection, slice-wise signal intensity was examined for attenuation resulting from motion, cardiac pulsation and other artifacts using the automated DTIprep quality control tool (http://www.nitrc.org/projects/dtiprep/). Four (~5%) additional datasets were excluded based on the DTIprep results, leaving 77 DTI datasets (patients = 23, controls = 54) for analysis.

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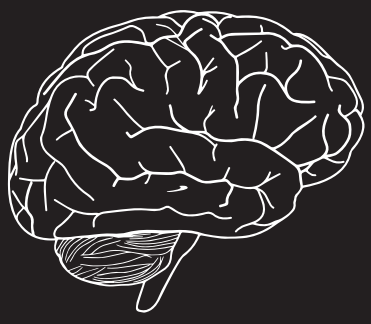
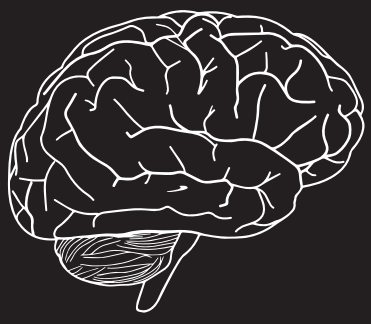


THE VULNERABLE BRAIN AFTER NEONATAL CRITICAL ILLNESS

CHAPTER 9



MEMORY DEFICITS FOLLOWING NEONATAL CRITICAL ILLNESS: A COMMON NEURODEVELOPMENTAL PATHWAY

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SUMMARY

Over the last decade, knowledge has emerged that children growing up after neonatal critical illness, irrespective of underlying diagnosis, are at risk of memory impairment and school problems. Strikingly, these problems are manifest even when intelligence is normal. In this review, we propose a common neurodevelopmental pathway following neonatal critical illness by demonstrating that the survivors of preterm birth, congenital heart disease, and severe respiratory failure, share an increased risk of long-term memory deficits and associated hippocampal alterations. Rather than being a consequence of underlying diagnosis, we suggest that this shared vulnerability is most likely related to common conditions associated with neonatal critical illness. These include hypoxia, neuroinflammation, stress, exposure to anaesthetics, or a complex interplay of these factors at different postconceptional ages. Future work should be aimed at improving early identification of patients at risk and evaluating intervention modalities, such as cognitive or exercise training.

INTRODUCTION

Over the last decade, the number of children admitted to neonatal intensive care units has increased significantly worldwide.[1,2] Due to medical improvements, the majority of these children now survive to discharge[1,2], necessitating our focus to broaden from prevention of mortality to long-term outcome. Fortunately, a significant number of children survive without overt brain abnormalities, such as cerebral haemorrhage or periventricular leukomalacia.[3-5] However, knowledge has emerged that children growing up after neonatal critical illness, irrespective of gestational age or underlying diagnosis, are at risk of neuropsychological impairments and school problems. Strikingly, these problems exist even when intelligence is within the average range.[6-9]

Memory deficits are frequently reported following neonatal critical illness. The hippocampus is the critical hub for memory formation.[10] Interestingly, the hippocampus is particularly vulnerable to conditions associated with critical illness, such as hypoxia and neuroinflammation.[11,12] We therefore speculate that a common neurodevelopmental pathway exists across critically ill neonates, where early hippocampal alterations result in long-term memory deficits. This 'growing into deficit' phenomenon[13] – where subtle brain injuries acquired in early life only become evident later in life when those brain regions are required for higher cognitive functioning – can potentially be delineated following three major causes of neonatal critical illness: preterm birth, congenital heart disease (CHD), and severe respiratory failure (necessitating neonatal extracorporeal membrane oxygenation (ECMO) treatment).

In this review, we describe the abnormalities in long-term memory functioning, and summarize findings on memory and its neurobiological substrates, specifically those pertaining to the hippocampus, in children following preterm birth, CHD and neonatal ECMO treatment. Next, we evaluate why the hippocampus may be particularly vulnerable in these children. Taken together, we propose a common neurodevelopmental pathway following neonatal critical illness. We conclude with the potential clinical implications and future directions of research.

SEARCH STRATEGY AND SELECTION CRITERIA

PubMed was searched for articles published between January 1, 2000 and June, 2017 with the search terms in the title or abstract: ("brain imaging" OR "brain" OR "neuroimaging" OR "magnetic resonance imaging" OR MR* OR hippocamp* OR "limbic") AND ("memory" OR "learning") AND (("preterm" OR "preterm birth" OR "premature birth") OR ("congenital heart disease" OR complex heart anomal* OR "complex heart disease") OR ("neonatal respiratory failure" OR "acute respiratory failure" OR "neonatal extracorporeal membrane oxygenation"

OR "neonatal ECMO")). This search resulted in 348 references. We reduced the number to 258 by restricting findings to human studies. Studies that did not assess the hippocampus specifically and/or did not assess memory, and studies including patients with severe neurologic abnormalities or genetic syndromes known to affect neurodevelopmental outcome were excluded. Searches were supplemented by hand searching of reference lists of published articles. The final reference list was generated on the basis of relevance to the scope of this review. In total, 27 studies were included.

MEMORY AND THE HIPPOCAMPUS FOLLOWING NEONATAL CRITICAL ILLNESS

Despite generally average intelligence, the incidence of academic difficulties is strikingly high following preterm birth, CHD and severe respiratory failure.[6,7,9,14-16] This is highly suggestive of an alternative explanation related to specific neuropsychological deficit rather than general intellectual functioning (Table 1). Memory deficits can greatly affect daily activities and academic achievement, and have been reported in 19-41% of children born preterm[7,19], in 28-64% of children with CHD[20], and in 50-70% of children with severe respiratory failure, treated with or without neonatal ECMO[18], compared to 16% in the general population.[17] These deficits become particularly evident as children get older, suggesting that they 'grow into their deficits'.

Table 1. Neuropsychological impairments following neonatal critical illness

	IQ	Attention	Verbal memory	Visuospatial memory	Executive functioning	Visuospatial processing	Academic difficulties
Preterm	x	x	x	x	x	x	x
CHD		x	x	?	x	x	x
Neonatal ECMO[1]		x	x	x	*		x
CDH		x	x	x			x

Frequently reported neuropsychological impairments following neonatal critical illness, an impairment regarded to be significantly lower than healthy controls. In case of intelligence (normal mean IQ(SD) = 100(15), reported mean IQ score of ≤ 85 (i.e. ≤ -1 SD) is regarded impaired.

[1]Neonatal ECMO treatment applied in case of severe respiratory failure, such as meconium aspiration syndrome or congenital diaphragmatic hernia.

*Only working-memory impairments.

? Indicates equally impaired and normal outcomes reported in studies.

Abbreviations: IQ, intelligence quotient; CHD, congenital heart disease; ECMO, extracorporeal membrane oxygenation; CDH, congenital diaphragmatic hernia.

Memory encoding, consolidation and retrieval are highly dependent on the hippocampus and its connections, which are embedded within the brain's limbic system.[10] The hippocampus is thought to be highly involved in the ability to store and retrieve information about an event as well as about the context in which the event took place, and in the de-

layed recall of verbal and visuospatial information.[10,21] In utero, changes in hippocampal morphology occur and the hippocampus is thought to resemble adult shape by 25 weeks of gestation. A crucial period of hippocampal development is in the first two years of life when it undergoes a growth spurt. Hippocampal volume is thought to peak between 9 years and 11 years of age, after which it resembles adult size (Figure 1).[22] Various studies have examined memory and the underlying neurobiology of memory impairments following neonatal critical illness and have shown that, just as in healthy children and adults[10,11], the hippocampus is essential for memory functioning in survivors of neonatal critical illness (please refer to the online Appendix for an overview of these studies).

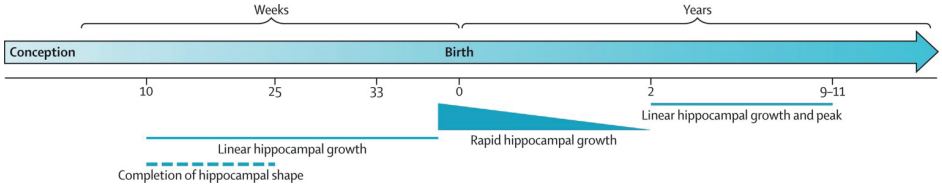

Figure 1. Schematic overview of the major phases of hippocampal development
Hippocampal morphological and positional changes occur in utero. A growth spurt occurs between the perinatal months and two years of age. Peak volume reached between 9-11 years, resembling adult size.

Preterm birth

Preterm birth is defined as birth before 36 completed weeks of gestation and accounts for 10% of all births in high-income countries.[2] Preterm birth is increasing in Europe and is the major cause of death in neonates.[2] However, survival of preterm infants is also increasing[2], and hence long-term outcomes are becoming increasingly important.

In school-age and adolescent survivors of preterm birth, impairments in speed of information processing, attention, visuospatial processing, language, executive functioning, and memory have been reported.[7] Short-term and long-term verbal and visuospatial memory deficits have been identified, even in young adults who were born preterm.[7,21,23-25] The neurobiological substrates of memory have been assessed in various developmental stages following preterm birth (online appendix). In infancy, left and right hippocampal volumes as well as shape were altered in preterm neonates compared to term-born controls at term-equivalent age.[26,27] Although hippocampal shape in infants was not related to memory function, bilateral hippocampal volume was positively associated with verbal memory at seven years of age.[23] Preterm children have consistently altered hippocampal shape and smaller left and right hippocampal volumes than do term-born controls between 7 years and 11 years of age.[19,23,28-30] Studies that have also assessed the association between the hippocampus and memory are scarce, but have shown no association between hippocampal alterations and memory impairments in school-age survivors.[19,29] This absence of association might be due to

the type of memory tests used or the involvement of other brain regions in the assessed memory functions, or both. In adolescents and young adults who were born preterm, alterations have been found in the hippocampus and surrounding brain regions, such as the hippocampal fornix and parahippocampal gyrus, have been found. In these studies, consistent associations were demonstrated between the hippocampal alterations, as well as alterations to the areas surrounding the hippocampus, that were associated with impaired memory.[24,25,31-33] One study, however, found hippocampal alterations but normal memory performance.[28] These findings might reflect brain plasticity or compensatory mechanisms, causing other brain regions to take over the function of the affected regions in the preterm brain.[21]

One study found similar hippocampal volumes in school-age children born preterm and term-born controls, despite poor memory outcome in preterm children.[34] These memory impairments may be explained by alterations in other unassessed brain areas responsible for memory functioning. However, methodological issues, such as small sample size, assessment of only one type of memory, and use of two different MRI scanners within the same cohort, might also explain these contradictory results. The severity of prematurity could also affect findings as previous studies have shown positive associations between gestational age and hippocampal volume.[35-37]

Congenital heart disease

Children with CHD who have been critically ill in the neonatal period and have had major cardiac surgery are at risk of significant neurodevelopmental problems later in life. The Boston Circulatory Trial assessed children with dextro-transposition of the great arteries (d-TGA) who underwent the arterial switch operation and found below average academic achievement, visuospatial skills, working-memory and attention at school-age and during adolescence. These impairments were found despite normal intelligence.[8,38] A meta-analysis in 5-8 year-olds who had heart surgery for CHD found similar impairments in executive functioning, attention and visuomotor integration. Furthermore, generally lower verbal memory was identified in survivors compared to healthy controls, whereas non-verbal memory was normal.[16] In CHD children tested four years after heart surgery treated with tight glucose control, worse working-memory and immediate verbal memory were found compared to healthy controls.[16] A study in children with d-TGA between the ages 8-16 years, reported specific deficits in both verbal and visual delayed memory.[39] Similar deficits have been found in adolescent survivors of CHD of differing complexity as well, even persisting into young adulthood.[20] A small number of studies have examined memory and its neurobiological correlates in survivors of CHD (online appendix). Smaller bilateral hippocampal volumes were demonstrated in 40% of school-age children who had d-TGA and cyanosis when compared to healthy controls. Hippocampal reductions were associated with worse verbal and visuospatial memory.[39]

Furthermore, 13-year-old children who had cardiopulmonary bypass surgery in infancy had smaller bilateral hippocampal volumes as well as volume loss in other parts of the limbic system's grey matter than did healthy controls.[40]

It is important to note that underlying cardiac anomaly and treatment may influence neurodevelopmental outcome. For instance, adolescents who underwent the Fontan and Norwood procedures scored below the population norm on general memory, whereas patients who underwent a different operation had normal outcomes.[15] Although assessed in a small sample size, cyanotic CHD patients had more pronounced hippocampal volume loss than acyanotic patients. Memory was not assessed.[40]

Neonatal ECMO in case of severe respiratory failure

Since the first neonatal ECMO treatment applied in 1975, nearly 40,000 neonates were treated with ECMO worldwide.[41] The annual number of neonatal ECMO runs has decreased over the years and treatment has shifted from respiratory to cardiac runs. However, the most frequent underlying diagnoses for neonatal ECMO remain meconium aspiration syndrome (MAS) and congenital diaphragmatic hernia (CDH). The survival rate following MAS is over 90%. CDH is a rare congenital anatomical malformation associated with significant mortality and morbidity due to pulmonary hypoplasia and pulmonary hypertension. In the most severe cases of CDH necessitating treatment with ECMO, mortality rates are 49%.[41] Over the past decade, standardised treatment protocols for CDH patients have reduced mortality and the need for ECMO.[42]

In school-age neonatal ECMO survivors, extensive neuropsychological assessment has shown mainly attention, verbal and visuospatial memory deficits.[17] Similar deficits were found in adolescent survivors, while other domains remained relatively unaffected.[9] Interestingly, memory deficits have also been found in children with CDH who were not treated with ECMO.[37]

Studies have examined the neurobiological substrates of long-term neuropsychological outcome following severe respiratory failure with neonatal ECMO treatment (online appendix). We found global white matter microstructure alterations and regional alterations in the limbic system in school-age neonatal ECMO survivors compared to healthy controls.[43] Specifically, hippocampal volume reductions were associated with worse delayed verbal memory in the neonatal ECMO survivors.[17] White matter microstructure alterations in the parahippocampal region of the cingulum – a white matter tract connecting the medial temporal lobe with the parietal and occipital lobes – were associated with worse visuospatial and verbal memory.[17] Similar structure-function relationships were demonstrated in CDH patients not treated with ECMO.[17] In line with these findings, in school-age survivors of acute hypoxic respiratory failure, either treated with conventional ventilator management or ECMO, smaller hippocampal volumes were identified when compared to healthy controls. These were associated with impaired memory for everyday events, and verbal and visuospatial memory deficits.[37]

HIPPOCAMPAL VULNERABILITY AND NEONATAL CRITICAL ILLNESS

The above-described findings demonstrate that memory deficits are associated with hippocampal alterations following neonatal critical illness, irrespective of underlying diagnosis. These hippocampal alterations are likely a result of both the timing and type of insults critically ill neonates are exposed to. The brain, including the hippocampus and the rest of the limbic system, develops rapidly in the third trimester and throughout the neonatal period.[44] During this period, critically ill infants are at risk of exposure to hypoxia, neuroinflammation, stress, and clinical procedures requiring general anaesthesia. The hippocampus has been found to have a selective vulnerability to these conditions associated with critical illness. The hippocampus consists of the cornu ammonis (CA) regions 1 (CA1) and 3 (CA3), the dentate gyrus (DG) and subiculum (Sub). Animal and *in vitro* models of the hippocampus have demonstrated that these subregions show differential vulnerability[11,45], which might explain why it is affected by such a wide range of conditions (Figure 2). In the next section, we will explore why the hippocampus shows a pronounced and selective vulnerability to hypoxia, neuroinflammation, stress, and anaesthetics.

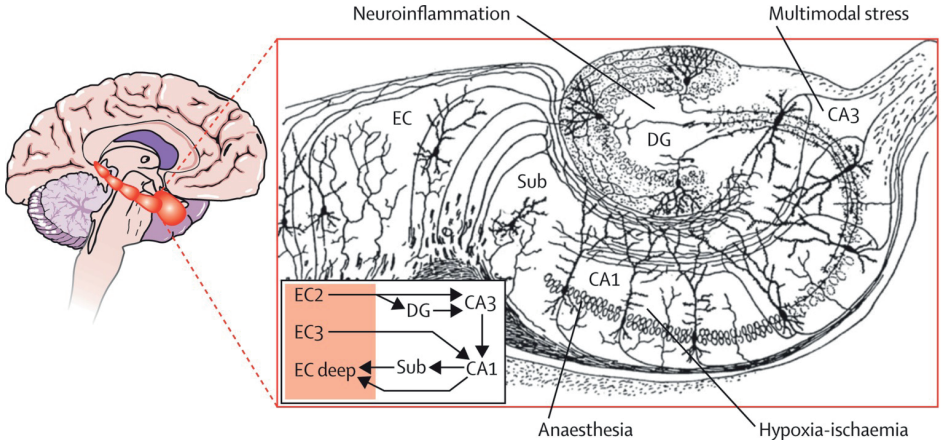

Figure 2. Vulnerability of the hippocampus to conditions associated with neonatal critical illness
Differential vulnerability of the hippocampal cornu ammonis (CA) regions 1 (CA1) and 3 (CA3), the dentate gyrus (DG) and subiculum (Sub) to hypoxic-ischaemia, neuroinflammation, stress and anaesthetics is shown.

Hypoxia

Cerebral hypoperfusion and/or hypoxemia resulting in hypoxia are common complications in preterm infants, or (near) term infants with CHD, or severe respiratory failure, either treated with or without neonatal ECMO. Studies using animal and *in vitro* models have demonstrated that the hippocampus shows more pronounced changes follow-

ing hypoxia-ischaemia than other brain structures (reviewed by Schmidt-Kastner).[47] Furthermore, differential vulnerability for hypoxia-ischaemia in the hippocampal subregions has been suggested; CA1 seems more vulnerable to acute hypoxic-ischaemia than CA3 and DG, in which relative sparing has been found. However, prolonged periods of cerebral ischaemia have been shown to damage CA3 and DG as well.[47] Differential vulnerability within the hippocampus is suggested to result from regional differences in antioxidant enzymes, inflammatory reaction and/or in the distribution of glutamatergic N-methyl-D-aspartate (NMDA) receptors.[11] This variation in vulnerability might also lead to different types of memory impairments since differential functional organisation within the hippocampal formation has been suggested as well.[44] However, this needs further study in humans.

In addition to the hippocampus, its surrounding white matter, and in particular the periventricular white matter, seems specifically susceptible to hypoxic-ischaemic insults. Using animal models, premyelinating oligodendrocytes (pre-OLs) in cerebral white matter have been identified as selectively targeted by oxidative stress. These cells account for approximately 90% of the total oligodendroglial population at 28 weeks of gestation and approximately 50% at term.[48] Increased regional susceptibility of the periventricular white matter is suggested to be due to the distribution of these pre-OLs and relative underdevelopment of distal arterial fields in these areas.[48,49] Therefore, neonates exposed to hypoxic-ischaemic injuries, even at term, might be at increased risk of myelin and axonal disruptions resulting in white matter abnormalities, which have been shown to correlate with impaired neurodevelopmental outcome.[50]

Inflammation

Critical illness is often accompanied by a marked proinflammatory response to underlying factors such as stress, infection or hypoxia-ischaemia.[51] The hippocampus has been shown to be involved in the regulation of inflammation due to its high density of microglia. Microglia in the (immature) central nervous system respond rapidly to infection or injury. In case of deleterious conditions, microglia in the hippocampus show a dynamic process of neuroprotective and pro-inflammatory responses, producing pro-inflammatory and neurotoxic factors. Using rodent models, the latter mechanism has been shown to negatively affect hippocampal neurogenesis and cellular composition in the developing brain.[52] Clinical studies in neonates have shown an elevated risk of brain injury following inflammation[51], but its specific effects on the hippocampus need further research. Behavioural effects of inflammatory damage to the hippocampus have been studied, though mostly using experimental models, demonstrating an association between pro-inflammatory cytokines in the hippocampus and memory impairments.[52]

Elevated glucocorticoid levels

Endogenous glucocorticoids

Environmental stressors from the neonatal ICU have been found to elicit physiological stress responses in critically ill infants and affect brain structure and function.[53] In response to stress, the brain's hypothalamus-pituitary-adrenal (HPA) axis is activated, causing the release of cortisol into the blood by the adrenal gland. The hippocampus is a key regulator in this system by reducing HPA axis activity following stress exposure. Cortisol binds to mineralocorticoid receptors (MRs) and glucocorticoid receptors (GRs), which are highly expressed in the hippocampus and excess cortisol results in hippocampal dendritic atrophy and inhibited neurogenesis.[54] Animal models have shown pronounced effects of acute stress on CA1. However, in reaction to chronic and/or multimodal stress (e.g. hours-long light, loud noise), which might be more similar to experiences in the NICU, the CA3 region showed synapse reductions, leading to poorer object recognition memory.[55] One study[54] showed that stressors in the neonatal ICU environment were associated with altered brain microstructure and functional connectivity within the temporal lobes, but not in the frontal lobe of the brain, in preterm infants (born <30 weeks). Although memory functioning was not assessed, increased stress exposure resulted in more neurobehavioral problems at term-equivalent age.[56] Stress may also be experienced by the mother during pregnancy in case of prenatally identified congenital anomalies and/or in the NICU period. Increased pre- and postnatal maternal stress has shown to affect infants' hippocampal growth in the first six months of life.[54] Maternal stress exposure is thought to have 'programming' effects on the foetal HPA axis activity. Increased maternal cortisol secretion may partly reach the foetus, increasing foetal HPA activity by reducing the number of MRs and GRs in the hippocampus.[54] Although more research in humans is needed, maternal stress may contribute to an increased risk of long-term memory impairments in critically ill neonates.[54]

Exogenous glucocorticoids

Studies in preterm born children have shown that postnatal treatment with dexamethasone – a corticosteroid used especially in preterm children to accelerate lung development – negatively affected hippocampal morhpology.[23,26] However, dexamethasone might not affect children treated with neonatal ECMO and CHD as much, as corticosteroids are generally used less frequently in these patients.[43]

Anaesthetics

Possible negative effects on the developing brain of prolonged, general anaesthesia has recently resulted in an FDA warning regarding its use in children younger than three years.[57] Although clinical studies in humans are scarce and findings are mostly based on experimental studies, hippocampal development may be affected by the use of com-

monly used anaesthetic agents, resulting in long-term memory deficits. Commonly used anaesthetics bind either to γ-aminobutyric acid (GABA) or NMDA receptors. The GABA and NDMA receptor systems are crucial for neuronal connection and communication in the developing brain, and if unavailable lead to neuroapoptosis.[57] Various types of anaesthesia, such as midazolam, propofol and ketamine, have been suggested to disrupt memory formation through its effects on the hippocampus.[58] Memory formation and recall are dependent on a system of persistent strengthening of synapses following high levels of stimulation, called Long-Term Potentiation (LTP). LTP, which mainly happens in the hippocampus, relies heavily on NMDA. In a rat model, midazolam was found to affect pyramidal neurons in the CA1 region and memory by suppression of LTP.[59] However, translating findings from animal models to the developing human brain is restricted[60] and research in humans and/or specific disease models is crucial. In very preterm neonates (24-32 weeks of gestation), high exposure to midazolam was negatively associated with hippocampal growth from birth to term-equivalent age, adjusted for clinical confounders including gestational age, days of mechanical ventilation, and number of surgeries.[61] However, memory outcome was not assessed. In school-age children who underwent general anaesthesia before one years of age, significantly worse memory recall was found compared to untreated controls, whereas IQ remained unaffected.[57] The association between memory deficits and altered hippocampal morphology was not assessed.

A COMMON NEURODEVELOPMENTAL PATHWAY FOLLOWING NEONATAL CRITICAL ILLNESS

In this Review we present evidence to suggest a shared vulnerability of the hippocampus that is associated with long-term memory impairments across critically ill neonates. On the basis of this evidence, we speculate that a final common neurodevelopmental pathway exists following neonatal critical illness (Figure 3). It is important to note that the patient groups described in this review have varying brain development trajectories due to variations in illness onset (e.g. congenital anomaly developing in utero versus postnatal sepsis necessitating neonatal ECMO), gender, and gestational age. These factors are likely to interact with the exposure to harmful conditions associated with neonatal critical illness, and may influence how and when the hippocampus is affected. The exact pathophysiological mechanisms of the hippocampal alterations in each of these patient groups remains unknown and needs further research.

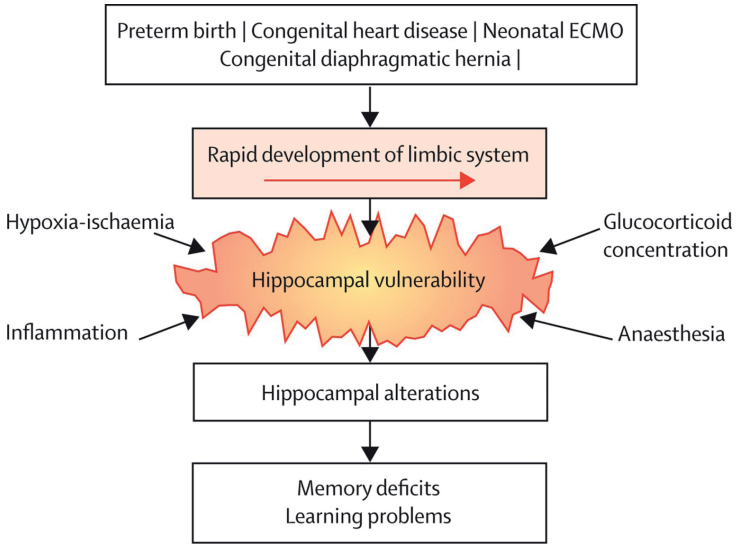

Figure 3. A common neurdevelopmental pathway following neonatal critical illness
Neonatal critical illness survivors share an increased risk of hippocampal alterations due to vulnerability to common conditions associated with neonatal critical illness, leading to long-term memory deficits**.**

CLINICAL IMPLICATIONS AND FUTURE DIRECTIONS

In the previous sections, the shared memory impairments and the role of the hippocampus across critically ill infants was highlighted. In this section, we describe how the findings outlined in this review guide the direction of future research and contribute to the realisation of early identification of patients at risk as well as the development of targeted intervention or treatment modalities.

Early risk prediction

Currently, the identification of patients at risk of school problems relies solely on neuropsychological assessment. However, evaluating higher-order cognitive functions such as memory cannot be reliably conducted until school-age.[7] The identification of patients at risk of academic problems is as such often too late. The neuropsychological deficits, that may have remained unidentified or unspecified, may by then have already led to school problems. Neuropsychological assessment should therefore be primarily used as a diagnostic tool, rather than as a prediction tool. Patients at risk should be identified before academic difficulties are present. In order to do so, early predictors of long-term memory impairments, favourably measurable in infancy, are needed.

Firstly, hippocampal volume alterations, if detected in infancy, could potentially serve as such a predictor of impaired long-term memory. MRI is a non-invasive neuroimaging technique and therefore a useful tool to assess the hippocampus in infants. Findings

in preterm infants have shown that MRI can be reliably performed without sedation and that infant hippocampal volumes, measured at term-equivalent age, correlate with memory outcomes both at two years and seven years of age.[23,27] Future studies are needed to find cut-off points to separate normal from abnormal hippocampal volume in infancy using a healthy control group. Furthermore, differences in the timing and type of hippocampal injury are likely to exist across critically ill infants because of differences in brain development associated with disease aetiologies. For instance, brain alterations have been found as early as in the third trimester in CHD patients[5] and the immature preterm brain may respond differently than the term brain to the strenuous conditions associated with neonatal critical illness.[49] Longitudinal studies are therefore imperative to accurately delineate the longitudinal hippocampal growth trajectories across these patient groups. Also, given the rapid hippocampal growth during the first two years of life[22], longitudinal studies will help to determine the best time to assess hippocampal morphology as an early predictor of memory.

Up to this point, the hippocampus has been primarily quantified through volumetry using structural MRI. This method has been shown to be very robust and useful to accurately parcellate hippocampal volume. In future studies, it would be interesting to focus on details in the hippocampal parcellation by obtaining finer resolution images and multi-contrast imaging, or using alternative modalities such as diffusion imaging. This may contribute to a better understanding of the differential vulnerability of the hippocampal subregions, and, if combined with neuropsychological assessment, its suggested differential involvement in various memory processes.[11,45] However, importantly, MRI does not provide information on the exact anatomical or molecular mechanisms underlying the hippocampal alterations. The specific contribution of the risk factors outlined in this review to the hippocampal alterations and long-term memory deficits therefore remains speculative.

Secondly, hypoxia is consistently shown to affect hippocampal morphology. The severity of cerebral hypoperfusion sustained in the perinatal period may therefore be another risk factor of long-term memory problems following neonatal critical illness. Adequate ways to monitor cerebral metabolism, haemodynamics and autoregulation in the NICU are urgently needed since current methods, such as transcranial Doppler ultrasonography or near-infrared spectroscopy, do not have enough resolution for targeting the brain region of interest in this context.

Treatment opportunities

Although the hippocampus is a highly vulnerable brain structure, it has also been shown to exhibit more plasticity and capability of long-term neurogenesis than other brain structures.[11] This makes it a promising target for intervention strategies to improve long-term memory following neonatal critical illness.

Neuroprotection

Another group of critically ill neonates at high risk of hypoxic-ischaemic injuries are survivors of perinatal asphyxia. In contrast to the patient groups described in this review, in a significant number of neonates with perinatal asphyxia overt, chronic neurological abnormalities are present as well as long-term severe morbidities such as cerebral palsy and intellectual disability.[62] Whole body therapeutic hypothermia is the standard of care in full-term neonates with perinatal asphyxia and has shown to improve neurologic outcomes.[63] Using animal and *in vitro* models, mild to moderate therapeutic hypothermia has been shown to reduce hippocampal cell death following an hypoxic or ischaemic insult.[64] However, while patients with perinatal asphyxia experience an acute phase of hypoxia[65], more prolonged exposure to hypoxia may exist in the patient groups described in this review. Furthermore, hypothermia likely does not protect the hippocampus against the other types of harmful conditions associated with critical illness. Future randomized controlled trials are needed to assess if and to what extent therapeutic hypothermia affects the hippocampus and memory in these groups before it can be recommended as a routine neuroprotective strategy.

Stress prevention

As demonstrated, stress may negatively affect the hippocampus and memory.[54] Reducing stress exposure during NICU stay could therefore be beneficial for critically ill infants as well as relatively feasible in clinical practice. Indeed, decreasing light and sound in the NICU, as well as encouraging physical parent-infant contact during hospital stay, have been shown to positively affect development.[66] Future studies assessing whether such stress reduction measures specifically influence hippocampal development and memory improvements in neonatal critical illness survivors are needed.

Cognitive interventions

Studies evaluating 'brain training' or computerized cognitive training programs have increased over the last decade. However, its effectiveness remains controversial. Cognitive training programs are based on the idea that repetitive mental exercise of one cognitive task will result in improved functioning that may generalize to other tasks with a similar underlying system. A widely evaluated cognitive training for both children and adults is Cogmed working-memory training.[67] Studies have fairly consistently shown improvements in the trained verbal and visuospatial working-memory skills, but less consistently in untrained functions such as delayed memory recall.[68] Working-memory, the main targeted function of Cogmed, relies primarily on frontal-parietal networks.[67] It is unclear how, or if, Cogmed influences the plastic nature of the hippocampus. Neuroimaging studies assessing the exact effects of Cogmed on hippocampal function and different types of memory are needed in survivors of neonatal critical illness. Cur-

rently, randomized controlled trials are being performed with school-age survivors of neonatal ECMO (Trial Registration Number: NTR4571) and children and adolescents with CHD (Clinical Trial Numbers: NCT03023644 and NCT02759263). Other types of cognitive training programs used specifically for memory rehabilitation may be effective but need further research in neonatal critical illness survivors.[69]

An important part of effectively using cognitive intervention is the identification of neonatal critical illness survivors that have significant memory deficits, and are thus in need of treatment. While early identification of these patients is ideal, in today's practice, identifying children with such deficits remains reliant on neuropsychological assessment at school-age. It is therefore crucial to conduct neuropsychological assessment in which, in addition to intelligence, specific neuropsychological outcomes are the primary focus following neonatal critical illness.

Exercise interventions

Exercise might enhance memory and learning by targeting the hippocampus. Greater aerobic fitness has been associated with hippocampal volume as well as improvements in memory in children.[70] Whether improvements persist in the long-term remains largely unknown and needs further study in survivors of neonatal critical illness.

CONCLUSION

With this review, we propose a common neurodevelopmental pathway following neonatal critical illness by demonstrating that survivors of preterm birth, CHD, and severe respiratory failure share an increased risk of long-term memory deficits and related hippocampal alterations. Rather than being a consequence of underlying diagnosis, we suggest the shared vulnerability to be related to common complications or conditions associated with neonatal critical illness. These include hypoxia, neuroinflammation, stress, anaesthetics, or a complex interplay of these factors at different postconceptional ages. Our findings underline the need of broadening our focus from prevention of mortality to long-term outcome of critically ill infants. Follow-up should incorporate standardized assessment of specific neuropsychological functions, such as memory, at school-age. Early identification of patients at risk may be feasible using infant hippocampal volumes assessed with non-invasive structural MRI. Future prospective studies on memory rehabilitation with the use of cognitive or exercise training are needed in neonatal critical illness survivors. Lastly, increased awareness of the vulnerability of the hippocampus and memory deficits following neonatal critical illness is crucial to prepare survivors for future academic problems and participation in society.

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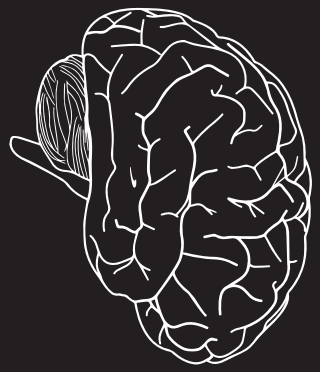
CHAPTER 10



ANALGESICS AND SEDATIVES IN CRITICALLY ILL NEWBORNS AND INFANTS: THE IMPACT ON LONG-TERM NEURODEVELOPMENT

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SUMMARY

Inadequate pain and/or stress management in preterm and term born infants has been associated with increased morbidity and even mortality. However, exposure to analgosedatives during early infancy may also be one of the risk factors for subsequent neurodevelopmental impairment, at least in animal studies. Since infants admitted to neonatal or pediatric intensive care units may receive very high amounts of these drugs for prolonged periods of time and the majority of these infants nowadays survive to discharge, this is of major concern. A balanced approach that incorporates the assessment and quantification of both wanted effects as well as unwanted side effects is therefore needed. In this paper, the optimum dose determination of commonly used analgosedative drugs as well as their potential long-term effects on the developing human brain and neuropsychological functioning are reviewed.

INTRODUCTION

Over the last decades, phenomena such as pain, stress, and anxiety/agitation have intrigued a variety of clinicians and scientists. The paradigm that immaturity protects neonates from pain and its negative effects has been questioned years ago by Anand et al. who demonstrated that untreated perioperative pain was associated with increased morbidity and even mortality.[1] These negative effects have been described in greater detail in recent reviews, showing that insufficient pain management in (pre)term infants during painful interventions alters physiological responses, pain thresholds and pain or stress-related behavior.[1-3] However, evidence showing a link between exposure to analgosedatives (analgesics and sedatives) in early infancy and subsequent neurodevelopmental impairment is accumulating as well.[4-6] As the majority of infants admitted to neonatal or pediatric intensive care units now survive to discharge[7,8], this is of major concern. Although there may be long-term effects of anesthetic agents on the brain as well, this has been extensively described and reviewed in the past few years[2,9-13] due to a recent FDA warning about its use in children younger than three years[14], and is therefore beyond the scope of the current review.[2,9-13]

The balance between adequate pain management and the risk of long-term neurodevelopmental impairments reads like a catch-22, but at least indicates that a balanced approach that incorporates assessing and quantifying both wanted effects as well as unwanted side effects is needed. To avoid both pain and stress with all negative consequences as well as over-exposure (too much or too long) to analgosedatives, health care providers should select the most appropriate intervention (either pharmacological or non-pharmacological) based on the best available evidence for a given indication, with adjustments to reach the most effective and shortest exposure.[15] Over the last five years, a number of guidelines have been published and trials have been performed to find the optimum dosage for analgosedative drugs.[16-20] However, we need to be aware that the level of evidence is unfortunately still limited. Data on maturational pharmacokinetics (PK) are a crucial, yet first, step of a drug development program, especially in neonates and young infants. Therapeutic dose-finding studies, even for commonly used drugs, are needed to come up with a valid study design in neonates and infants before comparative phase 3 efficacy and safety trials can be conducted.[21]

In pain management of infants admitted to the neonatal or pediatric intensive care unit, the ultimate goal is to achieve adequate pain management with minimal short and long-term side effects. In reaching this goal, it is imperative to also increase our understanding of how exposure to sedatives and analgesia may affect the developing brain. Based on the existing literature combined with our own research, we will summarize the present knowledge and level of evidence of a number of commonly used

analgosedative drugs which serve as model drugs and their potential long-term effects on the developing brain and neuropsychological functioning in humans.

DOSE DETERMINATION OF COMMON ANALGOSEDATIVE DRUGS

Analgesics

Morphine

Morphine is the most commonly used intravenous opioid to provide potent analgesia in neonates, infants and children. However, age-related differences in both pharmacokinetic (PK) and pharmacodynamic (PD) responses during development pose challenges for selection of an appropriate dose. As sensitivity of the central nervous system to morphine is increased in neonates, a lower initial dose of morphine is recommended which is then adjusted based on individual responses. In addition, the elimination half-life of morphine is more than twice as long as that observed in adults (6-12 hours in neonates versus 3-4 hours in adults[22]), due to immaturity of the neonatal hepatic drug metabolizing enzyme system. Morphine clearance increases in accordance with the maturation of the glomerular filtration rate and by 1 year of age, the ratio of plasma to cerebral spinal fluid concentration is comparable to that of adults. A number of studies have described optimal dosage of morphine. Four studies only included neonates[23-26] and four studies consisted of data solely based on children[27-30] (ranging from 10-40 μg/kg). The use of morphine is, especially in the preterm, still a matter of debate. In the cohort of Simons et al., preterm neonates in need of mechanical ventilation and with a median gestational age of 29 weeks, received either morphine 10 μg/kg per hour or placebo, and additional morphine in case of pain or distress.[31] In the NEOPAIN study of Anand et al., ventilated preterm born children received placebo or morphine as well, but in different dosages. The doses of morphine used in this study were based on gestational age; children born at 23-26 weeks of gestation received 10 μg/kg per hour; 27-29 weeks received 20 μg/kg per hour; and 30-32 weeks received 30 μg/kg per hour.[32] From these two randomized controlled trials it was concluded that the routine use of intravenous morphine is not beneficial in the short term in ventilated preterm newborns.[31,32]

Fentanyl

Fentanyl is commonly used due to its high lipid solubility and potency. Its half-life time is short compared to morphine (ranging between 188-570 minutes in infants and neonates versus 219 minutes in adults[33]), but longer than its derivatives sufentanil, alfentanil or remifentanil.[33] In the search for optimal dosing, using population pharmacokinetic (pop-PK) approaches, large variations were observed in the dose of fentanyl used across studies (ranging between 1-5 μg/kg). In four studies, neonates were included (range

1-71 days) with weight ranging between 1.4 kg and 4.0 kg.[34-37] Two studies included only infants and children (range 1 month-4.5 years) with weight ranging between 3.7 kg and 17.3 kg.[38,39] Only one study included neonates, infants, children and adolescents, although the total number of included subjects (n=17) was limited.[36] Intravenous bolus administration was used in most studies (n=5) followed by continuous infusion (n=2), whereas one study only applied continuous infusion.[34] Only limited PK data are available in preterm neonates[33], which warrants future research.

Acetaminophen

Acetaminophen (paracetamol) is the most commonly prescribed analgesic to treat mild to moderate pain to be administered by rectal, oral or intravenous route. In an attempt to avoid or reduce opioid exposure, intravenous acetaminophen is increasingly used in preterm and term-born neonates.[15] Pooled data with subsequent external validation of intravenous acetaminophen PK in neonates are available.[40,41] The same holds true for the maturation of the different routes (glucuronidation, sulfation and oxidation) involved in acetaminophen metabolism.[42] Flint et al.[43] recently reported a gestational-age-dependent increase in glucuronidation without evidence for saturation of a specific pathway as there was a proportional increase in exposure of acetaminophen and its metabolites in extreme preterm neonates between 24 and 32 gestational age. Despite the availability of these PK data, intravenous acetaminophen is still used off label for specific subpopulations (limited from term neonates onwards in Europe, and still off label in children under the age of 2 years in the United States).

As reported by Laughon et al.[44], this is because efficacy could not be documented in the registration studies perhaps because we miss 'common' models similar to the third molar surgery model, to assess the analgesic effect of non-steroidal anti-inflammatory drugs, including acetaminophen, in adults. The relevance of the study model is also reflected in the fact that the available observations on acetaminophen analgesia during procedures (heel prick, retinopathy of prematurity screening) suggest that acetaminophen is a very poor *procedural* analgesic anyhow.[45] In contrast, there is a proven and clinically relevant (-66%) morphine sparing effect of intravenous acetaminophen after major neonatal non-cardiac surgery.[46] The morphine sparing effect has also been observed in a retrospective analysis on morphine consumption in very low gestational age infants (<32 weeks) before and after introduction of intravenous acetaminophen.[47] Intravenous acetaminophen is also effective for moderate pain relief following traumatic delivery or medical conditions, with an effect compartment concentration similar to children and adults.[48]

Sedatives

Propofol

Propofol is used in many clinical settings in both adult and pediatric populations. However, given the increased risk of metabolic derangements and organ system failures, known as propofol infusion syndrome, propofol should preferably only be used for short-duration sedation.[49] Gestational and postnatal age both contribute to propofol clearance, with very fast maturation of clearance in early infancy. This implicates that neonates in the first week of postnatal life are at an increased risk for accumulation during either intermittent bolus or continuous administration of propofol, irrespective of the age or weight at birth.[50] This PK knowledge was subsequently integrated in a propofol dose-finding study (effective dose for 50% of patients, ED_{50}) through 8 patient strata (postmenstrual and postnatal age) to attain optimal effects for endotracheal intubation in neonates for the INSURE (*intubation, surfactant administration, extubation*) indication.[51,52] It turned out that the ED_{50} dose for preterm neonates varied between 0.7 and 1.4 mg/kg. This is significantly lower than initially suggested in literature.[53,54] Even with these lower doses, clinical recovery was accompanied by permissive hypotension (no clinical shock and no treatment).[51]

Midazolam and clonidine

A recent Cochrane analysis found no arguments for the use of midazolam as drug of choice for sedation in newborns compared with other medications because it did not seem to make the participants more sedated, nor did it reduce anxiety or pain, or made it the procedure easier to perform.[55] However, the evidence was rated to be of low-quality as many trials did not explain how participants were randomized to either midazolam or a different treatment.[55] In a systematic review, Vet et al.[56] identified 25 studies evaluating the level of sedation in pediatric intensive care unit (PICU) patients receiving continuous sedation, the most frequently used drug being midazolam. Of these studies, 23 used sedation level as the secondary outcome, and concluded that the level of sedation in critically ill children is often suboptimal. In particular, over sedation was found to be more common than under sedation, which has been associated with adverse long-term outcomes.[56]

As an alternative for midazolam, many institutions nowadays prefer clonidine. Three randomized controlled trials assessing the use of clonidine in the PICU have recently been published. The first compared the opioid and sedative sparing effect of placebo versus clonidine (fixed at 1 mcg/kg/h; median duration: 168 hours) in ventilated medical and surgical patients.[57] The trial indicated decreased sedative and analgesic requirements in neonates, although this was not found in older children of up to two years of age. In the clonidine group, a mild and well-tolerated decrease in systolic and mean blood pressure was observed compared to the placebo group, while heart rate was

similar between groups.[57] In a pilot multicenter trial, the opioid and benzodiazepine sparing effects of oral clonidine (5mcg/kg every 6 hours) compared to placebo were analyzed. The incidence of significant hypotension and bradycardia was similar between groups.[58] The SLEEPS study, compared the efficacy of clonidine (0.75-3 µg/kg/hour) and midazolam (50-200 µg/kg/hour) for sedation in ventilated children (1 month-15 years), concluding non-inferiority of clonidine to midazolam.[59] Alternative ways of providing sedation such as the concept of daily sedation interruption have not been shown to be effective in critically ill children.[60] In neonates, a recent Cochrane systematic review found only one trial that met the inclusion criteria to assess the efficacy and safety of clonidine used in term and preterm newborn infants for sedation during ventilation, and this evidence was therefore deemed insufficient.[61]

Dexmedetomidine

An increasing number of publications deals with the use of dexmedetomidine mainly in the pediatric ICU population – in particular for sedation in hemodynamic unstable patients, such as post-cardiac surgery. A recent review of the use of dexmedetomidine in the pediatric population showed that evidence favoring dexmedetomidine in children is mainly extrapolated based on adult studies, small randomized controlled trials, and observational studies.[62,63] Pediatric trials are therefore needed with a specific focus on newborns and infants, taking into account the major side-effects documented in the literature for dexmedetomidine being hypotension and bradycardia for which continuous cardiac monitoring is needed. Apart from its sedative effect, dexmedetomidine is also used as an adjuvant analgesic drug as recently published in a systematic review by Schnabel et al.[64]. As many studies deal with optimal dosing and comparative effectiveness of dexmedetomidine, evaluating long-term effects of dexmedetomidine on the developing brain are essential as well.

NEURODEVELOPMENT FOLLOWING COMMON ANALGOSEDATIVE DRUGS

Despite a generally average intelligence, the incidence of academic difficulties is extremely high following neonatal critical illness.[65-70] This finding is highly suggestive of an alternative explanation related to specific neuropsychological deficits rather than general intellectual functioning. Although a complex interplay of deleterious factors associated with neonatal critical illness is likely to be the underlying cause of these long-term neuropsychological deficits, an important first step is to evaluate different potential contributors independently. Recently, more studies have evaluated the negative effects of analgosedatives on the developing brain.[71-73] This is of interest because the adjustment of pain management in order to minimize short and long-term side effects may

improve neuropsychological outcome following neonatal critical illness. Therefore, the clinical endpoints of our review were defined as specific neuropsychological functions, such as memory, attention and executive functioning.

The ontogeny of the nervous system is based on a complex pattern of cell proliferation, migration, differentiation and selective cell survival and includes apoptosis and synaptogenesis. Its development relates to a balance of ongoing excitatory and inhibitory signals.[74] Furthermore, the brain matures in a nonlinear fashion from childhood into adulthood, indicating that the timing of microstructural changes differs per brain region.[75,76] Consequently, the timing of injuries is likely to also have specific effects on the development of the brain.[77] Exposure of nociceptive and non-nociceptive nervous circuits to analgosedatives during this period may modulate receptor-signaling-related brain development, as demonstrated by various animal experimental studies.[74] Alterations are in part drug and dose dependent, and there is an age-related window of vulnerability for apoptosis on the one hand or dendritic changes on the other hand.[74] Moreover, the balance between pain and exposure to analgosedatives may also play an important role.[2] Specifically, the limbic system undergoes rapid development in the third trimester and neonatal period.[78] Embedded within the brain's limbic system, the hippocampus and its connections are essential for memory encoding, consolidation and retrieval.[79] Given the high incidence of memory deficits following neonatal critical illness[80], this gray matter structure may be particularly vulnerable in these children. In utero, hippocampal morphology and positional changes occur and the hippocampus is thought to resemble adult shape at 25 weeks of gestation. A critical period of hippocampal development is from the third trimester throughout the first two years of life when it undergoes a growth spurt.[81] The hippocampus and other limbic system regions may therefore be particularly vulnerable in critically ill preterm and term newborns.[78,82]

In the next section, studies describing the effects of commonly used sedatives and analgesics on the developing brain and neuropsychological functioning later in life following neonatal critical illness are summarized.

Analgesics

Worldwide, opioids such as morphine and fentanyl are regularly used in preterm and term born neonates during admission to the NICU.[73] Neonatal opioid therapy to mitigate the effects of painful and stressful procedures may affect the developing brain as well.[71,83,84] Interestingly, animal models have demonstrated some neuroprotective effects of morphine as a pre-treatment for pain and in case of certain levels of pain or stress.[71,84] However, concerns have been raised as a result of studies showing that opioids such as morphine may induce apoptosis in human microglial cells and neurons, and long-term changes in brain function and memory.[71,85]

Morphine

The long-term effects of morphine administration in preterm born children are comprehensively evaluated in follow-up studies among children from two well-defined cohorts.[31,32] Follow up studies of the cohort of Simons et al. found that morphine exposure was significantly, negatively correlated with only one IQ subtest at the age of 5 years.[86] At 8/9 years of age, however, this negative effect disappeared and morphine was even positively correlated with executive functioning.[5] In line with these findings, a third study did not find major negative effects in children from this cohort who did receive morphine compared to healthy term born children without neonatal morphine exposure at10 years of age with respect to neuropsychological functioning or pain sensitivity.[87] However, whether morphine itself or the underlying condition causes long-term adverse events remains a topic of debate.[88,89]

With regards to brain morphology, van den Bosch et al. found strong, negative correlations between neonatal opioid exposure and volumes of pain-related brain regions, total gray volume and cerebral white matter at school-age in children born preterm (26-36 weeks). However, neuropsychological outcome of those born preterm did not differ from the norm population and was not associated with morphine exposure.[87] In contrast, Ferguson et al. demonstrated differences at term-equivalent age and during childhood in head circumference between 14 morphine treated and 5 placebo treated children born at 23-32 weeks of gestation.[90] At 5-7 years, overall IQ and academic achievement did not differ between these groups, however, short-term memory was significantly worse in children treated with morphine compared to the placebo-treated children.[90] A recent neuroimaging study assessing the effects of morphine on brain development in preterm infants (24-32 weeks), demonstrated that increased neonatal morphine exposure was associated with smaller cerebellar volume at term equivalent age, but not with cerebral volume.[89] Furthermore, greater morphine exposure was associated with poorer cognitive and motor outcomes at 18 months.[89]

A few follow-up studies have been conducted in term-born survivors of neonatal critical illness as well. In a neuroimaging study by van den Bosch et al. school-age ECMO survivors were less sensitive to the detection of cold but showed similar pain sensitivity and chronic pain compared to healthy controls. Importantly, no differences in brain activation during pain or in pain-related brain regions were observed.[91] However, associations with morphine and midazolam exposure were not assessed in this study.[91] Children treated with neonatal ECMO are often exposed to prolonged continuous opioids and sedatives in the absence of major pain.[91] This may contribute to the long-term neuropsychological deficits and brain alterations observed in these patients at school-age and even in adolescence.[66,67,92] In 8-year-old ECMO survivors, specific attention problems were found irrespective of generally average intelligence.[65] However, outcome at eight years was not associated with the time children had been exposed to opioids or other sedatives.[65]

Fentanyl

Fentanyl is another commonly used analgesic in the NICU and may have effects on the developing brain as well. In preterm infants (23-30 weeks), higher cumulative fentanyl dose was associated with a higher incidence of cerebellar hemorrhage as well as lower cerebellar diameter.[93] However, two studies that have analyzed the association between cumulative fentanyl dose and developmental outcome at 2 years of age found no relation between the two.[93,94] This may be due to the fact that at 2 years of age, only general mental functioning can be assessed. The cerebellum has been shown to be involved in various higher-order cognitive functions, such as visuospatial processing, attention, and executive functioning.[95] As these higher-order cognitive functioning cannot be reliably assessed before school-age[68], long-term neuropsychological follow-up of these children is imperative to better understand the impact of morphine and fentanyl exposure on neurodevelopmental outcome in these children.

Acetaminophen

The safety and potential long-term effects of acetaminophen warrant additional exploration. The shift from opioids to acetaminophen is largely driven by the perceived better safety profile.[15] This is likely the case when we compare short term aspects like hemodynamics or respiratory depression following either opioid or acetaminophen administration.[74] However, intriguingly, a recent Cochrane review concluded that acetaminophen given after assisted vaginal birth may lead to an increased response to later painful exposures.[96] Data on long term safety following acetaminophen exposure are based on epidemiological association type studies. These associations suggest a link between fetal exposure and subsequent risks for atopy, fertility or neurobehavioral problems and these links are further supported by animal experimental observations.[45] In particular when it comes to neurodevelopment, concerns have been raised about an increased risk of attention-deficit-hyperactivity disorder, autism spectrum symptoms and neurocognitive deficits following early exposure to acetaminophen, although most studies are based on prenatal exposure or animal studies (reviewed by De Fays et al.[4] and Avella-Garcia et al.[97]). A recent study on the long-term effects of acetaminophen in mice, showed that adverse effects on adult behavior and cognitive function occurred in both male and female mice exposed to paracetamol on postnatal days (PND) 3 and 10, but not when exposed on PND 19. These neurodevelopmental time points in mice correspond to the beginning of the third trimester of pregnancy and the time around birth in humans. These findings suggest particular sensitivity of the brain in the preterm and neonatal brain.[98] Future clinical studies are needed before conclusions can be drawn on the effect of neonatal exposure to acetaminophen on long-term neuropsychological outcome.

Sedatives

Propofol and midazolam

Exposure to propofol and midazolam in the neonatal period has been suggested to negatively affect the developing brain in animals and humans.[6,99] Although studies in humans are scarce, findings from experimental studies suggest that, in particular, hippocampal development and long-term memory are affected after exposure to these agents.[100,101] Interestingly, studies have shown long-term hippocampal alterations and associated memory deficits across survivors of neonatal critical illness, irrespective of gestational age or underlying disease.[92,102,103] The hippocampus is the brain's central hub for memory encoding, consolidation and retrieval.[79] Benzodiazepines, such as midazolam, potentiate the neuronal inhibitory pathways or inhibit the excitatory pathways by binding to gamma-Aminobutyric acid (GABA) or glutamatergic N-methyl-D-aspartate (NMDA) receptors in the brain.[6,99] In the hippocampus, NMDA receptors are highly involved in Long-Term Potentiation (LTP), a system of persistent strengthening of synapses following high levels of stimulation, that results in the ability to form memories.[100,104] If these NMDA receptors become occupied due to the presence of for instance midazolam, memory formation will be disrupted.[14]

In preterm infants, the acute effects of midazolam on the brain have been studied directly. Injection with midazolam led to a decrease in middle cerebral artery blood flow velocity and transient cerebral hypoperfusion.[72] As the hippocampus is selectively vulnerable to cerebral hypoperfusion[105], this may contribute to the specific effects of midazolam on the hippocampus, and thus on memory. A recent clinical study in preterm-born neonates (24-32 weeks of gestation) evaluated how midazolam exposure affected the hippocampus using MRI. They demonstrated a selective, negative effect of midazolam on hippocampal growth from birth to term-equivalent age, even after adjusting for the number of invasive procedures and other common clinical care practices.[6] Long-term outcome studies are needed to evaluate whether the hippocampal growth reductions in response to midazolam exposure are associated with memory deficits, and whether this exists in both preterm and term born survivors.

Dexmedetomidine

Interestingly, rodent studies have shown that dexmedetomidine and clonidine may reduce anesthetic-induced apoptosis, specifically in the hippocampus[106], and diminish subsequent cognitive decline.[107] However, future clinical studies are needed to assess whether these agents are neuroprotective in neonates as well.

DISCUSSION

Although animal studies have fairly consistently demonstrated brain development to be negatively affected by various classes of drugs, such as anesthetics, benzodiazepines and to some extent opioids[71,85], associations in survivors of neonatal critical illness seem less obvious.[6,14] This may be due to differences between animal and human studies, resulting in more contradicting conclusions in humans (for a review on this matter, please refer to van den Bosch et al.[2]). Nonetheless, our findings seem to suggest a link between the use of analgesics such as morphine and fentanyl, and cerebellar volume in critically ill infants.[89,93] Concerning sedatives, direct links between midazolam exposure and hippocampal alterations have been described in preterm infants.[6] Commonly used sedatives such as midazolam and propofol bind either to GABA or NMDA receptors. The GABA and NDMA receptor systems are crucial for neuronal connection and communication in the developing brain, and if unavailable lead to neuroapoptosis.[14] These agents have been suggested to disrupt memory formation through its effects on the hippocampus.[104] Memory formation and recall are dependent on LTP, a system of persistent strengthening of synapses following high levels of stimulation. LTP, which mainly happens in the hippocampus, relies heavily on NMDA. Although based on animal studies, midazolam was found to affect pyramidal neurons in the CA1 region and memory by suppression of LTP.[100] This mechanism may be underlying the negative effects of midazolam found on the hippocampus in preterm infants[6], and may subsequently lead to memory deficits later in life in these children. (Please also refer to a recent review on other proposed mechanisms underlying the effects of analgosedatives and anesthetics on the brain by van den Bosch et al.[2].) As both the cerebellum and hippocampus are important for higher-order cognitive functioning and seem to be targeted by analgosedatives, damage to these brain regions may be (partly) underlying the long-term deficits following neonatal critical illness.[79,95]

The indications that exposure to analgosedatives in neonates and infants may affect specific brain regions responsible for higher-order cognitive functioning warrant future research. Specifically, as these functions, such as memory, do not fully develop until later in childhood, it is imperative that long-term neuropsychological outcomes are measured when studying the effect of analgosedatives following neonatal critical illness. Therefore, studies described in this review that only include the assessment of general intellectual ability at 24 months (corrected age), e.g. with the commonly used Bayley Scales of Infant and Toddler Development–Third Edition[108], as well as general intellectual outcome at a later age are less informative in this respect. Future studies on the effect of commonly used analgosedatives that include neuropsychological assessment and neuroimaging later in childhood are needed to more reliably assess the potential clinical

effect of cerebellar and hippocampal alterations on cognitive outcome before definitive conclusions can be drawn.

Problematically, analgosedatives will remain a necessary treatment as the experience of pain itself has shown to negatively impact neurodevelopment as well. Ranger et al. showed that pain related stress predicted cortical thickness at 7 years in preterm born children, independent of morphine exposure. Also, a higher number of painful (skin-breaking) procedures in preterm born children was found to be associated with reduced white and subcortical gray matter.[2,109] In addition to pain, studying the association between analgosedatives and neurodevelopmental outcome is likely to be affected by differences in dosages. Moreover, critically ill infants are exposed to other factors such as stress, hypoxia-ischemia and neuroinflammation during a vulnerable and critical period of brain development. A complex interplay amongst these factors may lead to (subtle) brain injuries early in life, which become evident only later in life when those brain regions are required for higher cognitive functioning. In particular, the hippocampus, the main hub for memory formation in the brain, has been found to show pronounced vulnerability to factors associated with critical illness, including exposure to sedatives.[6,80] This 'growing into deficit' phenomenon[110], where early hippocampal alterations lead to memory deficits later in life, has recently been identified by our group across survivors of neonatal critical illness.[80] To what extent exposure to analgosedatives contribute to early brain injury in these patients needs further research that combines information on analgosedative exposure and exposure to confounding factors, such as pain, with neuroimaging and elaborate neuropsychological assessment.

Elaborate neuropsychological assessment is essential as an increasing number of studies has found that survivors of neonatal critical illness are at risk of specific memory and attention deficits, rather than intellectual disability.[66-68,111] The majority of clinical studies described in the current review only used general neurodevelopmental outcome measures such as intelligence at pre- or school-age. Therefore, it is difficult to draw any definitive conclusions on how and to what extent analgosedatives affect the brain in critically ill neonates. Better understanding of the association between the brain, cognition and analgosedatives exposure in early life is needed to optimize adequate pain management in neonates. Future studies combining elaborate neuropsychological assessment with multimodal neuroimaging techniques, such as (functional) MRI and Diffusion Tensor Imaging, are therefore needed.

In this vulnerable population, there is a significant need for early predictors of long-term neuropsychological deficits and school problems. As of now, identification of patients at risk relies solely on neuropsychological assessment which cannot be reliably conducted until school-age.[68] By this time, the cognitive deficits may have already hampered school performance in a number of children.[67] Predictors that can be measured as early as during first admission ('biomarkers') are therefore of utmost importance. A

better understanding of how and to what extent analgosedatives affect the brain may contribute to identifying which patients are most at risk. Within this context, interaction with age and developmental stage are essential. Furthermore, given the high incidence of memory deficits following neonatal critical illness[80,112], early identification of patients at risk of memory deficits may become possible using hippocampal volume as a neurobiological marker. The hippocampus can be accurately and non-invasively delineated using structural MRI and is the brain's critical hub for long-term memory formation.[79,81] Because of these features, the hippocampus is an important target for future studies aimed at improving long-term outcomes following neonatal critical illness. In preterm infants, studies have shown that MRI can be reliably performed without sedation and that infant hippocampal volumes, measured at term-equivalent age, correlated with memory outcomes later in childhood.[113,114] However, it is important to note that even the more advanced MR techniques are not sensitive enough to provide us with information on the exact anatomical or molecular mechanisms underlying neuropsychological deficits. The exact contribution of exposure to analgosedatives on neuropsychological outcome therefore remains speculative.

In neonatal pain management, the ultimate goal is to achieve adequate pain management with minimal short and long-term side effects. The search for the lowest, most effective exposure to analgosedatives becomes even more relevant when taking into account their potential effect on long-term neurocognitive outcome.[6,14,71,85] For both new compounds as well as for the already used drugs, dose-finding studies (phase 2) can be very instructive before conducting phase 3 efficacy and safety trials.[74] The earlier mentioned study on morphine sparing acetaminophen in neonates and infants[46] used a much lower dose of morphine in neonates and infants and still resulted in further reduction of the morphine maintenance dose (-66%) in cases co-exposed to acetaminophen. Similarly, the ED_{50} of propofol (0.5-1.5 mg/kg) to enable endotracheal intubation turned out to be significantly lower compared to the routine practices.[51,53,54] Furthermore, the increased use of (new) drugs such as dexmedetomidine will only result in evidence-based pharmacotherapy when assessed using appropriate and comparative effectiveness trials. Together with advanced analysis techniques such as pop-PK and physiology based/PK on sparse datasets, we will reach a higher level of evidence-based dosing of analgosedative drugs in neonates. The same holds true for future application of principles of pharmacovigilance studies. This is especially of interest since experimental studies have suggested that dexmedetomidine may have neuroprotective effects as well.[106,107]

CONCLUSION

Indications from both animal and clinical studies that early exposure to analgosedatives may have long-term effects on the brain and cognition warrant future research. As the number of critically ill neonates admitted to intensive care units grows worldwide and more and more of these patients survive to discharge due to medical improvements[7,8], the long-term outcome after surviving neonatal critical illness can no longer be ignored. It is therefore of utmost importance that we continue to gain insight into how pharmacotherapy affects the developing brain. In future studies, potential long-term sequelae should be primary outcome parameters and this information should be used when optimizing pain management in neonates.

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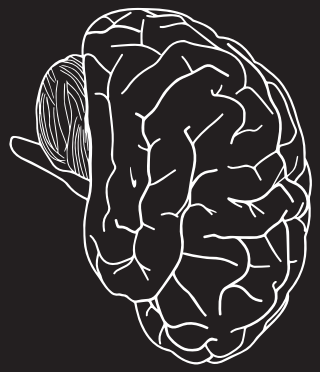
CHAPTER 11



GENERAL DISCUSSION

AIMS AND MAIN FINDINGS

In this thesis, the long-term neurodevelopmental sequelae following two common causes of neonatal critical illness were studied: severe respiratory failure in need of neonatal extracorporeal membrane oxygenation (ECMO) treatment and congenital diaphragmatic hernia (CDH). To improve (early) *identification* of patients at risk, we aimed to delineate the specific neuropsychological profile and its underlying neurobiology in these survivors. Our results showed that survivors of neonatal ECMO and/or CDH have specific memory and attention deficits, despite average intelligence (chapters 2, 3, 4). These deficits are associated with alterations in the brain's limbic system, in particular the hippocampus, and global white matter microstructure (chapters 5 & 6). The second aim of our thesis was to evaluate whether Cogmed Working-Memory Training (CWMT) could be an effective *treatment* strategy in school-age survivors of neonatal ECMO and/or CDH with (working)memory deficits. We found that CWMT may be beneficial for patients who have visuospatial memory deficits by showing long-term gains in this domain following CWMT (chapter 7). Furthermore, we found training-induced changes in white matter microstructure immediately following CWMT, demonstrating neuroplasticity in these children (chapter 8). Our findings taken together led to the postulation of a common neurodevelopmental pathway across survivors of neonatal critical illness, where early hippocampal alterations result in memory deficits later in life. This 'growing into deficit' phenomenon seems to exist across survivors of neonatal critical illness, irrespective of underlying diagnosis or gestational age, and may be due to common factors associated with neonatal critical illness such as hypoxia-ischemia, neuroinflammation, stress and exposure to common analgosedatives (chapters 9 & 10).

The work presented in this thesis has shown that, even in absence of major neurological abnormalities such as hemorrhage or periventricular leukomalacia, the brain of critically ill neonates is vulnerable. Our results have increased our understanding of the long-term neurodevelopmental sequelae following neonatal critical illness. Inevitably, our findings have also proven to us that '*the more we know, the more we do not know*' (Aristotle). Two important issues remain:

1) The need for predictors or risk stratification tools to identify patients at risk early, i.e. before the neuropsychological deficits affect school performance and daily life activities.
2) Treatment strategies that prevent or specifically target (all) altered brain regions and/or neuropsychological deficits following neonatal ECMO and/or CDH.

In the following section, our main findings will be placed into a broader perspective. Furthermore, directions of future research will be discussed, aimed at improving early

identification of children at risk as well as prevention or reduction of long-term neurodevelopmental impairment following neonatal critical illness.

IDENTIFICATION OF PATIENTS AT RISK

Neuropsychological assessment

Over the last decade, increasing awareness and knowledge has emerged about the long-term neuropsychological outcome following neonatal ECMO and/or CDH[1-3], which inspired the work presented in this thesis. In initial reports on long-term neuropsychological outcome in school-age survivors of neonatal ECMO and/or CDH, sustained attention deficits and a high incidence of school problems were found, despite normal intelligence.[1-4] However, elaborate assessment of all major neuropsychological domains was lacking. In this thesis, we showed that general intellectual outcome was normal in the majority of survivors of neonatal ECMO and/or CDH from two, five, to eight years of age (chapter 2). Using elaborate neuropsychological assessment, we replicated the findings on sustained attention deficits, but also found specific short- and long-term memory deficits in over half of these children at school-age (chapter 3). Strikingly, using a similar test battery, we demonstrated similar deficits in short- and long-term visuospatial and verbal memory in a group of 17-year-old adolescent survivors of neonatal ECMO, while other neuropsychological domains remained relatively unaffected (chapter 4). Although longitudinal assessment will be needed to increase our understanding of the neurodevelopmental trajectories following neonatal ECMO and/or CDH, this was the first study that performed elaborate neuropsychological assessment in adolescent survivors, indicating that memory deficits following neonatal critical illness are persistent from childhood into adolescence. Confirming earlier findings by our group in different cohorts of neonatal ECMO and CDH survivors[1,2], we found that a significantly higher number of survivors were in need of extra help in school compared to the general population at both 8 and 17 years of age (chapters 2 & 4), We found that these school problems were related to the specific neuropsychological deficits, rather than to general intellectual outcome (chapter 2).

Comparing our findings to neuropsychological outcome in other children who survived a period of neonatal critical illness without serious neurological sequelae shows striking similarities. Although lower IQ has been reported in children born preterm (< 37 weeks of gestation)[5], intelligence has generally been shown to be within the low average to average range across survivors of neonatal critical illness.[5-7] In children growing up after preterm birth and complex cardiac anomalies, impairments have been demonstrated across multiple neuropsychological domains, such as attention, visuospatial processing, executive functioning, and memory and learning.[5-10] Just as in survivors of

neonatal ECMO and/or CDH, memory deficits, both short- and long-term verbal and visuospatial memory are among the most frequently reported neuropsychological sequelae. Following preterm birth, memory deficits have been found to persist from childhood into adolescence and even into young adulthood.[5,11-14] In survivors of cardiac anomalies of differing complexity, short- and long-term verbal and visuospatial memory deficits have been demonstrated as well, becoming increasingly evident with age.[6,8,15] Unsurprisingly, the incidence of school problems is strikingly high across these patient groups[5-7], making long-term neuropsychological sequelae following neonatal critical illness a major concern.

While intelligence is generally in the low-average to average range in these children, the assessment of IQ often remains the main outcome parameter in the few long-term follow-up protocols that are available.[16,17] This is problematic as specific neuropsychological deficits are difficult to pick up using a global outcome measure such as an IQ test.[18] As such, problems in school and/or daily life remain misunderstood and targeted intervention strategies cannot be implemented. Standardized, problem-oriented neuropsychological follow-up that includes all major neuropsychological functions is therefore highly recommended following neonatal critical illness. Important to note is that, as we have shown that IQ at 5 years of age is highly predictive of IQ at 8 years of age, it may be sufficient to conduct a full-scale IQ test at 5 years and a short-form test at 8 years of age to increase efficiency.[19] As higher-order cognitive functions, such as attention and memory, continue to develop throughout childhood and into adolescence[11,20], follow-up assessments should take place both at school-age and into adolescence. In line with this, assessment of general intellectual ability at 24 months (corrected age), e.g. with the commonly used Bayley Scales of Infant and Toddler Development–Third Edition[21], will not identify those patients at risk of neuropsychological deficits. Preferably, we would like to identify children at risk of memory and attention deficits at this time, i.e. well before the neuropsychological deficits have interfered with school performance and activities in daily life. Studies using eye-tracking in infants to assess long-term memory and attention show promising results.[22,23] Future longitudinal studies are needed in survivors of neonatal critical illness that compare memory and attention assessed with eye-tracking in infancy to outcomes from neuropsychological assessment later in childhood to determine the utility of outcomes in infancy as early predictors.

Neuroimaging

Our findings of specific attention and memory deficits emerging in childhood and persisting into adolescence following neonatal ECMO and/or CDH, suggested a 'growing into deficit' phenomenon where subtle brain injuries acquired at a young age become functionally evident over time when demands on cognitive functioning increases.[24] This 'growing into deficit' is nested within different developmental processes that occur in the

brain (e.g. myelination, synaptic pruning and neurogenesis[25]). An important next step in understanding neurodevelopment following neonatal critical illness was therefore to study the underlying neurobiology of long-term neuropsychological impairments.

Using Diffusion Tensor Imaging (DTI) in neonatal ECMO survivors, we demonstrated global as well as specific white matter alterations in the cingulum bundle and parahippocampal part of the cingulum in school-age neonatal ECMO survivors compared to healthy controls (chapter 5). White matter microstructure has previously been found to be particularly vulnerable in the neonatal period, a time when it is undergoing rapid development.[26] In particular the limbic system fibers (i.e. cingulum bundle and parahippocampal part of the cingulum) develop rapidly in the first six months of life, causing fractional anisotropy (FA) to increase and mean diffusivity (MD) to decrease in these tracts.[26] As our subjects were critically ill in the first weeks of life, the development of these specific fibers may therefore be at increased risk.

Since white matter is important for high-speed transmission of neuronal signals between distant brain regions, aberrations in white matter development could affect the orchestration of specific cognitive functions.[26] Indeed, in another cohort of school-age survivors of neonatal ECMO and/or CDH in which we combined neuroimaging with neuropsychological assessment, we found that lower global FA, potentially indicative of reduced coherence of white matter fibers[27], was associated with sustained attention deficits (chapter 6). Global white matter abnormalities have been found in preterm born infants with attention deficits as well.[28] This shared vulnerability in both preterm and term-born neonates may be due to the increased susceptibility of white matter, in particular in the periventricular regions, to hypoxic-ischemic insults – a common complication in critically ill infants. Using animal models, premyelinating oligodendrocytes (pre-OLs) in cerebral white matter have been found to be selectively targeted by oxidative stress. These cells account for approximately 90% of the total oligodendroglial population at 28 weeks of gestation and approximately 50% at term.[29] Increased regional susceptibility of the periventricular white matter is suggested to be due to the distribution of these pre-OLs and relative underdevelopment of distal arterial fields to these areas.[29,30] Neonates exposed to hypoxic-ischemic injuries, both born preterm and at term, may therefore be at increased risk of myelin and axonal disruptions, resulting in white matter abnormalities. As more widespread white matter networks have been found to be underlying attention, this may explain the attention deficits in these children.[31] In addition to the association between global white matter alterations and attention, we found that higher MD in the parahippocampal part of the cingulum, suggestive of decreased integrity in axonal membranes, packing, or myelin[27], was related to long-term visuospatial memory deficits in survivors of neonatal ECMO and/or CDH (chapter 6). This is in line with earlier findings demonstrating that the parahippocampal part of the cingulum, which is bidirectionally connected to the hippocampus, forms

a larger memory circuit together with cortical structures and is thus highly important for intact functioning of various memory types.[32] Taken together, our results suggest that white matter microstructure alterations acquired early in life may have long-lasting implications in survivors of neonatal critical illness.

Given the high incidence of memory deficits (chapters 3 & 4), we also investigated the hippocampus using structural MRI in school-age neonatal ECMO survivors compared to healthy controls. The hippocampus, a gray matter structure within the brain's limbic system, is the critical hub for memory formation and rapidly develops within the first two years of life.[33,34] We found smaller bilateral hippocampal volume in the ECMO survivors compared to healthy controls, which was negatively associated with the ability to recall information from a story that the children had just heard, a measure of episodic memory (chapter 5). In a different cohort of school-age survivors of both neonatal ECMO as well as CDH treated without ECMO, we found these same structure-function relationships (chapter 6). Interestingly, neither the underlying diagnosis (such as meconium aspiration syndrome or CDH) or the type of ECMO-cannulation (venoarterial or venovenous) affected these associations (chapter 6). Although these findings should be interpreted with caution due to the small sample size, they suggest that factors other than diagnosis and treatment determine long-term neurodevelopmental outcomes in these patients.

Placing our findings in a broader perspective, we found that other groups of critically ill infants without overt neurological abnormalities had similar neurodevelopmental outcomes. As described previously, memory deficits are frequently reported across survivors of neonatal critical illness, such as in children following preterm birth and complex cardiac anomalies.[5,6,8,11-15] We therefore wondered whether these memory deficits would be associated with hippocampal alterations in these patients as well (chapter 9). In school-age children who experienced neonatal hypoxia, structural MRI combined with memory assessment demonstrated specific smaller bilateral hippocampal volumes associated with memory deficits in patients compared to healthy controls.[35] These structure-function relationships existed in both children treated with and without ECMO[35], confirming findings in our study population (chapter 6). In this population, the term "developmental amnesia" has been used to describe markedly impaired event, or episodic, memory and relatively preserved fact, or semantic, memory following hypoxic-ischemic insults sustained within the first year of life.[36,37] This has been suggested to be due to relatively selective bilateral hippocampal pathology.[36,38] This pattern of memory deficits and hippocampal pathology, despite relatively intact intellectual abilities, is remarkably similar to the significant impairments in delayed recall and hippocampal volume loss observed following neonatal ECMO and/or CDH (chapter 6), as well as to other groups of critically ill infants. In children born preterm, abnormalities in the hippocampus with impaired long-term memory have been reported as well.[12,39-42] In children with complex congenital heart disease, smaller bilateral hippocampal volumes were demonstrated

in 40% of school-age children who had dextro-Transposition of the Great Arteries and cyanosis compared to healthy controls. These hippocampal reductions were associated with memory deficits.[43] In line with this, 13-year-old children who had undergone cardiopulmonary bypass surgery in infancy had smaller bilateral hippocampal volumes as well as volume loss in other parts of the limbic system's gray matter compared to healthy controls.[44 43,44] The findings reported in these four common causes of neonatal critical illness taken together led to the postulation of a common neurodevelopmental pathway following various types of neonatal critical illness, where early hippocampal alterations result in memory deficits later in life, irrespective of underlying disease or gestational age (chapter 9). As memory problems can greatly affect daily life activities and academic achievement, this is of major concern. Early identification of patients at risk of memory deficits may become possible using hippocampal volume as a neurobiological marker. The hippocampus can be accurately and non-invasively delineated using structural MRI and is the brain's critical hub for long-term memory formation.[33,34] Because of these features, the hippocampus is an important target for future studies aimed at improving long-term outcomes following neonatal critical illness. In preterm infants, studies have shown that MRI can be reliably performed without sedation and that infant hippocampal volumes, measured at term-equivalent age, correlated with memory outcomes later in childhood.[12,45] A critical period of hippocampal development is in the first two years of life when it undergoes a growth spurt.[34] The hippocampus would thus ideally be measured after the first two years of life. However, unless with the use of sedation, it will be difficult to perform a reliable MRI scan at this time, let alone desirable for the patient. Since sedatives may have an additive negative effect on the developing brain (chapter 10), this should be avoided. Furthermore, the rapid development of the hippocampus within the first two years of life likely interacts with the exposure to deleterious factors associated with neonatal critical illness. Therefore, longitudinal assessment may lead to finding the optimal time to assess hippocampal volume and identify specific periods of sensitivity. Such longitudinal data should be coupled with memory assessment later in childhood to evaluate the utility of hippocampal volume as a prediction tool.

Pathophysiological mechanisms

Standardized, problem-oriented neuropsychological assessment can be used to understand why survivors experience difficulties at school or in daily life activities. In its present form, neuropsychological assessment is therefore a diagnostic tool rather than a prediction tool. Furthermore, the use of neurobiological correlates as early predictors, such as smaller hippocampal volume, is promising but will only become feasible once normative hippocampal volumes become available. As we should strive to identify and treat patients at risk well before the neuropsychological deficits have hampered their

school performance, it is imperative that we understand why and how patients develop these deficits.

In chapter 3 of this thesis, we analyzed associations between clinical characteristics at the time of hospitalization and neuropsychological outcome at 8 years of age. We found a specific negative association between the maximum dose of vasoactive medication received during first admission (measured by the Vasoactive Inotropic Score; VIS) and long-term verbal and visuospatial memory following neonatal ECMO and/or CDH. In these analyses, we adjusted for diagnosis and various measures of severity of illness, suggesting that a specific association exists between the VIS and memory later in life (chapter 3). Although currently speculative, receiving high levels of vasoactive medication in the first period of life may be an indirect marker of temporarily (regional) inadequate brain perfusion. As the hippocampus is particularly vulnerable for hypoperfusion and/or hypoxia, the association between the VIS and memory may be the indirect result of this pathophysiological mechanism. In addition, previous findings from both preclinical and clinical studies have shown that the hippocampus shows pronounced vulnerability to hyperoxia.[46-50] Hyperoxia, experienced by critically ill infants in need of oxygen supplementation[51,52], may therefore also play a role in the hippocampal alterations and memory deficits observed following neonatal critical illness. Importantly, the current lack of insight into how hypoxia/hyperoxia may affect hippocampal development in these children has direct clinical implications as the optimum resuscitation strategy in critically ill infants remains a topic of debate.[53,54] Future research that collects dense and detailed data on continuous oxygen saturation and supplemental oxygen supply will allow us to closely monitor oxygen fluctuations in critically ill neonates. Analyzing a combination of absolute values and the area under the curve[55] will provide more detailed information on the exposure to different levels of oxygen throughout the infant's hospital stay. Coupled with outcome parameters such as hippocampal volume and/or memory functioning, this data can provide insight into the extent to which hypoxia, hyperoxia or a combination of both, influences the hippocampus and memory in later life. To further understand the molecular, cellular and behavioral consequences of exposure to hypoxia/hyperoxia on the pathophysiology of the neonatal hippocampus, experimental studies using *in vitro* and *in vivo* animal models are of interest. In these types of experiments, the effect of hypoxia and/or hyperoxia on the hippocampus and memory at different stages of development can also be taken into account by including both a preterm model and a full-term model.[47,56,57] Such translational studies may eventually contribute to earlier identification of critically ill infants at risk of long-term deficits. Moreover, they may also lead to adjustments in the resuscitation strategy and thus the prevention or reduction of damage to the developing brain in critically ill neonates.

Importantly, in addition to hypoxia-ischemia, critically ill infants are exposed to many other potentially deleterious factors (chapter 9). A complex interplay amongst different factors associated with the underlying disease, (pharmacological) treatment and "iatrogenesis" are therefore likely to determine a child's neurodevelopment, further complicated by the child's genetic predisposition[58] and social economic status[59]. Using comprehensive literature reviews, in chapters 9 and 10 of this thesis we explored how these factors may affect the developing brain, and in particular the hippocampus, in critically ill infants. Because of its highly excitable and plastic nature, the hippocampus shows pronounced sensitivity to both internal and external influences and may therefore be particularly vulnerable in critically ill infants.[49] However, the exact pathophysiological mechanisms underlying hippocampal alterations and subsequent memory impairments in these children remain largely unknown. A first step will be to collect dense data on factors associated with neonatal critical illness, such as oxygen fluctuations, analgesic and sedative use, metabolic profiles[60,61], and neuromonitoring data[62] throughout hospital stay. Since children following neonatal critical illness 'grow into their deficits', longitudinal assessment is imperative. The detailed clinical information collected during initial hospital stay should therefore be coupled with neuroimaging and problem-oriented, standardized neuropsychological assessment at later stages of development. Such detailed data collection in combination with longitudinal assessment of neurodevelopment may allow us to identify which specific pathophysiological conditions lead to alterations in hippocampal volume and memory deficits later in life in these children. Furthermore, with a longitudinal design, windows of sensitivity (i.e. when infants are most at risk of impairment, the optimal timing of MRI or neuropsychological assessment) and opportunity (i.e. when it is best to implement therapy or interventions) can be identified. As differences in brain development and the timing of critical illness (e.g. preterm versus full-term brain) are likely to influence how and when the hippocampus is affected, a longitudinal design that starts collecting data in the prenatal period, for instance with the use of prenatal ultrasounds[63], is of interest as well. Favorably, the outcome parameters, such as the neuroimaging data and neuropsychological data, are compared to healthy control data to understand when hippocampal volumes and memory are different from the norm. The Generation R study, a pediatric population study in which over 3000 healthy children have undergone longitudinal neuroimaging, is a good example of such a healthy control cohort[64], which may make the use of normative brain parameters feasible in the future. In our institution, a longitudinal follow-up program in children who have been critically ill in the neonatal period has recently been initiated (Systematic Hospital-based Assessment of Rotterdam's (critically) Ill Infants' Neurodevelopment and Growth: S.H.A.R.I.N.G.). It is important to keep in mind that when comparing neuroimaging data, the image acquisition and type of analyses[65,66] as well as the scanner itself[67] may influence the results and should thus be similar or controlled for

in the analyses. Therefore, large sample sizes are needed. In this respect, multicenter collaborations should be a goal for future studies assessing long-term neurodevelopment following neonatal critical illness as well.

TREATMENT

Neurorehabilitation

Given the complex interplay of factors that are likely to affect the developing brain in critically ill infants (chapters 9 & 10), rehabilitation strategies aimed at improving impaired neuropsychological functions are of great interest as well. Cogmed Working-Memory Training (CWMT) is a widely evaluated cognitive training for both children and adults.[68] In this thesis, we reported the results of a nationwide, single-blind randomized controlled trial on the immediate and long-term effectiveness of CWMT in school-age survivors of neonatal ECMO and/or CDH with (working)memory deficits. Neuropsychological outcome was assessed before, immediately and one year after CWMT, and white matter microstructure was assessed before and immediately after CWMT (chapters 7 & 8).

Immediately after CWMT, we found significant improvements in verbal and visuospatial working-memory in the CWMT group compared to the non-training group (chapter 7). These findings are in line with the effects demonstrated in other clinical and non-clinical groups after CWMT.[69-72] Coupling our data with the neuroimaging findings immediately post-intervention, we found that the improvements in verbal working-memory were associated with an increase in FA in the left superior longitudinal fasciculus (chapter 8). The frontoparietal network has been consistently shown to be affected by working-memory training and is thus a common finding following CWMT.[68,73,74] The specific association between the superior longitudinal fasciculus in the left hemisphere and verbal working-memory may be due to the fact that working-memory is lateralized, i.e. verbal working-memory corresponds with the left hemisphere while visuospatial working-memory corresponds with the right hemisphere.[75,76] Furthermore, as the majority of children in our cohort were right handed (80%), which is generally associated with left hemispheric dominance for language[77], this may also explain this association. Nonetheless, after one year, we found that the improvements in working-memory had disappeared (chapter 7). This is in contrast with two previous studies that have assessed long-term outcome following CWMT in children. Gains in working-memory performance have been found in very low birthweight children seven months post-intervention[69] and in healthy children with working-memory problems one year post-training.[78] However, working-memory was found to be within the average range in our population at baseline (chapter 7). As the children studied in the other two long-term studies did have significant working-memory deficits[69,78], this may explain the incongruent findings. Although speculative,

the benefits of the training – such as an increased capacity to learn and manipulate information – may be more prone to subside after a while in our population because an increase in this particular function was not needed to begin with, i.e. the "use it or lose it" principle. Furthermore, rehabilitation treatment may be disease-dependent, e.g. have different effects in survivors of childhood cancer than in children treated with neonatal ECMO.[79,80]

One year after CWMT, we did find significant and sustained improvements in long-term visuospatial memory in the CWMT group compared to non-trained controls (Chapter 7). These improvements were not associated with microstructural changes immediately after CWMT (chapter 8). At baseline, we found an association between long-term visuospatial memory deficits and higher MD in the parahippocampal part of the cingulum (chapter 5). We therefore expected that improvements in this domain might have been accompanied by training-induced changes in this white matter tract. Since the improvements in long-term visuospatial memory found in our cohort increased from the assessment immediately post-intervention to one year later in the CWMT group compared to the control group, neurobiological changes may have only become detectable one year post-intervention. In line with this, a recent study has shown that a reverse relationship can exist between the brain and behavior, where behavior is actually shaping the brain, rather than the commonly assumed direction of the brain shaping behavior.[81] Such a downstream mechanism may explain why the improvements in long-term visuospatial memory in the CWMT group were not associated with changes in white matter microstructure immediately post-intervention. For instance, an increased ability following CWMT to memorize the location of information within a certain visual context may lead to increased use of this tactic to improve the ability to encode and recall information in various situations, thereby affecting the white matter connections underlying these abilities. However, this remains speculative as the MRI exam was unfortunately not repeated at this time. It is also important to note that neurobiological changes underlying these memory improvements may simply not have been detectable using DTI.[82] Nonetheless, given the fact that over 50% of children following neonatal ECMO and/or CDH has long-term visuospatial and verbal memory deficits at school-age (chapter 3), improving memory in these children is of great importance. We found that larger improvements in long-term visuospatial memory were significantly associated with higher scores on self-rated school functioning and parent-rated attention one year after CWMT (chapter 7). Additionally, the majority of children trained with CWMT and their parents reported to be happy with the results and to see improvements in memory and attention (data not shown). These findings taken together may suggest that the improvements in long-term visuospatial memory in the CWMT group have generalized to daily life activities. If so, intervention before memory problems have interfered with school performance should be strived for. In children with very low birthweight,

memory improvements have been found six months after CWMT at preschool-age[69], suggesting earlier intervention may lead to similar results. However, these results need to be replicated in preschool survivors of neonatal ECMO and/or CDH with long-term visuospatial memory deficits, as well as in other survivors of critical illness such as following complex cardiac anomalies, before any definitive conclusions can be drawn. In future trials, neuropsychological assessment and neuroimaging should be conducted both immediately and one year post-intervention.

Overall, our findings demonstrate that cognition and white matter microstructure are malleable with CWMT in survivors of neonatal ECMO and/or CDH. Working-memory was the primary outcome measure in our trial, which had been based on initial reports of neuropsychological outcome in our study population.[1-3] However, ongoing research, as described in this thesis, led to new insights of primarily short- and long-term memory and sustained attention deficits following neonatal ECMO and/or CDH. Although CWMT may be beneficial for survivors of neonatal ECMO and/or CDH with visuospatial memory deficits, it is not the (complete) answer to the long-term neuropsychological deficits observed in these children. Importantly, our results demonstrated that it is essential to conduct an elaborate neuropsychological assessment before initiating CWMT in survivors of neonatal critical illness to determine its clinical utility. Furthermore, if multiple domains are affected in a child, treatment strategies should ideally affect multiple domains as well. A combination of different intervention programs may therefore be of interest. Findings from both experimental and clinical studies have suggested that multimodal training leads to better results compared to a single training program.[83,84] Exercise training in children has been found to affect memory and learning by targeting the hippocampus.[85] Combining such a physical program with cognitive training aimed at improving attention or memory, may strengthen the results and be beneficial in survivors of neonatal critical illness.

However, these are future perspectives and of little use in today's clinical practice. Currently, survivors of neonatal critical illness with long-term neuropsychological deficits may have to manage with practical tools to improve school performance and daily life activities. To improve long-term outcome in these children, we recommend that survivors of neonatal critical illness receive information on the practical implications of the deficits they may experience (e.g. difficulty remembering homework that is due tomorrow or appointments with friends), as well as learn about compensatory techniques or external (memory) aids that may be used to improve their activities of daily living (e.g. errorless learning, mental imagery to improve recall, writing important things down and using a schedule book[80]). Ideally, this information is personalized to the patient's specific impairments and needs. Personalized information and practical tools can be realized by conducting neuropsychological assessment to evaluate the degree of neuropsychological deficits, as well as by evaluating the degree to which these deficits affect activities of

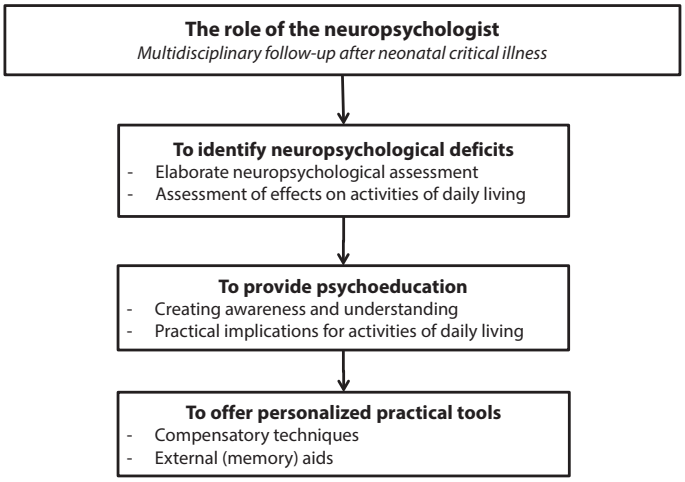


Figure 1. The role of the neuropsychologist in multidisciplinary follow-up after neonatal critical illness

daily living in the patient.[80] The neuropsychologist as such can play an essential role in (the improvement of) long-term outcome following neonatal critical illness (figure 1). A multidisciplinary approach to long-term follow-up after neonatal critical illness should therefore be strived for.

Prevention

Ideally, the deleterious effects of neonatal critical illness on the neonatal brain should be prevented. This may be (partly) accomplished by fine-tuning therapy or treatment strategies, but may also be achieved with the use of neuroprotective agents in the future. While it remains unknown which pathophysiological mechanisms are most detrimental to the developing brain, we do know that the hippocampus is highly vulnerable in critically ill infants (chapters 9 & 10). Besides hypothermia (chapter 9), the use of pharmacological agents that may have neuroprotective effects may therefore be of great value in critically ill infants. For instance, the effect of maternal allopurinol, which may protect the fetus against hypoxic-ischemic brain injury, is currently being investigated.[86] Here, we mention two other agents that are commonly used in the NICU and may have neuroprotective effects.

Dexmedetomidine, used in particular for sedation in the pediatric ICU population, may have neuroprotective effects on the hippocampus, in particular against hypoxic-ischemic damage.[87] These effects have been suggested to result from an activation of α_2-adrenergic receptors by dexmedetomidine, which inhibits inflammation following brain ischemia.[88] As the hippocampus has been found to be vulnerable to both hypoxia-ischemia as well as inflammation (chapter 9), this specific mechanism of action is of interest. However, these findings are mostly based on animal models and studies in adult

populations.[87,88] Future clinical trials that assess the efficacy and safety of dexmedetomidine in critically ill neonates that also include neurobiological outcome parameters, such as hippocampal volume, are therefore needed. Another agent that may be of interest in this respect is erythropoietin. Erythropoietin is produced by various cell types in the developing brain as a growth factor and as an endogenous neuroprotective response to hypoxia.[89] As previously mentioned, in addition to hypoxia, high oxygen concentrations as a result of supplementary oxygen may lead to neonatal brain damage as well.[46-50] A recent study in 6-day-old rat pups showed that a single dose of erythropoietin at the onset of hyperoxia (24 hours 80% oxygen) improved memory impairment and reduced acute oligodendrocyte degeneration up to the adolescent and adult stage.[90] Given the vulnerability of pre-oligodendrocytes in the periventricular white matter during the perinatal period[29], which may potentially be (partly) underlying the attention and memory deficits observed later in life in survivors of neonatal critical illness (chapter 6), reducing microstructural abnormalities in these fibers would have direct clinical benefits. In addition, studies have found that erythropoietin may have neurotrophic effects as well by increasing synaptic plasticity in the hippocampus and improving memory formation.[90,91] The hippocampus shows a uniquely high degree of neuroplasticity, which means it has the ability to adapt and reorganize in response to internal or external stimuli.[49] Although this unfortunately seems to result in more pronounced vulnerability than plasticity – the mechanisms underlying this (im)balance remain largely unknown – its ability to generate new neurons throughout life does make it a promising target in this respect.[49] Trials on potentially neurotrophic agents such as erythropoietin are therefore of interest. In infants with extreme prematurity, hypoxic-ischemic encephalopathy, perinatal stroke, and complex cyanotic heart disease, trials have demonstrated safety, and the potential for efficacy of erytrhopoietin.[92] However, the optimal dose and regimen for neuroprotection in neonates remains largely unknown.[93] Future clinical intervention trials assessing the effects of neuroprotective agents before, during or after exposure to both hypoxia and hyperoxia are needed in critically ill neonates.

CONCLUSION

In this thesis, we have demonstrated that survivors of neonatal critical illness are at risk of sustained attention and verbal and visuospatial memory deficits, despite generally average intelligence. These neuropsychological deficits seem to be associated with specific brain alterations that are mainly located in the brain's limbic system. In particular, we demonstrated that hippocampal alterations and associated memory deficits exist across survivors of neonatal critical illness, irrespective of underlying disease or gestational age. We suggest that this common neurodevelopmental pathway across survivors

of neonatal critical illness is due to factors associated with neonatal critical illness, such as hypoxia-ischemia, inflammation, stress, and analgosedatives, or a complex interplay amongst these factors. Our findings have further demonstrated that CWMT could be considered for school-age survivors of neonatal ECMO and/or CDH who have long-term visuospatial memory deficits. It is therefore important that, in today's practice, neuropsychological assessment is conducted before a child starts an intervention program such as CWMT to establish its clinical utility. Although it is promising that we find neurodevelopmental outcome to be malleable following neonatal critical illness, CWMT is not the optimum solution as multiple neuropsychological domains are affected in these children. To improve outcome following neonatal critical illness in current practice, providing psychoeducation, compensatory techniques and external (aids) should become a standard part of (long-term) care following neonatal critical illness.

The findings described in this thesis have underlined the necessity of broadening our focus from short-term to long-term outcome following neonatal critical illness. Given the increasing number of critically ill infants that survive today[94,95], future research directed at improving long-term outcome by protecting the vulnerable brain, particularly in the newborn period, is of utmost importance.

FUTURE RESEARCH DIRECTIONS

- Future research should aim to gain insight into the exact pathophysiological mechanisms underlying long-term neuropsychological deficits following neonatal critical illness. This may lead to the identification of clinical, iatrogenic or therapeutic factors that are most detrimental to the developing hippocampus in critically ill infants, which will improve risk stratification and better targeted treatment.
- As critically ill infants 'grow into their deficits', longitudinal studies that combine dense data collection in the perinatal period with follow-up neuroimaging and neuropsychological assessments are imperative. This will allow us to find 'windows of sensitivity' (i.e. when infants are most at risk of impairment, the optimal timing of MRI or neuropsychological assessment) as well as 'windows of opportunity' (i.e. when it is best to implement therapy or interventions).
- To improve outcome, future studies on the efficacy and safety of pharmacological therapy in critically ill infants should include the assessment of its effects on the developing brain in addition to other outcome parameters.
- Future trials on cognitive interventions following neonatal critical illness should be problem-oriented, i.e. focused on those neuropsychological domains or brain areas most at risk in survivors of neonatal critical illness.

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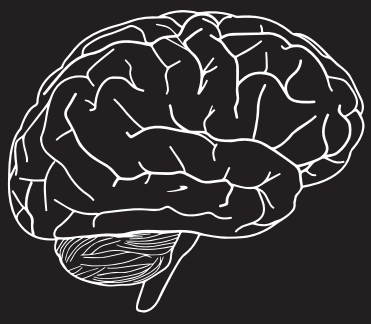
CHAPTER 12



SUMMARY

Introduction

Children growing up after neonatal critical illness are at risk of long-term neuropsychological deficits and school problems. However, the specific neuropsychological profile and its underlying neurobiology have remained largely unknown. Furthermore, strategies to prevent or diminish impaired outcome have remained unstudied. These knowledge gaps are tackled in a clearly delimited group of survivors of neonatal critical illness: children treated with neonatal extracorporeal membrane oxygenation (ECMO) and children with congenital diaphragmatic hernia (CDH) treated without ECMO.

Cognition and the brain after neonatal critical illness

In **chapter 2**, the developmental trajectory of IQ in survivors of neonatal ECMO is described at the ages of 2, 5, and 8 years. We show that IQ is stable and average in these children. Despite average IQ, neonatal ECMO survivors are more often in need of extra help in school compared to the general population. Specific neuropsychological deficits may be underlying these school problems. Indeed, we show that increased need for help in school is associated with sustained attention deficits, irrespective of IQ. We further demonstrate that children with CDH who needed ECMO treatment are at highest risk of long-term neuropsychological deficits.

In **chapter 3**, we use elaborate neuropsychological assessment to assess the neuropsychological outcome profile following neonatal ECMO and/or CDH at 8 years of age. Despite a generally average IQ, we show significant deficits in sustained attention, and short- and long-term verbal and visuospatial memory in survivors of neonatal ECMO and/or CDH compared to the general population. We find that children with CDH who needed ECMO have lower IQ than those not treated with ECMO and those treated with ECMO following diagnoses other than CDH. However, the specific neuropsychological deficits are similar across these groups. We also explore potential risk factors of long-term neuropsychological deficits. Our results show that a higher maximum vasoactive-inotropic score (maximum dose of vasoactive medication required during hospital stay) is specifically associated with long-term verbal and visuospatial memory deficits at 8 years of age. These findings may indicate a relationship between early cerebral hypoperfusion and memory in later life.

In **chapter 4**, we assess neuropsychological outcome in 17-year-old survivors of neonatal ECMO. We find similar deficits as those found at 8 years of age. Adolescents have significantly worse short- and long-term verbal and visuospatial memory compared to the norm. Sustained attention is not assessed at this age. These findings suggest memory deficits to persist until adolescence following neonatal ECMO.

In **chapter 5**, Diffusion Tensor Imaging and structural MRI are used to compare white matter microstructure and hippocampal volume between neonatal ECMO survivors and healthy controls (8-15 years). We find that neonatal ECMO survivors have significantly lower global fractional anisotropy, a measure of coherence of white matter fibers, which may indicate long-term injury to neural tracts. In addition to these global differences, we find significantly lower fractional anisotropy in the cingulum bundle, and higher mean diffusivity – suggestive of decreased integrity in axonal membranes, packing, or myelin – in the parahippocampal part of the cingulum. Furthermore, we show smaller hippocampal volume in neonatal ECMO survivors compared to controls, which is associated with worse verbal memory in these children. These results help define the underlying neurobiology involved in the long-term neuropsychological deficits following ECMO.

In **chapter 6**, we assess whether the neuropsychological deficits observed following neonatal ECMO are specifically associated with the brain alterations described in chapter 5. In a different cohort of survivors of neonatal ECMO and/or CDH (8-12 years), we show that lower global fractional anisotropy and lower fractional anisotropy in the cingulum bundle are associated with sustained attention deficits. Higher mean diffusivity in the parahippocampal part of the cingulum is associated with visuospatial memory deficits, whereas smaller hippocampal volume is associated with verbal memory deficits. Our findings indicate specific neurobiological correlates of attention and memory deficits in school-age survivors of neonatal ECMO and CDH. The structure-function relationships are observed irrespective of diagnosis or type of ECMO-cannulation. Interestingly, our results are in line with findings in survivors of other types of critical illness in early life, such as neonatal hypoxia and congenital heart disease.

Can we train the damaged brain after neonatal critical illness?

In **chapter 7**, we describe neuropsychological functioning immediately and one year after Cogmed Working-Memory Training (CWMT), assessed using a nationwide, single-blind randomized controlled trial in school-age (8-12 years) survivors of neonatal ECMO and/or CDH. In children trained with CWMT, we find immediate improvements in verbal and visuospatial working-memory compared to non-trained controls. However, these improvements do not persist until one year post-intervention. Sustained improvements in long-term visuospatial memory in the CWMT group are found one year post-intervention compared to the non-training group. Given the high risk of visuospatial memory deficits in this population, CWMT may be beneficial for survivors of neonatal critical illness with these specific deficits.

In **chapter 8**, white matter microstructure assessed using Diffusion Tensor Imaging is compared between school-age (8-12 years) neonatal ECMO and/or CDH survivors im-

mediately after CWMT and compared to non-trained survivors. We find training-induced changes in both global white matter microstructure as well as in the left superior longitudinal fasciculus in the CWMT group compared to non-trained children. Increased fractional anisotropy in the superior longitudinal fasciculus is significantly associated with improved verbal working-memory in the CWMT group. However, the global increase in fractional anisotropy is not associated with any cognitive improvements. Nonetheless, our findings demonstrate neuroplasticity following neonatal ECMO and/or CDH. Future studies that include neuroimaging and neuropsychological assessment both immediately and one year post-intervention are needed.

The vulnerable brain in critically ill infants

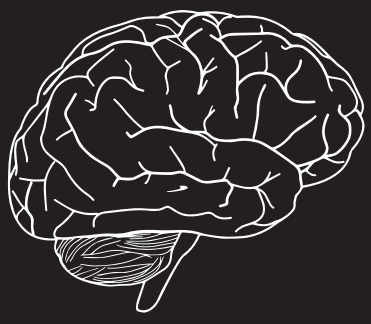
In **chapter 9**, we describe the results of a comprehensive literature review on memory and its neurobiological substrates, specifically the hippocampus, in children following preterm birth, congenital heart disease, neonatal ECMO treatment and/or CDH. We propose a common neurodevelopmental pathway following neonatal critical illness where early hippocampal alterations are associated with memory deficits later in life across survivors of neonatal critical illness, irrespective of underlying diagnosis or gestational age. We suggest that this shared hippocampal vulnerability is probably related to common conditions associated with neonatal critical illness, including hypoxia, neuroinflammation, stress, exposure to anesthetics, or a complex interplay of these factors at different postconceptional ages. The clinical implications of these findings and future perspectives are discussed.

In **chapter 10**, we describe the results of a literature review on the effects of commonly used analgesics and sedatives (analgosedatives) on the brain and neuropsychological outcome in critically ill neonates. Infants admitted to neonatal or pediatric intensive care units receive very high amounts of these drugs, which may contribute to the neurodevelopmental impairments observed in these children. Although less obvious in human studies than animal studies, results indicate that exposure to analgosedatives in neonates and infants may have long-term effects on the brain and cognition. Specifically, the hippocampus and memory may be affected by sedatives such as midazolam and propofol. A balanced approach that includes the assessment and quantification of both wanted effects and unwanted effects of analgosedatives is needed. Therefore, long-term neuropsychological outcome should be assessed as well when evaluating drug efficacy and safety.

In **chapter 11**, we discuss our main findings, compare them with the current literature and make recommendations for future research to improve long-term outcome following neonatal critical illness. Our findings emphasize the need for long-term neuropsycho-

logical follow-up in these patients and the need for early identification of patients at risk for long-term neuropsychological deficits and school problems. As MRI is a non-invasive technique, using hippocampal volume in early infancy as a neurobiological marker of memory deficits in later life may in the future lead to earlier identification of patients at risk. Longitudinal studies in which neurodevelopment is assessed using both neuroimaging and neuropsychological assessment are therefore imperative following neonatal critical illness, favorably using a healthy control group. Furthermore, an important area of future research is to gain insight into the exact pathophysiological mechanisms. This insight may lead to diminished or even prevention of long-term adverse outcomes by adjustments in neonatal critical care and/or application of neuroprotective strategies. Intervention strategies that aim to improve impaired neuropsychological outcome at a later stage in survivors of neonatal critical illness remain of interest as well. Assessing the effectiveness of multimodal interventions, such as cognitive training combined with an exercise program, may be beneficial in survivors of neonatal critical illness. Finally, to improve outcome following neonatal critical illness in current practice, providing psychoeducation, compensatory techniques and external (aids) should become a standard part of (long-term) care following neonatal critical illness.

CHAPTER 13



DUTCH SUMMARY

Introductie

Kinderen die in de neonatale periode zeer ernstig ziek zijn geweest, hebben op latere leeftijd een verhoogd risico op neuropsychologische problemen en schoolproblemen. Het specifieke neuropsychologische profiel en de onderliggende neurobiologie is echter onbekend. Daarbij zijn behandelingsmethoden om de neuropsychologische uitkomsten van deze kinderen te verbeteren nog altijd niet bestudeerd. Dit is daarom onderzocht bij kinderen die zijn behandeld met neonatale extracorporale membraan oxygenatie (ECMO) en bij kinderen met een congenitale hernia diafragmatica (CDH; congenital diaphragmatic hernia) die niet zijn behandeld met ECMO.

Cognitie en het brein

In **hoofdstuk 2** wordt de ontwikkeling van het IQ na behandeling met neonatale ECMO beschreven op de leeftijden van 2, 5 en 8 jaar. We laten zien dat het IQ van deze kinderen stabiel en gemiddeld is. Ondanks dit gemiddelde IQ hebben kinderen die zijn behandeld met ECMO in vergelijking met leeftijdsgenoten vaker extra hulp nodig op het regulier basisonderwijs. Specifieke neuropsychologische problemen liggen hier mogelijk aan ten grondslag. We laten zien dat kinderen die extra hulp nodig hebben op school vaker problemen hebben met de volgehouden aandacht. Deze problemen zijn niet gerelateerd aan het IQ. Ook zien we dat kinderen met CDH die zijn behandeld met ECMO het hoogste risico hebben op deze neuropsychologische langetermijnproblemen.

In **hoofdstuk 3** gebruiken we uitgebreid neuropsychologisch onderzoek om het neuropsychologisch profiel na behandeling met neonatale ECMO en/of CDH te onderzoeken op 8-jarige leeftijd. Hoewel deze kinderen een gemiddeld IQ hebben, zien we in vergelijking met gezonde leeftijdsgenoten significante afwijkingen in de volgehouden aandacht, en in het verbaal- en visueel-ruimtelijke, korte- en langetermijngeheugen. We zien dat kinderen met CDH die zijn behandeld met ECMO over het algemeen een lager IQ hebben dan kinderen met CDH die niet zijn behandeld met ECMO en dan kinderen die zijn behandeld met ECMO na andere diagnoses. Onze resultaten laten zien dat een hogere maximale vasoactieve-inotropische score (maximum dosering vasoactieve medicatie tijdens de ziekenhuisopname) specifiek geassocieerd is met problemen in het verbaal- en visueel-ruimtelijk langetermijngeheugen op 8-jarige leeftijd. Deze bevindingen duiden mogelijk op een relatie tussen cerebrale hypoperfusie tijdens de eerste levensmaanden en het geheugen op latere leeftijd.

In **hoofdstuk 4** beschrijven we neuropsychologische uitkomsten bij 17-jarige adolescenten die zijn behandeld met neonatale ECMO. We tonen aan dat de adolescenten problemen hebben die vergelijkbaar zijn met de geconstateerde problemen van de 8-jarige kinderen. Adolescenten die zijn behandeld met neonatale ECMO hebben een

significant slechter verbaal- en visueel-ruimtelijk korte- en langetermijngeheugen dan gezonde leeftijdsgenoten. Volgehouden aandacht is niet onderzocht op deze leeftijd. Deze resultaten suggereren dat de geheugenproblemen na behandeling met neonatale ECMO ook in de pubertijd nog aanwezig zijn.

In **hoofdstuk 5** gebruiken we Diffusion Tensor Imaging en structurele MRI om de microstructuur van de witte stof en het hippocampaal volume bij kinderen na behandeling met neonatale ECMO te vergelijken met gezonde leeftijdsgenoten (8-15 jaar). We laten zowel globale als specifieke afwijkingen zien in de microstructuur van witte stof bij kinderen die zijn behandeld met ECMO, in vergelijking met gezonde leeftijdsgenoten. Daarnaast zien we dat het hippocampusvolume kleiner is bij kinderen die zijn behandeld met ECMO dan bij gezonde kinderen. Een kleiner hippocampaal volume is eveneens geassocieerd met een verminderd verbaal geheugen bij kinderen die zijn behandeld met ECMO. Deze resultaten dragen bij aan het definiëren van de onderliggende neurobiologie van neuropsychologische problemen na behandeling met ECMO.

In **hoofdstuk 6** onderzoeken we of de neuropsychologische problemen na neonatale ECMO geassocieerd zijn met de veranderingen in het brein die zijn beschreven in hoofdstuk 5. In een ander cohort van kinderen die zijn behandeld met neonatale ECMO en/of CDH (8-12 jaar), vinden we dat globale afwijkingen in de microstructuur van witte stof geassocieerd zijn met problemen in de volgehouden aandacht. Specifieke afwijkingen in het parahippocampale deel van de cingulum zijn geassocieerd met slechter visueel-ruimtelijk langetermijngeheugen, terwijl een kleiner hippocampaal volume geassocieerd is met problemen in het verbaal langetermijngeheugen. De associaties tussen het brein en cognitie zijn onafhankelijk van de onderliggende diagnose of het type ECMO-canulatie dat gebruikt is. Opmerkelijk is dat onze bevindingen overeenkomen met bevindingen bij kinderen die om andere redenen in de neonatale periode op de Intensive Care hebben gelegen, zoals kinderen met een aangeboren hartafwijking of met hypoxie in de neonatale periode.

Kunnen we het beschadigde brein trainen?

In **hoofdstuk 7** beschrijven we het neuropsychologisch functioneren direct en één jaar na Cogmed werkgeheugentraining. Dit onderzoeken we met een gerandomiseerde, gecontroleerde trial onder 8- tot 12-jarige kinderen die zijn behandeld met neonatale ECMO en/of CDH. We vergelijken kinderen die zijn getraind met Cogmed met kinderen die niet deze training hebben gehad, zowel direct als een jaar na de training. De Cogmed-kinderen laten verbeteringen zien in zowel verbaal- als visueel-ruimtelijk werkgeheugen direct na de training in vergelijking met de niet-getrainde kinderen. Deze verbeteringen verdwijnen echter na een jaar. De Cogmed-groep laat wel blijvende ver-

beteringen zien in het visueel-ruimtelijk langetermijngeheugen een jaar na de training, in vergelijking met niet-getrainde kinderen. Omdat deze populatie een verhoogd risico heeft op dit soort geheugenproblematiek, is Cogmed werkgeheugentraining mogelijk een waardevolle interventie voor kinderen met problemen in het visueel-ruimtelijk langetermijngeheugen.

In **hoofdstuk 8** wordt het effect van de Cogmed werkgeheugentraining op de microstructuur van witte stof onderzocht door middel van Diffusion Tensor Imaging in 8- tot 12-jarige kinderen die zijn behandeld met neonatale ECMO en/of CDH. In vergelijking met de ongetrainde groep zien we direct na Cogmed zowel globale als specifieke veranderingen in de microstructuur van de witte stof. De specifieke veranderingen zijn geassocieerd met verbeteringen in het verbaal werkgeheugen direct na Cogmed. De globale veranderingen zijn niet geassocieerd met cognitieve verbeteringen direct of een jaar na Cogmed. Onze resultaten laten desalniettemin zien dat er neuroplasticiteit bestaat bij kinderen die zijn behandeld met ECMO en/of CDH. Studies die zowel direct als een jaar na Cogmed het neuropsychologisch functioneren en het brein onderzoeken zijn nodig om beter te begrijpen hoe Cogmed precies werkt.

Het kwetsbare brein

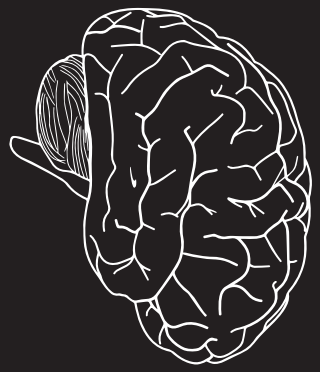
In **hoofdstuk 9** beschrijven we de resultaten van een literatuurstudie naar het geheugen en de hippocampus van kinderen die prematuur geboren zijn, kinderen die een aangeboren hartafwijking hebben, kinderen die behandeld zijn met neonatale ECMO, en kinderen met CDH. Op basis van deze resultaten veronderstellen wij dat er mogelijk een 'common neurodevelopmental pathway' bestaat na een periode van zeer ernstig ziek zijn in de eerste levensmaanden: vroege afwijkingen in de hippocampus leiden tot geheugenproblemen, onafhankelijk van de onderliggende diagnose of zwangerschapsduur. Deze gedeelde hippocampale gevoeligheid is mogelijk gerelateerd aan algemene factoren die horen bij zeer ernstig ziek zijn, zoals hypoxie, neuroinflammatie, stress, blootstelling aan sedativa en analgetica, of een complexe interactie tussen die factoren. De klinische implicaties en perspectieven voor toekomstige studies gebaseerd op deze bevindingen worden besproken.

In **hoofdstuk 10** worden de uitkomsten besproken van een literatuurstudie naar het effect van veelgebruikte analgetica en sedativa op het brein en de neuropsychologische ontwikkeling bij kinderen die zeer ernstig ziek zijn geweest. Kinderen die worden behandeld op de neonatale of kinder-intensive care unit krijgen zeer hoge doseringen van deze medicamenten, die mogelijk de neurocognitieve ontwikkeling negatief beïnvloeden. Hoewel de resultaten in studies met mensen minder duidelijk zijn dan in dierstudies, hebben analgetica en sedativa mogelijk een negatief effect op het brein

en op het cognitief functioneren van deze kinderen. Met name de hippocampus en het geheugen lijken gevoelig voor sedativa zoals propofol of midazolam. Een evenwichtige aanpak, waarin zowel de gewilde als de ongewilde effecten van deze middelen worden geanalyseerd, is nodig. Hierin dienen de effecten op de neurocognitieve ontwikkeling meegenomen te worden.

In **hoofdstuk 11** worden de belangrijkste bevindingen besproken en in perspectief geplaatst op basis van de bestaande literatuur. Ook worden er aanbevelingen gedaan voor toekomstige studies om zo de uitkomsten van kinderen die in de neonatale periode zeer ernstig ziek zijn geweest te verbeteren. Onze resultaten laten zien dat kinderen die zijn behandeld met ECMO en/of CDH een verhoogd risico hebben op geheugen- en aandachtsproblemen, terwijl ze een normaal IQ hebben. Omdat dit soort neuropsychologische functies zich pas op latere leeftijd ontwikkelt, is het van groot belang dat deze kinderen herhaald gezien worden op de lange termijn. We laten zien dat specifieke afwijkingen in het brein, zoals een kleiner hippocampaal volume, geassocieerd zijn met neuropsychologische problemen die deze kinderen hebben. Het in kaart brengen van het hippocampaal volume in de eerste levensjaren via MRI, een non-invasieve techniek, zou mogelijk kunnen bijdragen aan vroegere identificatie van kinderen met een verhoogd risico op geheugenproblemen. Longitudinale studies waarin de neurocognitieve ontwikkeling onderzocht wordt met zowel MRI als neuropsychologisch onderzoek, en waarbij een vergelijking wordt gemaakt met gezonde kinderen, is daarom essentieel. Een belangrijke volgende stap is het bestuderen van de exacte pathofysiologische mechanismen die aan de neurocognitieve problemen bij deze kinderen ten grondslag liggen. Deze kennis zal mogelijk leiden tot verbetering van de uitkomsten doordat behandelmethoden kunnen worden aangepast of specifieke neuroprotectieve middelen kunnen worden toegepast. Interventies gericht op het verbeteren van neuropsychologische problemen zijn echter ook van belang. Verder onderzoek naar de effectiviteit van multimodale interventies, zoals de combinatie van een cognitieve en lichamelijke training, is nodig bij deze kinderen. In de huidige praktijk is het noodzakelijk dat kinderen en ouders psychoeducatie, compensatietechnieken en handvatten wordt aangeboden. Dit zou een standaard onderdeel moeten zijn van de zorg voor kinderen die in de neonatale periode zeer ernstig ziek zijn geweest.

CHAPTER 14



APPENDICES

PHD PORTFOLIO

Name PhD student:	Raisa Schiller
Erasmus MC Department:	IC Children/Pediatric Surgery and Child and Adolescent Psychiatry/psychology
PhD period:	April 2014 – April 2018
Promotor(s):	Prof. dr. Tibboel and prof. dr. Verhulst
Supervisor(s):	Dr. Hanneke IJsselstijn and dr. Tonya White

Training program	Year	Workload
General academic skills		
EndNote and Pubmed courses	2014	1.0 EC
Open Clinica course, Erasmus Medical Center	2014	0.2 EC
Research management for PhD students	2014	1.0 EC
BROK	2015	1.0 EC
How to present your poster	2015	0.1 EC
Workshop Presenting Skills for junior researchers	2016	1.0 EC
Scientific Integrity	2017	0.3 EC
Research skills		
MRI safety course and technical training	2014	0.6 EC
Biostatistics Part II: Classical Regression Models	2015	2.8 EC
Advanced topics in MR imaging of the brain, Donders Institute	2015	1.0 EC
Introduction in GraphPad Prism, MolMed	2015	0.3 EC
FSL course	2016	2.0 EC
Presentations		
Research meeting neuroradiology (AMBER)	2014	0.3 EC
Research meeting Pediatric Surgery/IC	2014-2017	1.2 EC
Conference Verona, Italy on behalf of Ped. Surgery Dept.	2015	0.3 EC
Grand Round	2016	0.3 EC
KNICR Symposium	2016	0.3 EC
OHBM Conference Vancouver, Canada	2017	0.3 EC
Pediatric Psychology research meeting	2017	0.3 EC
Great Ormond Street Hospital, London	2018	0.3 EC
Conferences		
Conference Verona, Italy	2015	1.0 EC
Neuroimaging conference, Amsterdam	2015	0.3 EC
Pump Your Career, NWO, Amersfoort	2015	0.3 EC
Young Researchers Day, Tulips, Maarssen	2015	0.3 EC
Child Health Symposium, Noordwijk	2016	1.0 EC
KNICR symposium, Erasmus MC – Sophia	2016	0.3 EC
OHBM Conference Vancouver, Canada	2017	1.0 EC

Training program	Year	Workload
Seminars and workshops		
PhD Day Erasmus MC, several workshops	2014+2015	0.5 EC
Research meetings pediatric surgery (monthly)	2014-2018	2.0 EC
Research meetings neuroradiology (monthly)	2014-2018	2.0 EC
Research meetings neuroimaging (biweekly)	2014-2018	4.0 EC
Research meetings CHIL (monthly)	2014-2018	2.0 EC
Teaching tasks		
Tutor neuropsychological diagnostics, EUR	2014	1.3 EC
Tutor psychological diagnostics, EUR	2014	1.3 EC
Supervisor minor Neurobiology, Erasmus MC	2015	1.0 EC
Other		
Committee Sophia Research Day 2015	2014-2015	2.0 EC
Committee Sophia Research Day 2016	2015-2016	2.0 EC
Committee Sophia Research Day 2017	2016-2017	2.0 EC
TULIPS PhD curriculum	2016-2018	4.0 EC

CURRICULUM VITAE

Raisa Schiller was born on April 24th 1988 in Nijmegen, The Netherlands. She grew up in Bilthoven, where she received her Gymnasium degree in 2006. That same year, she moved to the United States to study Psychology and play Division-1 Field Hockey on a full scholarship at Wake Forest University in Winston-Salem, North Carolina. After graduating in 2010, she moved to Amsterdam to continue her studies. In August 2013, she received her master's degrees after completing the two-year research master Cognitive Neuropsychology and master Clinical Neuropsychology (cum laude).

From September 2013, Raisa started working as a neuropsychologist at the outpatient clinic of the Department of Child- and Adolescent Psychiatry/Psychology at the Erasmus MC-Sophia Children's Hospital in Rotterdam for six months. She then commenced her PhD-project in April 2014 on neurodevelopmental outcome following neonatal critical illness at the departments of Intensive Care and Pediatric Surgery (Prof. dr. D. Tibboel (promotor), Dr. Hanneke IJsselstijn (copromotor)) and Child and Adolescent Psychiatry/Psychology (Prof. dr. Frank Verhulst (promotor), Dr. Tonya White (copromotor)).

Raisa aims to combine research with clinical work. In January 2018, she started working part-time as a neuropsychologist at the outpatient clinic of the Child and Adolescent Psychiatry/Psychology department while finishing her PhD-project. After her PhD-project, she has been given the opportunity to continue as a postdoctoral researcher at the Intensive Care and Pediatric Surgery department, which she will combine with her clinical work.

DANKWOORD

Hoewel alleen mijn naam op de voorkant van dit boekje staat, is het verre van een solo-project. De totstandkoming van dit proefschrift heb ik te danken aan velen.

Alle kinderen en ouders die vanuit het hele land bereid waren meerdere malen naar het Sophia te komen om mee te doen aan de Cogmedstudie: de weekenden waarin de MRI- en neuropsychologische onderzoeken plaatsvonden, waren ontzettend leuk door jullie enthousiasme en interesse! Ook alle kinderen en ouders die deelnemen aan de Chirurgische Lange Termijn Follow-up wil ik bedanken.

Mijn promotoren: prof. dr. D. Tibboel en prof. dr. F. Verhulst.

Beste professor Tibboel, wie had kunnen denken dat het afdelingshoofd Intensive Care Kinderen en een beginnend neuropsycholoog een goede match zouden zijn? Ik niet direct. Toch heeft u een ontzettend belangrijke rol gespeeld in mijn promotietraject. Dank voor uw interesse, uw vertrouwen, uw altijd kritische houding, en de goede en stimulerende gesprekken. Ik ben blij en vereerd dat ik hier gebruik van mag blijven maken. Op naar nieuwe ideeën en projecten!

Beste Frank, zonder jou was ik überhaupt niet bij dit project terechtgekomen. Dank voor het duwtje in de juiste richting!

Mijn copromotoren: dr. H. IJsselstijn en dr. T. White.

Beste Hanneke, jij staat elk moment van de dag voor jouw promovendi klaar om hen van advies te voorzien. Je bent gestructureerd, efficiënt en van alles op de hoogte. Dit maakt je tot een fijne begeleider bij wie ik voor mijn gevoel altijd terechtkon. Dank voor al je hulp de afgelopen vier jaar!

Beste Tonya, dank voor het mogelijk maken van één van de meest interessante onderdelen van mijn promotie de afgelopen vier jaar: het MRI-onderzoek. De door jou in het leven geroepen KNICR-groep zorgt voor een goed netwerk van mede-neuroimagers, waar ik veel aan heb gehad!

Dr. M. Madderom, lieve Marlous, officieel geen co-promotor maar zo voelde het wel – zeker in de eerste jaren van mijn promotie. Zonder jou was de Cogmedstudie er niet geweest. Dank voor al je hulp en begeleiding. Het was gezellig zo nauw met je samen te werken!

De commissieleden:
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Over the last decade, the number of children admitted to specialized intensive care units has increased significantly worldwide. The majority of critically ill infants nowadays survive. This development requires our focus to broaden from minimizing mortality rates to maximizing long-term quality of life following neonatal critical illness.

The findings presented in this thesis demonstrate the importance of long-term neurodevelopmental follow-up in survivors of neonatal critical illness and stress the need for early risk stratification and targeted intervention strategies for these children.