

Born just a few weeks early... is this relevant?

- Insights into brain lesions, brain growth, and outcome in moderate-late preterm infants -



UMC Utrecht Brain Center

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Born just a few weeks early... is this relevant?

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Slechts een paar weken te vroeg geboren... is dat relevant?

*-Inzicht in hersenletsel, hersengroei en ontwikkeling van matig-laag prematuur geboren kinderen-
(met een samenvatting in het Nederlands)*

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The family tree will always grow
Venice – The Family Tree

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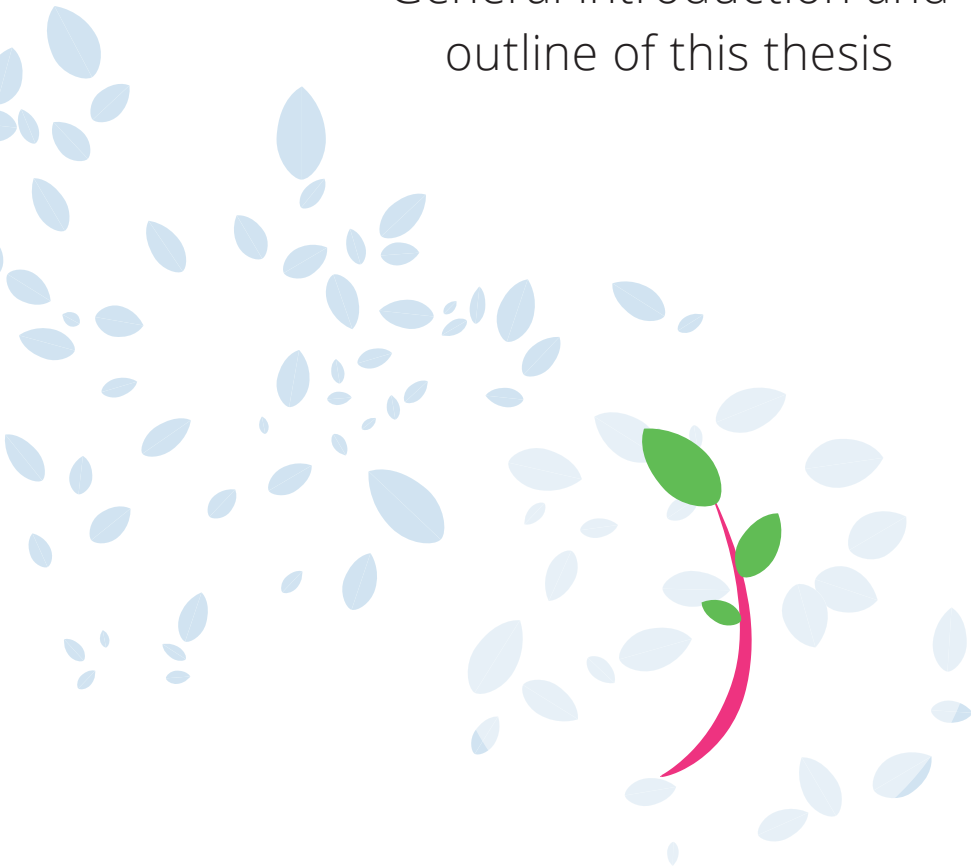
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CHAPTER 1

General introduction and
outline of this thesis



GENERAL INTRODUCTION AND OUTLINE OF THIS THESIS

With the wide spread use of cranial ultrasound (cUS) and magnetic resonance imaging (MRI), knowledge of the newborn brain has advanced rapidly in the past decades. Imaging the newborn brain can improve recognition of underlying diagnoses, aid to determine management and may predict neurodevelopmental outcome. Within this field, numerous scientific articles are published and contribute to the current understanding and knowledge of the developing newborn brain.

The scope of this thesis includes moderate-late preterm (MLPT) infants and cerebellar hemorrhage (CBH). Although not obvious at first glance, there is a similarity between these two topics. Within the field of neonatal neuroimaging, one of the most discussed topics is brain injury and neurodevelopmental delay in very preterm infants (who are born at a gestational age (GA) less than 32 weeks). Furthermore, infants born after 37 weeks of gestation (i.e. full-term), who suffer oxygen deprivation at birth resulting in hypoxic ischemic encephalopathy, are frequently discussed. Infants born after 32 weeks, but before 37 weeks, also known as MLPT infants, have received less attention. In addition, the most discussed types of brain injury are intraventricular hemorrhage (IVH) and white matter injury (WMI), which are both forms of supratentorial injury. Infratentorial injury, the most frequently occurring injury being CBH, is less frequently investigated. In conclusion, although at first there appear to be no common ground between MLPT infants and CBH, both topics received relatively less attention. Therefore, MLPT infants, and to a lesser extent CBH, will be the main focus of this thesis.

In this chapter, we will first start with the position of MLPT infants within the preterm population, and will give a brief overview of what is known about neurodevelopmental and behavioral problems in MLPT infants. Secondly, we will discuss known factors associated with neurodevelopmental and behavioral problems. In addition, we will describe why the developing brain of MLPT infants is vulnerable for brain injury and what types of brain injury may be encountered in these infants. Furthermore, the use of cUS and MRI in (preterm) infants will be briefly explained. Finally, we will give a short overview of the current knowledge on neurodevelopmental outcome in infants with cerebellar hemorrhage.

PRETERM BIRTH AND GLOBAL BURDEN

Preterm birth is defined as birth before 37 completed weeks of gestation (1). In 2014, the estimated global preterm birth rate was 10.6%, accounting for 14.8 million preterm births worldwide (2). In the Netherlands, each year approximately 11.000 infants are born prematurely (3), and represent about 6.5% of all live births.

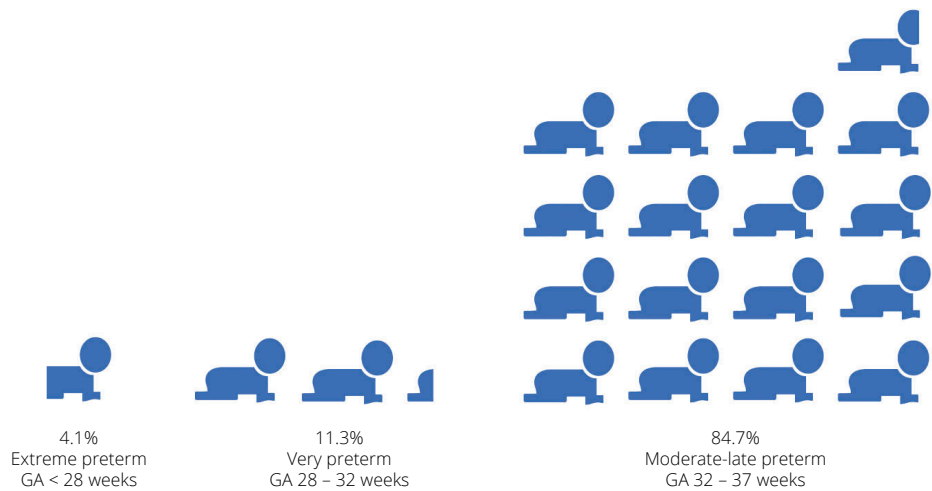
Based on gestation, preterm infants can be divided into three groups (1):

- Extremely preterm (GA <28+0 weeks);
- Very preterm (GA 28+0 - 31+6 weeks);
- Moderate-late preterm (MLPT; GA 32+0 - 36+6 weeks), who can be further subdivided in:
 - Moderate preterm (MP; GA 32+0 – 33+6 weeks);
 - Late preterm (LP; GA 34+0 – 36+6 weeks).

All preterm infants have an increased risk of several short- and long-term complications due to anatomical and/or functional immaturity. Both mortality and morbidity are inversely related to GA at birth and birth weight (4). It seems reasonable that most researchers and clinicians have focused on improving outcomes of extreme and very preterm infants, since they are most vulnerable. However, the global burden of developmental problems also depends on the number of infants born at each GA group (Figure 1). Since more than 80% of preterm infants is born MLPT, the global burden is the greatest for this group. (2,5,6).

Figure 1. Preterm birth proportions by GA group

Numbers are based on the estimated global proportions of preterm birth in 2014 by Chawanpaiboon et al. (2).



DEVELOPMENTAL AND BEHAVIORAL OUTCOMES IN MLPT INFANTS

Several methods can be used to monitor developmental and behavioral outcomes and may enable early detection of neurological and/or functional abnormalities. These methods include standardized neurological examinations, developmental tests and questionnaires.

So far, most studies have assessed developmental and behavioral outcome at 24 months of corrected age (corrected for prematurity). Widely used developmental tests at this age are the Bayley Scales of Infant Development (BSID) (7) and the Griffiths Mental Development Scales (GMDS) (8). Both tests are used by professionals to assess cognitive and motor domains. A good alternative for these two labor-intensive, and therefore expensive, tests is the validated parent-reported Ages and Stages Questionnaire (ASQ) (9). Furthermore, the Modified Checklist for Autism in Toddlers (M-CHAT) (10) and Child Behavior Checklist (CBCL) (11), which are also parent-reported questionnaires, can be used to screen for autism and behavioral problems.

Until a few years ago, it was assumed that MLPT infants had a similar risk of developmental problems as infants born full-term. However, this assumption has been proven incorrect. Recent studies report higher rates of neurodevelopmental and behavioral problems in MLPT infants compared to full-term infants (12,13).

Preschool age (up to 4 years of age)

Romeo et al. provided an overview of the literature concerning motor developmental outcomes of LP infants from term equivalent age (TEA) up to two years of age. The authors concluded that LP infants had a higher risk of developing cerebral palsy, motor delays and coordination disorders compared to full-term infants (14). Cheong et al. (15) and others (16,17), found lower scores for cognitive, language and motor domains in MLPT infants at 24 months corrected age. Furthermore, MLPT infants showed more internalizing behavioral problems and had an increased risk for a positive autism screening (18-20). Kerstjens et al. showed that MLPT infants were more likely to have problems with fine motor skills, communication and personal-social functioning at school entry (43 – 49 months of age) (21).

School age

At age 5-10 years, grade retention and need for special educational were more commonly seen in MLPT children than in full-term children (22,23). Seven-year-old MLPT children had lower scores ($<p10$) on tests of total intelligence quotient (IQ), performance IQ, visuospatial reasoning, attention, and executive functioning than full-term controls (24). These problems were more frequently seen in MP infants than in LP infants (25,26). In addition, MLPT children were also more likely to exhibit behavioral attention problems compared to full-term peers (27-29).

Adolescence and adulthood

Knowledge on long-term outcomes of MLPT born adults is scarce and has, so far, only been described in Scandinavian cohorts. Moster et al. found a higher risk of several serious medical disabilities (such as cerebral palsy and mental retardation) in MLPT individuals than in full-term controls (30). Heinonen et al. reported that especially LP born individuals who were born small for gestational age (SGA; birth weight $< - 2$ SD) had an increased risk of poorer neurocognitive functioning in adulthood, compared to LP born individuals with an

appropriate weight for GA and to full-term controls (31). Furthermore, MLPT born individuals were more likely to have a lower performance and lower job related income compared with full-term controls (6,30,32).

Despite the increased risk of neurodevelopmental, behavioral and school problems, there are currently no specific (inter)national accepted guidelines aimed at special care and/or follow-up in MLPT infants.

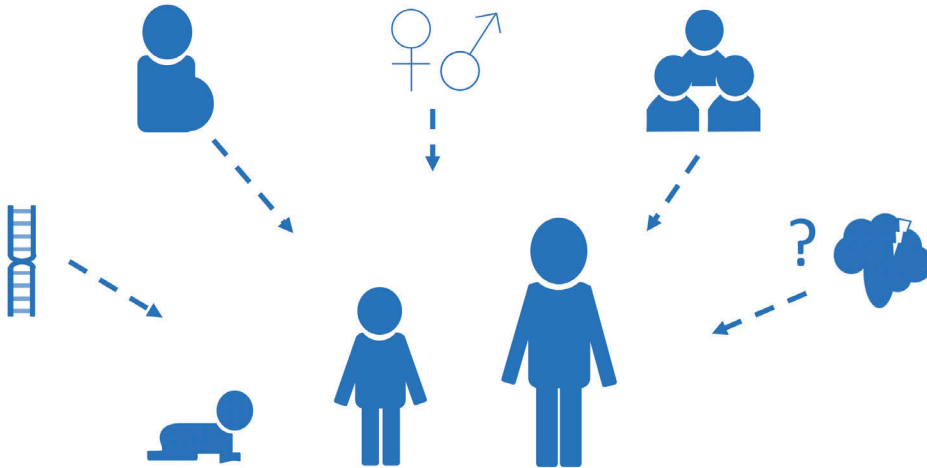
FACTORS ASSOCIATED WITH NEURODEVELOPMENTAL AND BEHAVIORAL PROBLEMS

To be able to improve neurodevelopmental outcome in MLPT infants, understanding of factors associated with neurodevelopmental and behavioral problems is needed. Improving knowledge on these factors could support early screening and targeted interventions to prevent (severe) disabilities and may improve neurodevelopmental outcome.

The etiology of neurodevelopmental and behavioral problems in (very) preterm infants is multifactorial. Multiple factors such as pre-existing genetic, maternal, pregnancy-related, fetal, perinatal, postnatal and sociodemographic factors have been associated with neurodevelopmental and behavioral problems (33-35). Most knowledge on these factors is derived from the very preterm population, but in the last decade, insights into the impact of these factors on the MLPT population have increased. Recent studies found an association between socio-economic status, maternal pre-pregnancy obesity, preeclampsia, multiple birth, male sex, ethnicity, SGA, hypoglycemia and an increased risk of developmental delay at the age of four years (Figure 2) (36-39).

While in very preterm populations brain injury acquired around birth, delayed brain development and impaired brain growth are also associated with neurodevelopmental and behavioral problems (40-42), knowledge on the impact of this factor on MLPT infants is scarce (13,43).

Figure 2. Schematic overview of factors associated with neurodevelopmental and behavioral problems in MLPT infants.



BRAIN DEVELOPMENT

To understand why the brain of MLPT infants is susceptible to injury, delayed development and impaired growth, we need to take a look at the development and growth of the brain in-utero.

Brain development is a highly complex and intriguing process. The second half of gestation is characterized by remarkable brain growth. At 20 weeks' gestation, the brain weighs only 10% of the volume of the brain at term. By 32 weeks, merely 60% of term volume is reached, and the brain thus still needs to gain 40% of the overall volume to reach term volume (44,45). At the same time, the cortical surface increases rapidly thanks to formation of gyri and sulci. At 20 to 27 weeks' gestation, the brain is still very smooth and only the Sylvian fissure, central sulcus and superior temporal sulcus are present. From GA 29 weeks onwards, secondary sulci and gyri rapidly develop. At term, tertiary sulci and gyri are also present. During this period, the cortical gray matter volume shows a fourfold increase (44,46). In addition, the cerebellar volume increases fivefold and the cerebellar cortical surface increases more than 30-fold from 24 weeks to term (47). (Pre-) myelination may start as early as 28 weeks, but a significant increase in myelinated white matter volume starts only after 36 weeks and continues until early adolescence (44,48).

To conclude, in the moderate and late preterm period, the brain is still immature and many maturational processes still need to take place. Thus, for MLPT infants, an important part of these processes takes place after birth. This likely makes their brains susceptible for injury.

BRAIN INJURY

At present, the incidence of brain injury in the MLPT population is largely unknown. In addition, it is unknown what type of injury occurs in this population. On the other hand, we do know that brain injury is frequently encountered in extreme and very preterm infants. In these infants, brain injury is strongly related to neurological impairment, including motor and cognitive deficits, sensorineural hearing loss, cerebral visual impairment and behavioral problems (49-51). In the past, neuro-imaging studies in preterm infants were mainly focused on evident and severe brain injury such as IVH, post-hemorrhagic ventricular dilatation, periventricular hemorrhagic infarction and cystic periventricular leukomalacia (Figure 3). With decreasing incidence of these serious brain lesions, focus has now shifted towards more subtle, diffuse and mild forms of brain injury, such as punctate white matter lesions, widening of the interhemispheric fissure and delayed myelination (Figure 4). These and other milder forms of brain injury are now frequently reported in extreme and very preterm infants and are, although to a lesser extent, also associated with neurodevelopmental delays and behavioural problems (40,52,53).

Figure 3. Examples of severe brain injury.

A) Coronal T2-weighted image in a preterm infant (GA 31+4 weeks, scanned at 43+6 weeks) showing a remnant of an intraventricular haemorrhage (arrow). Post - hemorrhagic (ventricular) dilatation is seen of the frontal and temporal horns of the lateral ventricles and the third and fourth ventricle; B) Transverse T2-weighted image in another preterm infant (GA 31+5 weeks, scanned at 35+3 weeks) showing bilateral cystic periventricular leukomalacia (arrows).

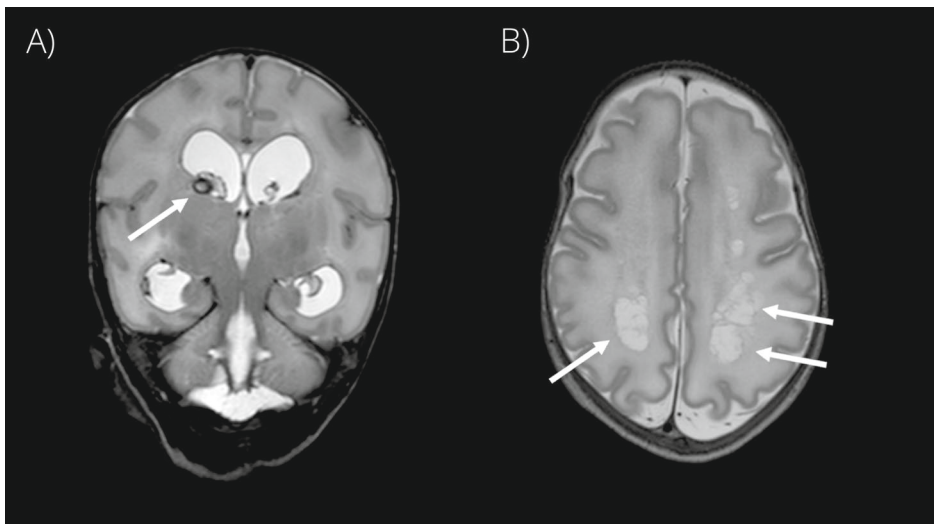


Figure 4. Examples of more subtle/milder brain injury.

A) T1- weighted image in a preterm infant (GA 35+3 weeks, scanned at 39+6 weeks) showing several punctate white matter lesions (arrows); B) Coronal T2-weighted imaging in a preterm infant (GA 32+0 weeks, scanned at 40+3 weeks) showing a somewhat widened interhemispheric fissure, white line = measurement of interhemispheric distance; C) T1-weighted image of an infant scanned at 43+4 weeks showing delayed myelination (arrows) of the posterior limb of the internal capsule (PLIC). At this age, the PLIC should be at least 1/3 myelinated.



NEUROIMAGING

The primary neuroimaging modality to detect brain injury in newborns is cUS. However, the previously mentioned subtle forms of brain injury may be missed with cUS (54). MRI can therefore be helpful to detect these subtle forms of brain injury. At present, it is not well known what type of brain injury can be expected in MLPT infants, both imaging techniques will be used in this thesis. Therefore, we will briefly describe both cUS and MRI, including their advantages and disadvantages.

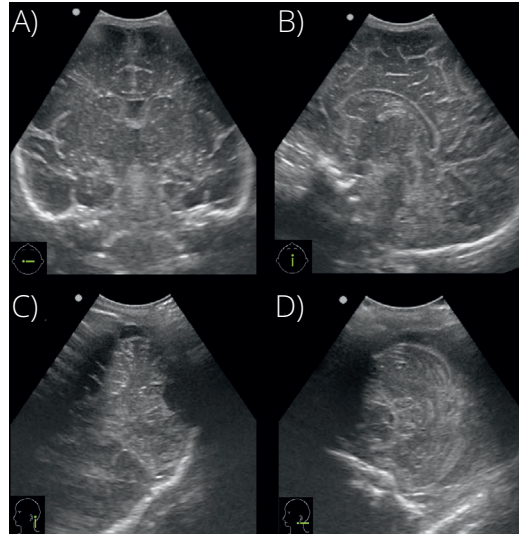
Cranial ultrasound (cUS)

CUS uses high-frequency sound waves to produce images of the brain. The cUS transducer sends the sound waves into the brain through one of the acoustic windows (e.g. the fontanelles). The sound waves are reflected back to the transducer by boundaries between and within tissues. The returned sound waves are transformed into images, which are displayed on the monitor of the ultrasound machine.

The anterior fontanelle is the most commonly used acoustic window and allows visualization of (almost) the whole brain. In a standard cUS examination, images of at least six coronal and five sagittal planes are recorded. The mastoid window is used to detect abnormalities in the posterior fossa and midbrain (Figure 5). Complementary, the posterior and temporal window can be used. The posterior fontanelle enables better visualization of the occipital and temporal parenchyma and the posterior fossa structures. The temporal window allows visualization of the brain stem and the circle of Willis (55).

Figure 5. Example of images through the anterior and mastoid fontanelle.

A and B) CUS image through the anterior fontanelle showing A) a coronal plane and B) the mid-sagittal plane. C and D) CUS image through the mastoid fontanelle showing C) a coronal plane and D) a transverse plane.



CUS has several advantages. It is a safe, reliable, relatively cheap and patient-friendly tool. Furthermore, it can be repeated as often as necessary and can be performed at the bedside or on the lap with little disturbance to the infant (55). It is therefore the first neuroimaging technique in clinical practice. However, there are also some limitations. The quality of cUS depends highly on the ultrasound system, the transducers used and the skills of the ultra-sonographer. Some parts of the cortex cannot be detected using cUS. Moreover, abnormalities in the posterior fossa are sometimes difficult to detect and subtle changes may not be recognized. In addition, cUS cannot be used to evaluate the myelination process.

Magnetic resonance imaging (MRI)

MRI produces three-dimensional detailed anatomical images using a strong magnetic field and radiofrequency pulses. The magnetic field forces protons in the body to align in the same direction. Short bursts of radiofrequency pulses are sent to the body, forcing the protons out of alignment.

When the radiofrequency pulses stop, the protons return to the alignment of the magnetic field. When the protons return, they send out a signal that is detected by the MR scanner. As a result, different types of structures realign in different speeds and produce different signals. This gives a detailed reconstruction of the structures inside the body.

The most common used MRI sequences are T1- and T2-weighted sequences. T1-weighted images are mainly used to assess the anatomy of the brain and the myelination process; while T2-weighted images enable even better detection of abnormalities. Both sequences can also be used to calculate brain volumes. In addition, diffusion weighted imaging (DWI) and susceptibility weighted imaging (SWI) are frequently used to detect respectively acute ischemic and hemorrhagic lesions (Figure 6).

Compared to cUS, MRI enables a more precise determination of the site and extent of brain abnormalities. Furthermore, MRI can be used to assess the entire cortex and to assess myelination of the white matter. In the past two decades, MRI has become increasingly important and more widely available for clinical imaging, including neonatal neuro-imaging.

However, MRI also has its disadvantages. MRI is very sensitive to movement of the patient. To obtain an MRI scan in a (preterm) infant can therefore be challenging. During the scan procedure, infants need to lie still for approximately 20 to 30 minutes. In the past 'conscious sedation' with chloral hydrate or phenobarbital was frequently used to prevent infants from moving during scanning. Nowadays, preference is given to the so-called 'feed and sleep' method (56-58). This method implies feeding and swaddling the infant prior to scanning. At the MRI department, the infant is placed in a vacuum-bag immobilizer (Figure 7). Using this method, good quality MRI can be completed without the need for sedation.

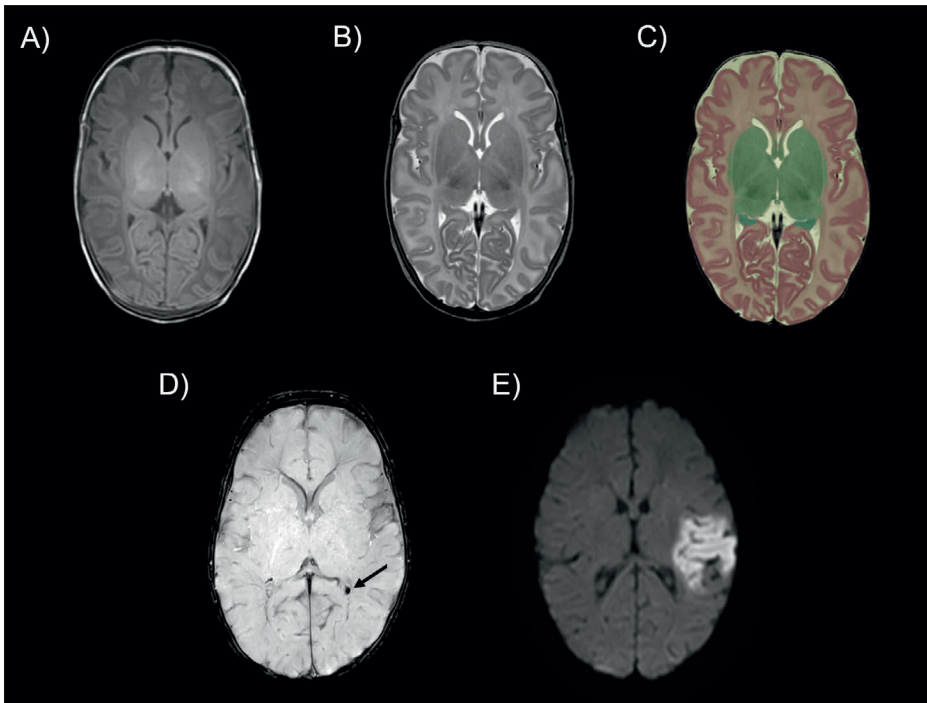
In addition, the infant needs to be monitored during the procedure with an MRI-compatible monitor, and specially trained personnel needs to be present during the scan procedure. Performing an MRI including extra preparations, and the presence of specialized trained personnel is therefore expensive and time-consuming. It is also more burdensome for the patient than cUS.

CEREBELLAR HEMORRHAGE (CBH) AND NEURODEVELOPMENTAL OUTCOME

As mentioned before, supratentorial brain injury (e.g. IVH and WMI) is considered the main cause for impaired neurologic outcome of children who were born prematurely. More recently, more attention is drawn to injury of the developing cerebellum, which can be an additional complication of prematurity and may also affect neurodevelopmental outcome (59-61). CBH is the most common form of neonatal cerebellar injury. The incidence ranges from 2.2% to 19.0%, depending on the study sample and imaging techniques used (59,62-64). CBH mostly originates from the external granular cell layer of the cerebellum. This is a transient, vulnerable, highly vascularized region, which may easily be injured (47,65,66). Three patterns of CBH have been described: punctate CBH (≤ 4 mm.) limited CBH (> 4 mm but smaller than 1/3 of the cerebellar hemisphere) and massive CBH ($\geq 1/3$ of the cerebellar hemisphere (55,67).

Figure 6. Commonly used MRI sequences.

A - C) Images of an infant without brain injury (born at GA 34+6 weeks, scanned at 42+3 weeks); A) T1-weighted image; B) T2-weighted image; C) Color map of calculated brain volumes. D) SWI of an infant (GA 35+3 weeks, scanned at 41+3 weeks) showing a small hemorrhagic lesion in the choroid plexus (arrow); E) DWI of an infant (born at GA 41+3 weeks, scanned at 55 weeks) showing diffusion restriction in the territory of the middle cerebral artery indicating an ischemic event.



The size of the CBH may be of importance with respect to neurodevelopmental outcome. Massive CBH can be easily detected with both cUS and MRI and is associated with cerebral palsy, language and cognitive delays and behavioral problems (59). Punctate CBH are frequently noted on MRI, but are usually not detected with cUS and are associated with a more favorable prognosis (63,68). Up to now, infants with limited CBH have been rarely described and the effect on neurodevelopmental outcome is unknown. The association between the size of CBH and neurodevelopmental outcome will be described in **Chapter 8**.

Figure 7. The infant is swaddled and placed in a vacuum-bag immobilizer at the MR scanner.



AIMS AND OUTLINE OF THIS THESIS

The general aim of this thesis is to provide more insights into two topics that are relatively less discussed: 1) MLPT infants and 2) CBH. We will focus on neuroimaging and neurodevelopmental follow-up. To investigate the first population, we initiated an observational prospective cohort study: 'Brain Imaging in Moderate-late Preterm infants - The BIMP study'. To gain more knowledge on the effect of cerebellar injury on neurodevelopmental outcome, we performed a retrospective multicenter study named 'Cerebellar Hemorrhage and Outcome in Preterm Infants - The CHOPIn study'.

1

PART I. CURRENT KNOWLEDGE AND PRACTICE REGARDING BRAIN INJURY IN MODERATE-LATE PRETERM INFANTS

Chapter 2

In this chapter, the literature on incidence of brain injury and altered brain development in MLPT infants is reviewed. Besides the incidence of IVH and WML, we evaluate current knowledge on other forms of brain injury, including CBH. In addition, we review the use of more advanced techniques such as volumetric analysis and diffusion tensor imaging (DTI) in the MLPT population.

Research question:

- What is known about brain injury and altered brain development in MLPT infants as compared to very preterm and term infants?

Chapter 3

Currently, specific neurological surveillance in MLPT infants, such as neuroimaging and follow-up, has generally not been included in (inter)nationally accepted guidelines. In this chapter, we provide an overview of current practice in neonatal centers the Netherlands and Canada.

Research question:

- What are the current local clinical practices concerning neurological surveillance in MLPT infants in neonatal centers in the Netherlands and Canada?

PART II. INCIDENCE OF BRAIN LESIONS IN MODERATE-LATE PRETERM INFANTS

Chapter 4

In this chapter, we describe the imaging results of the BIMP study. An overview of the incidence and characteristics of brain lesions, as encountered on serial cUS and MRI, in MLPT infants is given.

Research questions:

- What is the incidence of brain lesions in MLPT infants?
- What type of brain lesions are seen in MLPT infants?
- Can brain lesions in MLPT infants be detected with both cUS and MRI?

PART III. QUANTIFICATION OF STRUCTURAL BRAIN GROWTH IN MODERATE-LATE PRETERM INFANTS

In very preterm infants, alterations in structural brain growth have been associated with suboptimal neurodevelopmental outcome. Although MLPT infants have mainly been approached as one group, the risks for developmental problems are not the same. Risks for developmental problems are higher in MP than in LP infants. We therefore explore if the brains of MP infants may be more vulnerable to impaired growth than the brains of LP infants. In addition, we explore if mild brain injury was also associated with reduced brain volumes.

Chapter 5

To explore if simple linear cUS measurements can be used to examine impaired brain growth, we compared the size of several brain structures at TEA between MP, LP and term infants.

Research question:

- What are normal sizes of brain structures in MP and LP infants as measured with cUS at TEA?
- Are brain structures measured with cUS at TEA different between MP, LP and full-term infants?

Chapter 6

Based on the results described in Chapter 4, we explored whether the frequently encountered mild brain lesions affect brain volumes. We used T2-weighted images at TEA to calculate brain volumes in MLPT infants. The brain volumes were compared between MP and LP infants, and between MLPT infants with and without mild brain injury.

Research questions:

- Do brain volumes differ between MP and LP infants?
- Do brain volumes differ between infants with and without mild brain injury?

PART IV. NEURODEVELOPMENTAL AND BEHAVIORAL OUTCOME IN MODERATE-LATE PRETERM INFANTS AND IN PRETERM INFANTS WITH CEREBELLAR HEMORRHAGE

Chapter 7

Up to now, only two studies investigated possible associations between brain injury and neurodevelopmental outcome in MLPT infants. These studies did not include behavioral outcomes and did not make a distinction between MP and LP infants. In this chapter, we present a preliminary descriptive analysis of the neurodevelopmental and behavioral outcome of MP and LP infants enrolled in the BIMP-study.

Research questions:

- What is the incidence of neurodevelopmental and behavioral problems in MP and LP infants enrolled in the BIMP study?
- Which types of abnormal neonatal MRI findings are present within the group of infants with normal and suboptimal neurodevelopmental and behavioral outcome?

Chapter 8

CBH is frequently seen in extreme preterm, very preterm infants and - as we recently encountered in Chapter 4 - also in MLPT infants. Although the impact of CBH on neurodevelopmental outcome is increasingly recognized, the effect of the pattern of CBH (i.e. location and size) is still less well known. In this chapter, we investigate the neurodevelopmental outcome at 24 months corrected age in a large number of preterm infants (GA \leq 34 weeks) with different patterns of CBH.

Research questions:

- What is the effect of the size and location of CBH on the neurodevelopmental outcome around 24 months corrected age?
- Is there an association between the pattern of CBH and cerebellar atrophy?
- Is there an association between the pattern of CBH and perinatal factors?

In **Chapter 9**, the results of the previous chapters are discussed, future perspectives are outlined and a conclusion is given. **Chapter 10** summarizes the results of the studies in this thesis in English and Dutch.

REFERENCES

1. Blencowe H, Cousens S, Chou D, Oestergaard M, Say L, Moller A, et al. Born Too Soon: The global epidemiology of 15 million preterm births. *Reproductive Health* 2013;10(1):S2.
2. Chawanpaiboon S, Vogel J, Moller A, Lumbiganon P, Petzold M, Hogan D, et al. Global, regional, and national estimates of levels of preterm birth in 2014: a systematic review and modelling analysis. *Lancet Glob Health* 2019;7(1):e37-e46.
3. Perined. Perinatale zorg in Nederland anno 2019: landelijke perinatale cijfers en duiding. 2020.
4. Saigal S, Doyle L. An overview of mortality and sequelae of preterm birth from infancy to adulthood. *Lancet* 2008;371(9608):261-269.
5. National Institute for Health and Care Excellence. Preterm labour and birth [NICE guideline NG25]. 2015; Available at: <https://www.nice.org.uk/guidance/ng25>.
6. Lindström K, Winbladh B, Haglund B, Hjern A. Preterm infants as young adults: a Swedish national cohort study. *Pediatrics* 2007 Jul;120(1):70-77.
7. Bayley N. Bayley Scales of Infant and Toddler Development. 3rd ed. San Antonio, TX: Harcourt Assessment; 2006.
8. Griffiths R, Huntley M. The Griffiths mental development scales-revised manual: from birth to 2 years. High Wycombe: Association for Research in Infant and Child Development; 1996.
9. Squires J, Bricker D. Ages & Stages Questionnaires®, Third Edition (ASQ®-3): A Parent-Completed Child Monitoring System. Baltimore: Paul H. Brookes Publishing Co., Inc; 2009.
10. Robins DL, Fein D, Barton M. The Modified Checklist for Autism in Toddlers, Revised with Follow-Up (M-CHAT-R/F). 2009.
11. Achenbach TM, Rescorla L. Manual for the Child Behavior Checklist. Preschool Forms and Profiles. Burlington VT: University of Vermont Department of Psychiatry; 2000.
12. Allotey J, Zamora J, Cheong-See F, Kalidindi M, Arroyo-Manzano D, Asztalos E, et al. Cognitive, motor, behavioural and academic performances of children born preterm: a meta-analysis and systematic review involving 64 061 children. *BJOG* 2018 Jan;125(1):16-25.
13. Natarajan G, Shankaran S. Short- and Long-Term Outcomes of Moderate and Late Preterm Infants. *Am J Perinatol* 2016;33(3):305-317.
14. Romeo DM, Ricci M, Picilli M, Foti B, Cordaro G, Mercuri E. Early Neurological Assessment and Long-Term Neuromotor Outcomes in Late Preterm Infants: A Critical Review. *Medicina (Kaunas, Lithuania)* 2020;56(9):475.
15. Cheong J, Doyle L, Burnett A, Lee K, Walsh J, Potter C, et al. Association Between Moderate and Late Preterm Birth and Neurodevelopment and Social-Emotional Development at Age 2 Years. *JAMA Pediatr* 2017;171(4):e164805.
16. Voigt B, Pietz J, Pauen S, Kliegel M, Reuner G. Cognitive development in very vs. moderately to late preterm and full-term children: can effortful control account for group differences in toddlerhood? *Early Hum Dev* 2012 May;88(5):307-313.
17. Mirzakhani H, Kelly RS, Yadama AP, Chu SH, Lasky-Su JA, Litonjua AA, et al. Stability of developmental status and risk of impairment at 24 and 36 months in late preterm infants. *Infant Behav Dev* 2020 Aug;60:101462.

18. de Jong M, Verhoeven M, Lasham CA, Meijssen CB, van Baar AL. Behaviour and development in 24-month-old moderately preterm toddlers. *Arch Dis Child* 2015 Jun;100(6):548-553.
19. Guy A, Seaton SE, Boyle EM, Draper ES, Field DJ, Manktelow BN, et al. Infants born late/moderately preterm are at increased risk for a positive autism screen at 2 years of age. *J Pediatr* 2015 Feb;166(2):269-75.e3.
20. You J, Shamsi BH, Hao MC, Cao CH, Yang WY. A study on the neurodevelopment outcomes of late preterm infants. *BMC Neurol* 2019 May 30;19(1):108-0.
21. Kerstjens JM, de Winter AF, Bocca Tjeertes IF, ten Vergert EMJ, Reijneveld SA, Bos AF. Developmental delay in moderately preterm-born children at school entry. *J Pediatr* 2011;159(1):92-98.
22. Kirkegaard I, Obel C, Hedegaard M, Henriksen TB. Gestational age and birth weight in relation to school performance of 10-year-old children: a follow-up study of children born after 32 completed weeks. *Pediatrics* 2006 Oct;118(4):1600-1606.
23. van Baar AL, Vermaas J, Knots E, de Kleine, Martin JK, Soons P. Functioning at school age of moderately preterm children born at 32 to 36 weeks' gestational age. *Pediatrics* 2009;124(1):251-257.
24. Cserjesi R, Van Braeckel, KNJA, Butcher PR, Kerstjens JM, Reijneveld SA, Bouma A, et al. Functioning of 7-year-old children born at 32 to 35 weeks' gestational age. *Pediatrics* / 2012;130(4):838-46.
25. Chyi L, Lee H, Hintz S, Gould J, Sutcliffe T. School outcomes of late preterm infants: special needs and challenges for infants born at 32 to 36 weeks gestation. *J Pediatr* 2008;153(1):25-31.
26. Lipkind HS, Slopen ME, Pfeiffer MR, McVeigh KH. School-age outcomes of late preterm infants in New York City. *Obstet Gynecol* 2012 -3;206(3):1-6.
27. Bogicevic L, Verhoeven M, van Baar AL. Toddler skills predict moderate-to-late preterm born children's cognition and behaviour at 6 years of age. *PLoS One* 2019;14(11).
28. Faleschini S, Matte-Gagné C, Côté S, Tremblay R, Boivin M. Trajectories of behavioral problems among moderate-late preterm children from 4 to 10 years: A prospective population-based study. *Early Hum Dev* 2020;143.
29. Jin JH, Yoon SW, Song J, Kim SW, Chung HJ. Long-term cognitive, executive, and behavioral outcomes of moderate and late preterm at school age. *Clin Exp Pediatr* 2020 Jun;63(6):219-225.
30. Moster D, Lie RT, Markestad T. Long-Term Medical and Social Consequences of Preterm Birth. *N Engl J Med* 2008;359(3):262-273.
31. Heinonen K, Lahti J, Sammallahiti S, Wolke D, Lano A, Andersson S, et al. Neurocognitive outcome in young adults born late-preterm. *Dev Med Child Neurol* 2018 Mar;60(3):267-274.
32. Heinonen K, Eriksson JG, Kajantie E, Pesonen A, Barker DJ, Osmond C, et al. Late-Preterm Birth and Lifetime Socioeconomic Attainments: The Helsinki Birth Cohort Study. *Pediatrics* 2013;132(4):647-655.
33. Linsell L, Malouf R, Morris J, Kurinczuk JJ, Marlow N. Prognostic Factors for Poor Cognitive Development in Children Born Very Preterm or With Very Low Birth Weight: A Systematic Review. *JAMA Pediatr* 2015;169(12):1162-1172.
34. Linsell L, Malouf R, Morris J, Kurinczuk JJ, Marlow N. Prognostic factors for cerebral palsy and motor impairment in children born very preterm or very low birthweight: a systematic review. *Dev Med Child Neurol* 2016;58(6):554-569.
35. Linsell L, Malouf R, Johnson S, Morris J, Kurinczuk J, Marlow N. Prognostic Factors for Behavioral Problems and Psychiatric Disorders in Children Born Very Preterm or Very Low Birth Weight: A Systematic Review. *Journal of Developmental and Behavioral Pediatrics* 2016;37(1):88-102.

36. Kerstjens JM, Bocca-Tjeertes IF, de Winter AF, Reijneveld SA, Bos AF. Neonatal morbidities and developmental delay in moderately preterm-born children. *Pediatrics* 2012 Aug;130(2):265.
37. Potijk MR, Kerstjens JM, Bos AF, Reijneveld SA, de Winter AF. Developmental delay in moderately preterm-born children with low socioeconomic status: risks multiply. *J Pediatr* 2013 Nov;163(5):1289-1295.
38. Kerstjens JM, de Winter AF, Sollie KM, Bocca-Tjeertes IF, Potijk MR, Reijneveld SA, et al. Maternal and pregnancy-related factors associated with developmental delay in moderately preterm-born children. *Obstet Gynecol* 2013 Apr;121(4):727-733.
39. Johnson S, Evans TA, Draper ES, Field DJ, Manktelow BN, Marlow N, et al. Neurodevelopmental outcomes following late and moderate prematurity: a population-based cohort study. *Arch Dis Child Fetal Neonatal Ed* 2015;100(4):F301-F308.
40. Horsch S, Muentjes C, Franz A, Roll C. Ultrasound diagnosis of brain atrophy is related to neurodevelopmental outcome in preterm infants. *Acta Paediatr* 2005;94(12):1815-1821.
41. Anderson P, Cheong JLY, Thompson D. The predictive validity of neonatal MRI for neurodevelopmental outcome in very preterm children. *Semin Perinatol* 2015;39(2):147-158.
42. Brouwer MJ, Kersbergen KJ, van Kooij BJM, Benders MJNL, van Haastert IC, Koopman-Esseboom C, et al. Preterm brain injury on term-equivalent age MRI in relation to perinatal factors and neurodevelopmental outcome at two years. *PLoS ONE* 2017;12(5):e0177128.
43. Favrais G, Saliba E. Neurodevelopmental outcome of late-preterm infants: Literature review. *Arch Pediatr* 2019 Nov;26(8):492-496.
44. Hüppi PS, Warfield S, Kikinis R, Barnes PD, Zientara GP, Jolesz FA, et al. Quantitative magnetic resonance imaging of brain development in premature and mature newborns. *Ann Neurol* 1998 Feb;43(2):224-235.
45. Kinney H. The near-term (late preterm) human brain and risk for periventricular leukomalacia: a review. *Semin Perinatol* 2006;30(2):81-88.
46. Haynes RL, Sleeper LA, Volpe JJ, Kinney HC. Neuropathologic studies of the encephalopathy of prematurity in the late preterm infant. *Clin Perinatol* 2013 Dec;40(4):707-722.
47. Volpe JJ. Cerebellum of the premature infant: rapidly developing, vulnerable, clinically important. *J Child Neurol* 2009;24(9):1085-104.
48. Counsell S, Maalouf E, Fletcher A, Duggan P, Battin M, Lewis H, et al. MR imaging assessment of myelination in the very preterm brain. *AJNR Am J Neuroradiol* 2002;23(5):872-881.
49. De Vries LS, Van Haastert IC, Rademaker KJ, Koopman C, Groenendaal F. Ultrasound abnormalities preceding cerebral palsy in high-risk preterm infants. *Journal of Pediatrics*, The 2004;144(6):815-20.
50. Arulkumaran S, Tusur N, Chew A, Falconer S, Kennea N, Nongena P, et al. MRI Findings at Term-Corrected Age and Neurodevelopmental Outcomes in a Large Cohort of Very Preterm Infants. *AJNR. American journal of neuroradiology* 2020;41(8):1509-1516.
51. Parodi A, Govaert P, Horsch S, Bravo MC, Ramenghi LA, eurUS.brain group. Cranial ultrasound findings in preterm germinal matrix haemorrhage, sequelae and outcome. *Pediatr Res* 2020 Mar;87(Suppl 1):13-24.
52. Nguyen The Tich S, Anderson PJ, Hunt RW, Lee KJ, Doyle LW, Inder TE. Neurodevelopmental and Perinatal Correlates of Simple Brain Metrics in Very Preterm Infants. *Archives of Pediatrics & Adolescent Medicine* 2011 Mar 7;165(3):216-222.

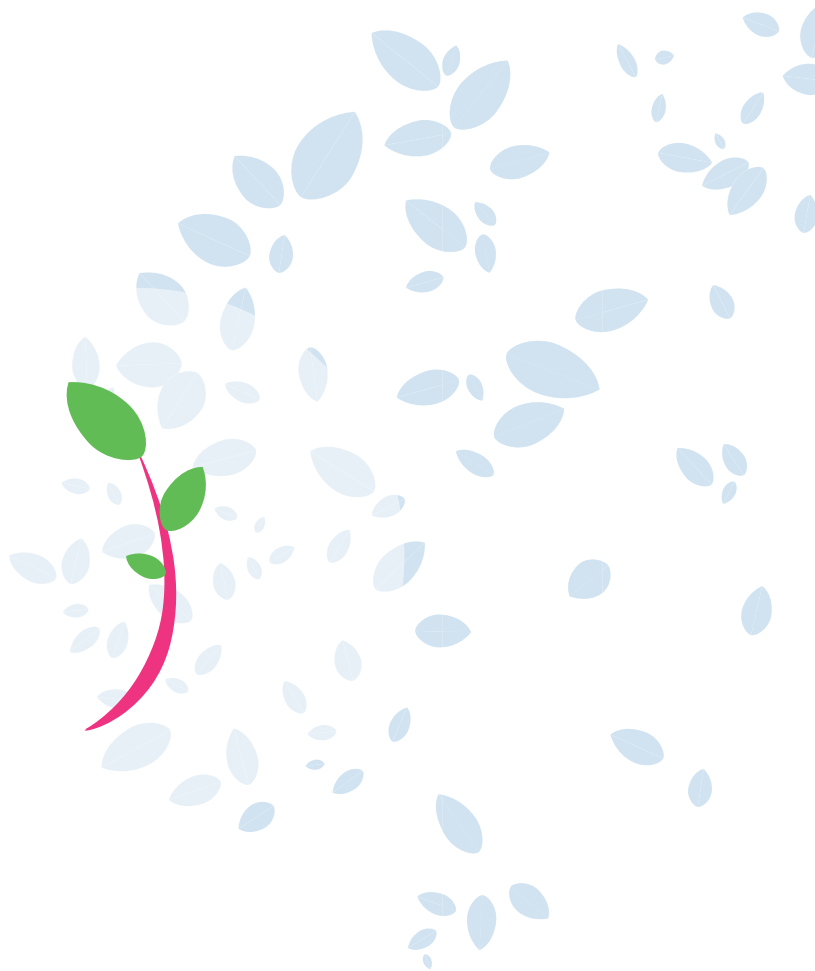
53. Kidokoro H, Neil J, Inder T. New MR imaging assessment tool to define brain abnormalities in very preterm infants at term. *Am J Neuroradiol* 2013;34(11):2208-14.
54. de Vries LS, Benders MJNL, Groenendaal F. Imaging the premature brain: ultrasound or MRI? *Neuroradiology* 2013;55 Suppl 2:13-22.
55. Meijler G, Steggerda SJ. *Neonatal Cranial Ultrasonography*, third edition. Cham: Springer; 2019.
56. Neubauer V, Griesmaier E, Baumgartner K, Mallouhi A, Keller M, Kiechl-Kohlendorfer U. Feasibility of cerebral MRI in non-sedated preterm-born infants at term-equivalent age: report of a single centre. *Acta Paediatr* 2011 Dec;100(12):1544-1547.
57. Windram J, Grosse-Wortmann L, Shariat M, Greer ML, Crawford MW, Yoo SJ. Cardiovascular MRI without sedation or general anesthesia using a feed-and-sleep technique in neonates and infants. *Pediatr Radiol* 2012 Feb;42(2):183-187.
58. Hughes EJ, Winchman T, Padormo F, Teixeira R, Wurie J, Sharma M, et al. A dedicated neonatal brain imaging system. *Magn Reson Med* 2017 Aug;78(2):794-804.
59. Limperopoulos C, Bassan H, Gauvreau K, Robertson RLJ, Sullivan NR, Benson CB, et al. Does cerebellar injury in premature infants contribute to the high prevalence of long-term cognitive, learning, and behavioral disability in survivors? *Pediatrics* 2007;120(3):584-593.
60. Messerschmidt A, Fuiko R, Prayer D, Brugger PC, Boltshauser E, Zoder G, et al. Disrupted cerebellar development in preterm infants is associated with impaired neurodevelopmental outcome. *Eur J Pediatr* 2008;167(10):1141-7.
61. Hortensius LM, Dijkshoorn ABC, Ecury Goossen GM, Steggerda SJ, Hoebeek FE, Benders MJNL, et al. Neurodevelopmental Consequences of Preterm Isolated Cerebellar Hemorrhage: A Systematic Review. *Pediatrics* 2018;142(5);e20180609.
62. Steggerda SJ, Leijser LM, Wiggers-de Bruïne FT, van der Grond J, Walther FJ, van Wezel-Meijler G. Cerebellar injury in preterm infants: incidence and findings on US and MR images. *Radiology* 2009;252(1):190-199.
63. Steggerda SJ, De Bruïne FT, van den Berg-Huysmans AA, Rijken M, Leijser LM, Walther FJ, et al. Small cerebellar hemorrhage in preterm infants: perinatal and postnatal factors and outcome. *Cerebellum* 2013 Dec;12(6):794-801.
64. Kidokoro H, Anderson PJ, Doyle LW, Woodward LJ, Neil JJ, Inder TE. Brain injury and altered brain growth in preterm infants: predictors and prognosis. *Pediatrics* 2014;134(2):444-53.
65. Fumagalli M, Bassi L, Sirgiovanni I, Mosca F, Sannia A, Ramenghi LA. From germinal matrix to cerebellar haemorrhage. *Journal of Maternal-Fetal and Neonatal Medicine* 2015;28:2280-5.
66. Pierson CR, Al Sufiani F. Preterm birth and cerebellar neuropathology. *Seminars in Fetal and Neonatal Medicine* 2016.
67. Parodi A, Rossi A, Severino M, Morana G, Sannia A, Calevo MG et al. Accuracy of ultrasound in assessing cerebellar haemorrhages in very low birthweight babies. *Fetal and Neonatal* 2015;100(4):289-92.
68. Tam EWY, Rosenbluth G, Rogers EE, Ferriero, DM, Glidden D, Goldstein RB et al. Cerebellar hemorrhage on magnetic resonance imaging in preterm newborns associated with abnormal neurologic outcome. *J Pediatr* 2011;158(2):245-50.





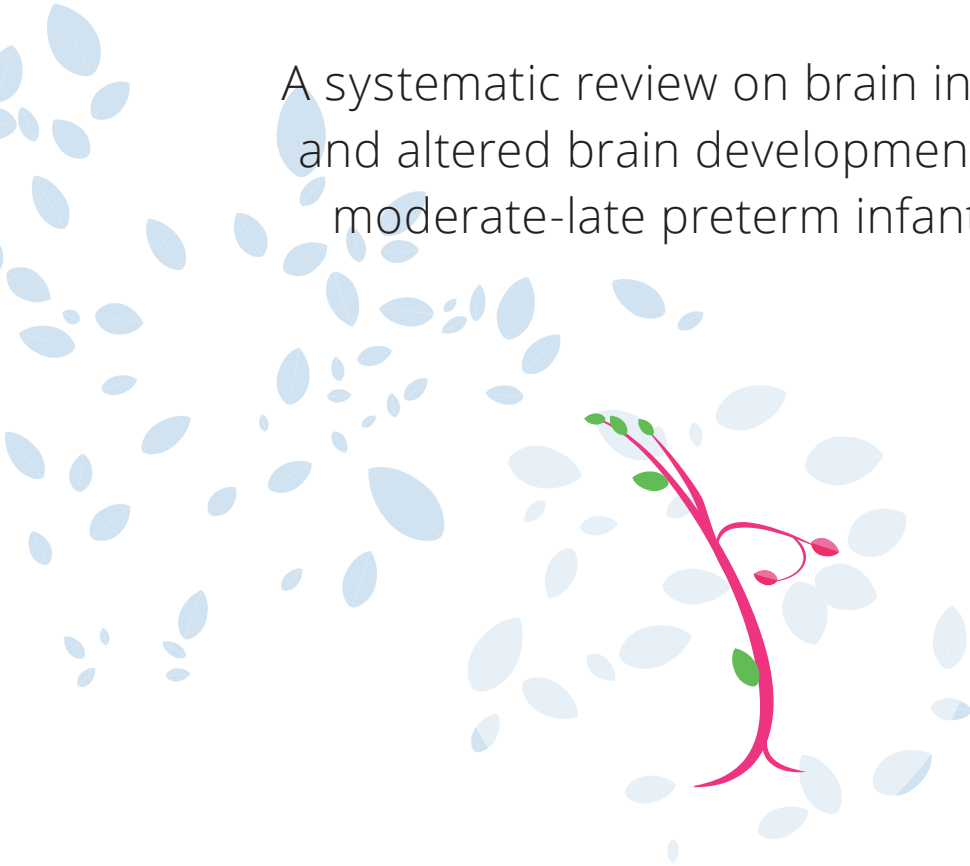
PART I

CURRENT KNOWLEDGE AND PRACTICE REGARDING BRAIN INJURY IN MODERATE-LATE PRETERM INFANTS



CHAPTER 2

A systematic review on brain injury and altered brain development in moderate-late preterm infants



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ABSTRACT

Objectives To provide a systematic review of brain injury and altered brain development in moderate-late preterm (MLPT) infants as compared to very preterm and full-term infants.

Study design A systematic search in five databases was performed in January 2020. Original research papers on incidence of brain injury and papers using quantitative data on brain development in MLPT infants were selected. The Johanna Briggs Institute 'Critical Appraisal Checklist for Studies Reporting Prevalence Data' was used for quality appraisal. Data extraction included: imaging modality, incidences of brain injury, brain volumes, 2D-measurements and diffusivity values.

Results In total, 24 studies were eligible. Most studies had a moderate quality. Twenty studies reported on the incidence of brain injury in MLPT infants. The incidence of intraventricular hemorrhage (IVH) ranged from 0.0% to 23.5% and of white matter injury (WMI) from 0.5% to 10.8%. One study reported the incidence of arterial infarction (0.3%) and one the incidence of cerebellar hemorrhage (4.5%). Eleven studies compared incidences of brain injury between MLPT infants and very preterm or full-term infants. Five studies reported signs of altered brain development in MLPT infants.

Conclusions The incidences of IVH and WMI in MLPT infants varied widely between studies. Other abnormalities were sparsely reported. Evidence regarding a higher or lower incidence of brain injury in MLPT infants compared to very preterm or term infants is weak due to moderate methodological quality of reported studies. There is limited evidence suggesting a difference in brain development between MLPT and full-term infants.

INTRODUCTION

Moderate-late preterm (MLPT) infants, born between 32+0–36+6 weeks gestational age (GA), have an increased risk for disabilities compared to full-term infants. They have a 2-fold higher prevalence of developmental delay, and more problems with fine motor skills, communication, and personal-social functioning (1–4). In adulthood, MLPT infants perform less well. Ekeus et al. (5) demonstrated that MLPT born men had a lower intellectual performance score at age 18–19 years compared to their term born peers. Furthermore, psychiatric disorders in adolescence and young adulthood were more often seen in MLPT infants compared to full-term infants (8.8–9.5% vs 7.4%) (6). More than 80% of the total preterm population (GA <37 weeks) is MLPT (7). Given the size of the MLPT population, even a small increased risk of disabilities is a considerable health concern. It can be assumed that these disabilities are at least partially related to 1) brain injury, acquired during the perinatal and/or neonatal period and/or 2) abnormal brain development.

Almost all knowledge about the preterm brain is, however, obtained from extensive research performed in the very preterm population (GA < 32 weeks). The most common forms of brain injury in this population are intraventricular hemorrhage (IVH) (8–10), white matter injury (WMI) (9,11,12) and cerebellar hemorrhage (CBH). (13–16) WMI can be subdivided in cystic (also known as cystic periventricular leukomalacia; cPVL) and non-cystic. Non-cystic WMI is histologically characterized by microscopic areas of necrosis and is more easily identifiable with magnetic resonance imaging (MRI) than with cranial ultrasonography (cUS). On cUS, non-cystic WMI can be seen as non-physiological periventricular echogenicity, but the sensitivity of cUS for non-cystic PVL is low. (9,17) These forms of preterm brain injury are associated with motor and cognitive deficits, sensorineural hearing loss, cerebral visual impairment and behavioral problems. (18)

In addition to brain injury, alterations in structural and functional brain development are associated with impaired neurodevelopment in the very preterm population (19–23). This can be quantified by 2-D measurements, or by more advanced techniques such as MR volumetric analysis or diffusion tensor imaging (DTI). Diffusion can be quantified using a mathematical tensor model, which provides diffusivity values (i.e. fractional anisotropy and mean-, axial- and radial diffusivity) (24). To enable early detection of brain injury and/or abnormal brain development, very preterm infants routinely undergo neuro-imaging: serial cUS during the neonatal period and in specific cases also MRI. In addition, they undergo standardized follow-up programs after discharge, enabling early diagnosis of neurological impairment and thus early interventions.

Aim

To provide a systematic review of brain injury and altered brain development in MLPT infants as compared to very preterm and full-term infants.

METHODS

This systematic review was conducted according to the guidelines of 'Preferred Reporting Items for Systematic Reviews and Meta-Analyses' (PRISMA). (25)

Type of studies

Prospective/retrospective cohort studies, case-control studies and randomized controlled trials were eligible for inclusion. Studies reporting on brain injury or brain development in MLPT infants were included in the analysis. If the imaging technique, the system used to classify brain injury or outcome parameters could not be retrieved from potentially relevant papers, the first author was contacted for further information. If authors did not respond or insufficient information was obtained, the paper was subsequently excluded from the analysis. Studies of populations restricted to specific diseases, conditions or metabolic disorders were also excluded, as well as reviews, poster presentations and conference papers.

Participants

MLPT infants GA 32+0–36+6 weeks.

Intervention/exposure

Imaging: cUS and/or MRI.

Comparator(s)/control

If available, very preterm infants, born before GA 32 weeks or full-term infants, born after GA 37 weeks.

Outcome measures

Primary outcome

1. Incidence of IVH, WMI, CBH and other forms of brain injury as detected by cUS or MRI.

Secondary outcome

2. Quantitative measurement of brain development in MLPT infants using
 - a. Brain volumes
 - b. 2D-measurements
 - c. White matter microstructure (diffusivity values)

Data collection and analysis

The literature search included electronic databases (from inception of each of the databases) Cochrane, Medline/PubMed, Embase, Scopus and Web of Science. No search filter for study type or publication date was used. The following search criteria were used: late preterm, moderate preterm, moderately preterm, near term, gestational age/gestation: 32, 33, 34, 35, 36 weeks, cranial ultrasound, cUS, cranial sonography, head ultrasound, HUS, head sonography, (nuclear) magnetic resonance imaging, MRI, cranial imaging, intraventricular

hemorrhage, IVH, periventricular leukomalacia, PVL, cerebellar hemorrhage, CBH, neonatal/perinatal morbidity (Appendix 1). To identify additional potentially relevant studies, reference lists from relevant reviews and papers were manually searched.

Study selection

Two authors (VB and JN-O) independently screened titles and abstracts. Only papers written in English or Dutch were selected. Full text papers were screened on criteria used for the classification of IVH, WMI and/or for other forms of brain injury (e.g. Papile et al. [10], Volpe et al. [8], Kidokoro et al. [26], de Vries et al. [17] or others).

Data extraction

The following data were independently extracted by two authors (VB and JN-O) from each study: cohort year, design, data source, imaging technique, GA and results. Any disagreement about data was resolved by consensus.

Quality appraisal

For the methodological quality appraisal, the Johanna Briggs Institute (JBI) "Critical Appraisal Checklist for Studies Reporting Prevalence Data" was used (Appendix 2) (27). Methodological quality score was assigned as follows: 0–6: low quality, 7–12: moderate quality, 13–18: high quality.

Definitions

Image oriented studies were defined as studies in which cUS and/or MRI images were (re) evaluated to determine brain injury. In studies based on medical records, the incidence of brain injury was determined by screening radiological reports in medical records.

RESULTS

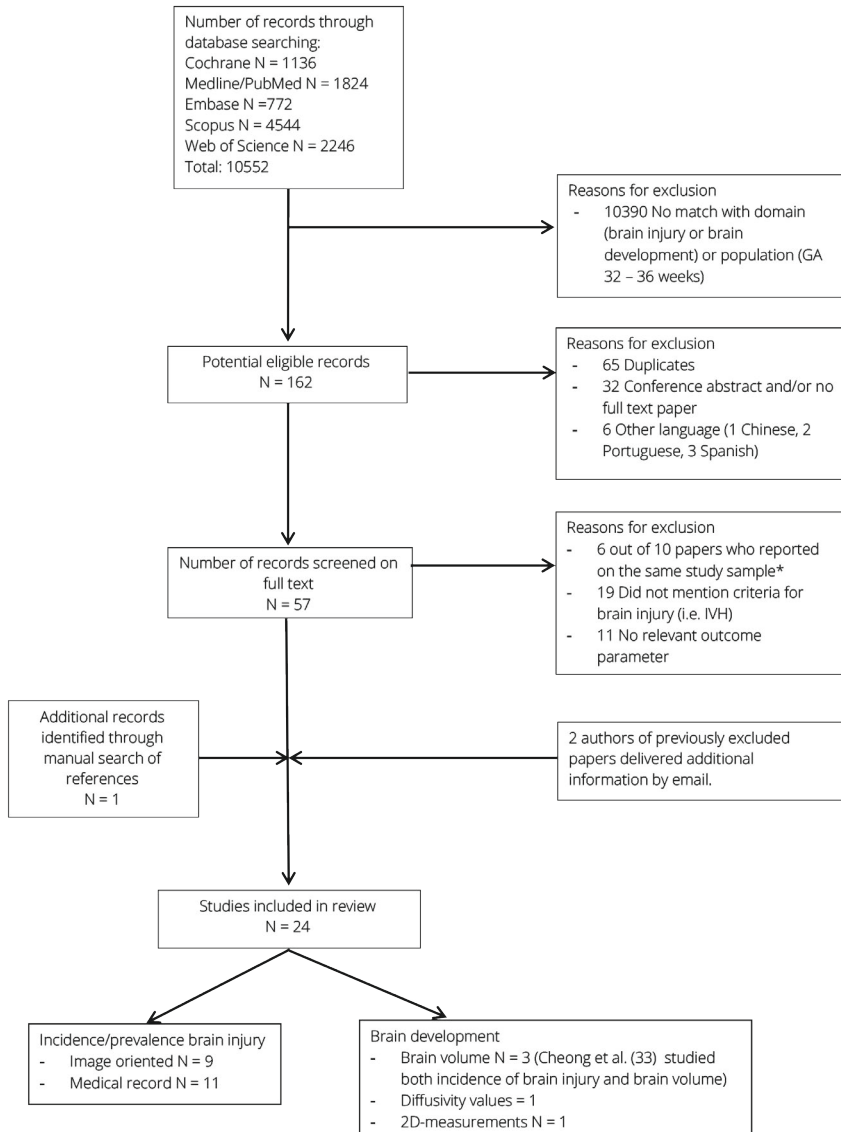
Search process and screening results

The electronic database search provided 10,552 records, of which 162 were potentially relevant after reviewing titles and abstracts (Figure 1). After removal of duplicates, full text papers in other languages, conference abstracts and abstracts without a full text paper, 57 studies potentially met our inclusion criteria and their full text was screened. After removal of studies on the same study samples and studies of which information on lacking data could not be retrieved, we included 24 studies in this review (Figure 1).

Figure 1. Flow diagram of search process and screening results.

GA = gestational age; IVH = intraventricular hemorrhage; WMI = white matter injury.

Search date 27-01-2020



* Ten papers published about the same study sample (i.e. Bajaj et al. [28], Natarajan et al. [29] and Trembath et al. [30] described the same study sample as Walsh et al. (2017) [31]). Thompson et al. (2019a [21] and 2019b [32]) described the same study sample as Cheong et al. (33) and Kelly et al. (34). Marret et al. (35) used the same study sample as Ancel et al. (36). Four of these ten studies were included (i.e. Walsh et al., Cheong et al. Kelly et al. and Ancel et al.), the other five publications had no additional content concerning brain injury or brain development and were thus excluded.

Methodological quality

The methodological quality score of the 24 included studies ranged from 7 to 16 (Table 1). Seven studies had a high and seventeen a moderate quality score. Many studies scored poor on coverage bias, statistics and response bias. Four medical record-based studies reported that not all infants had a cUS assessment (31,36–38). The authors considered infants without cUS assessment as infants without severe cerebral lesions (coverage bias). Twelve other studies reported incomplete statistical methods (31,36,39–48). These studies did not mention which tests were used, which variables and/or groups were tested, or which program for statistical analysis was used (statistical bias). Furthermore, many studies did not mention the number of potentially eligible subjects and/or did not describe efforts to address this potential source of response bias (31,36,38,39,43,48–52).

Study characteristics

Of the 24 included studies, 19 used cUS as imaging modality (31,36–50,53–55) and 11 had a prospective design (31,33,34,36,41,43,49–51,53,56). Five studies included infants with a GA 34+0 to 36+6 weeks (37,40,43,44,50) and five with a GA 32+0 to 36+6 weeks (33,34,38,55,56). The remaining studies used different GA ranges (31,36,39,41,42,45–49,51–54). Twenty studies reported on the incidence of brain injury (31,33,36–50,53–55) and four on quantitative measurements of brain development (34,51,52,56). Due to the high heterogeneity of the studies, a meta-analysis could not be performed, and only descriptive data are presented.

Incidence of brain injury

Of the twenty studies reporting on brain injury in MLPT infants, nine were image oriented (33,39,41–43,45,50,51,55). One study used MRI as neuro-imaging technique (33) and eight studies used cUS (39,41–43,45,50,53,55). In the other eleven studies, radiological reports in medical records were screened on the presence of brain injury (31,36–38,40,41,46–49,54). The study samples ranged from 63 to 21,771.

Table 1. Assessment of the methodological quality of included studies.

First author	Year of publication	Target Population	Participant recruitment	Sample size	Setting	Coverage bias	Classification bias	Measurement bias	Statistics	Re-sponse bias	Overall score (max 18 points)
Altman	2011	+	+	+	?	-	+	-	+	-	11
Ancel	2015	+	+	+	?	?	+	-	-	-	10
Ballardini	2014	+	+	+	?	-	+	+	?	?	13
Beltempo	2019	+	+	+	+	-	+	-	+	+	10
Bhat	2012	+	+	+	-	-	+	?	-	-	9
Bonnevier ^a	2018	+	-	+	+	-	+	-	+	?	11
Chen	2013	+	+	+	-	?	+	?	-	?	11
Cheong ^{a,b}	2016	+	+	-	+	-	+	+	+	?	13
Egwu	2019	+	+	-	+	-	+	+	+	+	14
Fumagalli	2015	+	+	+	+	+	+	+	+	-	16
Garcez	2016	+	+	+	-	-	+	-	-	?	9
Gleißner	2000	+	-	+	?	-	+	-	+	-	9
Harris	2006	+	+	+	-	-	?	+	-	?	10
Kadri	2007	+	+	-	-	+	+	-	-	-	8
Kelly ^b	2016	+	+	-	+	?	?	+	+	?	13
McIntire	2008	+	?	+	+	-	?	-	-	?	9
Munakata	2013	+	-	-	?	?	+	+	+	-	10
Niwa	2017	+	-	-	?	-	+	-	+	-	7
Resch ^a	2015	+	+	+	?	+	+	?	-	?	13
Khoshnood	2015	+	-	-	?	?	+	-	-	?	7
Shariati											
Stephen	2019	+	+	-	+	+	+	-	-	+	12
Sugiura	2012	+	-	+	-	+	+	?	-	-	9
Walsh ^b	2014	+	+	-	+	?	+	+	+	?	14
Walsh	2017	+	+	+	+	?	?	-	-	-	10

A plus sign (2 points) was assigned if the quality was good, a question mark if the quality was uncertain (1 point) and the minus sign was assigned if the quality was poor (0 points).

^a Additional information was obtained by email correspondence; ^b Cheong, Kelly and Walsh (2014) studied the same study sample.

Incidence of IVH

Incidence of IVH (all grades) ranged from 0.0% to 23.5% (Table 2). Cheong et al. (33) found the lowest incidence. None of the 199 included infants (GA 32+0–36+6 weeks) from their neonatal unit had IVH (classified according to Kidokoro et al. [26]). Kadri et al. (43) reported the highest incidence of IVH. They included 282 preterm infants (GA 30+0–36 +6 weeks) admitted to the neonatal intensive care unit (NICU), of which 68 were late preterm (GA 34+0–36+6 weeks). All underwent a daily cUS in the first postnatal week and one cUS in the second and the third postnatal week. They found IVH in 16/68 late preterm infants and in 62/130 of infants with a GA 30+0–33+6 weeks. Fumagalli et al. (50) conducted the largest image-oriented study. They included 1172 late preterm infants from the postnatal ward or NICU. All had at least two cUS examinations: the first within the first postnatal week and the second at five weeks of age. They found IVH grade I–II in four infants (0.3%). The largest medical record-based study, by Bonnevier et al. (37), selected 14,030 late preterm singleton infants (GA 34+0 –36+6 weeks) from the Perinatal Revision South Quality Register. They found IVH grade I–II in 6 and grade III–IV in 7 late preterm infants (overall IVH incidence [all grades]: 0.1%). We received additional information about this population: cUS was not routinely performed. It is therefore unknown how many infants had a cUS examination and if and how often this was repeated. Two studies compared the incidence of IVH between MLPT and full-term infants. Both studies (i.e. Bonnevier et al. [37] and McIntire et al. [44]) demonstrated a lower incidence of IVH in term infants (see Table 2). Nine studies compared the incidence of IVH with a group of very preterm infants, showing lower incidences of IVH in the MLPT group (Table 2).

Incidence of WMI

Ten studies reported the incidence of WMI, range 0.0% to 10.8% (Table 2). In a retrospective cohort study by Ballardini et al. (53) all infants with GA 33+0 and 36+6 weeks had at least one cUS examination. They reported an incidence of 10.6% (77/724) for non-cystic WMI (PVL grade I; classification according to De Vries et al. [17]). Chen et al. (41) included 672 infants (GA 33+0–34+0 weeks) in a prospective cohort study to explore brain injury. They found non-cystic WMI in 38 and cystic WMI in two infants using cUS. Resch et al. (45) published on cystic WMI in a large consecutive cohort study performed in infants ≤ 35 weeks. Through email correspondence, we learned that their cohort consisted of 3415 infants born between 32+0 and 34+6 weeks. Within this group, they found cystic WMI in 73 infants (2.0%). Two studies compared the incidence of WMI between MLPT and very preterm infants. Chen et al. (41) reported a similar and Sugiura et al. (48) a lower incidence of WMI in the MLPT group.

Other abnormalities

Three studies reported other brain abnormalities besides IVH and WMI (Table 3). Subependymal cysts were seen in 8/724 infants (Ballardini et al. [53]) and arterial infarction in 4/1172 infants. (Fumagalli et al. [50]) and CBH in 9/199 infants (Walsh et al. [56]).

Table 2. Overview of studies reporting the incidence of IVH and WMI in MLPT infants.

First author	Study design, cohort year and setting	Incidence based on	Imaging modality and timing of imaging	GA in weeks	Number of infants	Number of infants with imaging performed	Incidence of IVH	Incidence of WMI	Control group
Altman	Prospective database 2004 – 2008 Neonatal units, Sweden	Medical records	cUS, timing unknown	32 ⁺⁰ - 34 ⁺⁶	5229	Unknown	0.8%	0.5% (cystic WMI)	No.
Ancel	Prospective database 2011 NICU, France	Medical records	cUS during hospitalization, exact timing unknown	32 ⁺⁰ - 34 ⁺⁶	1175 (IVH) 1177 (WMI)	cUS performed in 88% of infants born at GA 33 weeks and in 66% born at 34 weeks	0.6%	0.8% (cystic WMI)	No.
Ballardini	Retrospective cohort 2007 – 2012 Setting unknown, Italy	Image oriented	cUS between postnatal day 3 – 7, repeated depending on GA and previous cUS findings	33 ⁺⁰ - 36 ⁺⁶	724	All	0.7%	non-cystic WMI: 10.6% cystic WMI: 0.1%	No.
Beltempo ^b	Retrospective cohort 2011 – 2016 Tertiary level NICU, Canada	Medical records	cUS during hospitalization, exact timing unknown	32 ⁺⁰ - 32 ⁺⁶	2576	All (infants without cUS were excluded)	1.3% (IVH grade III or IV)	1.5% (PVL defined as persistent periventricular echogenicity, duration unknown)	GA 30 ⁺⁰ - 31 ⁺⁶ weeks N = 5999 infants Severe neurologic injury (IVH gr III or IV and/or persistent periventricular echogenicity) Incidence: 3.2%

Table 2. Continued.

First author	Study design, cohort year and setting	Incidence based on	Imaging modality and timing of imaging	GA in weeks	Number of infants	Number of infants with imaging performed	Incidence of IVH	Incidence of WMI	Control group
Bhat	Retrospective cohort 1997 – 2007 Setting unknown, USA	Image oriented	cUS; for infants GA \leq 32 weeks between postnatal day 5 – 7; for infants GA 33–34 weeks based on clinical suspicion	32 ⁺⁰ – 34 ⁺⁶	304	All (infants without cUS were excluded)	2.5%	- (no classification criteria for WMI)	No.
Bonnevier ^a	Retrospective database 1995 – 2013 Obstetric and neonatal units, Sweden	Medical records	cUS, timing unknown	34 ⁺⁰ – 36 ⁺⁶	14030	Unknown	0.1%	-	GA 37 ⁺⁰ – 41 ⁺⁶ weeks N = 294814 infants incidence IVH = 0.01%
Chen	Prospective cohort 2005 – 2006 Third class/level A hospitals, China	Image oriented	cUS between postnatal day 3–7; most infants had one or more follow-up cUS	33 ⁺⁰ – 34 ⁺⁰	684 (IVH) 762 (WMI)	All	17.7%	non-cystic WMI: 4.7% cystic WMI: 0.3%	GA \leq 32 weeks Incidence IVH = 184/867 (21.2%) Incidence WMI = 51/1030 (4.9%)
Cheong ^a	Prospective cohort 2009 – 2012 Neonatal unit, Australia	Image oriented	MRI at TEA	32 ⁺⁰ – 36 ⁺⁶	199	All	0.0%	Non-cystic WMI: 5.0% cystic WMI: 0.0%	No.
Egwu	Prospective cross-sectional 2014 Special care baby unit, Nigeria	Image oriented	repeated cUS at: \leq 24 h, 24 – 72 h, 4 – 7 days, and 8 – 14 days after birth	32 ⁺⁰ – 36 ⁺⁶	63	All	6.3%	-	GA 28 ⁺⁰ – 31 ⁺⁶ weeks N = 36 infants incidence IVH: 33.3%

Table 2. Continued.

First author	Study design, cohort year and setting	Incidence based on	Imaging modality and timing of imaging	GA in weeks	Number of infants	Number of infants with imaging performed	Incidence of IVH	Incidence of WMI	Control group
Fumagalli	Prospective cohort 2010 – 2013 Tertiary center NICU, Italy	Image oriented	CUS; Two cUS examinations: postnatal 1 -7 and 28 – 35 days	34 ⁺⁰ - 36 ⁺⁶	1172	All	0.3%	2.0% (non-cystic and cystic WMI)	No.
Garcez	Retrospective database 2011 – 2013 Level III hospital including NICU, Portugal	Medical records	CUS, timing unknown	34 ⁺⁰ - 36 ⁺⁶	513	Unknown	3.3%	-	No.
Gleißner	Retrospective database 1994 – 1997 Setting unknown, Germany	Medical records	CUS; timing unknown	32 ⁺⁰ - 36 ⁺⁶	3011	In all infants <1500 g; in infants ≥ 1500 g if clinical symptoms suspicious for IVH	0.9%	-	GA < 32 weeks N = 636 infants Incidence IVH: 17.3%
Harris	Retrospective cohort 1999 – 2004 NICU, USA	Image oriented	CUS at postnatal day 7 – 10, or earlier if indicated	32 ⁺⁰ - 33 ⁺⁶	288	All	2.7%	- (no classification criteria for WMI)	GA < 32 weeks N = 198 infants Incidence IVH: 6.6%

Table 2. Continued.

First author	Study design, cohort year and setting	Incidence based on	Imaging modality and timing of imaging	GA in weeks	Number of infants	Number of infants with imaging performed	Incidence of IVH	Incidence of WMI	Control group
Kadri	Prospective cohort 2002 NICU, Syria	Image oriented	Repeated cUS; daily in the first postnatal week, one in the second and one in the third postnatal week	34 ⁺⁰ - 36 ⁺⁶	68	All	23.5%	-	GA < 30 weeks N = 84 Incidence IVH: 57.1% GA 30 ⁺⁰ - 33 ⁺⁶ weeks N = 130 Incidence IVH: 47.7%
McIntire	Retrospective database 1988 - 2005, all units including third level NICU	Medical records	cUS, timing unknown	34 ⁺⁰ - 36 ⁺⁶	21771	Unknown	0.2%	-	GA 39 weeks N = 84747 infants Incidence IVH 0.02%
Resch ^a	Retrospective cohort 1988 - 2012, Tertiary care center, Austria	Image oriented	Repeated cUS: postnatal day 1, 3 and 5; thereafter weekly in infants with pathological findings	32 ⁺⁰ - 34 ⁺⁶	3415	All	-	2.0% (cystic WMI)	Incidence of cystic WMI of infants < 32 weeks unknown
Khoshmood Shariati	Retrospective database 2011 - 2013 Setting unknown, Iran	Medical records	cUS during hospitalization, exact timing unknown	32 ⁺⁰ - 33 ⁺⁶	228	Unknown	0.9%	-	GA < 32 weeks N = 247 infants Incidence IVH: 1.2%

Table 2. Continued.

First author	Study design, cohort year and setting	Incidence based on	Imaging modality and timing of imaging	GA in weeks	Number of infants	Number of infants with imaging performed	Incidence of IVH	Incidence of WMI	Control group
Stephen	Retrospective database 2014 – 2017 Tertiary NICU, India	Medical records	cUS, timing unknown	32 ^a – 33 ^{a6}	80	Unknown	1.3%	-	GA < 32 weeks N = 76 infants Incidence IVH: 4.5%
Sugiura	Retrospective survey 2007 NICU, Japan	Medical records	CUS and MRI: CUS every 2 weeks; MRI before discharge	32 ^a – 32 ^{a6}	605 (CUS) 589 (MRI)	All	-	CUS: 1.7% MRI: 2.2% (cystic WMI)	GA < 32 weeks cUS (N = 2278) Incidence WMI: 2.9% MRI (N = 2235) Incidence WMI: 3.5%
Walsh (2017)	Prospective database 2012 – 2013 NICU, USA	Medical records	At least one cUS within the first 28 days	32 ^a – 33 ^{a6}	3921	In >75% of infants born at a GA < 32 weeks, in 48.9% of infants born at GA 32 weeks and in 28.2% born at 34 weeks	4.6%	-	GA < 32 weeks N = 2640 infants Incidence IVH: 13.5%

^a Additional information was obtained by email correspondence. ^b The exact incidence of IVH grade III/IV and WMI for infants with a GA 30+0–31+6 weeks could not be extracted from the study; DTI = diffusion weighted imaging; GA = gestational age; IVH = intraventricular hemorrhage; TEA = term equivalent age; WMI = white matter injury.

Table 3. Overview of other reported brain abnormalities in MLPT infants.

First author	Total infants	Subependymal cysts	LSV	Echogenicity of the thalami	Sinovenous thrombosis	Arterial stroke	Venous infarction	CBH	Miscellaneous
Ballardini	724	8	4	2	1	-	-	-	1 case: enlarged cisterna magna
Fumagalli	1172	-	-	-	-	4	2	-	4 cases: Blake's pouch, agenesis of the corpus callosum, arachnoid cyst, abnormalities due to CMV infection
Walsh (2014)*	199	-	-	-	-	-	-	9	2 cases: signal intensity abnormalities in the deep nuclear gray matter

CBH = cerebellar hemorrhage; CMV = cytomegalovirus; LSV = Lenticulostriate vasculopathy. * Walsh et al. (56) used the same study sample as Cheong et al. (33)

Brain development

Five studies investigated brain development in MLPT infants. Three studies reported on brain volume, one on linear measurements of brain structures and one on white matter microstructure.

Brain volumes

Munakata et al. (51) measured brain volumes at term equivalent age (TEA) MRI in 16 late preterm (GA 34+0–35+6 weeks) and 13 full-term infants. They found no differences in white matter and ventricular volumes between the two groups. However, gray matter volume and the percentage of gray matter volume were significantly smaller in the late preterm infants (respectively 162.0 ml vs. 190.4 ml; $p = 0.01$ and 53.1% vs. 57.4%; $p < 0.01$). The authors suggested that this altered gray matter development may partly contribute to the neurodevelopmental disabilities described in late preterm infants. Niwa et al. (52) performed TEA MRI in 27 MLPT infants and 13 very preterm infants (GA < 32 weeks). Mean total deep gray matter volume (i.e. basal ganglia and thalami) ranged from 14.2 ml in infants born at 30 weeks' gestation to 16.5 ml in infants of 35 weeks' gestation and tended to correlate with GA in weeks, but differences were small and not significant. No significant differences were found in cerebral hemispheric volumes. Cheong et al. (33) found a mean cortical gray matter volume of 177.4 cc and mean subcortical gray matter volume of 31.7 cc in a sample of 168 MLPT infants at TEA. They shared additional information about various mean brain volumes per GA week. Mean cortical gray matter volume was 169.8 cc in infants born at 33 weeks ($n = 35$) and 181.5 cc in infants born at 36 weeks ($n = 23$) of gestation.

Linear measurements

Walsh et al. (56) found smaller sizes in MLPT infants compared to full-term infants at TEA MRI for biparietal diameter, basal ganglia and thalami, midbody, genu and splenium of the corpus callosum, and larger sizes for interhemispheric- and extra-axial distance and left ventricular atrial width. Furthermore, these MLPT infants had features consistent with a more immature brain compared to their term-born peers, such as delayed gyral maturation 36% vs 2% ($p < 0.01$) and incomplete myelination of the posterior limb of the internal capsule 28% vs 6% ($p = 0.02$).

Diffusivity values

Kelly et al. (34) used DTI to study white matter microstructure in 193 MLPT and 83 full-term infants. They assessed the diffusivity values, which are an important reflection of brain white matter maturation as they are influenced by e.g. changes in water content and fiber density, and (pre)myelination. Compared to full-term infants, MLPT infants had different diffusivity values (lower fractional anisotropy and higher mean, axial and radial diffusivity) in nearly 70% of the white matter fiber tracts. This may indicate a loss of directional restriction of water diffusion and an overall increase in diffusion in all directions within the white matter, and may reflect delayed or disrupted white matter development.

DISCUSSION

This systematic review provides an overview of the incidence of brain injury and altered brain development in MLPT infants. The majority of the included 24 studies had a moderate quality score. Twenty studies reported on the incidence of IVH and/or WMI.

The incidences for IVH and WMI in MLPT infants varied widely between individual studies (IVH: 0.0 to 23.5%; WMI: 0.5 to 10.8%). The studies by Chen et al. (41) and Kadri et al. (43) reported high incidences of IVH in moderate preterm (Chen et al.) and late preterm infants (Kadri et al.) as well as in their very preterm cohorts. These high incidences may be explained by the fact that these studies were performed in different parts of the world, different cohort years (early 2000) and/or different settings (level 2 or level 3 units) which can all affect perinatal care. Furthermore, a high frequency of cUS examinations, and thus a higher chance to detect also the smallest hemorrhages, might be an explanation for the high incidence of IVH found in these two studies. However, their very high incidences in moderate and late preterm infants remain largely unexplained, as it is even much higher than those reported in very preterm populations. (9,57)

Very low incidences for IVH were found in the MRI-study by Cheong et al. (33) and in the retrospective cohort study by Bonnevier et al. (37). None of the included infants in the study by Cheong et al. had an IVH, while in all other studies IVH was reported in MLPT infants. This may be related to the fact that Cheong et al. performed a single MRI at TEA, when small hemorrhages may no longer be detectable, especially because susceptibility weighted images (SWI) were apparently not included in the scan protocol. IVH generally develop within the first three postnatal days and small IVH may have resolved after a few weeks (9,58).

The large retrospective cohort study by Bonnevier et al. (37) reported an incidence of IVH of 0.1% in 14,030 late preterm infants, but not all infants underwent cUS. Therefore, the true incidence of IVH in this study sample may be underestimated. In the studies by Cheong et al. (33) and Bonnevier et al. (37), the primary aim was not to investigate the incidence of IVH. Therefore, their study designs may not have been optimal to determine the incidence of IVH.

The study by Ballardini et al. (53) reported a relatively high incidence of non-cystic WMI (PVL grade I in 10.6%). However, they did not define periventricular echogenicity and may have reported both inhomogeneous and homogeneous echogenicity as PVL grade I. It is well known that homogeneous periventricular echogenicities which do not exceed the echogenicity of the choroid plexus, are physiological. (59) They may therefore have overestimated the incidence of WMI, even though they only diagnosed PVL grade I in those infants with echogenicities still present on the second cUS at 7–10 days after birth.

Only the studies by Bonnevier et al. (37) and McIntire et al. (44) compared the incidences of IVH between MLPT and full-term infants. Both studies had a moderate quality and were based on medical records. None of the included studies reported the incidence of WMI in full-term infants. Compared to existing literature, the reported incidences of IVH in full-term infants by Bonnevier et al. and McIntire et al. are low (respectively 0.01% and 0.02%). Literature on the incidence of brain injury in the apparently healthy full-term population is, however, scarce.

Heibel et al. (60) found IVH - using cUS - in 3.5% in a cohort of 1000 clinically normal, full-term infants. Looney et al. (61) reported an incidence of 2.2% for IVH in a sample of 77 full-term infants who underwent MRI. Mercuri et al. (62) evaluated brain injury using cUS in a sample of 177 full-term infants. They reported IVH in 5.1% and periventricular echogenicity (possibly indicating non-cystic WMI) in 6.7%. In the study by Luciano et al. (63), 1805 full-term infants underwent cUS within the second day after birth. IVH grade I or II was reported in 61 full-term infants (3.4%) and IVH grade III or periventricular venous infarction was seen in 5 infants (0.3%).

To date, a high quality imaging oriented cohort study comparing incidences of brain injury between MLPT and full-term infants is missing. Furthermore, none of the reviewed studies compared brain abnormalities on both cUS and MRI simultaneously. CBH - a nowadays well known brain injury in the very preterm population (16) - could not be studied, because there was only one paper that reported the incidence of CBH in the MLPT population.

Due to the high heterogeneity of all reviewed studies, a meta-analysis was not performed. The included studies had different designs and used various GA ranges. Furthermore, both cUS and MRI neuro-imaging techniques were used and frequency of cUS examinations differed between studies. It is well known that serial cUS performed on a regular basis until TEA increases the detection of cystic white matter lesions. As cysts take time to develop and will eventually disappear, the cystic phase of cystic WMI may be overlooked if cUS is not well-timed or discontinued too early (64). Moreover, cystic WMI has become rare with non-cystic WMI now being more common (9). The more subtle forms of WMI are not easily recognized with cUS, while better detected with MRI (9,65). Unfortunately, most papers only used cUS to assess WMI, and non-cystic WMI was not well defined. Timing of neuroimaging (first postnatal week vs TEA) and setting (postnatal ward vs NICU) also have contributed to the high heterogeneity.

Our secondary objective was to evaluate brain development in MLPT infants using quantitative measurements. In general, several differences between MLPT and full-term infants were found, suggesting that MLPT infants may have a delay or alteration in both gray and white matter development compared to their term born peers. Only one study reported linear measurements. Walsh et al. (56) found smaller sizes in MLPT infants compared to full-term infants of gray and white matter structures. Munakata et al. (51) demonstrated a

smaller volume and size of the deep gray matter at TEA in late preterm infants compared to term infants. However, in the papers by Niwa et al. (52) and Cheong et al. (33) no significant differences in (deep) gray matter volume were reported. A limitation of all three studies is the small sample size per GA week.

Strengths and limitations

To our knowledge, this is the first systematic review on brain injury and altered brain development in MLPT infants. This review can be used for future directives. Strengths of this review are the novelty of the subject and the detailed and structured description of the data collection. Furthermore, two reviewers performed the selection of the papers and used the JBI Critical Appraisal Checklist to assess the methodological quality. There are, however, some limitations. Firstly, literature bias may be possible because only original published studies were included. Secondly, selection bias due to language selection may have occurred. In addition, we excluded 30 potentially relevant papers without a classification system or relevant outcome parameters.

Implications for clinical practice

There is insufficient information and evidence regarding an increased risk of brain injury or altered brain development in MLPT infants compared to full-term infants. Therefore, whether MLPT infants should routinely undergo neuro-imaging and/or targeted standardized follow-up programs, remains unclear. In addition, perinatal risk factors for brain injury and/or altered brain development are not well known in this population. Finally, it is unclear whether cUS, being a patient friendly and relatively cheap bedside neuro-imaging technique is a reliable tool to detect the most common and clinically relevant forms of brain injury in MLPT infants.

Implications for research

Future research should focus on the incidence of brain injury in MLPT as compared to full-term infants and should include IVH as well as the whole spectrum of WMI, cerebellar injury and other abnormalities. As some of the encountered abnormalities may be subtle, the reliability of cUS for detecting brain injury and/or altered brain development in MLPT infants should be assessed using MRI as reference standard. Larger studies are needed to assess differences in brain development between MLPT and full-term infants.

Since the population of MLPT infants is much larger than the very preterm population, routine neuro-imaging in all MLPT infants may not be warranted. Therefore, future research should also focus on risk factors associated with brain injury and delayed brain development, in order to identify subgroups of MLPT infants with increased risk for brain injury and/or abnormal brain development. Furthermore, the association between brain injury and brain development, and neurodevelopmental outcome at school age should be evaluated. If an association is found, this may lead to better care and intervention programs for targeted subgroups of MLPT infants and thus eventually to better outcomes and reduction of costs.

CONCLUSION

The incidences of IVH and WMI in MLPT infants varied widely between studies. Other abnormalities, including CBH, were only scarcely reported. Evidence regarding a higher or lower incidence of brain injury in MLPT compared to respectively very preterm or full-term infants, is weak due to moderate methodological quality of reported studies. Furthermore, there is limited evidence suggesting a quantitative difference of white and gray matter structures between MLPT and full-term infants.

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ERRATUM

In the initial published version of this systematic review, the authors stated: “None of the included studies reported on cerebellar hemorrhage” (last sentence below section ‘Other abnormalities’ in the results). The authors recently found out that this statement is incorrect.

In a publication by J. Walsh and colleagues the number of moderate-late preterm infants with signal intensity abnormalities in the cerebellum (indicative for cerebellar hemorrhage) was mentioned in one of their tables (table 4). The reported incidence of cerebellar hemorrhage in moderate-late preterm infants was 9/199 (4.5%).

In this thesis, a correction has been made to the abstract, section ‘Other abnormalities’, Table 3 and minor changes in the discussion. These corrections do not alter the study’s overall interpretation of the study results. The authors regret to have missed the incidence of cerebellar hemorrhage reported by J. Walsh and colleagues and want to apologize for any inconvenience caused.

REFERENCES

1. Kerstjens JM, de Winter AF, Bocca Tjeertes IF, ten Vergert EMJ, Reijneveld SA, Bos AF. Developmental delay in moderately preterm-born children at school entry, *J. Pediatr.* 2011;159(1):92–98.
2. van Baar AL, Vermaas J, Knots E, de Kleine MJK, Soons P. Functioning at school age of moderately preterm children born at 32 to 36 weeks' gestational age. *Pediatrics* 2009;124(1):251–257.
3. Natarajan G, Shankaran S. Short- and long-term outcomes of moderate and late preterm infants. *Am. J. Perinatol.* 2016;33(3):305–317.
4. Woythaler M. Neurodevelopmental outcomes of the late preterm infant. *Semin. Fetal Neonatal Med.* 2019;24(1):54–59.
5. Ekeus C, Lindström K, Lindblad F, Rasmussen F, Hjern A. Preterm birth, social disadvantage, and cognitive competence in Swedish 18- to 19-year-old men. *Pediatrics* 2010;125(1):e67–e73.
6. Mathiasen R, Hansen B, Forman J, Kessing L, Greisen G. The risk of psychiatric disorders in individuals born prematurely in Denmark from 1974 to 1996. *Acta Paediatr.* 2011;100(5):691–699.
7. Chawanpaiboon S, Vogel J, Moller A, Lumbiganon P, Petzold M, Hogan D, et al., Global, regional, and national estimates of levels of preterm birth in 2014: a systematic review and modelling analysis. *Lancet Glob. Health* 2019;7(1):e37–e46.
8. Volpe JJ. Intraventricular hemorrhage in the premature infant—current concepts. Part II. *Ann. Neurol.* 1989;25(2):109–116.
9. Volpe JJ. *Volpe's Neurology of the Newborn*. Sixth edition, Elsevier, Philadelphia, PA, 2018.
10. Papile LA, Burstein J, Burstein R, Koffler H. Incidence and evolution of subependymal and intraventricular hemorrhage: a study of infants with birth weights less than 1,500 gm. *J. Pediatr.* 1978;92(4):529–534.
11. Martinez Biarge M, Groenendaal F, Kersbergen KJ, Benders MJNL, Foti F, Cowan FM et al. MRI based preterm white matter injury classification: the importance of sequential imaging in determining severity of injury. *PLoS One* 2016;11(6):e0156245.
12. Kersbergen KJ, Benders MJNL, Groenendaal F, Koopman-Esseboom C, Nivelstein RAJ, van Haastert IC et al., Different patterns of punctate white matter lesions in serially scanned preterm infants. *PLoS One* 2014;9(10):e108904.
13. Limperopoulos C, Bassan H, Gauvreau K, Robertson RLJ, Sullivan NR, Benson CB et al., Does cerebellar injury in premature infants contribute to the high prevalence of long-term cognitive, learning, and behavioral disability in survivors? *Pediatrics* 2007;120(3):584–593.
14. Hortensius LM, Dijkshoorn ABC, Ecury Goossen GM, Steggerda SJ, Hoebeek FE, Benders MJNL, et al. Neurodevelopmental consequences of preterm isolated cerebellar hemorrhage: a systematic review. *Pediatrics* 2018;142(5):e20180609.
15. Boswinkel V, Steggerda SJ, Fumagalli M, Parodi A, Ramenghi LA, Groenendaal F et al. The CHOPIn study: a multicenter study on cerebellar hemorrhage and outcome in preterm infants. *Cerebellum* 2019;18(6):989–998.
16. Steggerda SJ, Leijser LM, Wiggers-de Bruïne FT, van der Grond J, Walther FJ, van Wezel-Meijler G. Cerebellar injury in preterm infants: incidence and findings on US and MR images. *Radiology* 2009;252(1):190–199.

17. de Vries LS, Eken P, Dubowitz LM. The spectrum of leukomalacia using cranial ultrasound. *Behav. Brain Res.* 1992;49(1):1–6.
18. Volpe JJ. Brain injury in premature infants: a complex amalgam of destructive and developmental disturbances. *Lancet Neurol.* 2009;8(1):110–124.
19. Stipdonk L, Franken MJP, J. Dudink. Language outcome related to brain structures in school-aged preterm children: a systematic review. *PLoS One* 2018;13(6):e0196607.
20. Kidokoro H, Anderson PJ, Doyle LW, Woodward LJ, Neil JJ, Inder TE. Brain injury and altered brain growth in preterm infants: predictors and prognosis. *Pediatrics* 2014;134(2):444–453.
21. Thompson D, Kelly C, Chen J, Beare R, Alexander B, Seal M, et al. Characterisation of brain volume and microstructure at term-equivalent age in infants born across the gestational age spectrum, *Neuroimage Clin.* 2019;21:101630.
22. Young J, Vandewouw M, Morgan B, Smith M, Sled J, Taylor M. Altered white matter development in children born very preterm, *Brain Struct. Funct.* 2018;223(5):2129–2141.
23. Bouyssi Kobar M, Brossard Racine M, Jacobs M, Murnick J, Chang T, Limperopoulos C. Regional microstructural organization of the cerebral cortex is affected by preterm birth, *Neuroimage Clin.* 2018;18:871–880.
24. Pecheva D, Kelly C, Kimpton J, Bonthron A, Batalle D, Zhang H, et al. Recent advances in diffusion neuroimaging: applications in the developing preterm brain. 2018;F1000Res:7.
25. Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. *BMJ* 2015;350:g7647.
26. Kidokoro H, Neil J, Inder T. New MR imaging assessment tool to define brain abnormalities in very preterm infants at term. *Am J Neuroradiol* 2013;34(11):2208–14.
27. Munn Z, Moola S, Lisy K, Riitano D, Tufanaru C. Methodological guidance for systematic reviews of observational epidemiological studies reporting prevalence and cumulative incidence data. *Int J Evid Based Healthc* 2015;13(3):147–153.
28. Bajaj M, Natarajan G, Shankaran S, Wyckoff M, Laptook A, Bell E et al. Delivery room resuscitation and short-term outcomes in moderately preterm infants. *J. Pediatr.* 2018;195:33–38 (e2).
29. Natarajan G, Shankaran S, Saha S, Laptook A, Das A, Higgins R et al. Antecedents and outcomes of abnormal cranial imaging in moderately preterm infants. *J. Pediatr.* 2018;195:66–72 (e3).
30. Trembath A, Payne A, Colaizy T, Bell E, Walsh M. The problems of moderate preterm infants. *Semin. Perinatol.* 2016;40(6):370–373.
31. Walsh M, Bell E, Kandefer S, Saha S, Carlo W, D'angio C, et al. Neonatal outcomes of moderately preterm infants compared to extremely preterm infants. *Pediatr. Res.* 2017;82 (2):297–304.
32. Thompson DK, Kelly CE, Chen J, Beare R, Alexander B, Seal M et al. Early life predictors of brain development at term-equivalent age in infants born across the gestational age spectrum. *NeuroImage* 2019;185:813–824.
33. Cheong JLY, Thompson D, Spittle A, Potter C, Walsh J, Burnett A, et al. Brain Volumes at Term-Equivalent Age Are Associated with 2-Year Neurodevelopment in Moderate and Late Preterm Children. *J Pediatr* 2016;174:91–97.e1.
34. Kelly CE, Cheong JLY, Gabra Fam L, Leemans A, Seal M, Doyle L, et al. Moderate and late preterm infants exhibit widespread brain white matter microstructure alterations at term-equivalent age relative to term-born controls. *Brain Imaging Behav.* 2016;10(1):41–49.

35. Marret S, Ancel P, Marpeau L, Marchand L, Pierrat V, Larroque B et al. Neonatal and 5-year outcomes after birth at 30–34 weeks of gestation. *Obstet. Gynecol.* 2007;110(1):72–80.
36. Ancel P, Goffinet F, Kuhn P, Langer B, Matis J, Hernandorena X, et al. Survival and morbidity of preterm children born at 22 through 34 Weeks' gestation in France in 2011: results of the EPIPAGE-2 cohort study. *JAMA Pediatr.* 2015;169(3):230–238.
37. Bonnevier A, Brodzki J, Björklund L, Källén K. Underlying maternal and pregnancy- related conditions account for a substantial proportion of neonatal morbidity in late preterm infants. *Acta Paediatr.* 2018;107:1521–1528.
38. Gleissner M, Jorch G, Avenarius S. Risk factors for intraventricular hemorrhage in a birth cohort of 3721 premature infants. *J. Perinat. Med.* 2000;28(2):104–110.
39. Bhat V, Karam M, Saslow J, Taylor H, Pyon K, Kemble N, et al., Utility of performing routine head ultrasounds in preterm infants with gestational age 30-34 weeks. *J. Matern. Fetal Neonatal Med.* 2012;25(2):116–119.
40. Garcez C, Silva N, Pinheiro L, Costa M, Sá C, Abreu E, et al. Late-preterm birth in a level III hospital: incidence and associated morbidity. *J. Pediatr. Neonat. Individual. Med.* 2016;5:1.
41. Chen H, Wei K, Zhou C, Yao Y, Yang Y, Fan X, et al. Incidence of brain injuries in premature infants with gestational age = 34 weeks in ten urban hospitals in China. *World J Pediatr* 2013;9(1):17-24..
42. Harris NJ, Palacio D, Ginzel A, Richardson CJ, Swischuk L. Are routine cranial ultrasounds necessary in premature infants greater than 30 weeks gestation? *Am J Perinatol* 2007;24(1):17-21.
43. Kadri H, Mawla A, Kazah J. The incidence, timing, and predisposing factors of germinal matrix and intraventricular hemorrhage (GMH/IVH) in preterm neonates. *Childs Nerv Syst* 2006;22(9):1086-1090.
44. McIntire D, Leveno K. Neonatal mortality and morbidity rates in late preterm births compared with births at term. *Obstet Gynecol* 2008;111(1):35-41.
45. Resch B, Resch E, Maurer Fellbaum U, Pichler Stachl E, Riccabona M, Hofer N, et al. The whole spectrum of cystic periventricular leukomalacia of the preterm infant: results from a large consecutive case series. *Childs Nerv Syst* 2015;31(9):1527-1532..
46. Khoshnood Shariati M, Karimi Z, Rezaeinejad M, Basiri A, Torkestani F, Saleh Gargari S. Perinatal complications associated with preterm deliveries at 24 to 33 weeks and 6 days gestation (2011-2012): A hospital-based retrospective study. *Iran J Reprod Med* 2015;13(11):697-702.
47. Stephen ST, Vazhayil PP, Kandi NP. Mortality and morbidity pattern of severe and moderate preterm babies in a tertiary care hospital in Kerala- A Retrospective record based study. *J. Evolution Med. Dent. Sci* 2018;7(13):1625-1629.
48. Sugiura T, Goto T, Ueda H, Ito K, Kakita H, Nagasaki R, et al. Periventricular leukomalacia is decreasing in Japan. *Pediatr Neurol* 2012;47(1):35-39
49. Altman M, Vanpée M, Cnattingius S, Norman M. Neonatal morbidity in moderately preterm infants: a Swedish national population-based study. *J Pediatr* 2011;158(2):239-244.e1.
50. Fumagalli M, Ramenghi LA, De Carli A, Bassi L, Farè P, Dessimone F, et al. Cranial ultrasound findings in late preterm infants and correlation with perinatal risk factors. *Ital J Pediatr* 2015;41:65.
51. Munakata S, Okada T, Okahashi A, Yoshikawa K, Usukura Y, Makimoto M, et al. Gray matter volumetric MRI differences late-preterm and term infants. *Brain Dev* 2013;35(1):10-16.

52. Niwa T, Suzuki K, Sugiyama N, Imai Y. Regional volumetric assessment of the brain in moderately preterm infants (30–35 gestational weeks) scanned at term-equivalent age on magnetic resonance imaging. *Early Human Development* 2017;111:36-41.
53. Ballardini E, Tarocco A, Baldan A, Antoniazzi E, Garani G, Borgna Pignatti C. Universal cranial ultrasound screening in preterm infants with gestational age 33-36 weeks. A retrospective analysis of 724 newborns. *Pediatr Neurol* 2014;51(6):790-794.
54. Beltempo M, Wintermark P, Lemyre B, Shalish W, Martel Bucci A, Narvey M, et al. Predictors of Severe Neurologic Injury on Ultrasound Scan of the Head and Risk Factor-based Screening for Infants Born Preterm. *J Pediatr* 2019;214:27-33.e3.
55. Egwu CC, Ogala WN, Farouk ZL, Tabari AM, Dambatta AH. Factors associated with intraventricular hemorrhage among preterm neonates in Aminu Kano teaching hospital. *Niger J Clin Pract* 2019;22(3):298-304.
56. Walsh JM, Doyle LW, Anderson PJ, Lee KJ, Cheong JLY. Moderate and late preterm birth: effect on brain size and maturation at term-equivalent age. *Radiology* 2014;273(1):232-240.
57. Leijser LM, de Bruïne FT, Steggerda SJ, van der Grond J, Walther FJ, van Wezel-Meijler G. Brain imaging findings in very preterm infants throughout the neonatal period: Part I. Incidences and evolution of lesions, comparison between ultrasound and MRI. *Early human development* 2009;85(2):101-109.
58. de Vries LS, Benders MJNL, Groenendaal F. Imaging the premature brain: ultrasound or MRI? *Neuroradiology* 2013;55(Suppl. 2):13–22.
59. van Wezel-Meijler G, van der Knaap MS, Sie LT, Oosting J, van Amerongen AH, Cranendonk A, et al. Magnetic resonance imaging of the brain in premature infants during the neonatal period. Normal phenomena and reflection of mild ultrasound abnormalities. *Neuropediatrics* 1998;29(2):89-96.
60. Heibel M, Heber R, Bechinger D, Kornhuber HH. Early diagnosis of perinatal cerebral lesions in apparently normal full-term newborns by ultrasound of the brain. *Neuroradiology* 1993;35(2):85-91.
61. Looney C, Smith JK, Merck L, Wolfe H, Chescheir N, Hamer R, et al. Intracranial hemorrhage in asymptomatic neonates: prevalence on MR images and relationship to obstetric and neonatal risk factors. *Radiology* 2007;242(2):535-541.
62. Mercuri E, Dubowitz L, Brown SP, Cowan F. Incidence of cranial ultrasound abnormalities in apparently well neonates on a postnatal ward: correlation with antenatal and perinatal factors and neurological status. *Arch Dis Child Fetal Neonatal Ed* 1998;79(3):F185-F189.
63. Luciano R, Bersani I, Mancini G, Vento G, Mercuri E. Cranial ultrasound evaluation in term neonates. *Early Hum Dev* 2020;143:104983.
64. Meijler G, Steggerda SJ. *Neonatal Cranial Ultrasonography*, third edition. Cham: Springer; 2019.
65. Leijser LM, Bruïne FT, van der Grond J, Steggerda SJ, Walther FJ, van Wezel-Meijler G. Is sequential cranial ultrasound reliable for detection of white matter injury in very preterm infants? *Neuroradiology* 2010;52(5):397-406.

SUPPLEMENTAL MATERIAL

Appendix 1. Terms used for electronically search.**Cochrane N = 1136**

"moderate preterm" OR "late preterm" OR "near term" OR "32 weeks" OR "33 weeks" OR "34 weeks" OR "35 weeks" OR "36 weeks" in Title, Abstract, Keywords and "magnetic Resonance Imaging" OR "cranial ultrasound" OR "cranial sonography" OR "head ultrasound" OR "head sonography" OR "HUS" OR "cUS" OR "cranial imaging" OR "neonatal morbidity" OR "morbidity" OR "cerebral intraventricular hemorrhage" OR "leukomalacia, periventricular" OR "cerebellar hemorrhage" OR "CBH" in Title, Abstract, Keywords.

Medline/PubMed search N = 1824

((("32 weeks" OR "33 weeks" OR "34 weeks" OR "35 weeks" OR "36 weeks")) AND "Gestational age"[Mesh] OR ("late preterm" OR "near term" OR "moderate preterm" OR "moderately preterm"))

AND
 ("Magnetic Resonance Imaging"[Mesh] OR "cranial ultrasound" OR "cranial sonography" OR "head ultrasound" OR "head sonography" OR "HUS" OR "cUS" OR "cranial imaging")) OR
 ("neonatal morbidity" OR "morbidity" OR "cerebral intraventricular Hemorrhage"[Mesh] OR "Leukomalacia, Periventricular"[Mesh] OR "cerebellar hemorrhage" OR "CBH")

Embase N = 772

((("32 weeks" OR "33 weeks" OR "34 weeks" OR "35 weeks" OR "36 weeks")) AND "Gestational age") OR
 ("late preterm" OR "near term" OR "moderate preterm" OR "moderately preterm")

AND
 "nuclear magnetic resonance imaging" OR "cranial ultrasound" OR "cranial sonography" OR "head sonography" OR "HUS" OR "cUS" OR "cranial imaging" OR "new born morbidity" OR "perinatal morbidity" OR "neonatal outcome" OR "perinatal outcome" OR "brain hemorrhage" OR "periventricular leukomalacia" OR "cerebellar hemorrhage" OR "CBH"

Scopus N = 4544

TITLE-ABS-KEY (("late preterm" OR "near term" OR "moderate preterm" OR "moderately preterm") OR ("32 weeks" OR "33 weeks" OR "34 weeks" OR "35 weeks" OR "36 weeks")) AND "Gestational age")

AND
 TITLE-ABS-KEY ("neonatal morbidity" OR "perinatal morbidity" OR "morbidity" OR "intraventricular hemorrhage" OR "periventricular leukomalacia" OR "cerebellar hemorrhage" OR "CBH" OR "IVH" OR "PVL" OR "Magnetic Resonance Imaging" OR "MRI" OR "cranial ultrasound" OR "cranial sonography" OR "head ultrasound" OR "head sonography" OR "HUS" OR "cUS" OR "cranial imaging")

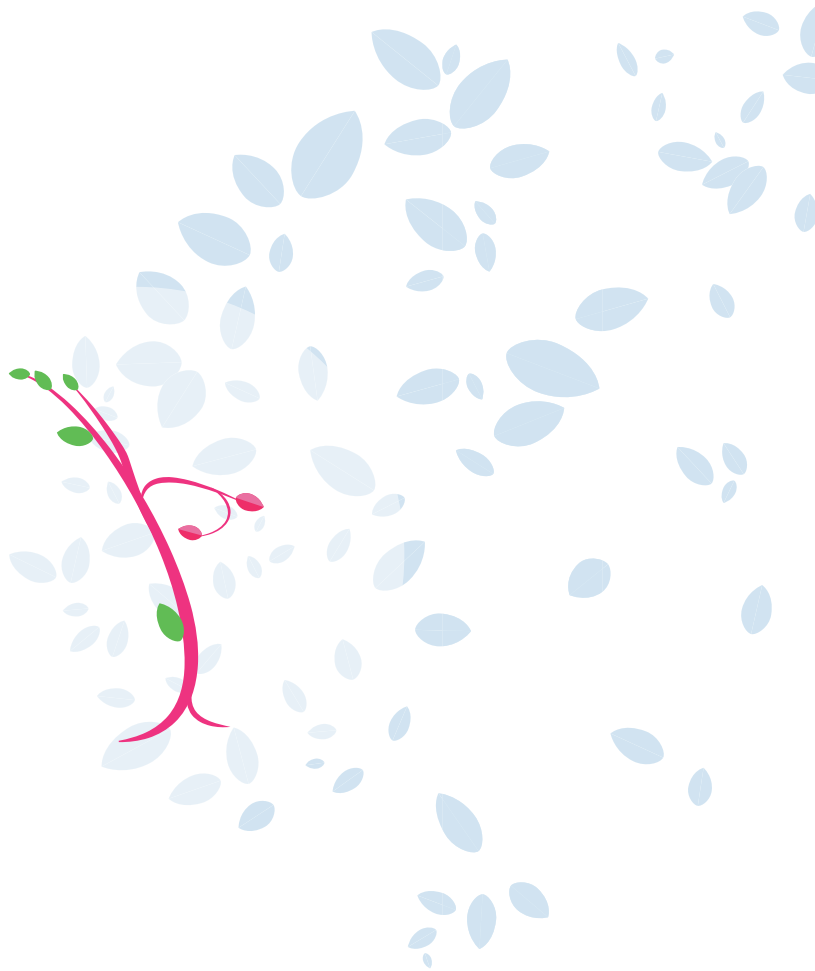
Web of Science N = 2246

("late preterm" OR "near term" OR "moderate preterm" OR "moderately preterm") OR ("32 weeks" OR "33 weeks" OR "34 weeks" OR "35 weeks" OR "36 weeks") AND Gestational age)

AND
 ("Magnetic Resonance Imaging" OR "MRI" OR "cranial ultrasound" OR "cranial sonography" OR "head ultrasound" OR "head sonography" OR "HUS" OR "cUS" OR "cranial imaging") OR
 ("neonatal morbidity" OR "morbidity" OR "cerebral intraventricular Hemorrhage" OR "IVH" OR "Leukomalacia, Periventricular" OR "PVL" OR "cerebellar hemorrhage" OR "CBH")

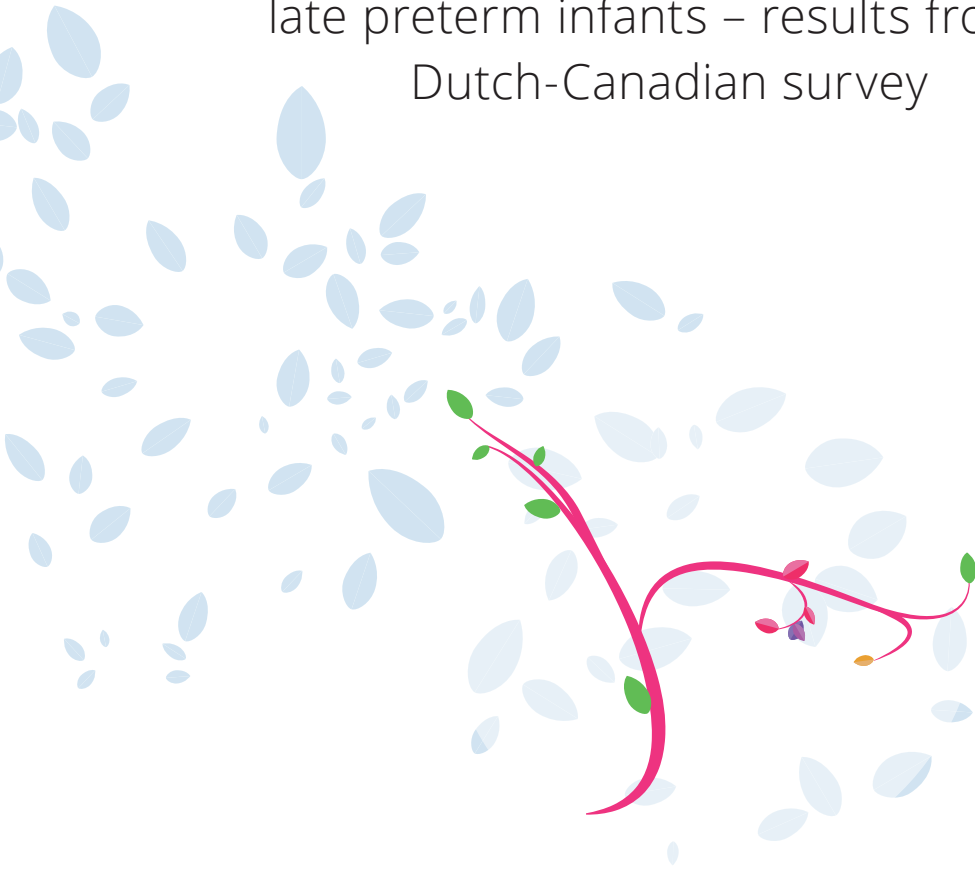
Appendix 2. Explanation of items of the quality assessment.

Items	Explanation
Target population	+ was assigned if subjects/subgroups were within the gestational age range of 32 – 36+6 weeks.
Participant recruitment	+ was assigned if enrolled subjects were not selected, inclusion/exclusion criteria were clearly declared.
Sample size	+ was assigned to studies with a number of sampled infants ≥ 286 . Based on numerous data in very preterm infants and the scarce data in full-term infants we expect to find some form of brain injury in about 20% of an unselected group of moderate preterm infants. For a precision of 5% with a confidence interval of 95%, when Daniel's formula $n = Z^2 * P(1 - P) / d^2$ was applied, a sample size of 286 subjects was sufficient.
Subject and setting description	+ was assigned if data regarding sex, birthweight and perinatal/neonatal complications of subjects as well as the hospital setting (NICU, high care, medium care, maternity ward) were reported.
Coverage bias	+ was assigned if low risk exists that some subgroups of the target population were under- or over represented. (Difference between groups < 15%)
Classification bias	+ was assigned if the identification technique used to detect the presence of brain injury (ea. IVH and/or WMI) was considered adequate and if the classification system was mentioned.
Measurement bias	+ was assigned if adequate procedures to avoid observer variation were taken into account.
Statistics	+ was assigned if statistical methods were adequate described.
Response bias	+ was assigned if a small number (<10%) of subjects potentially eligible for the study refused and if a small number (<10%) of infants were lost to follow-up.



CHAPTER 3

Neurological surveillance in moderate-late preterm infants – results from a Dutch-Canadian survey



Vivian Boswinkel*, Martine F. Krüse-Ruijter*, Anna Consoli, Ingrid M. Nijholt, Martijn F. Boomsma, Linda S. de Vries, Gerda van Wezel-Meijler, Lara M. Leijser

* both authors contributed equally

In preparation

ABSTRACT

Aim To gain insight into local clinical practices concerning neurological surveillance in moderate-late preterm (MLPT) infants, born at 32 – 37 weeks' gestation, in the Netherlands and Canada.

Methods An observational quantitative survey was conducted among Dutch and Canadian neonatal centers (June 2020 - March 2021). Each center was approached to designate one pediatrician/neonatologist to complete the survey.

Results 80/174 (46%) neonatal centers completed the survey. Admission of MLPT infants was guided by clinical criteria in 73/80 (91%) centers. Routine laboratory testing was performed in 45/80 (56%) centers. Cranial ultrasonography was routinely performed in 15/80 (19%) centers and on indication in 37/80 (46%) centers. In 55/80 (69%) centers, neurological examination was performed at least once in admitted infants. Fifty of 80 (63%) centers had a post-discharge follow-up program, with follow-up ranging from 1 to 52 months of age.

Conclusions This study showed a wide variety in neurological surveillance in MLPT infants among Dutch and Canadian neonatal centers. Given the risk for short-term morbidity and long-term neurodevelopmental disabilities, future studies should investigate best practices for and cost-effectiveness of in-hospital care and follow-up of MLPT infants. Given MLPT population size, being 80% of the preterm population, redundant use of advanced care should be avoided.

INTRODUCTION

Birth before 37 weeks of gestation is associated with lifelong health disabilities, including neurodevelopmental and behavioral problems (1). The vast majority (> 80%) of the preterm population is born moderate to late prematurely (MLPT) at a gestational age (GA) of 32-37 weeks (2). However, dedicated neurological surveillance in MLPT infants, such as neuroimaging and follow-up, has generally not been included in (inter)nationally accepted guidelines. An explanation for this might be that the needs of MLPT infants were considered comparable to those of full-term infants (GA \geq 37 weeks) (3). Therefore, until recently MLPT infants were managed the same way as their full-term counterparts.

Over the past two decades, several studies reported that MLPT infants have a higher risk for neurodevelopmental problems than previously considered. Compared to full-term infants, MLPT infants have a two-fold increased risk for cognitive, behavioral and motor problems during childhood, which may persist into adolescence (4-8). Immaturity of the brain and brain injury may contribute to these problems, but this association has yet to be thoroughly studied (9). In an ongoing multicenter Dutch-Canadian study, we are currently investigating the relationship between neonatal neuroimaging findings and neurodevelopmental outcome in MLPT infants (Registered at: <https://www.trialregister.nl/trial/6310>)

A few recommendations for neuroimaging and follow-up in MLPT infants have recently been published. In 2020, the American Academy of Pediatrics and the Canadian Pediatric Society advised to perform cranial ultrasound (cUS) in infants born at GA 32+0 to 36+6 weeks with additional risk factors for brain injury (e.g., placental abruption, prolonged mechanical ventilation, sepsis, major surgery, or abnormal neurological symptoms) (10,11). Currently, the Dutch Pediatric Society has not yet formulated its own recommendations for neuroimaging of the preterm brain.

The Spanish Society of Neonatology, in collaboration with the Spanish Association of Primary Paediatric Care, recommended to perform post-discharge neurodevelopmental follow-up in all preterm infants (including MLPT infants) or, if not feasible, at least in infants with risk factors for poor neurodevelopmental outcomes (e.g. respiratory distress, symptomatic hypoglycemia, hyper-bilirubinemia requiring phototherapy, intraventricular hemorrhage, periventricular leukomalacia) (12). Most of their recommendations were based on expert opinions, as qualitative research is currently lacking. The Dutch and Canadian pediatric societies are less specific on performing follow-up in MLPT infants (13,14).

As the above-mentioned recommendations are relatively new, mostly based on expert opinions and are probably not (yet) nationally and internationally adopted, we expect that local practices exist in the Netherlands and Canada. The aim of this study was to gain more insight into local clinical practices concerning neurological surveillance, with special attention to neuroimaging and standardized follow-up, by conducting a survey among pediatricians

and neonatologists in the Netherlands and Canada. As a result, we hope to encourage more research in the MLPT population and to stimulate a more uniform practice for MLPT infants between and within countries with the final aim to improve the care and outcomes of MLPT infants and their families.

METHODS

This study was an initiative of Isala Women and children's Hospital (Zwolle, The Netherlands) and the Calgary Zone Neonatal Intensive Care Units (NICUs) (Calgary, Canada). The prospective survey was conducted between June 2020 and March 2021. All Dutch and Canadian centers that provide care for MLPT infants (GA within the range of 32+0 to 36+6 weeks) were approached. In the Netherlands, this applies to 69 centers, of which 10 have a level III NICU. In Canada, this applies to 105 centers, of which 31 centers have a level III NICU. All neonatal centers were asked to designate one pediatrician or neonatologist to complete the survey.

The designated pediatrician or neonatologist received an invitation by email, containing a personal link to start the survey. The purpose of this survey was to explore possible differences in (local) clinical care practices between all qualifying centers and between Dutch and Canadian centers. The first part of the survey consisted of general questions (e.g. number of [incubator] beds, profession of the responder [pediatrician, neonatologist] and number of years of working experience as staff). The second part of the survey consisted of items concerning admission criteria and routine laboratory testing, neuroimaging (cUS and magnetic resonance imaging [MRI]), neurological examination and follow-up for MLPT infants. In addition, we asked questions about management of MLPT infants admitted to the maternity ward and participants' opinion on current overall management of MLPT infants (see Supplemental material). All questions were multiple choice, with options to specify the answer and free text for questions and remarks. Once the survey was completed, it could not be accessed again. All answers were automatically stored in Research Manager (version 5.56.0, Research Manager, The Netherlands). The study was approved by the Medical Ethics Committee at the Isala Hospital Zwolle (Ethics ID 200423) and the University of Calgary Conjoint Health Research Ethics Board (Ethics ID REB20-0442). Data were analyzed using SPSS software (version 26.0; SPSS inc, USA). Frequencies are reported as number and percentage.

RESULTS

Of the 174 centers, 122 (70%) centers designated a pediatrician or neonatologist to complete the survey. Eighty/174 (46%) pediatricians-neonatologists completed the survey (Figure 1) of whom 46/80 (58%) were Dutch and 34/80 (42%) were Canadian. Characteristics of participants and their neonatal centers (e.g., level II or III, number of incubator beds) are shown in Table 1.

Figure 1. Recruitment of participants.

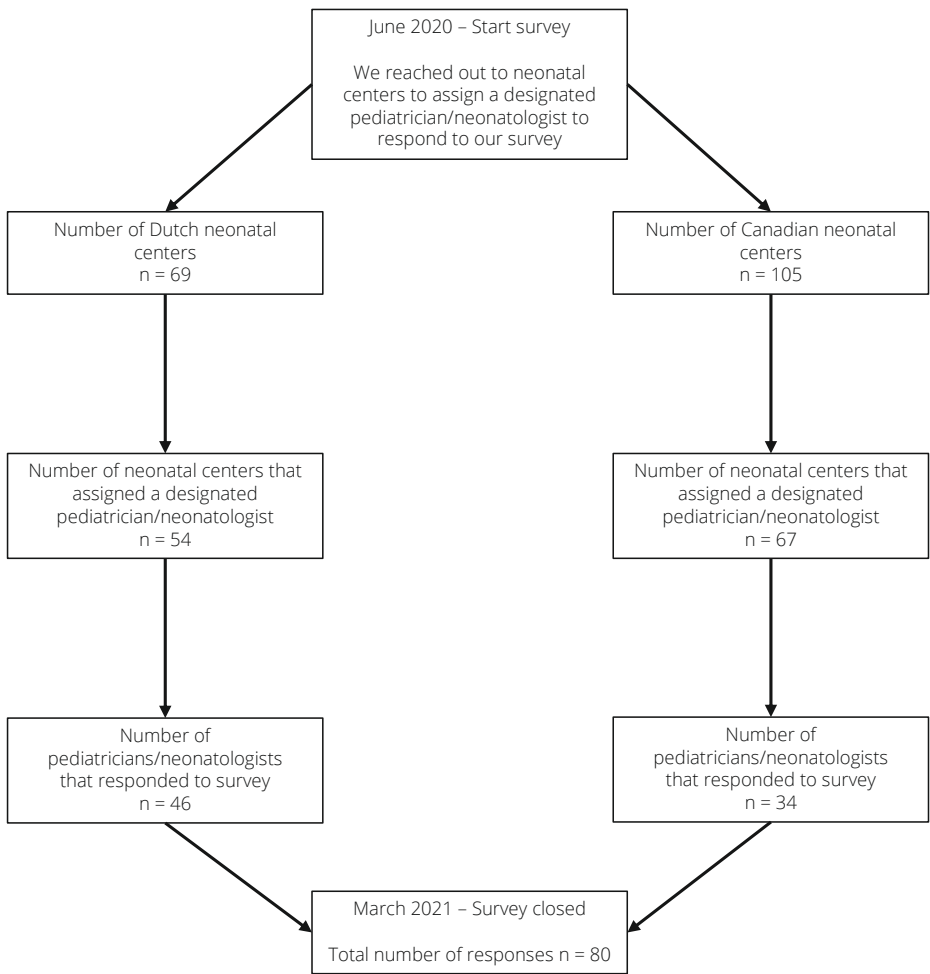


Table 1. Characteristics of participants and setting.

	Dutch centers	Canadian centers	Total
Response rate	46/69 (67)	34/105 (32)	80/174 (46)
	N = 46	N = 34	N = 80
Position			
Pediatrician	22 (48)	7 (21)	29 (36)
Neonatologist	23 (50)	26 (76)	49 (61)
Missing data	1 (2)	1 (3)	2 (3)
Number of years working as a pediatrician or neonatologist			
< 5 years	5 (11)	2 (6)	7 (9)
5 – 10 years	13 (28)	10 (29)	23 (29)
10 – 15 years	14 (30)	6 (18)	20 (25)
> 15 years	14 (30)	15 (44)	29 (36)
Missing data	0	1 (3)	1 (1)
Setting			
Level III neonatal center	6 (13)	18 (53)	24 (30)
Level I-II neonatal center	40 (87)	16 (47)	56 (70)
Number of incubators/beds (in total)			
< 5 beds	6 (13)	1 (3)	7 (9)
5 – 12 beds	12 (26)	4 (12)	16 (20)
10 – 15 beds	12 (26)	5 (35)	17 (21)
15 – 20 beds	13 (28)	6 (18)	19 (24)
> 20 beds	3 (7)	18 (53)	21 (26)

Numbers are reported as n (%)

Criteria for admission of MLPT infants

In 73 of 80 (91%) participating centers, admission of MLPT infants was guided by clinical criteria. Fifty-seven/80 (93%) centers used GA as a criterion for admission. The reported GA limits ranged from < 34 weeks to < 37 weeks. Most frequently reported GA limits were < 35 weeks (23/80 [29%]) and < 36 weeks (27/80 [34%]). In addition, 48/80 (60%) centers used birth weight as a criterion for admission. Three centers (3/80 [4%]) determined the need for admission based on birth weight percentile (below <P5 or <P10). Other centers used an absolute weight as cut-off for admission, ranging from < 1800 to < 2500 grams. Most frequently reported cut-off was a birth weight <2000 grams (17/80 [21%]).

Furthermore, clinical practices included admission criteria for hypoglycemia during postnatal day 1-3 (48/80 [60%]; cut-off value range: 1.6 – 3.3 mmol/l), hyperbilirubinemia needing treatment (51/80 [64%]), (suspected) sepsis (51/80 [64%]), feeding difficulties (44/80 [55%]), suboptimal start indicated by an APGAR score < 7 at five minutes (27/80 [34%]) and respiratory distress (61/80 [76%]). Other criteria mentioned by participants were maternal medication use (not specified), maternal diseases that may affect the infant (e.g., hypothyroidism), neonatal abstinence syndrome, congenital anomalies (e.g., congenital

heart disease) and (suspected) seizures. Almost all centers recorded their practices in local guidelines (69/80 [86%]).

Laboratory testing

Forty-five/80 (56%) centers indicated to perform routine laboratory testing in MLPT infants. Testing included: glucose (routine: 37/80 [46%]; only when indicated [i.e., risk factors or clinical symptoms]: 8/80 [10%]), bilirubin (routine: 26/80 [32%]; only when indicated: 17/80 [21%]), C-reactive protein (routine: 1/80 [1%]; only when indicated: 31/80 [39%]), hemoglobin (routine: 12/80 [15%]; only when indicated: 19/80 [24%]). Other routine laboratory tests mentioned included: ferritin measurement two weeks after birth (1/80 [1%]), complete blood count in small for gestational age infants (2/80 [3%]) and electrolytes (3/80 [4%]).

Neuroimaging

Local guidelines concerning neuroimaging (cUS/MRI) in MLPT infants were available in 40/80 (50%) centers.

CUS: Fifty-two/80 (65%) centers indicated to perform cUS in MLPT infants. In 15/80 (19%) centers, cUS was performed routinely in (a subgroup of) MLPT infants. GA cut-off for routine cUS differed between centers (< 33 weeks in 5/15 [33%]; < 34 weeks in 5/15 [33%]; < 35 weeks in 2/15 [13%]; 3/15 [20%] had no GA cut-off). Five of the 15 (33%) centers with routine cUS performed cUS on day 1 – 3, 5 (33%) on day 4 – 7, and 1 (7%) before discharge/around term equivalent age. In 4/15 (27%), the day of performing cUS was not provided. CUS was repeated based on findings of the first cUS in 9/15 (60%) centers; for 3/15 (20%) centers this question was not completed. The remaining 37/80 (46%) centers, did not routinely perform cUS but used other indications to perform cUS (Table 2).

MRI: In none of the centers, routine MRI was performed. Ten/80 (13%) centers indicated performing MRI in MLPT infants on indication, including abnormal cUS findings, hypoxic-ischemic encephalopathy or clinical signs suggestive of brain abnormalities (e.g., seizures, meningitis, microcephaly, suspected anomalies or dysmorphic features). The remaining 70 centers did not perform MRI or referred MLPT infants to a level III neonatal center for MRI (the latter was only mentioned by four centers).

Table 2. Indications for cUS in MLPT infants.

Indication	Number of centers performing cUS on indication N = 37
(Suspected) seizures	34 (92)
Other neurological symptoms (such as jitteriness, irritability, excessive crying, abnormal muscle tone, lethargy)	34 (92)
Suspected sepsis	7 (19)
Confirmed sepsis	16 (43)
Suspected meningitis	21 (57)
Confirmed meningitis	30 (81)
Anemia, infant needing blood transfusion	20 (54)
Hyperbilirubinemia, infant needing exchange transfusion	20 (54)
Antenatal diagnosis or suspicion of brain anomaly	35 (95)
Dysmorphisms	32 (86)
Other (multiple answers per center)	13 (35)
	Intrauterine growth restriction/dysmaturity (5 centers)
	Birth weight < 1500 gram (3 centers)
	Unexplained apnea (3 centers)
	Monochorionic twins (2 centers)
	Resuscitation (2 centers)
	Fetal therapy (1 center)
	Hydrocephaly (1 center)
	Micro-/macrocephaly (1 center)
	Severe hypoglycemia (1 center)
	Severe thrombocytopenia (1 center)
	Suspected congenital cytomegalovirus (1 center)

Numbers are reported as n (relative %)

Neurological examination

Routine neurological examination in admitted MLPT infants was performed in 55/80 (69%) centers. Sixteen/80 (20%) centers performed neurological examination on the first postnatal day and 17/80 (21%) centers prior to discharge, while 22/80 (28%) centers performed examinations on both the first postnatal day and prior to discharge, or even more frequently. The content of the neurological examination was not specified.

Follow-up program

Follow-up of MLPT infants was performed in 50/80 (63%) centers. In almost all centers, the infants were seen by a pediatrician or neonatologist (48/50 [96%]), mostly together with

other disciplines or allied services (e.g., vaccinations or repeating cUS examination) (42/50 [84%]) (Table 3). Duration of follow-up ranged from 1 to 52 months.

Care for MLPT infants admitted to the postpartum maternity ward

Questions about the care of MLPT infants staying in the postpartum maternity ward were interpreted differently. Six participants did not complete any of the questions, 14 used the options 'I don't know' and/or 'I don't wish to answer this question', and two completed the questions but commented that they did not fully understand the questions. Hence, we can not elaborate on these questions.

Comparison of Dutch and Canadian practices

Almost all Dutch and Canadian neonatal centers used criteria for admission of MLPT infants and recorded these in local guidelines (Dutch: 39/46 [85%]; Canadian: 30/34 [88%]). Routine laboratory testing was performed in 25/46 (54%) Dutch centers and 20/34 (59%) Canadian centers. Of the 46 Dutch centers, 34 (74%) had local guidelines for neuroimaging of the MLPT brain, compared to 6/34 (17%) of the Canadian centers. CUS examinations were performed in 32/46 (70%) of the Dutch and in 20/34 (59%) of the Canadian centers. Routine cUS (in a subgroup of MLPT infants) was performed in 10/46 (22%) Dutch and 5/34 (15%) Canadian centers. MRI was not routinely performed in either country. Two Dutch (2/46 [4%]) and eight Canadian (8/34 [23%]) centers performed MRI in MLPT infants on clinical indication. Routine neurological examinations were performed in 28/46 (61%) Dutch and 27/34 (79%) Canadian centers. In Dutch centers, 37/46 (80%) provided some form of follow-up for MLPT infants, while this was the case in 13/34 (37%) Canadian centers. Four Canadian centers (4/34 [12%]) without follow-up commented that follow-up was provided by others (i.e., general practitioners or by a local community pediatrician).

Participants' opinion on local management

We asked participants about their satisfaction regarding current practice on neuroimaging and neurological follow-up in their center during admission and after discharge. Thirteen/80 (16%) participants indicated to be very satisfied with screening during admission, while 47/80 (59%) were reasonably satisfied and 16/80 (20%) were neutral. Three/80 (4%) participants were unsatisfied for reasons including too little experience with MLPT infants, the perception that care by nurses could be improved and a preference to perform routine cUS. One participant refrained from expressing his/her opinion. In addition, 40/80 (50%) were very satisfied about their practices after discharge, while 17/80 (21%) were reasonable satisfied and 12/80 (15%) were neutral. Four/80 (5%) participants were not satisfied as they would like to see closer developmental follow-up in MLPT infants. Seven participants refrained from expressing their opinion.

Table 3. Indication for follow-up, content and duration of follow-up programs in MLPT infants.

Indication for follow-up n = 50	Follow-up performed by (multiple answers were possible) n = 50	
GA < 35 weeks	26 (52)	Pediatrician/ -neonatologist 48 (96)
GA < 34 weeks	8 (16)	Paediatric nurse 7 (14)
GA < 33 weeks	9 (18)	Paediatric resident 3 (6)
Other	Low birth weight (38 centers) Medical conditions (22 centers) < 37 weeks (3 centers) < 36 weeks (2 centers) Feeding difficulties (2 centers) Psycho-social circumstances (1 center)	Paediatric nurse practitioner/ physician 11 (22) assistant
Content of follow-up visit n = 50	Collaboration with other disciplines or services (multiple answers were possible) n = 50	
Measuring weight, length and height	48 (96)	No 9 (18)
Physical examination	47 (94)	Yes 41 (82)
Neurological examination	44 (88)	Physiotherapist 30/41 (73)
Answering parents' questions	48 (96)	Psychologist 7/41 (17)
Developmental assessment	45 (90)	Speech therapist 19/41 (46)
Other	Start iron supplementation (2 centers) Screening for postnatal depression (1 center)	Dietician (5 centers) Well baby doctor or nurse (3 centers) Social worker (3 centers) Occupational therapist (3 centers) Vaccinations (3 centers) Cranial ultrasound on indication (1 center)

Table 3. Continued.

Duration of follow-up program	n = 50
1 month	1 (2)
3 – 12 months	17 (34)
When the child starts walking	12 (24)
Unknown	2 (4)
Other	18 (36)
	Up till 18 months (1 center)
	Up till 24 months (4 centers)
	Between 24 – 36 months (1 center)
	Between 24 – 48 months (1 center)
	Up till 36 months (1 center)
	Up till 42 months (2 centers)
	Up till 48 months (2 centers)
	Up till 54 months (1 center)
	Depending on medical condition and history (4 centers)
	First year in neonatal center, after this the infant will enroll in a special program at the ‘well baby’ clinic (1 center)

Numbers are reported as n (relative %)

DISCUSSION

We conducted an online survey to gain better insight into current clinical practice concerning neurological surveillance in MLPT infants in neonatal centers in the Netherlands and Canada. We found various differences in clinical practices among individual neonatal centers and between Dutch and Canadian centers.

Different GA and birth weight limits for admission of MLPT infants to the neonatal unit were reported. This is in consistence with the paper by Fleming et al., who also demonstrated variability in limits for admission of late preterm infants (GA 34+0 – 36+6 weeks) using a national survey in England (15). The variation found is likely related to differences in local facilities, skills level of personnel in the different units (neonatal units or postpartum maternity ward) and lack of evidence for best practices in MLPT infants.

Nearly half of the centers participating in our survey did not perform routine laboratory testing in MLPT infants. Dutch and Canadian Pediatric Society guidelines advise to evaluate blood glucose levels within 2 hours of birth in all preterm infants (GA < 37 weeks) for early detection of hypoglycemia and thereby prevention of brain injury (16,17). Moreover, Kerstjens et al. reported that neonatal hypoglycemia was associated with an increased risk of developmental delay in MLPT infants at 4 years of age (18). As MLPT infants may benefit from strict/stringent monitoring of blood glucose levels, this should be better implemented in local clinical practices.

Some participants reported routine testing of other laboratory parameters (such as electrolytes, hemoglobin and C-reactive protein), although there is no evidence supporting this. For example, routinely testing of C-reactive protein has a low sensitivity, specificity and positive predictive value for neonatal sepsis (19,20). Further studies are necessary to evaluate whether, in addition to glucose monitoring, other routine laboratory tests may be beneficial in MLPT infants.

Only 17% of the Canadian centers had a local guideline in which the application of neuroimaging in MLPT infants was documented. As the recommendation from the Canadian Pediatric Society on neuroimaging of the preterm brain is recent, it may not have been implemented in local guidelines across Canadian centers yet. In contrast, up to 74% of the Dutch centers had a local guideline for neuroimaging of the MLPT brain while national Dutch recommendations on neuroimaging in preterm infants currently do not exist. Of note, routine cUS in MLPT infants was performed in ten Dutch and five Canadian centers, while this is not recommended by current (American or Canadian) reports (10,11). Furthermore, although American and Canadian recommendations advise cUS in MLPT infants with risk factors for brain injury, the level of evidence for this recommendation was moderate (grade B). Therefore, more research on risk factors for brain injury in MLPT infants and therewith risk of neurodevelopmental problems may help to target neuroimaging in a subgroup of

MLPT infants. This may avoid unnecessary diagnostic imaging in infants at low risk and therewith costs.

Our survey also demonstrated a wide variety in performing neurological examinations and follow-up in MLPT infants. Early identification of developmental delay is important to facilitate timely intervention and to optimize neurodevelopmental outcomes (21,22). So far, a few 'expert opinion based' recommendations for the follow-up of the vulnerable MLPT population have been suggested (12,23). However, as the MLPT population is large, this will place a considerable burden on health care services and will be of major cost to society (24,25). Therefore, there is a need for research on best practice and cost-effectiveness of follow-up for MLPT infants.

Our study highlights the knowledge gaps within the neurological surveillance of MLPT infants. Furthermore, it illustrates the need for more research in the MLPT population and a more uniform and targeted clinical practice. Ultimately, this will improve care for and outcome of MLPT infants. Our study also has some limitations. Firstly, participation in the survey was voluntary. Therefore, participants with a special interest in the matter may have been selected. The response rate of our survey (46%) was moderate. However, our response rate was comparable to those of other surveys on clinical practices in pediatrics (26,27). In addition, the study was conducted in two high-income countries and is probably not representative for the global situation. Furthermore, we acknowledge that there are other organizations, like primary care/community pediatricians, that provide care and follow-up in MLPT infants. This was, however, beyond the scope of this study.

In conclusion, this study demonstrated a wide variety in neurological surveillance of MLPT infants. National guidelines advise monitoring of blood glucose levels for early detection of hypoglycemia but are not well implemented in most neonatal centers. There is a lack of evidence for best practice concerning admission criteria, neuroimaging and follow-up. Future studies need to investigate whether subgroups of MLPT infants may benefit from advanced in-hospital care (such as neuroimaging and additional laboratory testing) and post-discharge neurodevelopmental follow-up. Given MLPT population size, being 80% of the preterm population, redundant use of advanced care should be avoided.

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REFERENCES

1. Blencowe H, Cousens S, Chou D, Oestergaard M, Say L, Moller A, et al. Born Too Soon: The global epidemiology of 15 million preterm births. *Reproductive Health* 2013;10(1):S2.
2. Chawanpaiboon S, Vogel J, Moller A, Lumbiganon P, Petzold M, Hogan D, et al. Global, regional, and national estimates of levels of preterm birth in 2014: a systematic review and modelling analysis. *Lancet Glob Health* 2019;7(1):e37-e46.
3. Boyle JD. Born just a few weeks early: does it matter? *Fetal and Neonatal* 2013;98(1):85-8.
4. van Baar AL, Vermaas J, Knots E, de Kleine MJK, Soons P. Functioning at school age of moderately preterm children born at 32 to 36 weeks' gestational age. *Pediatrics* 2009;124(1):251-257.
5. Cserjesi R, Van Braeckel KNJA., Butcher PR, Kerstjens JM, Reijneveld SA, Bouma A, et al. Functioning of 7-year-old children born at 32 to 35 weeks' gestational age. *Pediatrics* 2012;130(4):838-46.
6. Bogicevic L, Verhoeven M, van Baar AL. Toddler skills predict moderate-to-late preterm born children's cognition and behaviour at 6 years of age. *PLoS One* 2019;14(11).
7. Martínez-Nadal S, Bosch L. Cognitive and Learning Outcomes in Late Preterm Infants at School Age: A Systematic Review. *International Journal of Environmental Research and Public Health* 2021;18.
8. Kerstjens JM, de Winter AF, Bocca Tjeertes IF, ten Vergert EMJ, Reijneveld SA, Bos AF. Developmental delay in moderately preterm-born children at school entry. *J Pediatr* 2011;159(1):92-98.
9. Boswinkel V, Nijboer-Oosterveld J, Nijholt IM, Edens MA, Mulder - de Tollenaer SM, Boomsma MF, et al. A systematic review on brain injury and altered brain development in moderate-late preterm infants. *Early Hum Dev* 2020 -5-28;148.
10. Hand IL, Shellhaas RA, Milla SS, Committee on fetus and newborn, section on neurology, section on radiology. Routine Neuroimaging of the Preterm Brain. *Pediatrics* / 2020;146(5).
11. Guillot M, Sebastianski M, Lemyre B. Comparative performance of head ultrasound and MRI in detecting preterm brain injury and predicting outcomes: A systematic review. *Acta Paediatr* 2020;n/a.
12. García Reymundo M, Hurtado Suazo JA, Calvo Aguilar MJ, Soriano Faura FJ, Ginovart Galiana G, Martín Peinador Y, et al. Follow-up recommendations for the late preterm infant. *Anales de Pediatría (English Edition)* 2019;90(5):318.e1-318.e8.
13. Nederlands Centrum Jeugdgezondheid. Te vroeg en/of small for gestational age (SGA) geboren kinderen. 2013.
14. Jefferies AL, Canadian Paediatric Society, Fetus and Newborn Committee. Going home: Facilitating discharge of the preterm infant. *Paediatr Child Health* 2014 Jan;19(1):31-42.
15. Fleming PF, Arora P, Mitting R, Aladangady N. A national survey of admission practices for late preterm infants in England. *BMC Pediatrics* 2014;14.
16. Narvey MR, Marks SD. The screening and management of newborns at risk for low blood glucose. *Paediatr Child Health* 2019 Dec;24(8):536-554.
17. Nederlandse Vereniging voor Kindergeneeskunde, (NVK). Postnatale zorg in de algemene kindergeneeskunde. 2021.
18. Kerstjens JM, Bocca Tjeertes IF, de Winter AF, Reijneveld SA, Bos AF. Neonatal morbidities and developmental delay in moderately preterm-born children. *Pediatrics* 2012 Aug;130(2):265.

19. Sharma D, Farahbakhsh N, Shastri S, Sharma P. Biomarkers for diagnosis of neonatal sepsis: a literature review. *J Matern Fetal Neonatal Med* 2018 Jun;31(12):1646-1659.
20. Khan F. C-reactive Protein as a Screening Biomarker in Neonatal Sepsis. *J Coll Physicians Surg Pak* 2019 Oct;29(10):951-953.
21. Natarajan G, Shankaran S. Short- and Long-Term Outcomes of Moderate and Late Preterm Infants. *Am J Perinatol* 2016;33(3):305-317.
22. Romeo DM, Ricci M, Picilli M, Foti B, Cordaro G, Mercuri E. Early Neurological Assessment and Long-Term Neuromotor Outcomes in Late Preterm Infants: A Critical Review. *Medicina (Kaunas, Lithuania)* 2020;56(9):475.
23. Phillips RM, Goldstein M, Hougland K, Nandyal R, Pizzica A, Santa-Donato A, et al. Multidisciplinary guidelines for the care of late preterm infants. *Journal of Perinatology* 2013;33:5-22.
24. Khan KA, Petrou S, Dritsaki M, Johnson SJ, Manktelow B, Draper ES, et al. Economic costs associated with moderate and late preterm birth: a prospective population-based study. *BJOG* 2015 Oct;122(11):1495-1505.
25. Speer RR, Schaefer EW, Aholoukpe M, Leslie DL, Gandhi CK. Trends in Costs of Birth Hospitalization and Readmissions for Late Preterm Infants. *Children (Basel)* 2021 Feb 10;8(2):127. doi: 10.3390/children8020127.
26. Bridgemohan C, Bauer NS, Nielsen BA, DeBattista A, Ruch-Ross H, Paul LB, et al. A Workforce Survey on Developmental-Behavioral Pediatrics. *Pediatrics* 2018;141(3):e20172164.
27. Short HL, Taylor N, Thakore M, Piper K, Baxter K, Heiss KF, et al. A survey of pediatric surgeons' practices with enhanced recovery after children's surgery. *J Pediatr Surg* 2018;53(3):418-430.

SUPPLEMENTAL MATERIAL – QUESTIONS BIMP SURVEY

General Questions about responder:

1. In which country are you employed?
 - a. The Netherlands
 - b. Canada
2. Number of years working as a pediatrician (-neonatologist)?
 - a. < 5 years
 - b. 5 – 10 years
 - c. 10 – 15 years
 - d. > 15 years
3. Position
 - a. Pediatrician
 - b. Pediatrician-neonatologist
4. Hospital
 - a. With level III NICU
 - b. With level I-II neonatal unit
 - c. Other
5. Number of (incubator) beds
 - a. < 5
 - b. 5-10
 - c. 10-15
 - d. 15-20
 - e. > 20

Specific questions related to moderate and late preterm (MLPT) infants

A. Admission criteria

6. Are there guidelines for admission of MLPT infants to your unit?
 - f. Yes
 - g. No
 7. If yes, are these criteria recorded in hospital guidelines
- If yes to question 6 → please proceed to questions 8-16 about these criteria
8. Gestational age cut-off
 - a. Yes (<....weeks) please specify cut-off
 - b. No
 9. Birthweight cut-off
 - a. Yes (<.....gram); please specify cut-off
 - b. No
 10. Hypoglycemia during postnatal day 1-3 (including day 3)
 - a. Yes (<....mg/dl/ mmol/l); please specify cut-off
 - b. No

11. Hyperbilirubinemia needing treatment
 - a. Yes
 - b. No
12. (Suspected) sepsis
 - a. Yes
 - b. No
13. Feeding difficulties (not drinking well, vomiting, delayed meconium passage)
 - a. Yes
 - b. No
14. Suboptimal start (Apgar score < 7 at 5 minutes)
 - a. Yes
 - b. No
15. Respiratory distress
 - a. Yes
 - b. No
16. Other guidelines
 - a. Yes (please specify)
 - b. No
17. Do you have any additional comments regarding questions 8-16?

B. Laboratory testing

18. Do you perform routine laboratory testing in admitted MLPT infants?
 - a. Yes
 - b. No

If yes, which tests are performed → Please proceed to questions 19-24

19. Glucose
 - a. Yes
 - b. No
 - c. Only when indicated (clinical symptoms)
20. Bilirubin
 - a. Yes
 - b. No
 - c. Only when indicated (clinical symptoms)
21. CRP (or other inflammatory parameter)
 - a. Yes
 - b. No
 - c. Only when indicated (clinical symptoms)
22. Hemoglobin (and/or other hematological parameters)
 - a. Yes
 - b. No

23. Other
 - a. Yes (please specify)
 - b. No
24. Do you have any additional comments regarding questions 19-23?

C. *Clinical condition of MLPT infant who is not admitted to neonatal unit*

25. Is the clinical condition of MLPT infants monitored if they are not admitted to the neonatal unit?

If yes → please proceed to questions 26-31

26. MLPT neonates are staying on the maternity ward
 - a. On first postnatal day
 - b. Until postnatal day 2
 - c. Until postnatal day 3
 - d. Other (please specify)
27. Through telephone contact with parents
 - a. Yes; if yes: when and by whom?
 - b. No
28. Through contact with midwife and/or maternity/community nurse
 - a. Yes
 - b. No
29. Through out-patient check-up during the first week
 - a. Pediatrician
 - b. Family doctor
 - c. Well-baby clinic
 - d. No
30. Through routine laboratory tests
 - a. Yes (please specify)
 - b. No
31. Do you have any additional comments regarding questions 26-31?

D. *Screening the MLPT brain*

32. Does your unit have a guideline for screening of the brain of MLPT infants?
 - a. Yes
 - b. No
33. If yes, are these guidelines generally followed?
 - a. Yes
 - b. No
34. If not, please specify why these guidelines are not followed?
35. Do MLPT infants routinely undergo one or more cranial ultrasound (cUS) examinations?
 - a. Yes; please proceed to questions 36-35
 - b. Only when indicated; please proceed to questions 40-52
 - c. No; please proceed to question 53

Questions regarding routine cUS (36-39)

36. At what postnatal age is first cUS done?
 - a. 1-3 days
 - b. 4-7 days
 - c. > 7 days
 - d. Around term equivalent age / prior to discharge
37. How frequently is cUS done during hospital admission?
 - a. 1x
 - b. 2x
 - c. =/> 3x
38. Is there a gestational age cut-off to do cUS examinations? Only when:
 - a. < 33 weeks
 - b. < 34 weeks
 - c. < 35 weeks
 - d. < 36 weeks
 - e. No
39. Who performs the cUS examinations in MLPT infants? Tick all that apply
 - a. Radiologist or radiology technician
 - b. Pediatrician
 - c. (fellow)Neonatologist
 - d. (pediatric)neurologist
 - e. Nurse practitioner/ physician assistant/nurse specialist

Questions regarding specific indications cUS (40-52)

Do you perform cUS in case of the following conditions:

40. (Suspected) Seizures
 - a. Yes
 - b. No
41. Other neurological symptoms (such as jitteriness, irritability, excessive crying, abnormal muscle tone, lethargy)
 - a. Yes (please specify)
 - b. No
42. Suspected sepsis
 - a. Yes
 - b. No
43. Confirmed sepsis
 - a. Yes
 - b. no
44. Suspected meningitis
 - a. Yes
 - b. No

45. Confirmed meningitis
 - a. Yes
 - b. no
46. Anemia, needing RBC transfusions
 - a. Yes, please specify cut-off
 - b. No
47. Hyperbilirubinemia needing exchange transfusion
 - a. Yes
 - b. No
48. Antenatal diagnosis or suspicion of brain anomaly
 - a. Yes
 - b. No
49. Dysmorphies
 - a. Yes
 - b. No
50. Other
 - a. Yes (please specify)
 - b. No
51. Are these indications (40-50) adhered to the same by all your colleagues?
 - a. Yes
 - b. No
52. If no, please specify?
53. Do MLPT infants routinely undergo MRI?
 - a. Yes; please proceed to questions 54-55
 - b. No; please proceed to question 56
 - c. Only when indicated;

Questions regarding brain MRI

54. Is there experience with doing MRI in MLPT infants in your unit?
 - a. Yes (> 10 infants/year)
 - b. Limited (1-10 infants/year)
 - c. No, the infant is referred to a level III hospital for MRI
55. If yes, are there specific indications for neonatal MRI?
 - a. Yes; please specify
 - b. No

Questions regarding neurological examinations and follow-up programs

56. Do you perform routine neurological examinations in MLPT infants?
 - a. Yes
 - b. No

57. If yes, when is this performed?
 - a. 1st postnatal day
 - b. Before discharge
 - c. Other; please specify
 58. Do MLPT infants enroll in a standard follow-up program in your hospital?
 - a. Yes
 - b. No
 - c. If indicated
 59. If yes to question 58, what are the indications (please tick all that apply)?
 - a. Gestational age < 35 weeks
 - b. Gestational age < 34 weeks
 - c. Gestational age < 33 weeks
 - d. Low birthweight
 - e. Other (please specify)
 60. If answer a. or c. to question 58, who performs the follow-up care?
 - a. Pediatric nurse
 - b. Pediatrician
 - c. Pediatric resident
 - d. Nurse practitioner/ physician assistant/nurse specialist
 - e. Other (please specify)
 61. If answer a. or c. to question 58 the follow-up consists of (please tick all that apply):
 - a. Weight, length and head circumference measurements
 - b. Answering parents questions
 - c. Physical examination
 - d. Neurological examination
 - e. Nutritional advice
 - f. Developmental assessment
 - g. Other (please specify)
- If answer a. or c. to question 58, is there a collaboration with other disciplines or services?
62. Physiotherapist
 - a. Yes
 - b. No
 63. Other services (i.e. speech therapist, psychologist)
 - a. Yes (please specify)
 - b. No
 64. Are MLPT infants seen together with another discipline?
 - a. Yes
 - b. No
 65. If yes to question 64, please specify which discipline
 - a. Physiotherapist
 - b. General practitioner
 - c. Well baby doctor or "jeugdarts" (the Netherlands)

66. Until what age or milestone is follow-up performed in MLPT infants?
- a. 1 month
 - b. 2-3 months
 - c. 3-12 months
 - d. When the child has started walking
 - e. Other (please specify)

Concluding questions

67. Do you feel that current clinical practice/care on neuroimaging and neurological development in MLPT infants during admission to your unit is satisfactory?
- a. Very satisfied
 - b. Reasonably satisfied
 - c. Neutral
 - d. Unsatisfied
68. If you are not satisfied, how would you like to improve this?
69. Do you feel that current clinical practice/care on neuroimaging and neurological development in MLPT infants after discharge is satisfactory?
- a. Satisfactory
 - b. Reasonably satisfactory
 - c. Neutral
 - d. Unsatisfactory
70. If you are not satisfied, how would you want to improve this?
71. If you have any final comments, please note them here

This is the end of the survey. We would like to thank you for your cooperation and time.

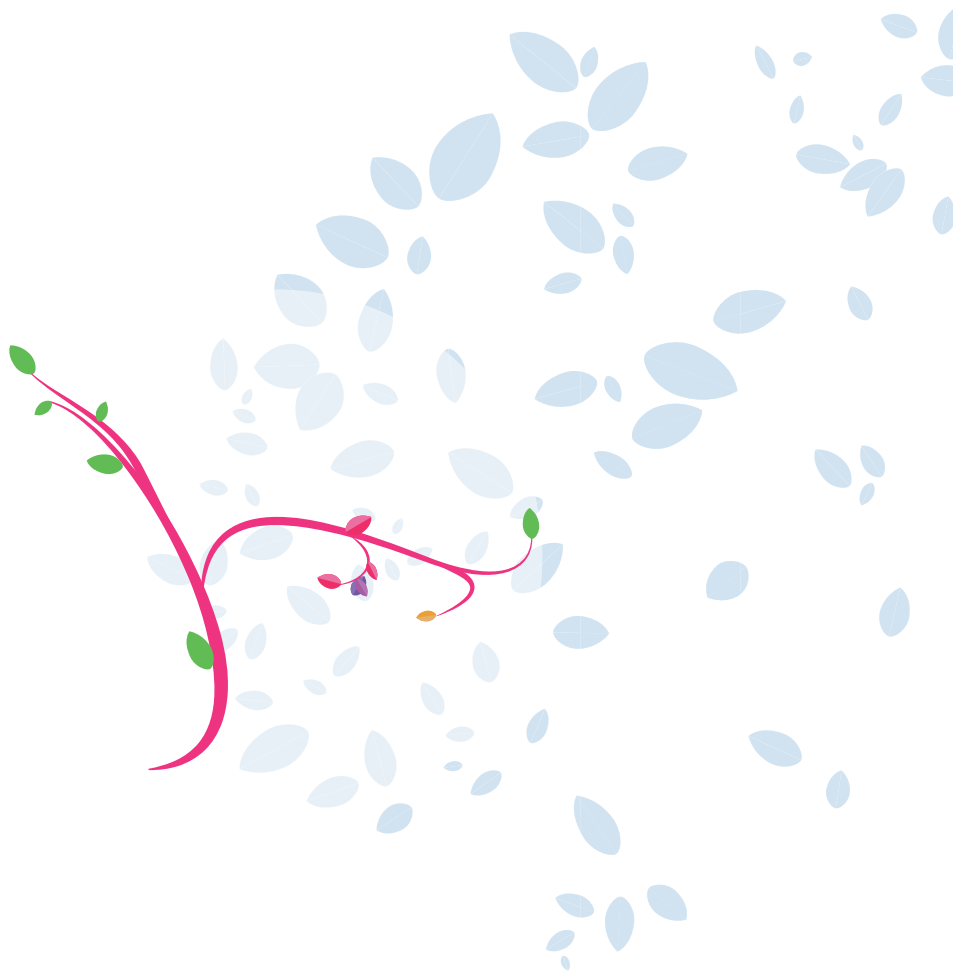
END of the survey





PART II

INCIDENCE OF BRAIN LESIONS IN MODERATE-LATE PRETERM INFANTS



CHAPTER 4

Incidence of brain lesions in moderate-late preterm infants assessed by cranial ultrasound and MRI: The BIMP-study



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ABSTRACT

Purpose To evaluate the incidence and characteristics of brain lesions in moderate-late preterm (MLPT) infants, born at 32–36 weeks' gestation using cranial ultrasound (cUS) and magnetic resonance imaging (MRI).

Methods Prospective cohort study carried out at Isala Women and Children's Hospital between August 2017 and November 2019. CUS was performed at postnatal day 3–4 (early-cUS), before discharge and repeated at term equivalent age (TEA) in MLPT infants born between 32+0 and 35+6 weeks' gestation. At TEA, MRI was also performed. Several brain lesions were assessed e.g. hemorrhages, white matter and deep gray matter injury. Brain maturation was visually evaluated. Lesions were classified as mild or moderate-severe. Incidences and confidence intervals were calculated.

Results 166 MLPT infants were included of whom 127 underwent MRI. One or more mild lesions were present in 119/166 (71.7%) and moderate-severe lesions in 6/166 (3.6%) infants on cUS and/or MRI. The most frequent lesions were signs suggestive of white matter injury: inhomogeneous echogenicity in 50/164 infants (30.5%) at early-cUS, in 12/148 infants (8.1%) at TEA-cUS and diffuse white matter signal changes (MRI) in 27/127 (23.5%) infants. Cerebellar hemorrhage (MRI) was observed in 16/127 infants (12.6%). Delayed maturation (MRI) was seen in 17/117 (13.4%) infants. Small hemorrhages and punctate white matter lesions were more frequently detected on MRI than on cUS.

Conclusions In MLPT infants mild brain lesions were frequently encountered, especially signs suggestive of white matter injury and small hemorrhages. Moderate-severe lesions were less frequently seen.

INTRODUCTION

Infants born prematurely (gestational age [GA] < 37 weeks) are at risk of abnormal neurodevelopmental outcome (1). The association between brain lesions and neurodevelopmental disabilities has extensively been investigated in very preterm infants (GA < 32 weeks) (2–4).

While there is increasing awareness of neurodevelopmental problems in moderate-late preterm (MLPT) infants, born at GA 32–36 weeks, there is only scarce attention for brain lesions in these infants. They have a two-fold higher incidence of developmental delay, and more problems with fine motor skills, communication and personal-social functioning at preschool age as compared to full-term infants. At school age, they have a higher incidence of grade retention and need for special education (5–7). Some of these problems may possibly be related to brain lesions acquired during the perinatal or neonatal period.

To optimize functional outcome of MLPT infants by prevention, intervention or supportive care, more knowledge about the incidence and characteristics of acquired brain lesions and of brain development is needed.

So far, only a few studies have described brain lesions in MLPT infants (8–11). These studies focused mainly on intraventricular hemorrhage (IVH) and cystic white matter lesions. Furthermore, these studies were generally performed in selected cohorts, consisting of infants admitted to the neonatal intensive care unit or not all included infants had a cranial ultrasound (cUS) assessment.

While magnetic resonance imaging (MRI) is most sensitive and reliable for the detection of brain injury in (preterm) infants (12), especially for small and/or subtle lesions, cUS is the primary neuro-imaging modality in this population. cUS is patient friendly, portable, relatively cheap and allows for early and serial imaging (13). It is, however, unknown whether cUS enables the detection of potentially clinically relevant (subtle) brain lesions in MLPT infants. None of the previous studies on brain lesions in MLPT infants combined cUS and MRI (11).

The primary aims of our study were 1) to evaluate the incidence of brain lesions including delayed brain maturation (myelination and gyration) and 2) to compare brain-imaging findings on cUS and MRI in MLPT infants. We hypothesized (subtle) brain lesions, including delayed brain maturation to be frequently present in MLPT infants.

METHODS

Study design and setting

This study is part of the BIMP-study ('Brain Imaging in Moderate-late Preterm infants', The Netherlands trial register; NL6310): a prospective cohort study conducted in MLPT infants at

Isala Women and Children's Hospital (Zwolle, the Netherlands). Ethical approval was given by the Central Committee in Research Involving Human Subjects, The Hague, The Netherlands (NL52323.075.15). Data were collected between August 2017 - November 2019.

Participants

Infants born at GA 32+0 - 35+6 weeks and admitted to the neonatal ward or intensive care unit were eligible. Exclusion criteria were congenital malformations of the central nervous system, chromosomal disorders, inborn errors of metabolism, congenital infections, and significant language barrier. Signed informed consent was obtained from both parents.

Data collection and outcome

Clinical characteristics (GA, birth weight, multiple gestation, admission to neonatal ward or intensive care unit) were collected from medical charts.

cUS-protocol

cUS was performed at three time-points: early-cUS (postnatal day 3–4), discharge-cUS (shortly before discharge) and term equivalent age (TEA)-cUS (preferably at postmenstrual age [PMA] 38–44 weeks). If discharge was within three days after early-cUS, discharge-cUS was omitted. Serial-cUS was defined as ≥ 2 cUS-examinations, one of which TEA. If the same lesion was seen on all serial cUS-examinations, this was treated as one lesion. cUS was performed by a research physician (VB) or pediatrician (MKR). They were intensively trained in neonatal cUS prior to the start of the study by GvWM, neonatologist with >25 years of experience in neonatal neuroimaging (VB and MKR both performed and assessed > 100 cUS under supervision, and both attended a course on cUS). Scans were performed with a Hitachi Aloka Prosound Alpha 7 Premier system using a multifrequency convex transducer, set at 8 MHz. The brain was visualized through the anterior and mastoid fontanelles. Images were recorded of six coronal and five sagittal planes using the anterior window and at least one coronal and one axial plane using the mastoid window (13). Additionally, (suspected) lesions were recorded in two image directions. Scans were assessed during and immediately after the procedure by the examiner (VB or MKR), checking for lesions with likely clinical consequences that might need medical intervention. Images were digitally stored for assessment with special attention to the brain lesions listed in Table 1. Inhomogeneous echogenicity was defined as areas within the periventricular white matter with inhomogeneous echogenicity, the echogenicity being equal to or exceeding that of the choroid plexus (20). Scans were classified by three investigators (VB, MKR and GvWM) using Clinical Assistant (version 6.1; RVC Medical IT BV, Baarn, the Netherlands). The cUS investigators were blinded to the MRI findings.

MRI-protocol

MRI was performed during natural sleep, <1 h after the TEA-cUS. Infants were fed shortly before scanning, swaddled and placed in a vacuum-bag immobilizer (MedVac® bag, CFI Medical Solutions/Contour Fabricators, Fenton, Michigan, USA). Noise protection consisted of MiniMuffs (Natus Medical Incorporated, Foster City, California, USA), a headphone (EMS for kids, Hornchurch, UK), and a polystyrene noise insulating coil cover. Infants were video-

monitored. Heart rate and oxygen saturation were continuously measured (Invivo Precess MRI compatible monitor, Invivo corporation, Orlando, FL, USA).

A 3 T Philips Ingenia MR system (Philips Medical Systems, Best, The Netherlands) was used. Three-dimensional T1- (slice thickness: 2 mm), coronal and transverse T2- (slice thickness 2 mm) weighted images, Diffusion Weighted Imaging (DWI; slice thickness: 3 mm), and Susceptibility Weighted Imaging (SWI; slice thickness: 1 mm) were performed.

Immediately after the procedure, the scans were assessed by MFB, a radiologist with >11 years of experience in pediatric imaging, checking for lesions with likely clinical consequences that might need medical intervention. Subsequently, three investigators (VB, GvWM and JN; the latter is a radiologist with >3 years of experience) evaluated the MRI scans by consensus. Investigators were unaware of (JN and MFB) or blinded to (VB, GvWM) the clinical course and cUS findings of the infants. The MRI scans were assessed with special attention to the brain lesions mentioned in Table 1. Diffuse white matter signal changes were defined as inhomogeneous and/or increased signal intensity within the white matter on T2-weighted images (21).

SWI and DWI are part of the standard scanning protocol for neonatal neuroimaging in our institution and support the accuracy of detection and investigation of brain injury. A distinction was made between hemorrhagic and ischemic punctate lesions using the SWI and DWI sequences. SWI was used to confirm hemorrhagic lesions, especially punctate lesions and the DWI sequence to determine timing of onset of ischemic lesions.

Classification of brain lesions

The following brain lesions were considered moderate-severe: IVH grade III (14), post-hemorrhagic ventricular dilatation (16), limited or massive cerebellar hemorrhage (CBH) (17), cystic white matter lesions, ≥ 6 punctate white matter lesions (PWML) (23), periventricular hemorrhagic infarction, arterial infarction, moderate-severe ex-vacuo ventricular dilatation, moderate-severe deep gray matter lesions. All other lesions mentioned in Table 1 were considered mild.

Statistics

Data-analyses were performed using SPSS software (version 26.0; SPSS inc, Chicago, Illinois, USA). Mean and SD were reported for normally distributed continuous variables. Differences in GA, birth weight and sex between participating and non-participating eligible infants were explored using the unpaired T-test or Chi-squared test. The incidences of brain lesions on cUS and MRI were calculated, including asymptotic continuity-corrected 95 % confidence intervals (CI). For both serial-cUS and MRI, the incidences of mild and moderate brain lesions were compared per GA week using the Chi-squared test. Furthermore, for each lesion we recorded whether it was seen on TEA-cUS and TEA-MRI, only/more on TEA-cUS or only/more on TEA-MRI. The level of significance was 5 %. Bias due to missing data for presence of lesions on cUS-discharge and to evaluate differences between infants with and without MRI was investigated using the Chi-squared test.

Table 1. Assessment of cUS and MRI.

cUS		MRI	
Hemorrhages		Hemorrhages	
-	Intraventricular hemorrhage (IVH) grade I-III According to Volpe et al. (14). Complications of IVH <ul style="list-style-type: none">- Periventricular hemorrhagic infarction Defined as an area of increased echogenicity adjacent to the lateral ventricle, co-occurring with and complicating IVH, evolving into a porencephalic cyst or several smaller cysts over a variable period of time (1-3 weeks) (15).	-	(Remnants of) Intraventricular hemorrhage (IVH) Defined as presence of hemosiderin deposits at the level of the germinal matrix and/or in the lateral ventricular wall and/or the occipital horn of the lateral ventricles. Complications of IVH <ul style="list-style-type: none">- Periventricular hemorrhagic infarction Defined as an area of abnormal signal intensity and/or cystic degeneration adjacent to or communicating with the lateral ventricle, co-existing with IVH or remnants of IVH (15).
-	Post-hemorrhagic ventricular dilatation Defined as ventricular index > P97 for PMA and/or anterior horn width > 6 mm (16).	-	Post-hemorrhagic ventricular dilatation Adapted for MRI from Brouwer et al. (16).
-	Choroid plexus hemorrhage Defined as a focal area of increased echogenicity within the choroid plexus.	-	Choroid plexus hemorrhage Defined as presence of hemosiderin deposits within the choroid plexus.
-	Cerebellar hemorrhage (CBH) Defined as increased echogenicity within the cerebellar parenchyma, categorized as 1) Punctate CBH: small lesions ≤ 4 mm; 2) Limited CBH: lesions > 4 mm but smaller than 1/3 of the cerebellar hemisphere; 3) Extensive CBH: lesions involving > 1/3 of the cerebellar hemisphere, according to Meijjer and Steggerda (13).	-	Cerebellar hemorrhage (CBH) Defined as hemosiderin deposits within the cerebellar parenchyma, categorized as 1) Punctate CBH: small lesions ≤ 4 mm 2) Limited CBH: lesions > 4 mm but smaller than 1/3 of the cerebellar hemisphere; 3) Extensive/massive CBH: lesions involving > 1/3 of the cerebellar hemisphere (17).
-	Subdural hemorrhage	-	Subdural hemorrhage

Table 1. Continued.

cUS		MRI	
White matter		White matter	
-	Cystic white matter lesions According to de Vries et al. (18).	-	Cystic white matter lesions According to Kidokoro et al. (19).
-	Inhomogeneous echogenicity Defined as inhomogeneous areas within the periventricular white matter with echogenicity equal to or exceeding that of the choroid plexus, adapted from Van Wezel-Meijler et al. (20).	-	Diffuse white matter signal changes Defined as inhomogeneous and/or increased signal intensity within the white matter on T2-weighted images, adapted from De Bruïne et al. (21).
-	Suspected punctate white matter lesions (PWML) – <i>only at cUS-TEA</i> Defined as small, focal echogenic spots within the white matter (22).	-	Punctate white matter lesions (PWML) Defined as focal areas of increased signal intensity within the brain white matter on T1-weighted images (adapted from Martínez-Biarge et al. [23]).
Arterial Infarction Defined as an area of focal or more diffusely increased echogenicity within the cerebral hemispheres in the territory of (branches of) one of the major cerebral arteries, followed by cystic evolution (24).		Arterial Infarction Defined as an area of focal abnormal signal intensity and/or volume loss within the cerebral hemispheres in the territory of (branches of) one of the major cerebral arteries (24).	
Deep gray matter lesions Defined as inhomogeneous increased and/or focal echogenicity within the basal ganglia and /or thalami, not indicating arterial infarction (see above), subdivided in small focal abnormality (mild) or moderate-serious abnormality (moderate-severe).		Deep gray matter lesions Defined as abnormal signal intensity in the basal ganglia and /or thalami, not indicating arterial infarction (see above), subdivided in small focal abnormality (mild) or moderate-serious abnormality (moderate-severe).	

Table 1. Continued.

cUS		MRI	
Miscellaneous		Miscellaneous	
-	Choroid plexus cysts ≥ 6 mm (25).	-	Choroid plexus cysts ≥ 6 mm (25).
-	Germinolytic/subependymal cysts ≥ 6 mm (25).	-	Germinolytic/subependymal cysts ≥ 6 mm (25).
-	Lenticulostriate vasculopathy (LSV) Defined as thick and hyperechoic lines within the basal ganglia and/or thalamus around the lenticulostriate vessels on coronal and parasagittal views (category D according to Sisman et al. [26]).		
Signs suggestive of brain atrophy due to white matter injury		Signs suggestive of brain atrophy due to white matter injury	
-	Ex-vacuo ventricular dilatation Mild dilatation: defined as uni- or bilateral ventricular index 13 - 15 mm; Moderate-severe dilatation: defined as uni- or bilateral ventricular index > 15 mm. The ventricular index was measured in the coronal plane at the level of the foramen of Monro. (27).	-	Ex-vacuo ventricular dilatation Mild dilatation: defined as uni- or bilateral ventricular index 13 - 15 mm; Moderate-severe dilatation: defined as uni- or bilateral ventricular index > 15 mm. The ventricular index was measured on a coronal T2-weighted scan at the level of the foramen Monro. Adapted for MRI (28).
-	Irregular shape of the lateral ventricles See figure 2-I and and Leijser et al. (12), the shape was best assessed on sagittal images.	-	Irregular shape of the lateral ventricles See figure 4-H and Leijser et al. (12).
-	Widened interhemispheric fissure Defined as interhemispheric fissure > 3 mm. The interhemispheric fissure was measured as the maximum width between the hemispheres from the surface of opposite gyri, in the coronal plane at the level of the foramen of Monro (27).	-	Widened interhemispheric fissure Defined as interhemispheric fissure > 3 mm. The interhemispheric fissure was measured as the maximum width between the hemispheres from the surface of opposite gyri, on a coronal T2-weighted image at the level of the foramen of Monro. Adapted for MRI (27).

Table 1. Continued.

cUS	MRI
	Delayed maturation
	- Delayed myelination of the posterior limb of the internal capsule Defined as absence or insufficient myelination of the posterior 1/3 part of the posterior limb of the internal capsule on T1-weighted images (29).
	- Delayed gyration Defined as visually assessed delayed gyration on T1- and T2-weighted images as compared to normal reference images (29).

RESULTS

Participants

During the study period, 404 MLPT infants were admitted to the neonatal ward ($n = 298$) or neonatal intensive care unit ($n = 106$). Consent was obtained from parents of 167 infants (Figure 1). One infant was excluded after informed consent (chromosomal disorder apparent at later stage). There were no significant differences in GA, sex and birth weight between participating and non-participating infants. See Table 2 for general characteristics of included infants.

Neuro-imaging

One or more brain lesion(s) were present on cUS and/or MRI in 125/166 infants (75.3 % [95 % CI 67.9–81.5 %]). In 119 infants these were considered mild (71.7 % [95 % CI 64.1–78.3 %]) and in six moderate to severe (3.6 % [95 % CI 1.5–8.1 %]).

Early-cUS

Imaging quality was insufficient in two infants. Therefore, early-cUS was assessed of 164 infants (median: 4 days; range: 2–6 days). Of these, 109 (66.5 % [95 % CI 58.6–73.5 %]) had no brain lesions, 48 (29.3 % [95 % CI 22.6–37.0 %]) one lesion, and seven (4.3 % [95 % CI 1.9–8.9 %]) two or more brain lesions. All lesions were considered mild (Figure 2).

Discharge-cUS

Forty-four infants did not have discharge-cUS: 37 were transferred to another hospital or discharged home within three days after early-cUS, in five infants discharge-cUS was not performed (missed cases) and in two cases image quality was insufficient. Altogether, discharge-cUS was performed in 122 infants. Mean PMA was 36.0 (SD 1.1) weeks. On discharge-cUS 88 infants (72.1 % [95 % CI 63.2–79.7 %]) had no, 29 (23.8 % [95 % CI 16.7–32.5 %]) had one, and five (4.1 % [95 % CI 1.5–9.8 %]) had two or more brain lesion(s). All lesions were considered mild (Figure 2).

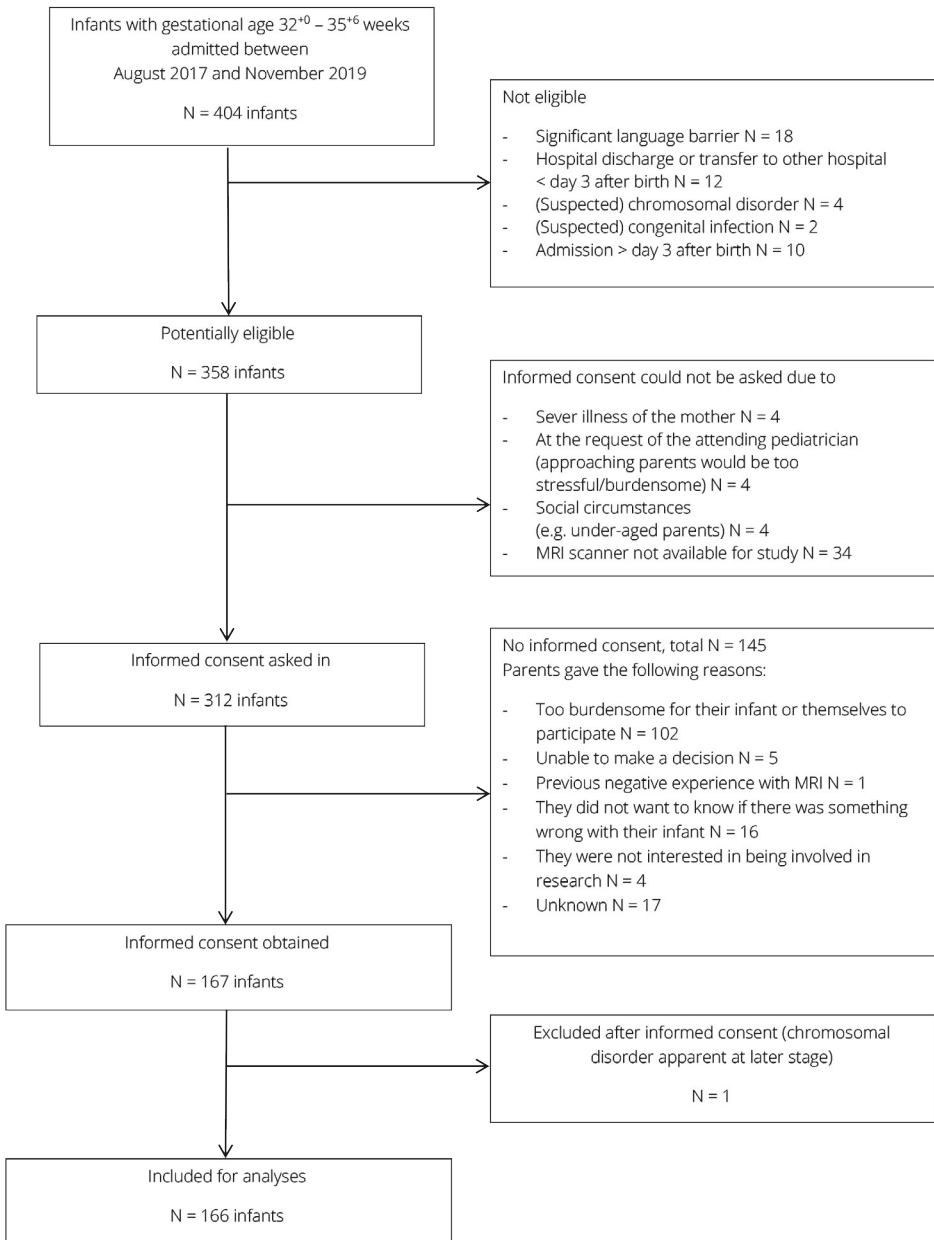
Figure 1. Flow-chart.

Figure 2. Coronal and sagittal/axial images of mild brain lesions on cUS in MLPT infants.

A) Subependymal germinal matrix hemorrhage (arrow); B) Punctate cerebellar hemorrhage (arrow); C) Bilateral inhomogeneous periventricular echogenicity (arrows); D) Subependymal germinal matrix hemorrhage (arrow); E) Punctate white matter lesion; F) Choroid plexus cyst (arrow); G) Germinolytic cyst (arrow); H) Mild ex-vacuo dilatation, dashed line = measurement of ventricular index; I) Irregular shape (angular instead of round) of the lateral ventricle (arrow), for comparison an image of a normally shaped ventricle is added; J) Widened interhemispheric fissure, dashed line = measurement of interhemispheric fissure.

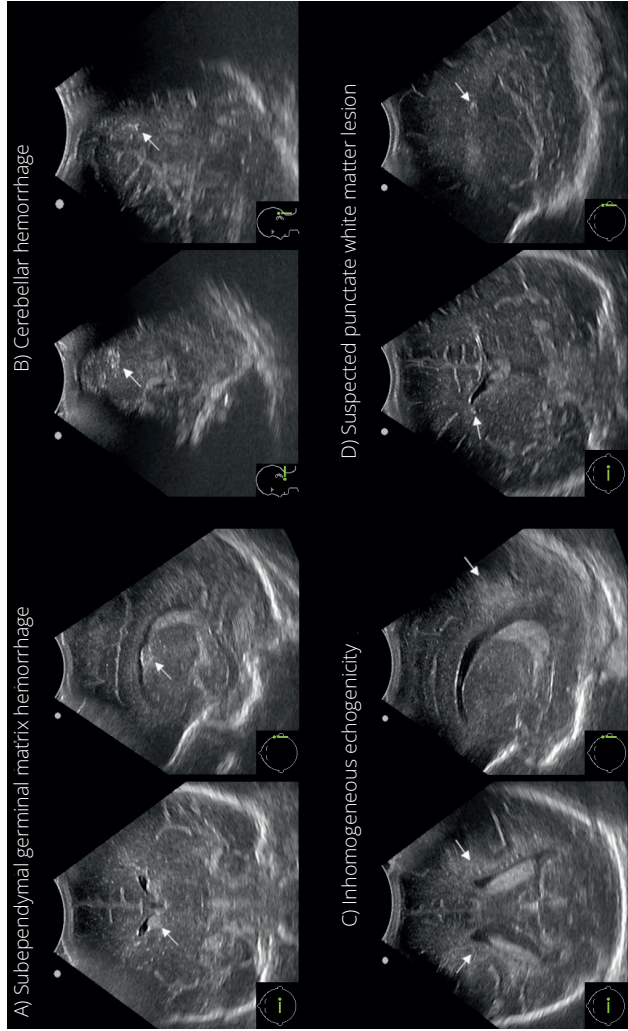
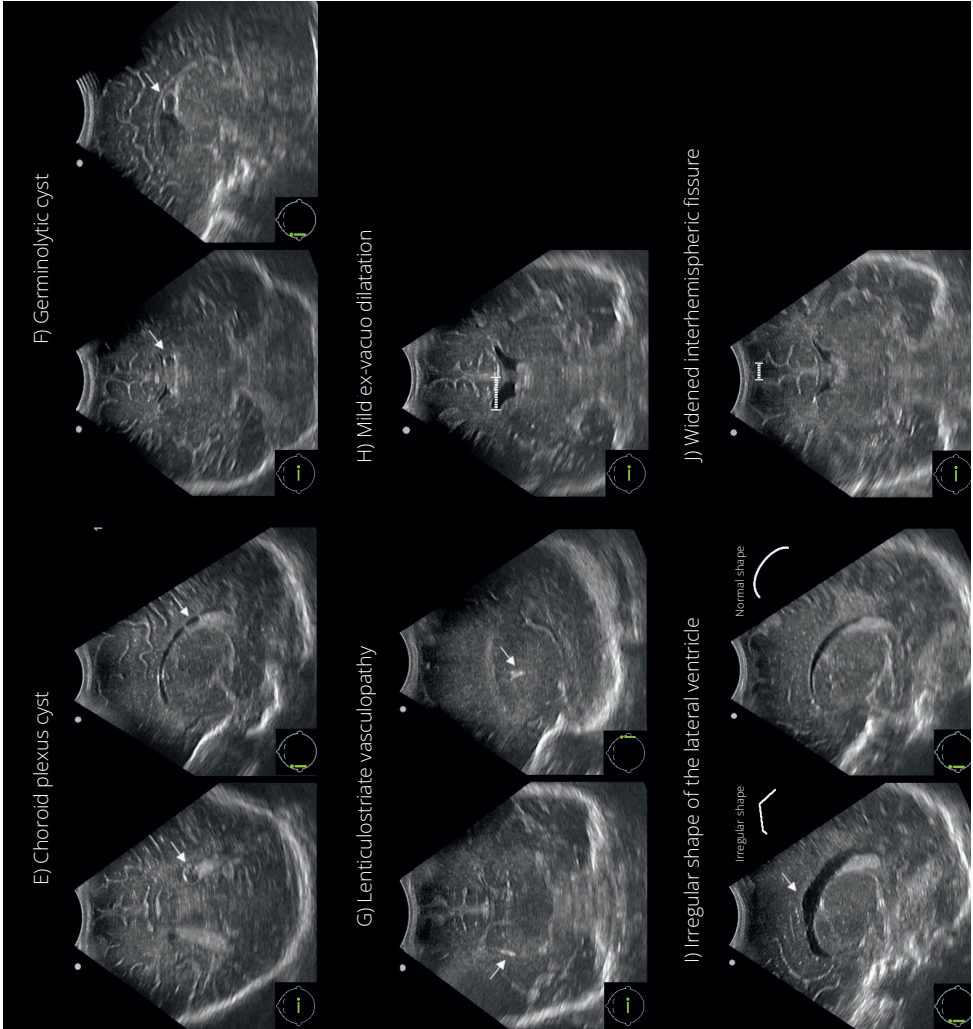


Figure 2. Continued.



TEA-cUS

Parents of eighteen infants considered the TEA-visit too burdensome. Therefore, 148/166 infants underwent TEA-cUS. Mean PMA was 41.3 (SD 2.0) weeks. On TEA-cUS, 71 infants (48.0 % [95 % CI 39.8–56.3 %]) had no, 44 (29.7 % [95 % CI 22.7–37.9 %]) had one, and 33 (22.3 % [95 % CI 16.1–30.0 %]) had two or more brain lesion(s). In three infants (2.0 % [95 % CI 0.5–6.3 %]) lesions were considered moderate-severe (i.e. one with periventricular hemorrhagic infarction, two with ex-vacuo ventricular dilatation) (Figure 3). The infant with periventricular hemorrhagic infarction had a normal early-cUS and unfortunately discharge-cUS was not performed due to an unexpected early discharge (one of the missed cases mentioned above).

Serial-cUS

Serial-cUS was available of 146 infants (36 infants with two and 110 infants with three cUS examinations). Of these, 43 infants (29.5 % [95 % CI 22.4–37.7 %]) had no, 59 (40.4 % [95 % CI 32.5–48.9 %]) had one, and 44 (30.1 % [95 % CI 23.0–38.4 %]) had two or more brain lesion(s). In three infants (2.1 % [95 % CI 0.5–6.4 %]) lesions were considered moderate-severe (the same as mentioned under TEA-cUS). Table 3 shows the incidences and characteristics of brain lesions on early-, discharge- and TEA-cUS.

MRI

In addition to the eighteen infants lost for TEA-visit, parents of 21 infants did not consent to MRI, resulting in 127 infants with MRI. PMA at MRI was 41.5 (SD 2.0) weeks. MRI quality was excellent in 102/127 infants. In 25/127 infants not all brain lesions (described in Table 1) could be scored due to movement artefacts or missing MRI sequences. SWI was available of 117/127 infants. The incidences of brain lesions including delayed brain maturation are shown in Table 4. Of 127 infants, 34 had no lesion (26.8 % [95 % CI 19.5–35.5%]), 43 (33.9 % [95 % CI 25.9–42.9 %]) had one, and 50 (39.4 % [95 % CI 30.9–48.5 %]) had two or more lesion(s). Figure 4 shows examples of mild brain lesions including delayed brain maturation. Six infants (4.7 % [95 % CI 1.9–10.4 %]) had moderate-severe brain lesions (i.e. one periventricular hemorrhagic infarction, one posterior cerebral artery infarction and ex-vacuo ventricular dilatation, one isolated ex-vacuo dilatation, and three ≥ 6 PWML) (Figure 3).

Comparison serial cUS and MRI per gestational age week

In infants born at 32 weeks' gestation we found the highest incidence of mild brain lesions on serial cUS and MRI, but differences with other GA groups were small and not significant ($p = 0.39$ and $p = 0.08$ respectively). Three out of the six moderate-severe lesions were seen in the 35 weeks GA group (Table 4)

Figure 3. Cases with moderate-severe brain lesions

A) CUS and MRI of an infant (GA 32⁺³ weeks) scanned at PMA 38⁺⁶ weeks with sagittal cUS-TEA showing inhomogeneous echogenicity (arrows) and T1-weighted MRI showing clusters of PWML (arrows); B) Coronal and sagittal view of TEA-cUS and T2-weighted transverse MRI of an infant (GA 33⁺⁴ weeks) performed at PMA 39⁺⁶ weeks showing remnants of IVH (long arrow) with cystic sequelae of PHVI (short arrow) and focal echogenicity in the caudate nucleus (arrowhead), corresponding with abnormal signal intensity on MRI (long arrow). C) Coronal view of TEA-cUS and transverse MRI of an infant (GA 35⁺⁶ weeks) performed at PMA 42⁺⁵ weeks. TEA-cUS shows moderate-severe ex-vacuo dilatation (ventricular index > 15 mm), but no clear signs of a posterior cerebral arterial infarction. On the transverse T2-weighted MRI a high intensity signal and some loss of grey-white matter differentiation (arrow) is seen in the territory of the left posterior cerebral artery, suggesting a posterior cerebral arterial infarction. No acute signs of diffusion restriction were seen on diffusion weighted imaging, we therefore assume the infarction developed around birth (> 1 week before the MRI was performed).

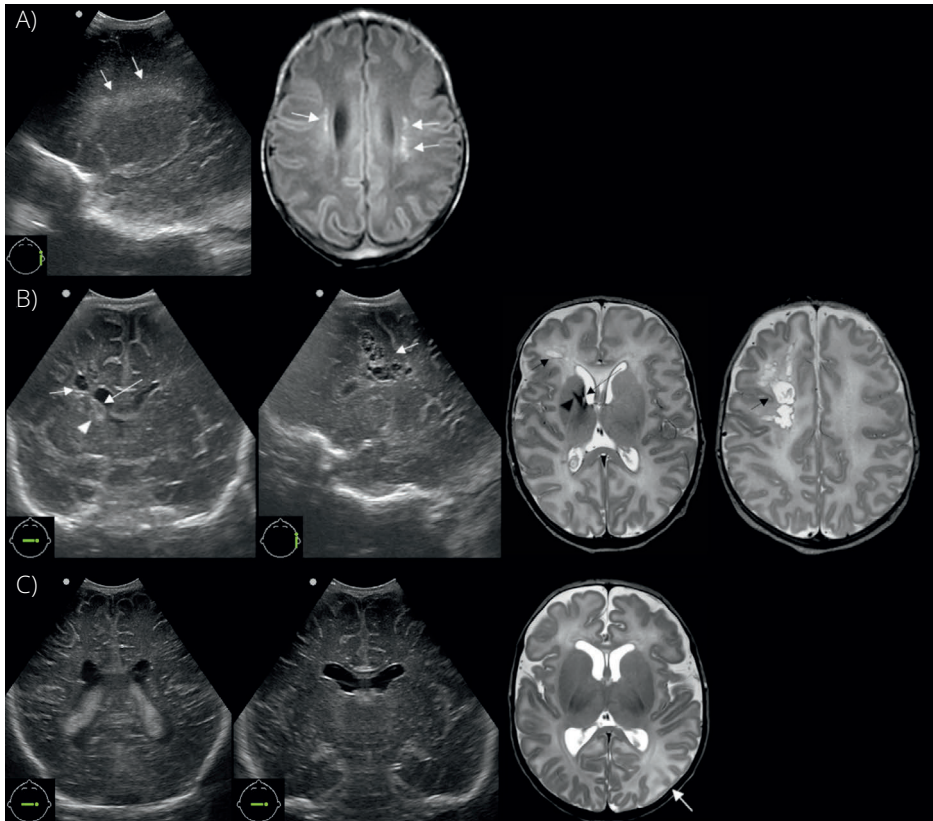


Table 3. Incidences of brain lesions on cUS.

Brain lesions		Early-cUS N = 164 n (%) [95% CI]	Discharge-cUS N = 122 n (%) [95% CI]	TEA-cUS N = 148 n (%) [95% CI]
Hemorrhages				
IVH	Total	8 (4.9 [2.3 – 9.7])	10 (8.2 [4.2 – 14.9])	7 (4.7 [2.1 – 9.9])
	Grade I	6 (3.7 [1.5 – 8.2])	8 (6.6 [3.1 – 12.9])	6 [#]
	Grade II	2 (1.2 [0.2 – 4.8])	2 (1.6 [0.3 – 6.4])	1 [#]
	Grade III	0	0	0
Periventricular hemorrhagic infarction		0	0	1 (0.7 [0.04 – 4.3])
Post-hemorrhagic ventricular dilatation		0	0	0
Choroid plexus hemorrhage		0	0	0
CBH [†]	Punctate	0	0	1 (0.7 [0.04 – 4.2])
	Limited	0	0	0
	Extensive/Massive	0	0	0
Subdural hemorrhage		0	0	0
White matter				
Cystic white matter lesions		0	0	0
Inhomogeneous echogenicity		50 (30.5 [23.7 – 38.2])	23 (18.9 [12.6 – 27.2])	12 (8.1 [4.5 – 14.1])
Suspected PWML		NA	NA	7 (4.7% [2.1 – 9.9])
Infarction				
Arterial infarction		0	0	0
Deep gray matter lesions [†]	Total	0	0	1 (0.7 [0.04 – 4.3])
	Small focal lesion	0	0	1 (0.7 [0.04 – 4.3])
	Moderate - severe lesion	0	0	0
Miscellaneous				
Choroid plexus cyst ≥ 6 mm		1 (0.6 [0.03 – 3.9])	1 (0.8 [0.04 – 5.2])	3 (2.0 [0.5 – 6.3])
Germinolytic or subependymal cyst ≥ 6 mm		4 (2.4 [0.8 – 6.5])	4 (3.3 [1.1 – 8.7])	11 (7.4 [4.0 – 13.2])
Lenticulostriate vasculopathy [†]		0	1 (0.8 [0.04 – 5.2])	1 (0.7 [0.04 – 4.3])
Signs suggestive of brain atrophy due to injury				

Table 3. Continued.

Brain lesions	Early-cUS N = 164 n (%) [95% CI]	Discharge-cUS N = 122 n (%) [95% CI]	TEA-cUS N = 148 n (%) [95% CI]
Ex-vacuo ventricular dilatation*	Mild (13 – 15 mm) Moderate-severe (> 15 mm)	NA NA	34 (23.1 [16.8 – 30.9]) 2 (1.4 [0.2 – 5.3])
Irregular shape of the lateral ventricles*	NA	NA	23 (15.6 [10.4 – 22.8])
Widened interhemispheric fissure*	NA	NA	24 (16.3 [10.9 – 23.5])

Percentages were adjusted for number of missing data. CBH = cerebellar hemorrhage; IVH = intraventricular hemorrhage; NA = not assessable; PWMML = punctate white matter lesions. * Considered as ‘remnants of IVH’ at TEA. In one infant both (remnants of) IVH and a germinolytic cyst were present. † CBH, echogenicity in the basal ganglia and/or thalami and lenticulostriate vasculopathy and could not be assessed due to poor imaging quality in 3 infants on early-cUS and in 1 infant on discharge-cUS. * Ex-vacuo ventricular dilatation, irregular shape of the lateral ventricles and widened interhemispheric fissure at TEA could not be determined in one infant due to poor imaging quality.

Table 4. Overview of infants with none, mild or moderate severe brain lesions on serial cUS and MRI per GA group.

GA group	32 weeks N = 27	33 weeks N = 38	34 weeks N = 48	35 weeks N = 53
	Serial cUS	Serial cUS	Serial cUS	Serial cUS
None, n (%)	7 (25.9%)	9 (23.7%)	18 (37.5%)	9 (17.0%)
[95% CI]	[11.8 – 46.6]	[12.0 – 40.6]	[24.3 – 52.7]	[8.5 – 30.3]
Mild, n (%)	17 (63.0%)	22 (57.9%)	27 (56.3%)	33 (62.3%)
[95% CI]	[42.5 – 79.9]	[39.0 – 77.0]	[41.3 – 70.2]	[47.9 – 74.9]
Moderate – severe, n (%)	0	1 (3.7%)[0.2 – 20.8]	1 (2.6%)	2 (3.8%)
[95% CI]		[0.1 – 15.4]	[0.1 – 15.4]	[6.5 – 14.1]
Missing data, n (%)	3 (11.1%)	6 (15.8%)	7 (14.6%)	9 (17.0%)
[95% CI]	[2.9 – 3.0]	[6.6 – 31.9]	[0.7 – 15.4]	[8.5 – 30.3]

Figure 4. MRI images of mild brain lesions in MLPT infants.

A) Subependymal hemorrhage/intraventricular hemorrhage grade I (arrow); B) Choroid plexus hemorrhage (arrow); C) Multiple punctate cerebellar hemorrhages; D) Diffuse white matter signal changes (arrows), for comparison a T2-weighted image with normal signal intensity is added; E) Small PWML (arrow); F) Germinolytic cyst (> 6 mm) (arrow); G) Mild ex-vacuo dilatation, dashed line = measurement of ventricular index; H) Irregular shape of the posterior limb of the lateral ventricle (arrows); I) Widened interhemispheric fissure, dashed line = measurement of interhemispheric fissure; J) Delayed myelination of the posterior limb of the internal capsule (arrow) in an infant scanned at PMA 41+6 weeks; K) T1-weighted image showing delayed gyration, especially in the frontal lobes (arrows), as compared to normal gyration reference images of Barkovich and Mukherjee (29). For comparison a T1-weighted image showing normal gyration in another infant, scanned at the same PMA. PMA = postmenstrual age; SWI = susceptibility weighted imaging.

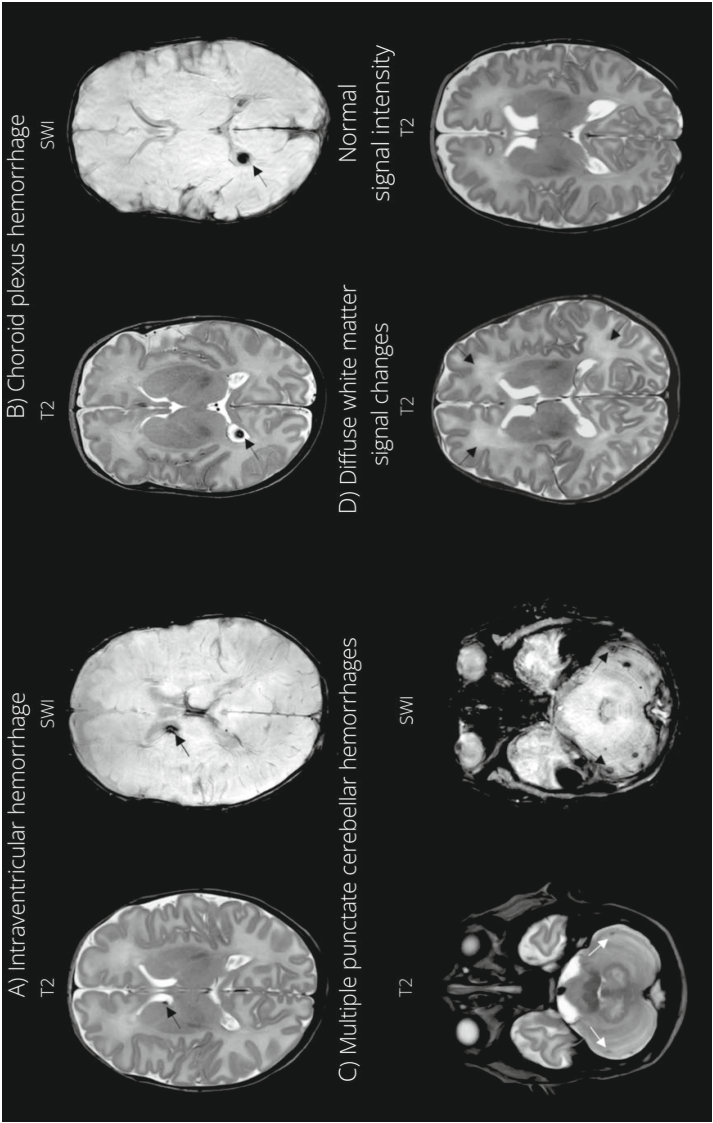
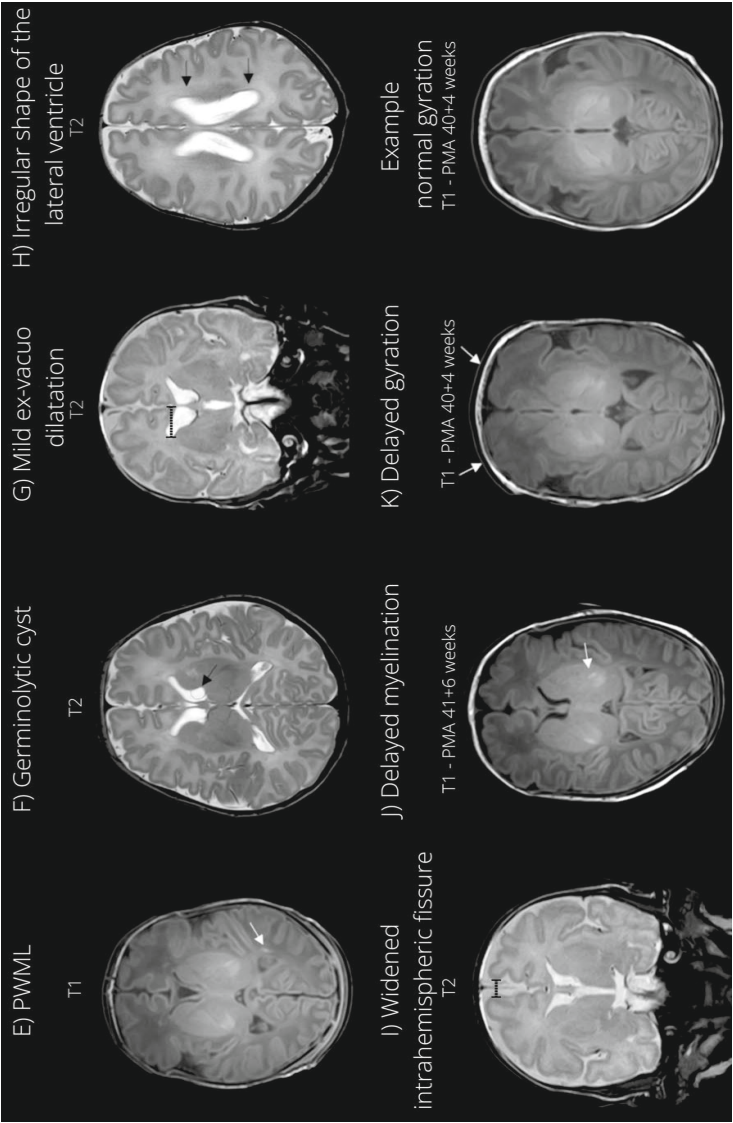


Figure 4. Continued.



Comparison cUS TEA and MRI per brain lesion

One hundred and twenty-seven infants had TEA-cUS and MRI. There were no significant differences between infants with and without MRI in number of brain lesions on early- (p = 0.55), TEA- (p = 0.66) or serial cUS (p = 0.92). Table 5 shows whether brain lesions were found on both TEA-cUS and MRI, only/more on TEA-cUS or only/more on MRI.

In three infants, moderate-severe lesions were found on both TEA-cUS and MRI (periventricular hemorrhagic infarction in one, moderate-severe ex-vacuo ventricular dilatation in two). One of the infants with ex-vacuo dilatation also had an arterial infarction, which was missed on TEA-cUS (Figure 3). Additionally, in three infants ≥ 6 PWML were detected with MRI.

Remnants of IVH were seen in four infants on TEA-cUS, and confirmed with SWI in three infants. In one infant with remnants of IVH on TEA-cUS, a small plexus hemorrhage was seen on MRI. In nine additional infants, hemorrhages undetected with cUS were encountered on MRI (remnants of IVH in five, choroid plexus hemorrhages in four infants; Figure 5). Except for one infant, punctate CBH was only seen on MRI.

Inhomogeneous echogenicity was seen in 47/127 infants on serial cUS. In 12/47 infants this was still present at TEA. At TEA-cUS this corresponded with diffuse white matter signal changes on MRI in 6/12 infants. Diffuse white matter signal changes were encountered in 21 additional infants without signs of inhomogeneous echogenicity on TEA-cUS. Fifteen of those had never experienced inhomogeneous echogenicity on serial cUS. PWML were seen in 20 infants on MRI and suspected in seven on TEA-cUS (Figure 5). Of these 20 infants, nine had inhomogeneous echogenicity on TEA-cUS. Fourteen of these 20 infants experienced inhomogeneous echogenicity on serial-cUS. Choroid plexus cysts (≥ 6 mm) were seen on TEA-cUS in two infants, while not on MRI (Figure 5). LSV was seen in one infant on TEA-cUS, but not on MRI. Signs suggestive of brain atrophy were more frequently seen on MRI.

Table 5. Incidences of brain lesions including delayed maturation on TEA-cUS and MRI and comparison between both techniques.

Brain lesions	TEA-cUS (N = 127) n (%) [95% CI]	MRI (N = 127) n (%) [95% CI]	Both cUS and MRI	Only/ more on cUS	Only/more on MRI
Hemorrhages					
Remnants of IVH [†]	4 (3.2 [1.0 – 8.4])	8 (6.3 [3.0 – 12.4])	3	2	5
Periventricular hemorrhagic infarction	1 (0.8 [0.04 – 5.0])	1 (0.8 [0.04 – 5.0])	1	0	0
Post-hemorrhagic ventricular dilatation	0	0	0	0	0
Choroid plexus hemorrhage [†]	0	5 (3.9 [1.5 – 9.4])	0	0	5
CBH	1 (0.8 [0.04 – 5.0])	16 (12.6 [7.6 – 19.9])	1	0	15
	0	0	0	0	0
	0	0	0	0	0
White matter					
Cystic white matter lesions	0	0	0	0	0
Inhomogeneous echogenicity (cUS)/diffuse white matter signal changes (MRI) [‡]	12 (9.4 [5.2 – 16.3])	27 (23.5 [16.3 – 32.5])	6	5	21
(suspected) PWML [#]		(N = 115)			
Total	7 (5.6 [2.5 – 11.6])	20 (16.4 [10.5 – 24.4])	7	0	13
< 6 PWML	NA	17 (13.9 [8.6 – 21.7])			
≥ 6 PWML	NA	3 (2.5 [0.6 – 7.6])			
	(N = 125)	(N = 122)			
Infarction					
Arterial infarction	0	1 (0.8 [0.04 – 5.0])	0	0	1
Deep gray matter lesions [§]					
Total	1 (0.8 [0.04 – 5.0])	1 (0.8 [0.04 – 5.0])	1	0	0
Small focal lesion	1 (0.8 [0.04 – 5.0])	1 (0.8 [0.04 – 5.0])	1	0	0
Moderate-severe lesion	0	0	0	0	0
		(N = 126)			
Miscellaneous					
Subdural hemorrhage	0	0	0	0	0
Choroid plexus cyst ≥ 6 mm	2 (1.6 [0.3 – 6.1])	0	0	2	0
Germinolytic or subependymal cyst ≥ 6 mm	10 (7.9 [4.1 – 14.4])	9 (7.1 [3.5 – 13.4])	8	2	1
Lenticulostriate vasculopathy	1 (0.8 [0.04 – 5.0])	0	0	1	0

Table 5. Continued.

Brain lesions	TEA-cUS (N = 127) n (%) [95% CII]	MRI (N = 127) n (%) [95% CII]	Both cUS and MRI	Only/ more on cUS	Only/more on MRI
Signs suggestive of brain atrophy due to injury					
Ex-vacuo ventricular dilatation ^β	Mild (13 – 15 mm)	30 (23.8 [16.9 – 32.4])	34 (28.8 [21.0 – 38.0])	20	7
	Moderate-severe (> 15 mm)	2 (1.6 [0.3 – 6.2]) (N = 126)	2 (1.7 [0.3 – 6.6]) (N = 118)	0	0
Irregular shape of the lateral ventricles ^α	22 (17.3 [11.4 – 25.3])	20 (15.9 [10.2 – 23.7]) (N = 126)	15	7	5
Widened interhemispheric fissure ^α	20 (15.9 [10.2 – 23.7]) (N = 126)	29 (24.6 [17.3 – 33.5]) (N = 118)	17	1	12
Delayed brain maturation [*]	NA	17 (14.5 [8.9 – 22.5]) (N = 117)	NA	NA	17
Myelination of the posterior limb of internal capsule < 1/3 [*]	Unilateral	NA	NA	NA	3
	Bilateral	NA	NA	NA	6
Delayed gyration	NA	6 (4.7 [1.9 – 10.4]) (N = 117)	NA	NA	6

Percentages were adjusted for number of missing data.

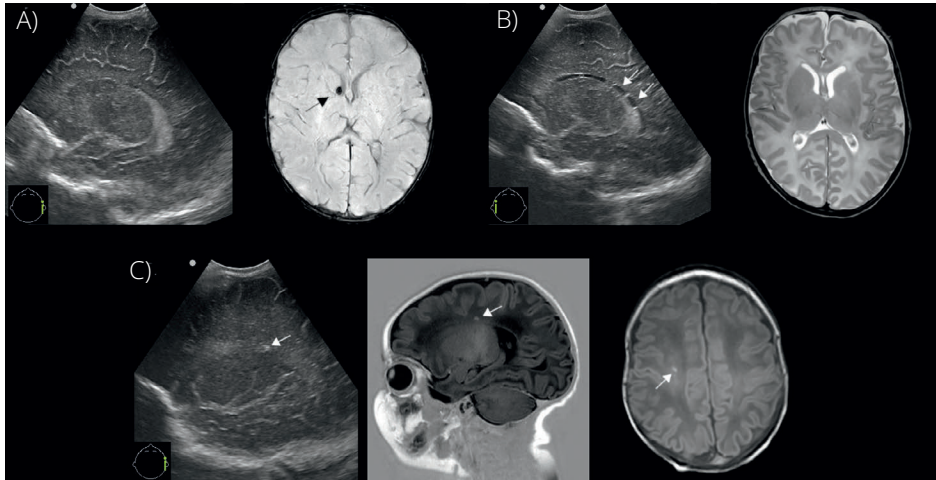
^{*} In one infant, remnants of IVH on cUS turned out to be a choroid plexus hemorrhage on MRI. ^α MRI assessment of diffuse white matter signal changes was missing in 12 infants due to poor imaging quality. One of these infants had inhomogeneous echogenicity on TEA-cUS.

^β Assessment of suspected PWML was missing in 1 infant on cUS and in 5 infants on MRI due to poor imaging quality. ^β cUS measurement was missing in 1 infant and MRI measurement was irregular shape of the lateral ventricles was missing in 1 infant due to poor imaging quality. In three infants with mild ex-vacuo ventricular dilatation on cUS, the lateral ventricles could not be measured on missing in 9 infants due to poor imaging quality. ^γ cUS measurement was missing in 1 infant, MRI measurement was missing in 9 infants due to poor imaging quality. Two infants MRI, due to missing MRI sequences. ^γ cUS measurement was missing in 1 infant, MRI measurement was missing in 9 infants due to poor imaging quality. Two infants with a widened interhemispheric fissure (> 3 mm) on cUS, had no coronal MRI to compare with. ^{*}Missing in 10 infants due to poor imaging quality on MRI.

CBH = cerebellar hemorrhage; IVH = intraventricular hemorrhage; NA = not assessable; PWML = punctate white matter lesions.

Figure 5. Comparisons between cUS and MRI.

A) Sagittal view of a TEA-cUS with no signs of IVH, while SWI shows a low signal intensity, representing remnants of an IVH. B) Several choroid plexus cysts are seen on the sagittal view of the TEA-cUS, while this is not depicted by T2-weighted MRI. C) A small punctate inhomogeneous lesion suspected for a PWML is seen in the frontal lobe on the sagittal view of the TEA-cUS, corresponding to a high signal on the sagittal inversion recovery T1-weighted image and transverse T1 weighted image.

**DISCUSSION**

Performing cranial ultrasound (cUS) and magnetic resonance imaging (MRI) in a cohort of moderate-late preterm (MLPT) infants, has demonstrated a high incidence of brain lesions in this population. Most of these lesions were considered mild. Six infants (3.6 %) had moderate-severe brain lesions.

The incidences of moderate-severe lesions are comparable to those reported by others. Fumagalli et al. found venous and arterial infarction in respectively 0.17 % and 0.34 % in a cohort of 1172 late preterm infants (GA 34+0 -36+6 weeks) (9). Walsh et al. found extensive/linear PWML in 10/199 (5 %) MLPT infants (30). Our and their incidences were, as expected, much lower than those found in very preterm infants (12,19). This is probably related to several factors: MLPT infants are generally vitally stable, the vast majority not needing respiratory support, and their brains are more mature and thus less vulnerable than the brains of very preterm infants (31).

The reported incidences of several types of hemorrhages in our MLPT cohort are different from incidences reported in very preterm and full-term cohorts. The incidence of (remnants of) IVH in our cohort was higher than that reported in full-term infants (range: 2.2 %-5.2

%) (32, 33), but in contrast to very preterm infants, severe IVH was rare. None of the MLPT infants in our cohort experienced post-hemorrhagic ventricular dilatation. Contrary to the low incidence of IVH, the incidence of CBH (12.6 % in our cohort) was similar to reported incidences in very preterm populations (34). Furthermore, subdural hemorrhage was not detected in our cohort, while this is the most often reported hemorrhage in otherwise healthy full-term infants (32,35). These differences with very preterm and full-term infants underpin that MLPT infants should be seen as a different group.

White matter injury or signs suggestive of white matter injury were most frequently seen in our cohort. The incidence of inhomogeneous echogenicity was relatively high, respectively 30.5 % at early-cUS and 8.1 % at TEA-cUS. Fumagalli et al. reported an incidence of 19.6 % at early-cUS and 1.8 % at cUS performed five weeks after birth in a cohort of 1172 late preterm infants (GA 34+0 – 36+6 weeks) (9). More than half of their cohort consisted of “more mature” infants born at GA 36+0 – 36+6 weeks. These “more mature” preterm infants were not included in our study, which may explain our higher incidence of inhomogeneous echogenicity. We also found a high incidence of diffuse white matter signal change on MRI. This was not previously reported for MLPT infants, while in very preterm infants it is frequently observed (12). It is suggested that diffuse white matter signal changes may represent mild white matter injury or delayed white matter maturation (36). However, neurodevelopmental consequences are unclear as available studies on the association with neurodevelopmental outcome are inconsistent (37).

Signs suggestive of brain atrophy due to injury, i.e. irregular shape of the lateral ventricles, ex-vacuo ventricular dilatation and a widened interhemispheric fissure, were frequently seen in our cohort. In very preterm infants these signs were associated with lower scores on neurodevelopmental outcome at preschool age (2,22). It may thus also be of clinical relevance in MLPT infants. The same may be applicable to delayed myelination and/or gyration. Especially delayed myelination may be a sequel of white matter injury and thus be of clinical importance (31).

In agreement with previous studies, small hemorrhages such as remnants of small IVH, plexus hemorrhages and punctate CBH were more frequently detected by MRI than by cUS (12,38). As expected, the incidence of PWML was higher on MRI. However, we suspected punctate lesions in the periventricular white matter on cUS in seven infants who had obvious PWML on MRI. Furthermore, of the 12 infants with inhomogeneous echogenicity at TEA-cUS, nine infants had PWML on MRI, suggesting that inhomogeneous PVE on cUS may represent PWML on MRI. In agreement with others (22,39), we have demonstrated that in some cases cUS does depict PWML, and that non-cystic white matter injury is not an exclusive MRI finding.

Strengths of our study are the serial cUS examinations, combining cUS and MRI around TEA, using SWI, and the special classification of and attention for subtle lesions. However,

some limitations also need to be addressed. Firstly, infants with a GA 36+0–36+6 weeks were not included because they are not routinely admitted at our hospital. Secondly, some data is missing: not all infants underwent cUS-discharge or MRI, and some infants were lost to TEA-visit. Although we found no significant differences in the number of brain lesions between these infants and infants who underwent all cUS and MRI examinations, this may have influenced our results.

Our study is the first to give a detailed overview of brain lesions detected on both cUS and MRI in a cohort of MLPT infants and to compare cUS and MRI in this population. Frequently encountered brain lesions were signs suggestive of white matter injury, small hemorrhages and delayed brain maturation. The reported brain lesions were mainly mild and some may be considered incidental findings. The majority of these lesions may not influence neurodevelopmental outcome, but long-term follow-up is needed to assess the clinical relevance.

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REFERENCES

1. Saigal S, Doyle L. An overview of mortality and sequelae of preterm birth from infancy to adulthood. *Lancet* 2008;371(9608):261-269.
2. Horsch S, Muentjes C, Franz A, Roll C. Ultrasound diagnosis of brain atrophy is related to neurodevelopmental outcome in preterm infants. *Acta Paediatr* 2005;94(12):1815-1821.
3. Anderson P, Cheong JLY, Thompson D. The predictive validity of neonatal MRI for neurodevelopmental outcome in very preterm children. *Semin Perinatol* 2015;39(2):147-158
4. Brouwer MJ, Kersbergen KJ, van Kooij, BJM, Benders MJNL, van Haastert IC, Koopman-Esseboom C, et al. Preterm brain injury on term-equivalent age MRI in relation to perinatal factors and neurodevelopmental outcome at two years. *PLoS ONE* 2017;12(5):e0177128..
5. van Baar AL, Vermaas J, Knots E, de Kleine MJK, Soons P. Functioning at school age of moderately preterm children born at 32 to 36 weeks' gestational age. *Pediatrics* 2009;124(1):251-257.
6. Kerstjens JM, de Winter AF, Bocca Tjeertes IF, ten Vergert EMJ, Reijneveld SA, Bos AF. Developmental delay in moderately preterm-born children at school entry, J. *Pediatr*. 2011;159 (1):92-98.
7. G Natarajan G, Shankaran S. Short- and Long-Term Outcomes of Moderate and Late Preterm Infants. *Am J Perinatol* 2016;33(3):305-317.
8. Ballardini E, Tarocco A, Baldan A, Antoniazzi E, Garani G, Borgna Pignatti C. Universal cranial ultrasound screening in preterm infants with gestational age 33-36 weeks. A retrospective analysis of 724 newborns. *Pediatr Neurol* 2014;51(6):790-794.
9. Fumagalli M, Ramenghi LA, De Carli A, Bassi L, Farè P, Dessimone F, et al. Cranial ultrasound findings in late preterm infants and correlation with perinatal risk factors. *Ital J Pediatr* 2015;41:65.
10. Cheong JLY, Thompson D, Spittle A, Potter C, Walsh J, Burnett A, et al. Brain Volumes at Term-Equivalent Age Are Associated with 2-Year Neurodevelopment in Moderate and Late Preterm Children. *J Pediatr* 2016;174:91-97.e1.
11. Boswinkel V, Nijboer-Oosterveld J, Nijholt IM, Edens MA, Mulder - de Tollenaer SM, Boomsma MF, et al. A systematic review on brain injury and altered brain development in moderate-late preterm infants. *Early Hum Dev* 2020 -5-28;148
12. Leijser LM, de Bruïne FT, Steggerda SJ, van der Grond J, Walther FJ, van Wezel-Meijler G. Brain imaging findings in very preterm infants throughout the neonatal period: Part I. Incidences and evolution of lesions, comparison between ultrasound and MRI. *Early human development* 2009;85(2):101-109.
13. Meijler G, Steggerda SJ. *Neonatal Cranial Ultrasonography*, third edition. Cham: Springer; 2019.
14. Volpe JJ. Intraventricular hemorrhage in the premature infant-current concepts. Part II. *Ann. Neurol*. 1989;25(2):109-116
15. Volpe JJ. *Volpe's Neurology of the Newborn*, sixth edition, Elsevier, Philadelphia, PA, 2018.
16. Brouwer AJ, van Stam C, Uniken Venema M, Koopman C, Groenendaal F, de Vries LS. Cognitive and neurological outcome at the age of 5-8 years of preterm infants with post-hemorrhagic ventricular dilatation requiring neurosurgical intervention. *Neonatology* 2012;101(3):210-216.
17. Boswinkel V, Steggerda SJ, Fumagalli M, Parodi A, Ramenghi LA, Groenendaal F et al. The CHOPIn study: a multicenter study on cerebellar hemorrhage and outcome in preterm infants. *Cerebellum* 2019;18(6):989-998.

18. de Vries LS, Eken P, Dubowitz LM. The spectrum of leukomalacia using cranial ultrasound. *Behav. Brain Res.* 1992;49(1):1–6.
19. Kidokoro H, Neil J, Inder T. New MR imaging assessment tool to define brain abnormalities in very preterm infants at term. *Am J Neuroradiol* 2013;34(11):2208–14.
20. van Wezel-Meijler G, van der Knaap MS, Sie LT, Oosting J, van Amerongen AH, Cranendonk A, et al. Magnetic resonance imaging of the brain in premature infants during the neonatal period. Normal phenomena and reflection of mild ultrasound abnormalities. *Neuropediatrics* 1998;29(2):89–96.
21. de Bruïne FT, van den Berg-Huysmans AA, Leijser LM, Rijken M, Steggerda SJ van der Grond J, et al. Clinical Implications of MR Imaging Findings in the White Matter in Very Preterm Infants: A 2-year Follow-up Study. *Radiology* 2011;261(3):899–906.
22. Agut T, Alarcon A, Cabañas F, Bartocci M, Martinez Biarge M, Horsch S. Preterm white matter injury: ultrasound diagnosis and classification. *Pediatr Res* 2020;87(Suppl1):37–49.
23. Martinez Biarge M, Groenendaal F, Kersbergen KJ, Benders MJNL, Foti F, Cowan FM et al. MRI based preterm white matter injury classification: the importance of sequential imaging in determining severity of injury. *PLoS One* 2016;11(6):e0156245.
24. Martinez Biarge M, Ferriero D, Cowan F. Perinatal arterial ischemic stroke. *Handb Clin Neurol* 2019;162:239–266.
25. Epelman M, Daneman A, Blaser S, Ortiz Neira C, Konen O, Jarrín J, et al. Differential diagnosis of intracranial cystic lesions at head US: correlation with CT and MR imaging. *Radiographics* 2006;26(1):173–196.
26. Sisman J, Chalak L, Heyne R, Pritchard M, Weakley D, Brown LS, et al. Lenticulostriate vasculopathy in preterm infants: a new classification, clinical associations and neurodevelopmental outcome. *J Perinatol* 2018;38(10):1370–1378.
27. Hagmann CF, Robertson NJ, Acolet D, Nyombi N, Ondo S, Nakakeeto M, et al. Cerebral measurements made using cranial ultrasound in term Ugandan newborns. *Early Human Development* 2011;87(5):341–347.
28. Levene MI. Measurement of the growth of the lateral ventricles in preterm infants with real-time ultrasound. *Arch Dis Child* 1981;56(12):900–4.
29. Barkovich AJ, Mukherjee P. Normal Development of the Neonatal and Infant Brain, Skull, and Spine. *Pediatric neuroimaging*. Fifth edition. ed. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2012. Chapter 2. p. 19–80.
30. Walsh JM, Doyle LW, Anderson PJ, Lee KJ, Cheong JLY. Moderate and late preterm birth: effect on brain size and maturation at term-equivalent age. *Radiology* 2014;273(1):232–240.
31. Volpe JJ. Brain injury in premature infants: a complex amalgam of destructive and developmental disturbances. *Lancet Neurol* 2009;8(1):110–124.
32. Looney C, Smith JK, Merck L, Wolfe H, Chescheir N, Hamer R, et al. Intracranial hemorrhage in asymptomatic neonates: prevalence on MR images and relationship to obstetric and neonatal risk factors. *Radiology* 2007;242(2):535–541.
33. Luciano R, Bersani I, Mancini G, Vento G, Mercuri E. Cranial ultrasound evaluation in term neonates. *Early Hum Dev* 2020;143:104983.
34. M.I. Levene, Measurement of the growth of the lateral ventricles in preterm infants with real-time ultrasound, *Arch. Dis. Child.* 56 (12) (1981) 900–904.

35. Steggerda SJ, Leijser LM, Wiggers-de Bruïne FT, van der Grond J, Walther FJ, van Wezel-Meijler G. Cerebellar injury in preterm infants: incidence and findings on US and MR images. *Radiology* 2009;252(1):190-199.
36. Kumpulainen V, Lehtola SJ, Tuulari JJ, Silver E, Copeland A, Korja R, et al. Prevalence and Risk Factors of Incidental Findings in Brain MRIs of Healthy Neonates-The FinnBrain Birth Cohort Study. *Frontiers in Neurology* 2019;10.
37. Volpe JJ. Confusions in Nomenclature: "Periventricular Leukomalacia" and "White Matter Injury"-Identical, Distinct, or Overlapping? *Pediatr Neurol* 2017;73:3-6.
38. Rath CP, Desai S, Rao SC, Patole S. Diffuse excessive high signal intensity on term equivalent MRI does not predict disability: a systematic review and meta-analysis. *Arch Dis Child Fetal Neonatal* Ed. 2021 Jan;106(1):9-16.
39. Parodi A, Rossi A, Severino M, Morana G, Sannia A, Calevo MG et al. Accuracy of ultrasound in assessing cerebellar haemorrhages in very low birthweight babies. *Fetal and Neonatal* 2015;100(4):289-92.
40. Leijser LM, Bruïne FT, van der Grond J, Steggerda SJ, Walther FJ, van Wezel-Meijler G. Is sequential cranial ultrasound reliable for detection of white matter injury in very preterm infants? *Neuroradiology* 2010;52(5):397-406.



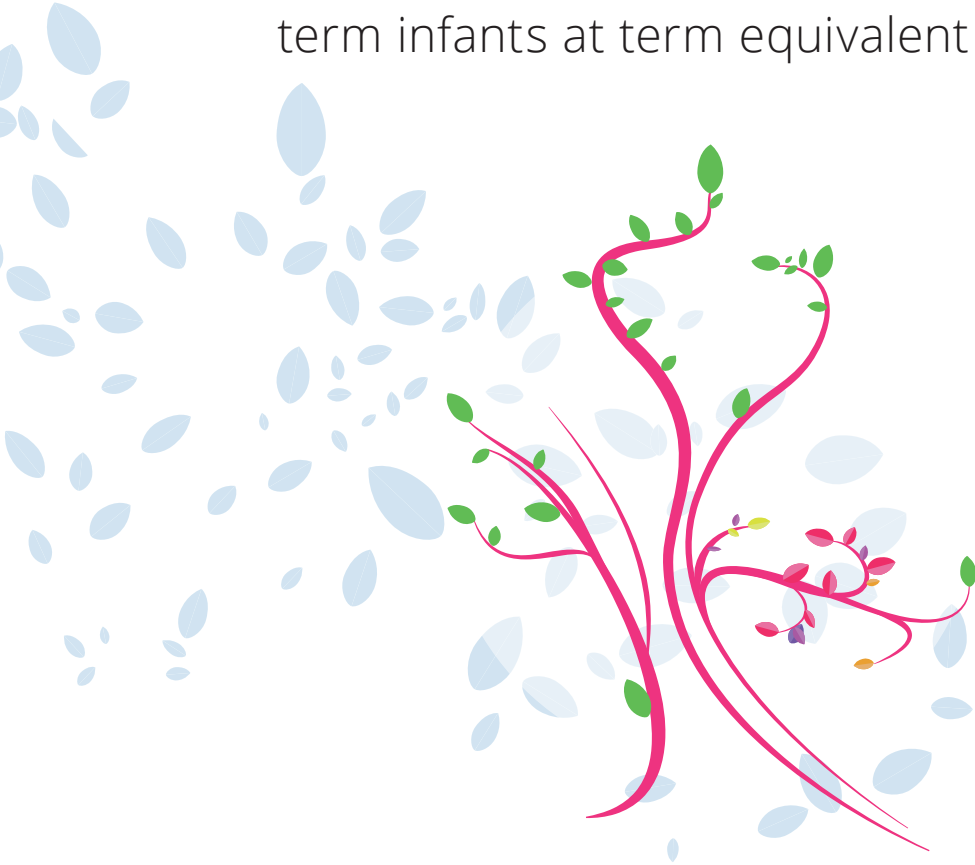
PART III

QUANTIFICATION OF STRUCTURAL BRAIN GROWTH IN MODERATE-LATE PRETERM INFANTS



CHAPTER 5

Ultrasound brain measurements differ
between moderate-late preterm and full-
term infants at term equivalent age



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ABSTRACT

Background Brain growth in moderate preterm (MP; gestational age (GA) 32⁺⁰ – 33⁺⁶ weeks) and late preterm infants (LP; GA 34⁺⁰ – 36⁺⁶ weeks) may be impaired, even in the absence of brain injury.

Aims The aims of this study were to assess brain measurements of MP and LP infants, and to compare these with full-term infants (GA > 37 weeks) using linear cranial ultrasound (cUS) at term equivalent age (TEA).

Study design cUS data from two prospective cohorts were combined. Two investigators performed offline measurements on standard cUS planes. Eleven brain structures were compared between MP, LP and full-term infants using uni- and multivariable linear regression. Results were adjusted for postmenstrual age at cUS and corrected for multiple testing.

Results Brain measurements of 44 MP, 54 LP and 52 full-term infants were determined on cUS scans at TEA. Biparietal diameter and basal ganglia-insula width were smaller in MP (-9.1mm and -1.7mm, $p < 0.001$) and LP infants (-7.0mm and -1.7mm, $p < 0.001$) compared to full-term infants. Corpus callosum – fastigium length was larger in MP (+2.2mm, $p < 0.001$) than in full-term infants. No significant differences were found between MP and LP infants.

Conclusions These findings suggest that brain growth in MP and LP infants differs from full-term infants. Whether these differences have clinical implications remains to be investigated.

INTRODUCTION

Each year more than 15 million infants are born preterm, i.e. before 37 weeks of gestation. More than 80% of the preterm population is born moderate to late preterm (MLPT) at a gestational age (GA) of 32 – 36 weeks (1). Overall, these infants more often demonstrate motor and cognitive delays than full-term infants (2-4). Within the MLPT population, neurodevelopmental problems are more frequently seen in moderate preterm (MP; GA 32⁺⁰ – 33⁺⁶ weeks) than late preterm (LP; GA 34⁺⁰ – 36⁺⁶ weeks) infants (5,6). Why and how MLPT infants develop these neurodevelopmental delays needs to be further elucidated.

During the third trimester of pregnancy, essential growth and development of the fetal brain take place (7,8). This includes major organizational events (e.g. establishment of connectivity and remodeling by synaptogenesis and apoptosis), cortical folding and myelination (9-11). In case of preterm birth, the brain is vulnerable to ischemia and inflammation during this period of impressive growth and development. This injury includes (cystic) periventricular leukomalacia and neuronal/axonal disease, for which the overall term ‘encephalopathy of prematurity’ has been introduced (12). Other reported injuries are germinal matrix hemorrhage – intraventricular hemorrhage (13) and cerebellar hemorrhage (14).

Although during the last decades, more knowledge has been reported on brain injury in MLPT infants (7,9,15), most information on encephalopathy of prematurity and its associations with neurodevelopmental delay is obtained from the very preterm population (GA < 32 weeks). In very preterm infants with encephalopathy of prematurity, reduced volumes of the white matter, cerebral cortex and deep gray matter have been described (16,17). In several studies, smaller brain sizes at term equivalent age (TEA) were related to poorer neurodevelopmental outcome at two years of age (18-21).

Studies investigating brain measurements in MLPT infants are scarce. To the best of our knowledge, only Walsh et al. performed linear brain measurements in MLPT infants. Using magnetic resonance imaging (MRI), they demonstrated that MLPT birth was associated with smaller brain sizes (22). No distinction was made between MP and LP infants.

Although MRI is the golden standard for detecting neonatal brain injury, cranial ultrasound (cUS) is the standard neuro-imaging modality in neonatal practice. cUS can be performed at the bedside with little disturbance to the infant and is therefore more patient friendly. In addition, cUS is a relatively inexpensive technique to evaluate development and growth of the neonatal brain (14). Whether smaller brain sizes in MLPT infants can be detected using simple linear cUS measurements is unknown.

The aims of this study were therefore 1) to measure sizes of several brain structures in MP and LP infants using cUS; 2) to compare brain measurements between MP, LP and full-term infants at TEA.

METHODS

Study population

CUS data from two prospective cohorts were combined. The first cohort consisted of 65 MP (GA 32⁺⁰ – 33⁺⁶ weeks) and 101 LP infants (GA 34⁺⁰ – 35⁺⁶ weeks) enrolled in the study ‘Brain Imaging in Moderate to late Preterm infants (BIMP)’ between August 2017 and November 2019 at Isala Women and children’s hospital (IVKC), Zwolle, The Netherlands (23). The second cohort consisted of 59 full-term infants born between August 2014 and May 2016 at the Leiden University Medical Center (LUMC), Leiden, The Netherlands. These full-term infants were recruited as a control group for a study investigating brain abnormalities in infants with prenatally detected congenital heart defects (24). Written informed consent was obtained from all parents. The present study, in which we compare linear cUS brain measurements, was filed as amendment and ethical approval was given by the Central Committee in Research Involving Human Subjects, The Hague, The Netherlands (NL52323.075.15).

Only infants in whom cUS was performed between a postmenstrual age (PMA) of 38 to 42 weeks were included. Infants with moderate-severe brain injury, as defined by Boswinkel et al., were excluded (23).

Baseline characteristics such as sex, GA, birth weight and head circumference at birth were collected from medical charts. In MP and LP infants, weight and head circumference were measured on the day of the cUS appointment. As almost all full-term infants were scanned within a week after birth, these measurements were not repeated at the cUS appointment, and weight and head circumference at birth were used. Birth weight percentile was calculated using the ‘Perined Hoftiezer’ Dutch birth weight charts (25).

Cranial ultrasound

CUS was performed around TEA (preferably at PMA of 38 – 42 weeks) by one of the investigators (IVKC: VB (research physician) or MKR (pediatrician); LUMC: FARJ (resident obstetrics & gynecology) or SJS (pediatrician-neonatologist). VB, MKR and FARJ were all intensively trained in neonatal cUS prior to the start of the initial studies. CUS in IVKC was performed under supervision of GvWM (with > 25 years of experience in neonatal neuroimaging) and in LUMC under supervision of SJS (with > 15 years of experience in neonatal neuroimaging). In IVKC an Aloka Prosound Alpha 7 Premier ultrasound system (Hitachi Medical Systems Holding AG, Switzerland) was used. In LUMC, cUS was performed with an Aloka Alpha 10 ultrasound system (Hitachi Medical Systems Holding AG, Switzerland) or a Toshiba Aplio 400 system (Canon Medical Systems Europe BV, The Netherlands). Images were recorded in six coronal and five sagittal planes using the anterior fontanelle window and at least one coronal and one axial plane using the mastoid fontanelle window (14). Scans were assessed during and immediately after the procedure by the investigator, checking for lesions with likely clinical consequences. On both locations, all images were digitally stored.

Linear cUS brain measurements

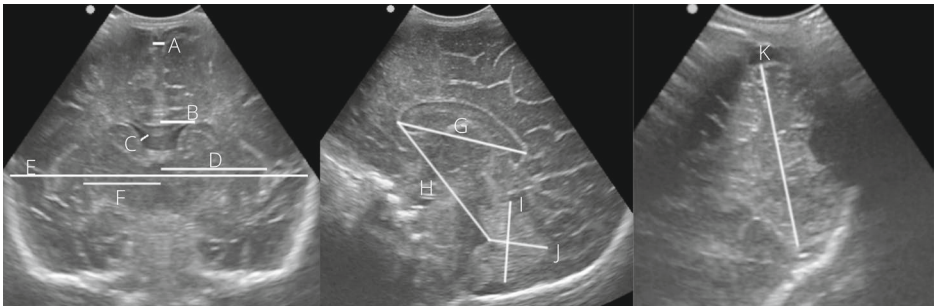
In both cohorts, all linear cUS brain measurements were performed offline and on site by one investigator (FIS). FIS had been trained to perform cUS measurements by VB and GvWM. The investigator was blinded to patient details. To establish the intra-observer reliability, measurements were repeated in 20 (13% of total) randomly selected scans. A second investigator (VB), blinded to the previous results, repeated measurements in 30 (20% of total) randomly selected scans for inter-observer reliability. Measurements were performed using the software program Clinical Assistant (RCV Medical IT BV Baarn, The Netherlands).

The following measurements were obtained using standard cUS planes (see Figure 1):

- 1) Anterior fontanelle – coronal plane:
Ventricular index (26), anterior horn width (27), interhemispheric distance (28), basal ganglia width (29), basal ganglia-insula width, biparietal diameter (21,30);
- 2) Anterior fontanelle – sagittal plane:
Corpus callosum length (29), corpus callosum – fastigium length (31), vermis height (29), vermis anterior-posterior diameter (29);
- 3) Mastoid fontanelle – coronal plane: Transcerebellar diameter (32).

Figure 1. Overview of linear cUS brain measurements.

A) Interhemispheric distance; B) Ventricular index; C) Anterior horn width; D) Basal ganglia-insula width; E) Biparietal diameter; F) Basal ganglia width; G) Corpus callosum length; H) Corpus callosum - fastigium length; I) Vermis height; J) Vermis anterior-posterior diameter; K) Transcerebellar diameter.



Statistics

To estimate if our sample size would be sufficient for the intended analysis, we conducted a sample size calculation. This calculation was performed in MedCalc Statistical Software version 19.0.5 (MedCalc Software bvba, Ostend, Belgium). No previous studies performed linear cUS measurements in MP and LP infants and thus we based the calculation on the results of the study by Walsh et al. (22). We took the biparietal diameter as representative measurement. In the study by Walsh et al. the biparietal diameter was 83.6 mm (SD ± 4.4)

in MLPT infants and 87.8 mm (SD ± 4.7) in term infants (22). Using these results for our sample size calculation, with an alpha of 0.05 and a power of 0.90, we need a sample with 26 infants per group.

Data analyses were performed using SPSS software (version 26.0; SPSS inc, Chicago, Illinois, USA). Continuous variables were assessed for normality and summarized with means (SD), or in case of non-normal distribution, with median (minimum – maximum). Frequency counts and percentages were given for categorical variables. Group differences in baseline characteristics were compared using ANOVA or Kruskal-Wallis for continuous and χ^2 -test or Fishers exact test for categorical variables. Inter- and intra-rater reliability were estimated with intraclass correlation coefficients (ICC). An ICC value of ≥ 0.90 was considered excellent, values between 0.75 and 0.89 good, between 0.50 and 0.74 moderate and < 0.50 poor (33). Correlations between confounding factors of interest were investigated using Person correlation coefficients. Using linear regression differences in linear cUS brain measurements were explored between 1) MP and full-term infants; 2) LP and full-term infants; and 3) MP and LP infants. We corrected for potential confounders using multivariable linear regression. A p-value < 0.05 was considered significant. We corrected for multiple testing using Holm-Bonferroni (34).

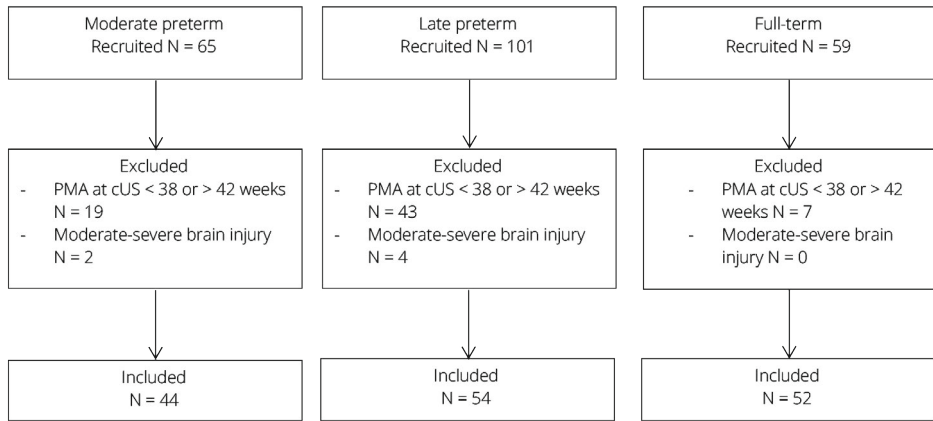
RESULTS

Study population

In total, 225 infants were enrolled in the two prospective cohort studies combined. Of these, 69 infants did not have a cUS between PMA 38 and 42 weeks. In addition, six MLPT infants were excluded due to presence of moderate-severe brain lesions: one infant with periventricular hemorrhagic infarction, one with arterial infarction, one with ex-vacuo dilatation (ventricular index > 15 mm), and three infants with ≥ 6 punctate white matter lesions. Of the remaining 150 infants, 44 were MP, 54 LP and 52 full term infants (Figure 2).

Figure 2. Inclusion flow chart for study population.

cUS = cranial ultrasound; PMA = postmenstrual age; TEA = term equivalent age.



Mean PMA at cUS for all groups was 39.97 (SD 0.99) weeks. PMA at time of cUS was slightly higher in LP infants compared to MP and full-term infants ($p=0.04$). Percentile birth weight and percentage male infants were not significantly different between groups (Table 1).

Table 1. Baseline characteristics.

	Moderate preterm N = 44	Late preterm N = 54	Full-term N = 52	p-value
GA in weeks, mean (SD)	33.08 (0.5)	34.80 (0.5)	39.81 (1.02)	<0.01
Male, N (%)	25 (56.8)	29 (53.7)	24 (46.2)	0.55
Birth weight in grams, mean (SD)	1996 (458)	2347 (413)	3495 (364)	<0.01
Perined Hoftiezer birth weight percentile	0.39	0.41	0.49	0.17
Weight at cUS in grams, mean (SD)	3256 (467)	3371 (520)	3495 (364)*	0.03
Head circumference at birth in cm, mean (SD) [†]	30.79 (1.8)	31.96 (1.4)	35.15 (1.07)	<0.01
Head circumference at cUS in cm, mean (SD)	35.4 (1.5)	35.5 (1.4)	35.15 (1.07)*	0.47
PMA at cUS in weeks, mean (SD)	40.09 (0.9)	40.57 (0.9)	40.34 (1.01)	0.04
Age at cUS in days, median, (min. – max.)	49 (36 – 63)	40 (21 – 56)	3 (1 – 11)	<0.01
cUS within 24 hours after birth, N (%)	NA	NA	10 (19%)	NA

GA = gestational age; cUS = cranial ultrasound; NA = not applicable; PMA = postmenstrual age. [†] Missing in 12 infants (3 MP, 5 LP and 4 full-term infants). * same as at birth.

Inter- and intra-observer reliability

Inter- and intra-observer reliability were good to excellent for most measurements. For the interhemispheric distance, basal ganglia, vermis height and vermis anterior-posterior diameter reliability was moderate (Table 2).

Table 2. Inter- and intra-rater correlation coefficients.

ICC = intraclass correlation coefficient; CI = confidence interval.

	Inter-rater N = 30		Intra-rater N = 20	
	ICC	95% CI	ICC	95% CI
Anterior fontanelle – Coronal plane				
Biparietal diameter	0.83	0.66 – 0.91	0.93	0.83 – 0.97
Interhemispheric distance	0.68	0.38 – 0.84	0.63	0.27 – 0.84
Ventricular index				
Right	0.86	0.73 – 0.93	0.97	0.92 – 0.99
Left	0.88	0.76 – 0.94	0.81	0.57 – 0.92
Anterior horn width				
Right	0.78	0.44 – 0.91	0.95	0.87 – 0.98
Left	0.83	0.40 – 0.94	0.91	0.73 – 0.96
Basal ganglia width				
Right	0.63	0.35 – 0.81	0.67	0.33 – 0.86
Left	0.66	0.40 – 0.82	0.74	0.46 – 0.89
Basal ganglia – insula width				
Right	0.72	0.49 – 0.86	0.95	0.87 – 0.98
Left	0.67	0.28 – 0.85	0.82	0.58 – 0.92
Anterior fontanelle – Sagittal plane				
Corpus callosum length	0.87	0.74 – 0.93	0.89	0.76 – 0.96
Corpus callosum – fastigium length	0.86	0.73 – 0.93	0.84	0.62 – 0.93
Vermis height	0.79	0.58 – 0.90	0.63	0.26 – 0.84
Vermis anterior-posterior diameter	0.55	0.19 – 0.77	0.65	0.12 – 0.87
Mastoid fontanelle – Coronal plane				
Transcerebellar diameter	0.93	0.83 – 0.97	0.97	0.92 – 0.99

Multivariable analysis

All baseline characteristics with a p-value < 0.20 were assumed potential confounding factors and were investigated. As (birth) weight, head circumference and Perined Hoftiezer birth weight percentile had a high correlation coefficient (>0.50), only the Perined Hoftiezer birth weight percentile was used in the analysis. In addition, PMA at cUS was investigated. The latter was the only confounder observed in the association between infant group (MP, LP or full-term) and the linear cUS brain measurements. Unadjusted and adjusted differences between 1) MP and full-term infants; 2) LP and full-term infants; and 3) MP and LP infants are shown in Supplemental tables 1 – 3.

Linear cUS brain measurements at TEA

The ranges of the sizes of several brain structures in MP, LP and full-term infants are shown in Figure 3.

Comparison of linear cUS brain measurements between MP and full-term infants

MP infants had a significantly smaller mean biparietal diameter compared to full-term infants (79.2 mm vs 88.5 mm, adjusted difference: -9.1 mm; $p < 0.001$). Both mean basal ganglia width and mean basal ganglia-insula width were also significantly smaller in MP infants (19.3 mm and 29.0 mm versus 20.6 mm and 30.7 mm in full-term infants, adjusted differences between -1.3 mm and -1.7 mm; $p < 0.001$ for all). Mean corpus callosum – fastigium length was larger in MP infants (52.3 mm vs 50.4 mm, adjusted difference: +2.2 mm; $p < 0.001$). See Figure 3 and Supplemental Table 1.

Comparison linear cUS brain measurements between LP and full-term infants

LP infants had a significantly smaller mean biparietal diameter compared to full-term infants (81.6 mm vs 88.5 mm, adjusted difference: -7.0 mm; $p < 0.001$). Both mean basal ganglia width and mean basal ganglia- insula width were also smaller in LP infants (19.7 mm and 29.0 mm vs 20.6 mm and 30.7 mm in full-term infants, adjusted differences between -1.0 and -1.7 mm, $p \leq 0.001$ for all). See Figure 3 and Supplemental Table 2.

Comparison linear cUS brain measurements between MP and LP infants

Small differences in linear cUS brain measurements were seen between MP and LP, but none of these were significantly different after Holm-Bonferroni correction. See Figure 3 and Supplemental Table 3.

Figure 3. Comparison of linear cUS brain measurements between MP, LP and full-term infants in mm (mean, error bars: +/- 2 SD).

** p-value is significantly different after adjustment for PMA at cUS and Holm-Bonferroni correction.

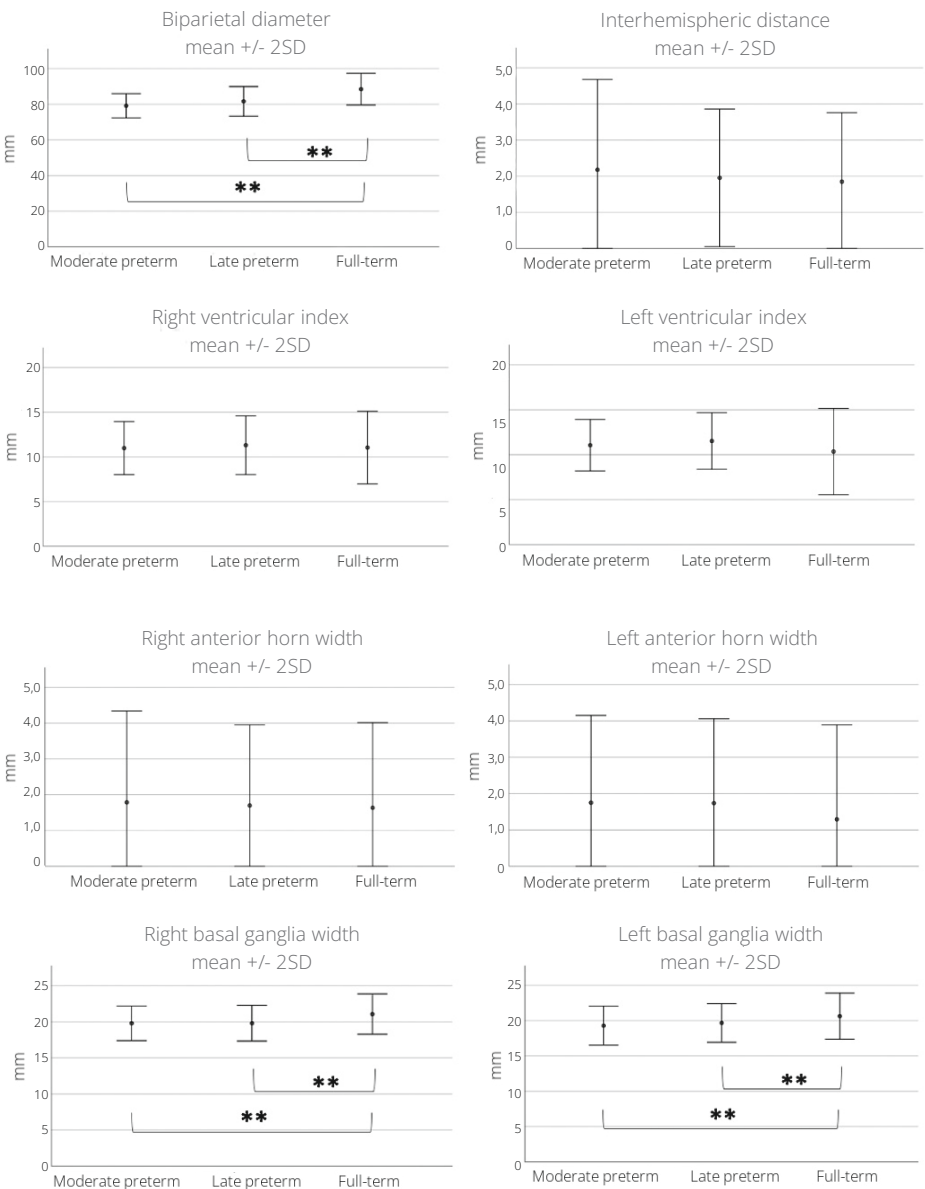
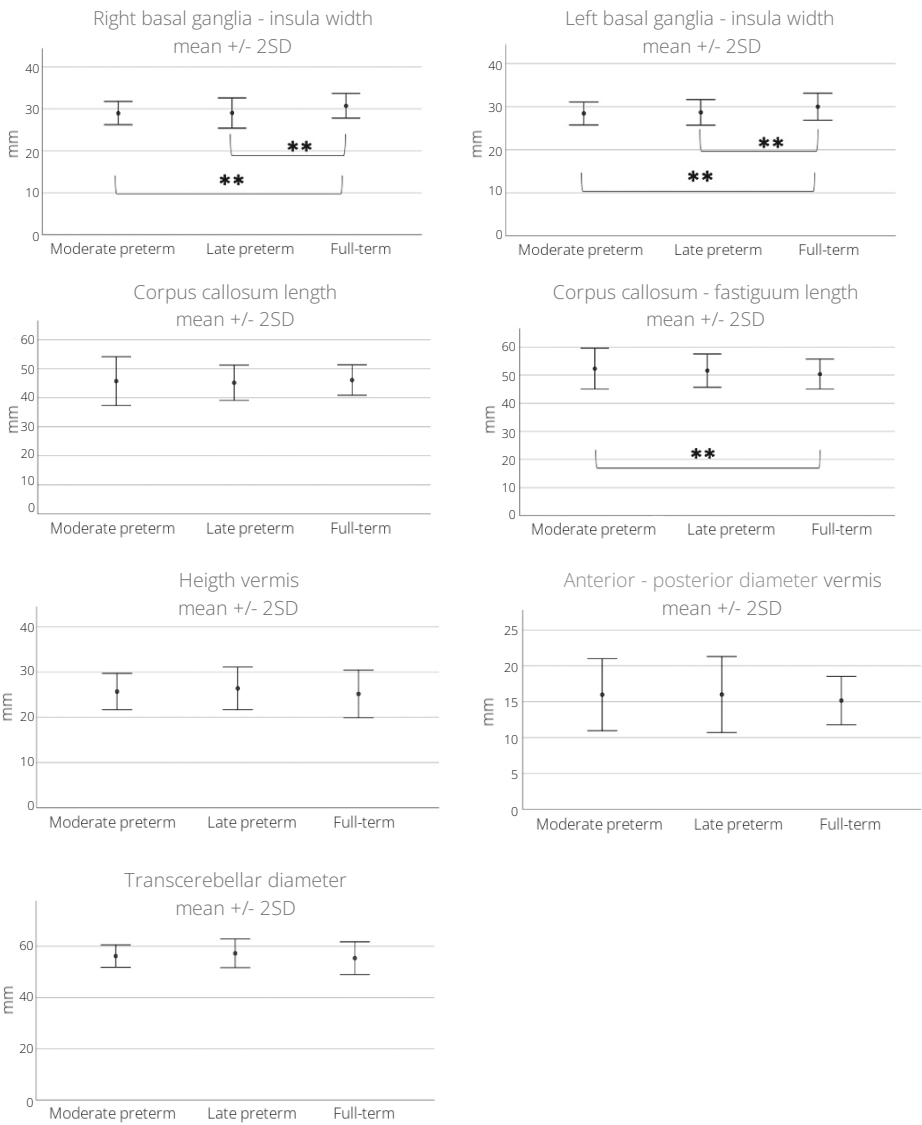


Figure 3 (continued)



DISCUSSION

We reported linear cUS brain measurements in MP and LP infants and demonstrated differences in the size of several brain structures between MP, LP and full-term infants. MP and LP infants had smaller biparietal diameter, basal ganglia width and basal ganglia-insula width compared to full-term infants. No significant differences were found between MP and LP infants.

To our knowledge, this is the first study reporting brain measurements for MP and LP infants at TEA using cUS. Although cUS is so far not routinely performed in this population, it is the primary neonatal neuro-imaging modality. Given the good to excellent (≥ 0.75) inter- and intrarater reliability of the biparietal diameter, ventricular index, anterior horn width, corpus callosum and transcerebellar diameter, the measurements give a good indication of the reference values of these structures at TEA in MP and LP infants.

In agreement with the MRI-study by Walsh et al., we found a smaller mean biparietal diameter and basal ganglia width in MP and LP infants compared to full-term infants (22). However, our differences in biparietal diameter (respectively -9.1 mm for MP and -7.0 mm for LP infants) were larger than the difference reported by Walsh et al. (respectively -3.0 mm for MLPT infants compared to full-term infants). This difference might be partially explained by differences in study samples and methods. While Walsh et al. also included infants born between 36+0 and 36+6 weeks' gestation, these infants were not included in our study, as these infants are not routinely admitted at IVKC. Infants born within this GA window may have larger brain structures at TEA, which may reduce the difference with the full-term population. In addition, the use of different imaging techniques (cUS versus MRI) may partly explain the differences. Even though Leijser et al. demonstrated that most structural linear cUS measurements were comparable with MRI, small differences between cUS and MRI measurements were found (29). Unfortunately, they did not measure the biparietal diameter. Likewise, the difference in basal ganglia width was larger in our study than in the study by Walsh et al. (22). However, as Walsh et al. measured the basal ganglia width in a different way (i.e. on a T2-weighted axial plane, while we measured this distance in a coronal plane), a reliable comparison between the results of that study and our study is not possible. Interestingly, our reported mean differences in biparietal diameter (respectively -9.1 mm for MP and -7.0 mm for LP compared to full-term infants) were similar to the reported mean difference in biparietal diameter for very preterm infants compared to full-term infants in other studies (30,35).

While we expected that most brain structures would be larger in full-term infants, we found one measurement that was smaller in full-term infants. The corpus callosum – fastigium length was significantly larger in MP infants than in full-term infants (mean difference +2.2 mm). A possible explanation for this finding and for the smaller biparietal diameter and basal ganglia-insula width might be that positioning of the preterm infant's head plays a role in the

direction of growth. Preterm infants are frequently positioned in prone position with their head rotated to either side, improving respiratory stability (36). This might reduce growth in the left-right direction and may be compensated by growth in cranial-caudal direction and thus a larger corpus – callosum fastigium length.

Graça et al. proposed a method to control for the differences in head shape between preterm and full-term infants (30). They used the biparietal diameter, occipito-frontal diameter and cranial height in a tri-dimensional ellipsoid model to estimate intracranial and cerebral volumes. They found a smaller biparietal diameter, larger occipito-frontal diameter and larger cranial height in preterm infants, but found no difference in intracranial volume between preterm and full-term infants. However, after adjustment for extracerebral space they demonstrated a significantly smaller cerebral tissue volume in preterm infants compared to full-term infants. Unfortunately, we were not able to measure the occipito-frontal diameter as (at IVKC) only images with a restricted field of view in the midsagittal plane were saved offline and thus, were not able to calculate the cerebral volume nor the extracerebral space.

In very preterm infants, a smaller biparietal diameter measured at TEA was related to poorer cognitive and psychomotor outcome at two years of age (18,20,21). This might indicate that differences in head shape are probably not the only explanation for the differences we found between MP and LP versus full-term infants, and a smaller biparietal diameter may be of clinical significance in this population as well. Follow-up is required to investigate whether an association between biparietal diameter and outcome also exists in MP and LP infants.

Another essential point is that we did not find a significant difference in cerebellar diameter between MP, LP and full-term infants. This is in contrast to the study of Walsh et al. who reported a smaller cerebellar diameter in MLPT infants compared to full-term infants after making adjustments for sex and PMA at MRI (22). However, conflicting results are reported when comparing the cerebellar diameter at TEA between very preterm and full-term infants (35,37-40). Again, intracranial and extracerebral volume may play a key role. Nguyen The Tich et al. found a significant difference in transcerebellar diameter between very preterm and full-term infants on MRI, but did not correct for intracranial volume (38). Graça et al. found a smaller transcerebellar diameter in very preterm infants on cUS, but also a larger cerebellar vermis volume. When they made adjustments for intracranial volume (i.e. head shape), the differences disappeared. This might indicate a relative preservation of the cerebellar size in very preterm infants (40).

We acknowledge several limitations of our study. Firstly, the group of LP infants was limited to infants born between 34+0 and 35+6 weeks. Secondly, although we used the standard coronal and sagittal planes to measure brain structures, even for well-trained sonographers it is difficult to capture the exact identical coronal or sagittal plane in each infant. This may have resulted in small differences between infants. In addition, a few structures had a moderate

inter- and intra-rater reliability. Agreements for these structures were likely moderate because the borders were often only vaguely visible, making it difficult to distinguish them from surrounding structures. Using a probe with higher frequencies and thus obtaining a higher near field resolution could have contributed to more precise measurements of some nearby structures, such as the interhemispheric distance and ventricular measurements. Furthermore, we used two-dimensional linear cUS measurements to represent three-dimensional structures and were not able to estimate brain tissue volumes. This should be taken into account while interpreting our data. However, Nguyen the Tich et al. previously reported a good correlation between biparietal diameter and total cerebral tissue volume (both measured using MRI), suggesting that this linear measurement can be used to get a good impression of the actual volume (38). Finally, we did not look at important markers for brain development such as gyration, which is not easy to quantify on CUS and myelination (not depicted by cUS).

Our findings suggest that not only in very preterm infants but also in MP and LP infants some brain structures have a different growth trajectory compared to full-term infants. The differences with full-term infants were smaller in LP infants than in MP infants. This is not surprising, since the brain has only reached 65% of full-term weight at 34 weeks of gestation. Accelerated growth in the last weeks of gestation makes the brain potentially vulnerable to events that may interfere with normal brain development e.g. suboptimal environmental factors or cerebral ischemia/reperfusion (7,9,12). Whether the reported smaller brain structures for MP and LP infants in our study are true reflections of suboptimal growth or rather related to differences in head shape and whether there is an effect on long-term neurodevelopmental outcome remains to be investigated.

CONCLUSIONS

In conclusion, biparietal diameter and basal ganglia width were significantly smaller in MP and LP infants compared with full-term infants. No significant differences between MP and LP infants were found. Some of the reported linear cUS brain measurements (i.e. those with good and excellent ICC values) obtained in MP and LP infants without moderate-severe brain injury can be considered reference values for brain sizes in this population.

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REFERENCES

1. Chawanpaiboon S, Vogel J, Moller A, Lumbiganon P, Petzold M, Hogan D, et al. Global, regional, and national estimates of levels of preterm birth in 2014: a systematic review and modelling analysis. *Lancet Glob Health* 2019;7(1):e37-e46.
2. Cheong J, Doyle L, Burnett A, Lee K, Walsh J, Potter C, et al. Association Between Moderate and Late Preterm Birth and Neurodevelopment and Social-Emotional Development at Age 2 Years. *JAMA Pediatr* 2017;171(4):e164805.
3. Kerstjens JM, de Winter AF, Bocca Tjeertes IF, ten Vergert EMJ, Reijneveld SA, Bos AF. Developmental delay in moderately preterm-born children at school entry. *J Pediatr* 2011;159(1):92-98.
4. Martínez-Nadal S, Bosch L. Cognitive and Learning Outcomes in Late Preterm Infants at School Age: A Systematic Review. *International Journal of Environmental Research and Public Health* 2021;18.
5. Chyi L, Lee H, Hintz S, Gould J, Sutcliffe T. School outcomes of late preterm infants: special needs and challenges for infants born at 32 to 36 weeks gestation. *J Pediatr* 2008;153(1):25-31.
6. Lipkind HS, Slopen ME, Pfeiffer MR, McVeigh KH. School-age outcomes of late preterm infants in New York City. *Obstet Gynecol* 2012 -3;206(3):1-6.
7. Kinney H. The near-term (late preterm) human brain and risk for periventricular leukomalacia: a review. *Semin Perinatol* 2006;30(2):81-88.
8. Guihard-Costa AM, Larroche JC. Growth velocity of some fetal parameters. I. Brain weight and brain dimensions. *Biol Neonate* 1992;62(5):309-16.
9. Haynes RL, Sleeper LA, Volpe JJ, Kinney HC. Neuropathologic studies of the encephalopathy of prematurity in the late preterm infant. *Clin Perinatol* 2013 Dec;40(4):707-722.
10. Budday S, Steinmann P, Kuhl E. Physical biology of human brain development. *Frontiers in cellular neuroscience* 2015;9:257.
11. Kostović I, Sedmak G, Judaš M. Neural histology and neurogenesis of the human fetal and infant brain. *Neuroimage* 2019 Mar;188:743-773.
12. Volpe J. Brain injury in premature infants: a complex amalgam of destructive and developmental disturbances. *Lancet Neurol* 2009;8(1):110-124.
13. Volpe J. Intraventricular hemorrhage in the premature infant--current concepts. Part II. *Ann Neurol* 1989;25(2):109-16.
14. Meijler G, Steggerda SJ. Neonatal Cranial Ultrasonography, third edition. Cham: Springer; 2019.
15. Boswinkel V, Nijboer-Oosterveld J, Nijholt IM, Edens MA, Mulder - de Tollenaer SM, Boomsma MF, et al. A systematic review on brain injury and altered brain development in moderate-late preterm infants. *Early Hum Dev* 2020 -5-28;148.
16. Inder TE, Warfield SK, Wang H, Hüppi PS, Volpe JJ. Abnormal cerebral structure is present at term in premature infants. *Pediatrics*. 2005 Feb;115(2):286-94.
17. Keunen K, Kersbergen KJ, Groenendaal F, Isgum I, de Vries LS, Benders MJNL. Brain tissue volumes in preterm infants: prematurity, perinatal risk factors and neurodevelopmental outcome: a systematic review. *J Matern Fetal Neonatal Med* 2012 Apr;25 Suppl 1:89-100.
18. Nguyen The Tich S, Anderson PJ, Hunt RW, Lee KJ, Doyle LW, Inder TE. Neurodevelopmental and Perinatal Correlates of Simple Brain Metrics in Very Preterm Infants. *Archives of Pediatrics & Adolescent Medicine* 2011 Mar 7;165(3):216-222.

19. Park HW, Yoon H, Han SB, Lee BS, Sung IY, Kim KS, et al. Brain MRI Measurements at a Term-Equivalent Age and Their Relationship to Neurodevelopmental Outcomes. *AJNR. American journal of neuroradiology* 2014 Mar;35(3):599-603.
20. Hammerl M, Zagler M, Zimmermann M, Griesmaier E, Janjic T, Gizewski ER, et al. Supratentorial Brain Metrics Predict Neurodevelopmental Outcome in Very Preterm Infants without Brain Injury at Age 2 Years. *Neonatology* 2020;117(3):287-293.
21. Cuzzilla R, Spittle AJ, Lee KJ, Rogerson S, Cowan FM, Doyle LW, et al. Postnatal Brain Growth Assessed by Sequential Cranial Ultrasonography in Infants Born 30 Weeks' Gestational Age. *AJNR Am J Neuroradiol* 2018;39(6):1170-1176.
22. Walsh JM, Doyle LW, Anderson PJ, Lee KJ, Cheong JLY. Moderate and late preterm birth: effect on brain size and maturation at term-equivalent age. *Radiology* 2014;273(1):232-240.
23. Boswinkel V, Krüse-Ruijter MF, Nijboer - Oosterveld J, Nijholt IM, Edens MA, Mulder - de Tollenaer SM, et al. Incidence of brain lesions in moderate-late preterm infants assessed by cranial ultrasound and MRI: The BIMP-study. *Eur J Radiol* 2021;136:109500.
24. Jansen FAR, Haak MC, van Wesemael MS, ten Harkel ADJD, van Lith JMM, Blom NA, et al. A low incidence of preoperative neurosonographic abnormalities in neonates with heart defects. *Early human development* 2020 Sep;148:105097.
25. Hoftiezer L, Hof MHP, Dijks-Elsinga J, Hogeveen M, Hukkelhoven, CWPM, van Lingen RA. From population reference to national standard: new and improved birthweight charts. *Obstet Gynecol* 2019;220(4):1-383.
26. Levene MI. Measurement of the growth of the lateral ventricles in preterm infants with real-time ultrasound. *Arch Dis Child* 1981;56(12):900-4.
27. Davies MW, Swaminathan M, Chuang SL, Betheras FR. Reference ranges for the linear dimensions of the intracranial ventricles in preterm neonates. *Fetal and Neonatal* 2000;82(3):218-23.
28. Hagmann CF, Robertson NJ, Acolet D, Nyombi N, Ondo S, Nakakeeto M, et al. Cerebral measurements made using cranial ultrasound in term Ugandan newborns. *Early Human Development* 2011;87(5):341-347.
29. Leijser LM, Srinivasan L, Rutherford MA, Counsell SJ, Allsop JM, Cowan FM.. Structural linear measurements in the newborn brain: accuracy of cranial ultrasound compared to MRI. *Pediatr Radiol* 2007;37(7):640-648.
30. Graça AM, Cardoso KR, da Costa JM, Cowan FM. Cerebral volume at term age: comparison between preterm and term-born infants using cranial ultrasound. *Early Hum Dev* 2013 Sep;89(9):643-648.
31. Roelants JA, Koning IV, Raets MMA, Willemsen SP, Lequin MH, Steegers-Theunissen RPM, et al. A new ultrasound marker for bedside monitoring of preterm brain growth. *American Journal of Neuroradiology* 2016 Aug 1;37(8):1516-1522.
32. Davies MW, Swaminathan M, Betheras FR. Measurement of the transverse cerebellar diameter in preterm neonates and its use in assessment of gestational age. *Australas Radiol* 2001;45(3):309-312.
33. Koo TK, Li MY. A Guideline of Selecting and Reporting Intraclass Correlation Coefficients for Reliability Research. *Journal of Chiropractic Medicine* 2016;15(2):155-63.
34. Holm S. A Simple Sequentially Rejective Multiple Test Procedure. *Scandinavian Journal of Statistics* 1979;6(2):65-70.

35. Kidokoro H, Neil J, Inder T. New MR imaging assessment tool to define brain abnormalities in very preterm infants at term. *Am J Neuroradiol* 2013;34(11):2208-14.
36. Picheansathian W, Woragidpoonpol P, Baosoung C. Positioning of Preterm Infants for Optimal Physiological Development: a systematic review. *JBI Library of systematic Reviews* 2009;7(7):224-259.
37. Wu Y, Stoodley C, Brossard-Racine M, Kapse K, Vezina G, Murnick J, et al. Altered local cerebellar and brainstem development in preterm infants. *Neuroimage* 2020;213:116702.
38. Nguyen The Tich S, Anderson PJ, Shimony JS, Hunt RW, Doyle LW, Inder TE. A novel quantitative simple brain metric using MR imaging for preterm infants. *AJNR.American journal of neuroradiology* 2009 -1;30(1):125-31.
39. Srinivasan L, Allsop J, Counsell SJ, Boardman JP, Edwards AD, Rutherford M. Smaller cerebellar volumes in very preterm infants at term-equivalent age are associated with the presence of supratentorial lesions. *AJNR Am J Neuroradiol* 2006;27(3):573-579.
40. Graça AM, Geraldo AF, Cardoso K, Cowan FM. Preterm cerebellum at term age: ultrasound measurements are not different from infants born at term. *Pediatr Res* 2013 Dec;74(6):698-704.

SUPPLEMENTAL MATERIAL

Supplemental table 1. Unadjusted and adjusted differences in linear cUS brain measurements between moderate preterm and full-term infants.

Brain measurement	Moderate preterm Mean (SD) in mm	Full-term Mean (SD) in mm	Unadjusted difference (95% CI)	p-value	Adjusted difference* (95% CI)	p-value
Anterior window – Coronal plane						
Biparietal diameter	79.2 (3.4)	88.5 (4.4)	-9.3 (-10.9 – -7.6)	<0.001	-9.1 (-10.8 – -7.5)	<0.001**
Interhemispheric distance	2.2 (1.3)	1.8 (0.9)	0.3 (-0.1 – 0.8)	0.13	0.3 (-0.1 – 0.8)	0.13
Ventricular index						
Right	11.0 (1.5)	11.0 (2.0)	-0.1 (-0.8 – 0.6)	0.87	0.1 (-0.6 – 0.8)	0.85
Left	11.1 (1.4)	10.4 (2.4)	-0.1 (-0.8 – 0.6)	0.88	0.1 (-0.6 – 0.8)	0.77
Anterior horn width						
Right	1.8 (1.3)	1.6 (1.2)	0.2 (-0.3 – 0.6)	0.54	0.2 (-0.2 – 0.7)	0.34
Left	1.8 (1.2)	1.3 (1.3)	0.5 (0.0 – 1.0)	0.07	0.5 (0.0 – 1.0)	0.05
Basal ganglia width						
Right	19.8 (1.2)	21.1 (1.4)	-1.3 (-1.8 – -0.8)	<0.001	-1.3 (-1.8 – -0.7)	<0.001**
Left [§]	19.3 (1.4)	20.6 (1.6)	-1.3 (-1.9 – -0.8)	<0.001	-1.3 (-1.9 – -0.7)	<0.001**
Basal ganglia – insula width						
Right	29.0 (1.4)	30.7 (1.5)	-1.7 (-2.4 – -1.1)	<0.001	-1.7 (-2.3 – -1.1)	<0.001**
Left [¶]	28.4 (1.3)	30.0 (1.6)	-1.6 (-2.2 – -1.0)	<0.001	-1.5 (-2.1 – -0.9)	<0.001**
Anterior window – Sagittal plane						
Corpus callosum length	45.7 (4.2)	46.1 (2.6)	-0.4 (-1.7 – 1.0)	0.59	-0.1 (-1.4 – 1.2)	0.83
Corpus callosum – fastigium length [†]	52.3 (3.7)	50.4 (2.7)	2.0 (0.7 – 3.2)	0.003	2.2 (1.0 – 3.4)	<0.001**
Vermis height [‡]	25.7 (2.0)	25.2 (2.6)	0.5 (-0.5 – 1.5)	0.31	0.6 (-0.3 – 1.6)	0.19
Vermis anterior-posterior diameter [•]	16.0 (2.5)	15.2 (1.7)	0.8 (-0.2 – 1.8)	0.10	0.8 (-0.1 – 1.8)	0.09
Mastoid window – Coronal plane						
Transcerebellar diameter ^β	56.2 (2.2)	55.4 (3.2)	0.8 (-0.3 – 2.0)	0.16	1.2 (0.1 – 2.2)	0.03

*Adjusted for post menstrual age. ** Significant after Holm-bonferroni correction for multiple comparison, total number of hypotheses: 45.

[§] Left basal ganglia width is missing in 1 full-term infant; [¶] Left basal ganglia till insula width is missing in 3 MP infants; [†] Corpus callosum fastigium length is missing in 2 MP and 1 LP infant(s); [‡] Vermis height is missing in 3 MP, 7 LP and 4 full-term infants; [•] Vermis anterior-posterior diameter is missing in 4 MP, 5 LP and 4 full-term infants; ^β Transcerebellar diameter is missing in 3 MP, 2 LP and 2 full-term infants.

Supplemental table 2 Unadjusted and adjusted differences in linear cUS brain measurements between late preterm and full-term infants.

Brain measurement	Late preterm		Full-term		Unadjusted difference		Adjusted difference#	
	Mean (SD)	in mm	Mean (SD)	in mm	(95% CI)	p-value	(95% CI)	p-value
Anterior window – Coronal plane								
Biparietal diameter	81.6 (4.1)		88.5 (4.4)		-6.9 (-8.4 – -5.3)	<0.001	-7.0 (-8.6 – -5.5)	<0.001**
Interhemispheric distance	2.0 (1.0)		1.8 (0.9)		0.1 (-0.3 – 0.5)	0.60	0.1 (-0.3 – 0.5)	0.62
Ventricular index								
Right	11.3 (1.6)		11.0 (2.0)		0.3 (-0.4 – 0.9)	0.44	0.1 (-0.5 – 0.8)	0.65
Left	11.5 (1.6)		10.4 (2.4)		1.2 (0.5 – 1.9)	<0.001	1.1 (0.4 – 1.8)	0.003
Anterior horn width								
Right	1.7 (1.1)		1.6 (1.2)		0.1 (-0.4 – 0.5)	0.79	0.0 (-0.5 – 0.4)	0.97
Left	1.7 (1.2)		1.3 (1.3)		0.4 (0.0 – 0.9)	0.06	0.4 (-0.1 – 0.9)	0.09
Basal ganglia width								
Right	19.8 (1.2)		21.1 (1.4)		-1.3 (-1.8 – -0.8)	<0.001	-1.3 (-1.8 – -0.8)	<0.001**
Left‡	19.7 (1.4)		20.6 (1.6)		-1.0 (-1.5 – -0.4)	<0.001	-1.0 (-1.5 – -0.4)	<0.001**
Basal ganglia – insula width								
Right	29.0 (1.8)		30.7 (1.5)		-1.7 (-2.3 – -1.1)	<0.001	-1.7 (-2.3 – -1.1)	<0.001**
Left	28.7 (1.5)		30.0 (1.6)		-1.3 (-1.9 – -0.8)	<0.001	-1.4 (-2.0 – -0.8)	<0.001**
Anterior window – Sagittal plane								
Corpus callosum length	45.2 (3.0)		46.1 (2.6)		-0.9 (-2.2 – 0.3)	0.15	-1.1 (-2.4 – 0.1)	0.07
Corpus callosum – fastigium length†	51.7 (3.0)		50.4 (2.7)		1.3 (0.1 – 2.5)	0.04	1.1 (0.1 – 2.2)	0.07
Vermis heightα	26.4 (2.4)		25.2 (2.6)		1.2 (0.3 – 2.2)	0.01	1.0 (0.1 – 2.0)	0.03
Vermis anterior-posterior diameter•	16.0 (2.7)		15.2 (1.7)		0.8 (-0.1 – 1.8)	0.08	0.8 (-0.1 – 1.7)	0.09
Mastoid window – Coronal plane								
Transcerebellar diameter	57.3 (2.8)		55.4 (3.2)		1.9 (0.8 – 3.0)	<0.001	1.7 (0.7 – 2.7)	0.002

Adjusted for post menstrual age ** Significant after Holm-bonferroni correction for multiple comparison, total number of hypotheses: 45.

‡ Missing in 1 full-term infant; † Missing in 2 MP and 1 LP infant(s); α Missing in 3 MP, 7 LP and 4 full-term infants; • Missing in 4 MP, 5 LP and 4 full-term infants; β

Missing in 3 MP, 2 LP and 2 full-term infants.

Supplemental table 3. Unadjusted and adjusted differences in two-dimensional cUS brain measurements between moderate preterm and late preterm infants.

Brain measurement	Moderate preterm Mean (SD) in mm	Late preterm Mean (SD) in mm	Unadjusted difference (95% CI)	p-value	Adjusted difference* (95% CI)	p-value
Anterior window – Coronal plane						
Biparietal diameter	79.2 (3.4)	81.6 (4.1)	-2.4 (-4.0 – -0.8)	0.004	-2.1 (-3.8 – -0.5)	0.01
Interhemispheric distance	2.2 (1.3)	2.0 (1.0)	0.2 (-0.2 – 0.6)	0.30	0.2 (-0.2 – 0.7)	0.29
Ventricular index						
Right	11.0 (1.5)	11.3 (1.6)	-0.3 (-1.0 – 0.4)	0.37	-0.1 (-0.8 – 0.6)	0.82
Left	11.1 (1.4)	11.5 (1.6)	-0.5 (-1.2 – 0.3)	0.21	-0.2 (-1.0 – 0.5)	0.56
Anterior horn width						
Right	1.8 (1.3)	1.7 (1.1)	0.1 (-0.4 – 0.6)	0.72	0.2 (-0.2 – 0.7)	0.33
Left	1.8 (1.2)	1.7 (1.2)	0.0 (-0.5 – 0.5)	0.96	0.1 (-0.4 – 0.6)	0.67
Basal ganglia width						
Right	19.8 (1.2)	19.8 (1.2)	0.0 (-0.5 – 0.5)	1.00	0.0 (-0.5 – 0.6)	0.92
Left	19.3 (1.4)	19.7 (1.4)	-0.4 (-1.0 – 0.2)	0.19	-0.3 (-0.9 – 0.3)	0.26
Basal ganglia – insula width						
Right	29.0 (1.4)	29.0 (1.8)	-0.1 (-0.7 – 0.6)	0.87	-0.0 (-0.6 – 0.7)	0.94
Left ^o	28.4 (1.3)	28.7 (1.5)	-0.2 (-0.8 – 0.4)	0.44	-0.1 (-0.7 – 0.5)	0.81
Anterior window – Sagittal plane						
Corpus callosum length	45.7 (4.2)	45.2 (3.0)	0.6 (-0.8 – 1.9)	0.40	1.0 (-0.3 – 2.3)	0.13
Corpus callosum – fastigium length [†]	52.3 (3.7)	51.7 (3.0)	0.7 (-0.6 – 1.9)	0.29	1.1 (-0.1 – 2.4)	0.08
Vermis height [‡]	25.7 (2.0)	26.4 (2.4)	-0.7 (-1.7 – 0.3)	0.17	-0.4 (-1.4 – 0.6)	0.45
Vermis anterior-posterior diameter [•]	16.0 (2.5)	16.0 (2.7)	0.0 (-1.0 – 0.9)	0.95	0.0 (-1.0 – 1.0)	0.93
Mastoid window – Coronal plane						
Transcerebellar diameter	56.2 (2.2)	57.3 (2.8)	-1.1 (-2.2 – 0.1)	0.07	-0.5 (-1.6 – 0.6)	0.37

*Adjusted for post menstrual age. ^o Missing in 3 MP infants; [†] Missing in 2 MP and 1 LP infant(s); [‡] Missing in 3 MP, 7 LP and 4 full-term infants; [•] Missing in 4 MP, 5 LP and 4 full-term infants; ^β Missing in 3 MP, 2 LP and 2 full-term infants.



CHAPTER 6

Mild brain lesions do not affect brain volumes in moderate-late preterm infants



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ABSTRACT

Purpose It is unknown whether frequently occurring mild brain lesions affect brain volumes in moderate preterm (MP; 32+0-33+6 weeks' gestation) and late preterm (LP; 34+0-35+6 weeks' gestation) infants. Therefore, we aimed to investigate the effect of mild brain lesions on brain volumes in moderate-late preterm (MLPT) infants and to compare brain volumes between MP and LP infants.

Methods From August 2017 to November 2019, eligible MLPT infants born at Isala Women and Children's Hospital were enrolled in a prospective cohort study (Brain Imaging in Moderate-late Preterm infants 'BIMP-study'). MRI was performed around term equivalent age (TEA). MRI scans were assessed for (mild) brain lesions. T2-weighted images were used for automatic segmentation of eight brain structures. Linear regression analysis was performed to compare absolute and relative brain volumes between infants with and without mild brain lesions and between MP and LP infants.

Results 36 MP and 68 LP infants were included. In infants with mild brain lesions, intracranial volume ($B=27.4\text{cm}^3$, $p=0.02$), cerebrospinal fluid ($B=8.78\text{cm}^3$, $p=0.01$) and cerebellar volumes ($B=1.70\text{cm}^3$, $p=0.03$) were significantly larger compared to infants without mild brain lesions. After correction for weight and postmenstrual age at MRI, these volumes were no longer significantly different. LP infants had larger brain volumes than MP infants, but differences were not significant. Relative brain volumes showed no significant differences in both analyses.

Conclusion Neither having mild brain lesions, nor being born moderate prematurely affected brain volumes at TEA in MLPT infants.

INTRODUCTION

Preterm birth, defined as birth before 37 weeks of gestation, is a growing public health concern. Worldwide annually 14.9 million infants are born prematurely (1). Eighty-five percent of them are born between 32 and 36 weeks of gestation (i.e. moderate-late preterm; MLPT) (1). Until recently, MLPT infants were thought to have a low risk of mortality, short- and long-term morbidities, such as neurodevelopmental problems (2, 3). However, recent studies have shown that they have a higher risk of developmental delay, cognitive impairment, behavioral and psychiatric problems as compared to term-born infants (4-9). These problems may at least partly be related to altered brain development and/or (mild) brain injury, acquired during the perinatal and/or neonatal period. To identify MLPT infants with an increased risk of abnormal or suboptimal neurodevelopmental outcome and to optimize functional outcome, more knowledge about their brain development and possible brain injury is needed (10).

Several studies have demonstrated a negative effect of brain injury on brain volumes in very preterm infants (< 32 weeks' gestation) (11). Especially moderate-severe brain lesions, such as intraventricular hemorrhage grade III, periventricular hemorrhagic infarction, post-hemorrhagic ventricular dilatation (>15mm), arterial infarction, and/or cystic white matter lesions are associated with reduced brain volumes (12-14). Moderate-severe brain lesions are only rarely encountered in MLPT infants, while mild brain lesions, such as intraventricular hemorrhage grade I, few punctate white matter lesions, irregularly shaped or mildly enlarged lateral ventricles, and punctate cerebellar hemorrhages are frequently seen (15). To the best of our knowledge, the effect of these mild brain lesions on brain volumes in MLPT infants is yet unknown.

Previously, several groups reported smaller brain volumes at term equivalent age (TEA) in MLPT infants compared to term-born infants (16-19). Smaller brain volumes in MLPT infants were associated with lower neurodevelopmental outcome scores at 2 years' corrected age (20). This suggests that brain volumes can be used as a biomarker to identify MLPT infants with increased risk of suboptimal neurodevelopmental outcome. Hitherto, moderate preterm (MP; 32+0 – 33+6 weeks' gestation) and late preterm (LP; 34+0 – 35+6 weeks' gestation) infants have mainly been approached as one group, but their risks for developmental problems are not the same (21, 22). The brains of MP infants may be more vulnerable to injury and altered development than the brains of LP infants. However, Niwa et al. and Thompson et al. did not find a significant difference between brain volumes of MP and LP infants (18, 23).

The aim of this study was to explore the effect of mild brain lesions on brain volumes in MLPT infants and to compare brain volumes at TEA between MP and LP infants. We hypothesized that around TEA brain volumes are smaller in infants with mild brain lesions than in infants without mild brain lesions and are smaller in MP infants than in LP infants.

METHODS

This study was part of a prospective cohort study, the 'BIMP-study' (the Netherlands trial register; NL6310). From August 2017 to November 2019, MLPT infants born between 32+0 and 35+6 weeks' gestation were recruited at the neonatal units (medium, high or intensive care) of Isala Women-Children's Hospital, Zwolle, The Netherlands (15). MP infants were defined as infants born between 32+0 and 33+6 weeks', and LP infants as infants born between 34+0 and 35+6 weeks' gestation. Infants with congenital anomalies of the nervous system, inborn errors of metabolism, congenital infections and chromosomal disorders, or whose parents did not speak sufficient Dutch or English were excluded. Signed informed consent was obtained from both parents. Ethical approval was given by the Central Committee in Research Involving Human Subjects, The Hague, The Netherlands (NL52323.075.15).

Data collection

All MRI scans were acquired around TEA with a 3 Tesla MRI scanner (Ingenia, Philips Healthcare, Best, The Netherlands). The scan protocol included 3-dimensional T1-weighted (voxel size 0.47x0.47x2mm), coronal and axial T2-weighted (voxel size 0.35x0.35x2mm), diffusion weighted (DWI; voxel size 0.80x0.80x3mm), and susceptibility weighted imaging (SWI; voxel size 0.31x0.31x1mm). Infants were scanned without sedation; natural sleep was induced by feeding and swaddling. Infants were subsequently immobilized with a MedVac vacuum-bag immobilizer (CFI Medical Solutions/Contour Fabricators, Fenton, MI, USA). Ear protection was provided by earmuffs (Natus MiniMuffs; Natus Medical Inc., San Carlos, CA, USA), headphones (EMS for kids, Hornchurch UK) and a polystyrene noise-insulating mattress covering the coil. Axial T2-images were acquired with a turbo spin-echo sequence with sensitivity encoding, repetition time 5483ms, echo time 110ms, flip angle 90°, matrix size 512x512, pixel spacing 0.35x0.35mm, 54 axial slices and slice thickness 2mm. Infant characteristics including sex, gestational age (GA), weight and head circumference at birth and MRI, and postmenstrual age (PMA) at MRI were collected.

Brain lesions

All MRI sequences were assessed for mild brain lesions as previously described by Boswinkel et al. (15). These included: <6 punctate white matter lesions (PWMLs) on T1-weighted images (24), inhomogeneous and/or increased white matter signal intensity on T2-weighted images (25), irregularly shaped lateral ventricles and/or ventricular index 13-15mm (26), increased width of the interhemispheric fissure (>3mm) (27), (remnants of) intraventricular hemorrhage (28), choroid plexus hemorrhage, punctate cerebellar hemorrhages (29), multiple smaller or single larger (≥ 6 mm) choroid plexus or germinolytic cysts (15). The ventricular index and interhemispheric fissure were measured on a coronal T2-weighted image at the level of the foramen of Monro.

Infants with more severe brain lesions were excluded from this part of the BIMP-study (e.g. ≥ 6 PWMLs, cystic white matter lesions, periventricular hemorrhagic infarction, post-hemorrhagic ventricular dilatation and/or arterial infarction). Immediately after the MRI procedure, scans were assessed by MFB (pediatric radiologist with >10 years of experience) for moderate-severe lesions with a potential need for intervention. Afterwards, scans were assessed by three investigators (VB: research physician, JN: radiologist with >3 years of experience and GvWM: neonatologist with >25 years of experience in pediatric neuro-imaging) by consensus. If no agreement could be achieved, the opinion of GvWM was leading. Investigators were unaware of (JN) or blinded to (VB and GvWM) the clinical course and head ultrasound findings.

MRI processing

FMRIB Software Library's (FSL) brain extraction tool (BET) (version 6.0.2, FMRIB, Oxford, UK) was used to remove skulls from the MRI scans (threshold = 0.5) (30-32). The brain extracted volume was used to calculate intracranial volume. Segmentation was performed on the axial T2-weighted images, using the morphologically adaptive neonatal tissue segmentation toolbox (MANTIS; Murdoch Children's Research Institute, Melbourne, Australia) (33). MANTIS was adapted to optimize segmentation in this specific MLPT cohort with mainly mild or no brain lesions. This adapted version of MANTIS is available at: <https://github.com/DevelopmentalImagingMCRI/mantis/tree/BIMP-NoLargeVentricles2>

Eight brain volumes were segmented: cortical gray matter (cGM), white matter (WM), cerebrospinal fluid (CSF), deep gray matter (dGM), hippocampus, amygdala, brainstem and cerebellum. Segmented structures, without CSF, were used to compute total tissue volume. A visual quality check of the segmentation was performed by MFB (radiologist), VB (research-physician) and ASV (technical medicine researcher). If no agreement could be achieved, the opinion of MFB was leading. In 25/104 scans mislabeling occurred due to severe motion artifacts. The motion affected MRI slices were re-estimated by cubic interpolation of adjacent slices (34). Subsequently, corrected scans were re-segmented and a second visual quality check was done by the same investigators (34). Scans with failed cubic interpolation attempts ($n=8$) or severe segmentation errors ($n=5$) were excluded from further analysis. Absolute and relative brain volumes were calculated with MATLAB (version 9.6, MathWorks, Natick, Massachusetts, USA). Relative volumes were defined as proportion of intracranial volume.

Statistics

Statistical analyses were executed with IBM SPSS statistics (version 25.0, IBM SPSS Statistics for Windows, IBM Corp. Released 2017). Descriptive characteristics were calculated for infants with and without mild brain lesions, and for MP and LP infants, and for excluded infants. Mean (SD) values were calculated for continuous variables and frequencies (%) for categorical variables. Differences in baseline characteristics between infants with and without brain lesions, between MP and LP infants, and between excluded and included infants were studied with the Fisher's exact (dichotomous variables) and independent-samples t-test (continuous

variables). Linear regression analyses were used to compare absolute and relative brain volumes of infants with and without mild brain lesions, and MP and LP infants. Confounders 'weight at MRI' and 'PMA' were included in the analyses. Results were expressed in difference in volume with 95% confidence interval (CI). Significance levels were set at $p < 0.05$.

RESULTS

Participants

A cohort of 167 MLPT infants was enrolled in the 'BIMP-study', of whom 127 infants (mean GA = 34 ± 2 [± 1] weeks, 69 boys) underwent MRI. Of these 127 infants, twenty-three (18.1%) subjects were excluded from this part of the BIMP-study (Figure 1). In total, MRI scans of 104 infants (mean PMA 41.1 [± 1.6] weeks) without or with only mild brain lesions were included for analysis. Neonatal descriptive characteristics were not significantly different for excluded infants compared to included infants. Due to the high PMA (>47 weeks) at TEA MRI of two excluded infants, PMA ($p < 0.001$), weight ($p = 0.01$) and head circumference ($p = 0.006$) at TEA were significantly higher for the excluded infants (Table 1). In the included group of infants, head circumference at MRI (unadjusted for PMA) was significantly larger in infants with mild brain lesions than in infants without mild brain lesions ($p = 0.02$). Mean PMA ($p = 0.003$), weight ($p = 0.03$) and head circumference ($p = 0.049$) at MRI were significantly higher for LP infants than MP infants (Table 1).

Brain lesions

Of the twenty-three excluded infants, four (17.4%) infants had no brain lesions (one infant with missing T2-weighted images, one infant with an MRI performed at PMA >47 weeks and two infants with irreparable motion artifacts) and thirteen (56.5%) had mild brain lesions (one infant with missing T2-weighted images, one infant with an MRI performed at PMA >47 weeks, five infants with severe segmentation errors and six infants with irreparable motion artifacts) (Table 2). As moderate-severe brain lesions were an exclusion criteria for this part of the BIMP-study, all six (5%) infants who had moderate-severe brain lesions on MRI were also excluded: one infant with a periventricular hemorrhagic infarction, one with a posterior cerebral artery infarction and moderate-severe ex-vacuo ventricular dilatation, one with isolated moderate-severe ex-vacuo ventricular dilatation, and three infants with ≥ 6 PWMLs.

Of the included infants, sixty-eight (65%) infants had mild brain lesions. Mild brain lesions were more frequently seen in MP than in LP infants (respectively 61% and 51%), but this difference was not significant ($p = 0.41$). There was no significant difference between boys and girls for presence of mild brain lesions ($p = 0.54$). The other general characteristics of infants with and without mild brain lesions were also not significantly different (Table 1).

MRI processing

The adapted version of MANTiS visually restored over-segmentation of CSF in the cerebellum of all infants ($n = 104$; example in Figure 2).

Figure 1. In- and exclusion flowchart.

Processing steps with number of participants and number of excluded infants are shown. PMA: Postmenstrual age.

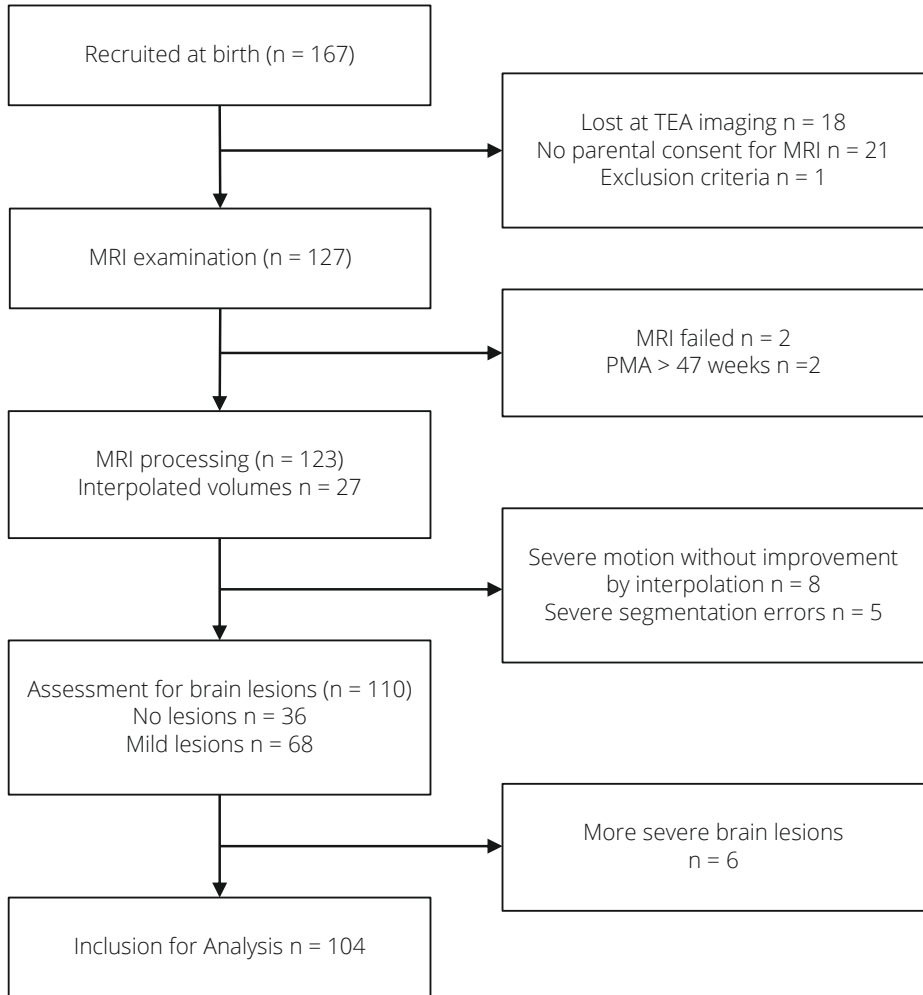


Table 1. Participant characteristics

Characteristics	Characteristics of infants without and with mild brain lesions			Characteristics of MP and LP infants			
	No lesions, n = 36	Mild lesions, n = 68	p-value	MP n = 36	LP n = 68	p-value	Excluded infants n = 23
Neonatal period							
Gestational age in weeks, mean (SD)	34.3 (1.1)	34.3 (1.2)	0.87	33.0 (0.6)	35.0 (0.5)	<0.001	34.3 (1.1)
Birth weight in grams, mean (SD)	2229 (486)	2332 (446)	0.24	2012 (397)	2442 (424)	<0.001	2188 (388)
Head circumference in cm, mean (SD)	31.4 (1.7)	31.7 (1.8)	0.33	30.4 (1.9)	32.2 (1.4)	<0.001	31.7 (1.3)
Boys, n (%)	17 (47.2)	37 (54.4)	0.54*	19 (52.8)	35 (51.5)	>0.99*	16 (69.6)
Girls, n (%)	19 (52.8)	31 (45.6)		17 (47.2)	33 (48.5)		7 (30.4)
Singleton, n (%)	27 (75.0)	47 (69.1)	0.84**	26 (72.2)	48 (70.6)	0.71**	16 (69.6)
Twin, n (%)	8 (22.2)	19 (27.9)		10 (27.8)	17 (25.0)		7 (30.4)
Triplet, n (%)	1 (2.8)	2 (2.9)		0 (0)	3 (4.4)		0 (0)
Admission to neonatal intensive care, n (%)	7 (19.4)	21 (30.9)	0.83	15 (41.7)	13 (19.1)	0.02	2 (8.7)
Characteristics at TEA MRI							
PMA in weeks, mean (SD)	41.0 (1.5)	41.2 (1.3)	0.53	40.6 (1.3)	41.5 (1.6)	0.003	42.9 (3.1)
Weight in grams, mean (SD)	3364 (592)	3588 (553)	0.06	3361 (411)	3589 (633)	0.03	3837 (784)
Head circumference in cm, mean (SD)	35.3 (1.5)	36.0 (1.4)	0.02	35.4 (1.3)	36.0 (1.5)	0.049	36.7 (1.8)

Values in bold are significant. PMA: postmenstrual age; MP: moderate preterm; LP: late preterm; TEA* term equivalent age; *p-value was calculated for sex, **p-value was calculated for plurality. # Excluded compared to included infants

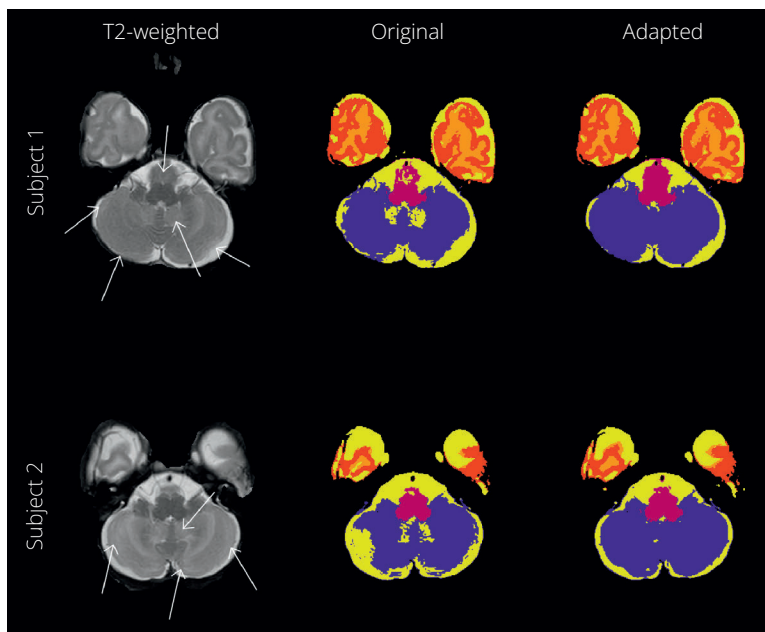
Table 2. Overview of brain lesions in included and excluded MLPT infants.

Severity		Included infants n = 104	Excluded infants n = 23
No brain lesions, n (%)		36 (34.6)	4 (17.4)
Mild brain lesions present, n (%)		68 (65.4)	13 (56.5)
Moderate-severe brain lesions present, n (%)		0 (0)	6 (26.1)
Type of brain lesion and frequency			
Hemorrhages			
Remnants of IVH (grade 1 – 2), n (%)		5 (4.8)	3 (13.0)
Periventricular hemorrhagic infarction, n (%)		0 (0)	1 (4.3)
Choroid plexus hemorrhage, n (%)		4 (3.8)	1 (4.3)
Punctate CBH, n (%)		12 (11.5)	4 (17.4)
White matter			
Cystic white matter lesions, n (%)		0 (0)	0 (0)
Inhomogeneous and/or increased diffuse white matter signal changes ^a , n (%)		(n = 99) 19 (19.2)	(n = 16) 8 (50.0)
PWML [#]	< 6, n (%)	(n = 101)	(n = 21)
	≥ 6, n (%)	15 (14.9)	2 (9.5)
		0 (0)	3 (14.3)
Infarction			
Arterial infarction, n (%)		0 (0)	1 (4.3)
Deep gray matter lesions	Small focal lesion, n (%)	0 (0)	1 (4.3)
	Moderate-severe lesion, n (%)	0 (0)	0 (0)
Miscellaneous			
Choroid plexus cyst ≥ 6 mm, n (%)			
Germinolytic or subependymal cyst ≥ 6 mm, n (%)		7 (6.7)	2 (8.7)
Signs suggestive of brain atrophy due to injury			
Ex-vacuo ventricular dilatation*	Mild (13 – 15 mm), n (%)	(n = 97)	(n = 21)
	Moderate-severe (> 15 mm), n (%)	26 (26.8)	8 (38.1)
Irregular shape of the lateral ventricles [‡] , n (%)		0 (0)	2 (9.5)
		(n = 103)	(n = 23)
Widened interhemispheric fissure*, n (%)		13 (12.6)	7 (30.4)
		(n = 97)	(n = 21)
		20 (20.6)	9 (42.9)

^a MRI assessment of diffuse white matter signal changes was missing in 12 infants due to poor imaging quality. [#] Assessment of PWML was missing in 5 infants on MRI due to poor imaging quality. [‡] MRI assessment of deep gray matter and irregular shape of the lateral ventricles was missing in 1 infant due to poor imaging quality. *MRI measurement was missing in 9 infants due to poor imaging quality. IVH: intraventricular hemorrhage; CBH: cerebellar hemorrhage; PWML: punctate white matter lesions

Figure 2. Results after adapting MANTiS segmentation toolbox for data collected in the 'BIMP-study'.

The left column shows T2-weighted MRI slices of two infants (subject 1: GA 35+6 weeks, MRI performed at PMA 40+6 weeks and subject 2: GA 35+4 weeks, MRI performed at PMA 38+4 weeks), the middle column shows segmentation results with the original MANTiS toolbox and the right column shows segmentation with the adapted MANTiS toolbox. Over-segmentation of CSF in the cerebellum is observed with the original MANTiS toolbox. Large errors are removed by adjusting the pipeline. Changes are indicated by arrows in the T2-weighted scans. Volumes are color coded as follows: red: cortical gray matter, orange: white matter, yellow: cerebrospinal fluid, blue: cerebellum, purple: brainstem. GA: gestational age, PMA: postmenstrual age.



Comparison of brain volumes between infants with and without mild brain lesions

Without correction for weight and PMA at MRI, intracranial volume (difference in volume = 27.44 cm³, $p = 0.02$), CSF (difference in volume = 8.78 cm³, $p = 0.01$), and cerebellar volume (difference in volume = 1.70 cm³, $p = 0.03$) were significantly larger in infants with mild brain lesions. Relative volumes were not different between infants with and without mild brain lesions. After correction for weight and PMA at MRI, none of the brain volumes were significantly different between infants with and without mild brain lesions (Table 3).

Comparison of brain volumes between MP and LP infants

Almost all absolute brain volumes were larger in LP infants than in MP infants. Only hippocampal and amygdala volumes were slightly smaller in LP infants. With regard to relative volumes, in LP infants only larger relative volumes of cGM and cerebellum were found. These differences between MP and LP infants were not significant, neither with nor without correction for weight at MRI and PMA (Table 4).

Table 3. Mean absolute and relative volumes in infants with and without mild brain lesions and linear regression analysis results before and after correction of confounding factors (i.e. weight and postmenstrual age at MRI).

Brain region	Mean volumes (cm ³)		Univariate analysis		Multivariate analysis	
	No lesions	Mild lesions	Coefficient (95% CI)	p-value	Coefficient (95% CI)	p-value
	n = 36 (±SD)	n = 68 (±SD)				
Intracranial volume	462 (63.5)	489 (50.9)	27.4 (4.74 - 50.1)	0.02	13.8 (-0.847 - 28.4)	0.07
Total brain tissue	398 (53.4)	417 (43.7)	18.7 (-0.658 - 38.0)	0.06	8.17 (-5.49 - 21.8)	0.24
cGM ^a	187 (34.0)	198 (31.6)	11.3 (-2.00 - 24.5)	0.10	4.45 (-4.74 - 13.6)	0.34
WM ^b	145 (16.1)	150 (12.1)	4.13 (-1.44 - 9.70)	0.14	2.05 (-3.17 - 7.26)	0.44
CSF ^c	64.3 (16.6)	73.0 (16.8)	8.78 (1.94 - 15.6)	0.01	5.62 (-0.290 - 11.5)	0.06
dGM ^d	26.3 (2.98)	27.3 (2.26)	1.03 (-0.007 - 2.06)	0.05	0.545 (-0.310 - 1.40)	0.21
Hippocampus	3.48 (0.80)	3.57 (0.82)	0.090 (-0.244 - 0.424)	0.59	0.027 (-0.309 - 0.364)	0.87
Amygdala	1.47 (0.25)	1.59 (0.35)	0.119 (-0.012 - 0.250)	0.08	0.089 (-0.041 - 0.281)	0.18
Cerebellum	27.5 (4.85)	29.2 (3.00)	1.70 (0.172 - 3.23)	0.03	0.856 (-0.119 - 1.83)	0.09
Brainstem	7.12 (0.77)	7.41 (0.75)	0.286 (-0.023 - 0.596)	0.07	0.124 (-0.143 - 0.39)	0.36
Ratio cGM	0.403 (0.030)	0.403 (0.027)	-4.6E-5 (-0.012 - 0.011)	0.99	-0.003 (-0.013 - 0.008)	0.63
Ratio WM	0.316 (0.026)	0.307 (0.027)	-0.009 (-0.020 - 0.001)	0.09	-0.005 (-0.014 - 0.004)	0.24
Ratio CSF	0.138 (0.027)	0.149 (0.028)	0.011 (-0.001 - 0.022)	0.07	0.008 (-0.003 - 0.019)	0.15
Ratio dGM	0.057 (0.004)	0.056 (0.004)	-0.001 (-0.003 - 0.0004)	0.16	-0.001 (-0.002 - 0.001)	0.44
Ratio Hippocampus	0.008 (0.002)	0.007 (0.002)	-0.0002 (-0.001 - 0.0005)	0.50	-0.0002 (-0.001 - 0.001)	0.67
Ratio Amygdala	0.003 (0.001)	0.003 (0.001)	0.00004 (-0.0002 - 0.0003)	0.78	0.0001 (-0.0002 - 0.0003)	0.62
Ratio Cerebellum	0.059 (0.004)	0.060 (0.004)	0.0005 (-0.001 - 0.002)	0.57	0.0004 (-0.001 - 0.002)	0.62
Ratio Brainstem	0.016 (0.002)	0.015 (0.001)	-0.0004 (-0.001 - 0.0002)	0.22	-0.0003 (-0.001 - 0.0002)	0.28

Values in bold are significant. cGM: cortical gray matter; CSF: cerebrospinal fluid; dGM: deep gray matter; WM: white matter.

Table 4. Mean absolute and relative volumes in MP and LP infants and linear regression analysis results before and after correction of confounding factors (i.e. postmenstrual age and weight at MRI).

Brain region	Mean volumes (cm ³)		Univariate analysis		Multivariate analysis	
	MP (±SD)	LP (±SD)	Coefficient (95% CI)	p-value	Coefficient (95% CI)	p-value
Intracranial volume	469 (47.6)	486 (60.6)	-171 (-40.2 - 5.95)	0.14	9.10 (-6.10 - 24.3)	0.24
Total brain tissue	400 (42.1)	416 (50.1)	-15.6 (-35.0 - 3.80)	0.11	5.18 (-8.92 - 19.3)	0.47
cGM ^c	187 (26.8)	198 (35.1)	-10.7 (-24.0 - 2.56)	0.11	4.14 (-5.30 - 13.6)	0.39
WM ^d	146 (15.2)	149 (12.8)	-3.19 (-8.78 - 2.40)	0.26	-0.333 (-5.71 - 5.04)	0.90
CSF ^e	69.0 (14.0)	70.5 (18.7)	-1.52 (-8.57 - 5.53)	0.67	3.92 (-2.20 - 10.1)	0.21
dGM ^f	26.8 (2.22)	27.0 (2.74)	-0.260 (-1.31 - 0.791)	0.63	0.676 (-0.199 - 1.55)	0.12
Hippocampus	3.56 (0.71)	3.53 (0.87)	0.027 (-0.307 - 0.361)	0.87	0.105 (-0.241 - 0.450)	0.55
Amygdala	1.57 (0.30)	1.53 (0.34)	0.038 (-0.095 - 0.171)	0.57	0.100 (-0.033 - 0.233)	0.14
Cerebellum	27.7 (3.05)	29.13 (4.09)	-1.48 (-3.02 - 0.057)	0.06	0.326 (-0.688 - 1.34)	0.53
Brainstem	7.25 (0.68)	7.34 (0.81)	-0.086 (-0.400 - 0.228)	0.59	0.096 (-0.178 - 0.371)	0.49
Ratio cGM	0.398 (0.026)	0.405 (0.029)	-0.007 (-0.019 - 0.004)	0.21	0.001 (-0.009 - 0.012)	0.83
Ratio WM	0.312 (0.025)	0.309 (0.028)	0.003 (-0.008 - 0.014)	0.61	-0.008 (-0.017 - 0.001)	0.08
Ratio CSF	0.147 (0.026)	0.144 (0.029)	0.003 (-0.008 - 0.014)	0.61	0.007 (-0.005 - 0.018)	0.25
Ratio dGM	0.058 (0.003)	0.056 (0.004)	0.001 (-0.0002 - 0.003)	0.09	0.0003 (-0.001 - 0.002)	0.72
Ratio Hippocampus	0.008 (0.002)	0.007 (0.002)	0.0004 (-0.0003 - 0.001)	0.30	0.0001 (-0.001 - 0.001)	0.77
Ratio Amygdala	0.003 (0.001)	0.003 (0.001)	0.0002 (-0.0001 - 0.0004)	0.15	0.0001 (-0.0001 - 0.004)	0.31
Ratio Cerebellum	0.059 (0.004)	0.060 (0.004)	-0.001 (-0.003 - 0.001)	0.25	0.0004 (-0.0002 - 0.001)	0.60
Ratio Brainstem	0.016 (0.001)	0.015 (0.001)	0.0003 (-0.0002 - 0.001)	0.24	-0.0001 (-0.001 - 0.0004)	0.63

cGM: cortical gray matter; CSF: cerebrospinal fluid; dGM: deep gray matter; LP: late preterm; MP: moderate preterm; WM: white matter.

DISCUSSION

Using an adapted MANTiS toolbox, we calculated brain volumes around TEA in MLPT infants and analyzed differences in brain volumes between MLPT infants with and without mild brain lesions, and between MP and LP infants. No differences were found between MLPT infants with and without mild brain lesions, or between MP and LP infants. These findings indicate that neither having mild brain lesions, nor being born MP had a measurable effect on brain volumes in MLPT infants.

In very preterm infants, moderate-severe brain injury is associated with a decrease in cGM, WM and cerebellar volumes, and an increase in CSF volumes (11, 35). Moderate-severe brain lesions are common in very preterm infants but are less frequently found in MLPT infants (15). Although mild brain lesions were frequently seen in MLPT infants, we did not find significant differences in brain volumes between MLPT infants with and without mild brain lesions. Kelly et al. reported that regional cortical gray matter and white matter volumes and, in addition, white matter microstructural alterations but not brain volumes, were associated with poorer cognitive and language scores in MLPT infants at two years of age (36). Future studies should therefore also investigate the effect of mild brain lesions on microstructural alterations in the MLPT preterm brain.

Our study supports the findings by Niwa et al. and Thompson et al. who did not find a significant difference between brain volumes of MP and LP infants (18, 23). Nevertheless, we saw some differences in absolute brain volumes between our and their studies. Niwa et al. reported smaller average brain volumes, which is probably related to lower PMA at MRI (mean PMA=38.6 versus 41.1 weeks in our study) (18). Furthermore, CSF volumes were respectively 23% and 26% lower in MP and LP infants in our study than as reported by Thompson et al. (23). These differences can probably be explained by MANTiS optimization, interpolation of motion artifacts and/or brain extraction threshold.

Contrary to our findings and the findings by Niwa et al. and Thompson et al., other studies found a significant linear association between GA at birth and brain volumes at TEA (37-39). An explanation for this might be that these studies used a wider GA spectrum (i.e. 24-42 weeks) and differences in brain volumes may have become more apparent between the two ends of the spectrum. However, as these studies used different statistical methods (i.e. student's independent t test, linear regression and linear mixed-effects model) a reliable comparison between the results of these studies and our study is not possible.

Strengths of our study are the prospective design, careful evaluation of mild brain lesions in our cohort and the application of methodological improvements to determine brain volumes. Segmentation was enhanced by using an interpolation technique to handle motion artifacts [34]. Additionally, adjustments to MANTiS optimized segmentation of the data. Nevertheless, limitations should be mentioned. Ideally, data should have been compared

to the data of a control group consisting of healthy full-term born infants, but inclusion of healthy full-term infants was not possible. Also, we were not yet able to investigate the association between brain volumes and neurodevelopmental outcome as follow-up is still ongoing. Secondly, infants born at GA 36+0 to 36+6 weeks were not routinely admitted and therefore not enrolled. Thirdly, motion artifacts were frequently encountered on MRI. Although infants were immobilized, fast imaging techniques were used and post-processing techniques were implemented, motion artifacts may still have influenced estimated brain volumes in some cases. Fourthly, inter-rater reliability testing of the assessment of brain lesions was not performed as MRI scans were assessed by consensus. Fifthly, the relative small sample size and low incidence of specific brain lesions hampered sub-analysis at lesion level. Finally, volumes of small structures such as hippocampus and amygdala should be interpreted with care, since Beare et al. showed a Dice agreement between manual and MANTiS segmentation of respectively 0.66 and 0.51 for these structures [33]. For such tiny brain structures segmentation accuracy may not be sufficient to draw valid conclusions.

Development of the neonatal brain is influenced by a wide variety of peri- and/or postnatal factors, including brain lesions (11, 40). More insight into brain development and neurodevelopmental outcome in MLPT infants may be provided by 1) investigating the effect of perinatal factors on brain volumes as well as the effect of (mild) brain lesions at lesion level in a larger cohort of MLPT infants and 2) investigating the association between these factors and neurodevelopmental outcome. We aim to perform short and long-term neurodevelopmental follow-up in this MLPT cohort to investigate whether brain volumes and/or mild brain lesions are associated with neurodevelopmental outcome.

To conclude, neither having mild brain lesions, nor being born moderate prematurely have a measurable effect on brain volumes in moderate-late preterm infants. Further research is required to fully understand the effect of brain lesions and other perinatal factors on brain development in moderate-late preterm infants, and to investigate the association with neurodevelopmental outcome.

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REFERENCES

1. Chawanpaiboon S, Vogel JP, Moller A, et al. Global, regional, and national estimates of levels of preterm birth in 2014: a systematic review and modelling analysis. *The Lancet Global Health* 2019;7(1):e37-e46.
2. Atkinson J, Braddick O, Anker S, et al. Coritcal vision, MRI and developmental outcome in preterm infants. *Archives of Disease in Childhood: Fetal and Neonatal Edition* 2008;93(4):F292-F297.
3. Davidoff MJ, Dias T, Damus K, et al. Changes in the Gestational Age Distribution among U.S. Singleton Births: Impact on Rates of Late Preterm Birth, 1992 to 2002. *Seminars in Perinatology* 2006;30(1):8-15.
4. Nepomnyaschy L, Hegyi T, Ostfeld BM, Reichman NE. Developmental Outcomes of Late-Preterm Infants at 2 and 4 Years. *Matern Child Health J* 2012;16(8):1612-1624.
5. Reuner G, Weinschenk A, Pauen S, Pietz J. Cognitive development in 7- to 24-month-old extremely/very-to-moderately/late preterm and full-term born infants: The mediating role of focused attention. *Child Neuropsychology* 2015;21(3):314-330.
6. Johnson S, Evans TA, Draper ES, et al. Neurdevelopmental outcomes following late and moderate prematurity: a population-based cohort study. *Arch Dis Child Fetal Neonatal Ed* 2015;100:F301-F308..
7. Woythaler M, McCormick MC, Mao W, Smith VC. Late Preterm Infants and Neurodevelopmental Outcomes at Kindergarten. *Pediatrics* 2015;136(3):424-431.
8. Kerstjens JM, de Winter AF, Bocca-Tjeertes IF, ten Vergert, EMJ, Reijneveld SA, Bos AF. Developmental Delay in Moderately Preterm-Born Children at School Entry. *Journal of Pediatrics*, The 2011;159(1):92-98.
9. Cheong JLY, Doyle LW, Burnett AC, et al. Association Between Moderate and Late Preterm Birth and Neurodevelopment and Social-Emotional Development at Age 2 Years. *JAMA Pediatrics* 2017;171(4):e164805.
10. Boswinkel V, Nijboer-Oosterveld J, Nijholt IM, Edens MA, Mulder - de Tollenaer SM, Boomsma MF, et al. A systematic review on brain injury and altered brain development in moderate-late preterm infants. *Early Hum Dev* 2020 -5-28;148
11. Keunen K, Kersbergen KJ, Groenendaal F, Isgum I, de Vries LS, Benders MJNL. Brain tissue volumes in preterm infants: prematurity, perinatal risk factors and neurodevelopmental outcome: a systematic review. *J Matern Fetal Neonatal Med* 2012 Apr;25 Suppl 1:89-100.
12. Kersbergen KJ, Makropoulos A, Aljabar P, et al. Longitudinal Regional Brain Development and Clinical Risk Factors in Extremely Preterm Infants. *The Journal of Pediatrics* 2016;178:93-100.e6.
13. Thompson DK, Wood SJ, Doyle LW, et al. Neonate hippocampal volumes: Prematurity, perinatal predictors, and 2-year outcome. *Annals of neurology* 2008;63(5):642-651.
14. Limperopoulos C, Soul JS, Haidar H, et al. Impaired Trophic Interactions Between the Cerebellum and the Cerebrum Among Preterm Infants. *Pediatrics* 2005;116(4):844-850.
15. Boswinkel V, Krüse-Ruijter MF, Nijboer - Oosterveld J, et al. Incidence of brain lesions in moderate-late preterm infants assessed by cranial ultrasound and MRI: The BIMP-study. *European journal of radiology* 2021;136(109500):109500.
16. Walsh JM, Doyle LW, Anderson PJ, Lee KJ, Cheong JLY. Moderate and Late Preterm Birth: Effect on Brain Size and Maturation at Term-Equivalent Age. *Radiology* 2014;273(1):232-240.
17. Hüppi PS, Warfield S, Kikinis R, et al. Quantitative magnetic resonance imaging of brain development in premature and mature newborns. *Annals of Neurology* 1998;43(2):224-235.

18. Niwa T, Suzuki K, Sugiyama N, Imai Y. Regional volumetric assessment of the brain in moderately preterm infants (30–35 gestational weeks) scanned at term-equivalent age on magnetic resonance imaging. *Early Human Development* 2017;111:36-41.
19. Kugelman A, Colin AA. Late Preterm Infants: Near Term But Still in a Critical Developmental Time Period. *Pediatrics* 2013;132(4):741-751.
20. Cheong JLY, Thompson D, Spittle A, Potter C, Walsh J, Burnett A, et al. Brain Volumes at Term-Equivalent Age Are Associated with 2-Year Neurodevelopment in Moderate and Late Preterm Children. *J Pediatr* 2016;174:91-97.e1.
21. Chyi LJ, Lee HC, Hintz SR, Gould JB, Sutcliffe TL. School Outcomes of Late Preterm Infants: Special Needs and Challenges for Infants Born at 32 to 36 Weeks Gestation. *Journal of Pediatrics*, The 2008;153(1):25-31.
22. Lipkind HS, Slopen ME, Pfeiffer MR, McVeigh KH. School-age outcomes of late preterm infants in New York City. *American Journal of Obstetrics and Gynecology* 2012;206(1):222.e1-6.
23. Thompson DK, Kelly CE, Chen J, et al. Characterisation of brain volume and microstructure at term-equivalent age in infants born across the gestational age spectrum. *NeuroImage: Clinical* 2019;21:101630.
24. Martinez Biarge M, Groenendaal F, Kersbergen KJ, Benders MJNL, Foti F, Cowan FM et al. MRI based preterm white matter injury classification: the importance of sequential imaging in determining severity of injury. *PLoS One* 2016;11(6):e0156245.
25. de Bruïne FT, van den Berg-Huysmans AA, Leijser LM, et al. Clinical Implications of MR Imaging Findings in the White Matter in Very Preterm Infants: A 2-year Follow-up Study. *Radiology* 2011;261(3):899-906.
26. Leijser LM, de Bruïne FT, Steggerda SJ, van der Grond J, Walther FJ, van Wezel-Meijler G. Brain imaging findings in very preterm infants throughout the neonatal period: Part I. Incidences and evolution of lesions, comparison between ultrasound and MRI. *Early human development* 2009;85(2):101-109.
27. Kidokoro H, Neil J, Inder T. New MR Imaging Assessment Tool to Define Brain Abnormalities in Very Preterm Infants at Term. *American journal of neuroradiology : AJNR* 2013;34(11):2208-2214.
28. Volpe JJ. Intraventricular hemorrhage in the premature infant -- current concepts. Part II. *Annals of Neurology* 1989;25(2):109-116.
29. Boswinkel V, Steggerda SJ, Fumagalli M, Parodi A, Ramenghi LA, Groenendaal F et al. The CHOPIn Study: a Multicenter Study on Cerebellar Hemorrhage and Outcome in Preterm Infants. *Cerebellum* 2019;18(6):989-998.
30. Jenkinson M, Beckmann CF, Behrens TEJ, Woolrich MW, Smith SM. FSL. *NeuroImage* 2012;782-790. DOI:10.1016/j.neuroimage.2011.09.015.
31. Smith SM, Jenkinson M, Woolrich MW, et al. Advances in functional and structural MR image analysis and implementation as FSL. *Neuroimage* 2004;23:S208-S219.
32. Smith SM. Fast robust automated brain extraction. *Human brain mapping* 2002;17(3):143-155.
33. Beare RJ, Chen J, Kelly CE, et al. Neonatal Brain Tissue Classification with Morphological Adaptation and Unified Segmentation. *Frontiers in neuroinformatics* 2016;10:12.
34. Verschuur AS, Boswinkel V, van Osch, J A C, et al. Cubic interpolation for automatic brain segmentation of MRI motion artefacts in moderate and late preterm infants (Abstract ID: 200144983). *Radiological Society of North America* 2020

35. Kersbergen KJ, Makropoulos A, Aljabar P, et al. Longitudinal Regional Brain Development and Clinical Risk Factors in Extremely Preterm Infants. *The Journal of Pediatrics* 2016;178:93-100.e6.
36. Kelly CE, Thompson DK, Spittle AJ, et al. Regional brain volumes, microstructure and neurodevelopment in moderate-late preterm children. *Archives of Disease in Childhood - Fetal and Neonatal Edition* 2020;0:F1-F7.
37. Inder TE, Warfield SK, Wang H, Hüppi PS, Volpe JJ. Abnormal Cerebral Structure Is Present at Term in Premature Infants. *Pediatrics (Evanston)* 2005;115(2):286-294.
38. Ball G, Boardman JP, Rueckert D, et al. The Effect of Preterm Birth on Thalamic and Cortical Development. *Cerebral cortex* 2012;22(5):1016-1024.
39. Makropoulos A, Aljabar P, Wright R, et al. Regional growth and atlas of the developing human brain. *NeuroImage* 2016;125:456-478.
40. Thompson DK, Kelly CE, Chen J, Beare R, Alexander B, Seal M et al. Early life predictors of brain development at term-equivalent age in infants born across the gestational age spectrum. *NeuroImage* 2019;185:813-824.





PART IV

NEURODEVELOPMENTAL AND BEHAVIORAL OUTCOME IN MODERATE-LATE PRETERM INFANTS AND IN PRETERM INFANTS WITH CEREBELLAR HEMORRHAGE



CHAPTER 7

Neonatal neuro-imaging findings and neurodevelopmental outcome at 24 months corrected age in moderate-late preterm infants: a preliminary descriptive analysis of the BIMP-study



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ABSTRACT

Purpose The aim of this preliminary descriptive overview was to assess the incidence of neurodevelopmental and/or behavioral problems in moderate preterm (MP; 32+0 – 33+6 weeks' gestation) and late preterm (LP; 34+0 – 35+6 weeks' gestation) infants. Furthermore, we will give an overview of abnormal neonatal MRI findings and how these are distributed within a group of infants with a normal and suboptimal neurodevelopmental and/or behavioral outcome.

Methods The BIMP (Brain Imaging in Moderate-late preterm infants) study is a prospective cohort study carried out at Isala Women and Children's hospital, the Netherlands. Infants born between 32+0 – 35+6 weeks' gestation were included from August 2017 to November 2019. MRI was performed at term equivalent age (TEA). Grading (none/mild/moderate-severe) and type of abnormal MRI findings were assessed. Follow-up consisted of three validated parent reported questionnaires regarding neurodevelopment, behavior and autism. Questionnaires were asked to be returned between 22 and 26 months of corrected age. The results of the three questionnaires were combined in a composite outcome. A suboptimal composite outcome score was defined as a score < -2 SD for the Ages and Stages questionnaire, ≥ 60 for the Child Behavior Check List or ≥ 3 for the Modified Checklist for Autism in Toddlers, Revised with Follow-Up. As the follow-up of the BIMP-study has not been finalized yet, we will only give a descriptive overview of the results. No statistical analysis will be performed at this stage.

Results At the time we conducted this preliminary descriptive overview, 98 of the 167 infants included in the BIMP study (58.7%) had reached 24 months corrected age and were eligible for follow-up. Parents of 85/98 (86.7%) infants completed the questionnaires, all were returned within the given time window. A suboptimal composite outcome was present in 12/30 (40%) MP and 14/55 (25.5%) LP infants. The incidence for the total group was 26/85 (30.6%). At TEA, an MRI had been performed in 68/85 (80%) of infants. Mildly abnormal neonatal MRI findings were present in 41/68 (66.2%). Of this group, 10 infants had a suboptimal composite outcome (10/41; 24%). Only 1 out of 4 infants (25%) with moderate-severe abnormal neonatal MRI findings had a suboptimal composite outcome. Mild ex-vacuo ventricular dilatation was the most frequently encountered abnormal MRI finding ($n = 22$). Most infants with this finding had a normal composite outcome (17/22; 77.3%).

Conclusion In this preliminary descriptive overview, almost a third of the infants had a suboptimal composite outcome at 24 months corrected age. Mildly abnormal MRI findings were frequently encountered but did not lead to a suboptimal outcome in all infants. At present, it is not possible to draw conclusions about a possible relation between neonatal MRI findings and neurodevelopmental outcome. Final results can be expected in one year from now.

INTRODUCTION

Globally, each year more than 15 million infants are born prematurely, before a gestational age (GA) of 37 weeks (1). Moderate-late preterm (MLPT) infants are born between 32 and 37 weeks of gestation and account for more than 80 percent of the total preterm population. However, so far only a relatively small number of studies has been dedicated to neonatal neuro-imaging and neurodevelopmental outcome in MLPT infants.

MLPT infants have a higher risk of neurodevelopmental and behavioral problems compared to full-term infants (GA > 37 weeks) (2-4). Also later in life, MLPT born teens and adolescents encounter more educational and health problems than their term born peers (5-7). The incidence of these problems is slightly higher in individuals born moderately prematurely (MP; GA 32+0 – 33+6 weeks) than late prematurely (LP; GA 34+0 – 36+6 weeks) (5). So far, little is known about the relation between neonatal neuro-imaging findings and these problems. During the last trimester of pregnancy, the fetal brain grows rapidly and important maturational changes take place, including rapid gyration and sulcation, establishment of connectivity and beginning of myelination (12-17). This rapid growth and development render the brain susceptible to injury.

Up to now, as far as we know, only two studies investigated possible associations between brain injury and neurodevelopmental outcome in MLPT infants. Nakasone et al. performed cranial ultrasound (cUS) in a group of infants born at GA 34+0 – 35+6 weeks and found intraventricular hemorrhages (IVH) in three out of 135 infants. All three infants had a suboptimal developmental score at 18 months corrected age (18). In a magnetic resonance imaging (MRI) study by Cheong et al. none of the infants (GA 32+0 – 36+6 weeks) had an IVH, while white matter signal abnormalities were seen in ten out of 199 (5%) infants. They found no association between brain lesions, brain maturation and neurodevelopmental outcome at 24 months corrected age (19). In another paper, by the same research group, associations were found between brain volumes, white matter microstructure and cognitive and language outcome at 24 months corrected age. No associations were found between brain volumes or white matter microstructure and behavioral problems (20). The association between brain lesions and behavioral problems was not investigated in this study and no distinction was made between MP and LP infants.

As much is still unknown in this large and vulnerable but relatively understudied population, we have attempted to fill-in some of the gaps in knowledge performing the BIMP-study (Brain Imaging in Moderate-late Preterm infants) in 2017. The BIMP-study is a prospective cohort study, in which we performed serial cUS and a single MRI in MLPT infants (GA 32+0 – 35+6 weeks). In Chapter 4 of this thesis, we reported that, besides IVH and punctate white matter lesions (PWML), several other (mainly mild) brain lesions and delayed brain development are frequently present (21). So far, it is unknown whether these lesions affect neurodevelopmental outcome. We are currently conducting neurodevelopmental follow-

up around 24 months corrected age, which is still ongoing. For the follow-up we use three validated parent reported questionnaires: the Ages and Stages Questionnaire (ASQ), the Child Behavior Checklist (CBCL) and the Modified Checklist for Autism in Toddlers, Revised with Follow-Up (M-Chat-R/F). These questionnaires have previously been used in MLPT infants at 24 months corrected age and results were reported by others (Table 1).

One of the final aims of the BIMP-study is to investigate if brain lesions are associated with neurodevelopmental and/or behavioral outcome. If so, it is important to perform neuro-imaging in MLPT infants with an increased risk of brain injury, as this enables early intervention, such as physiotherapy, speech therapy and/or other interventions in order to improve functional outcome. As the BIMP follow-up is still ongoing, we will present a preliminary descriptive overview of the neurodevelopmental and behavioral outcome at 24 months in this chapter. The aims of this preliminary descriptive overview are:

- 1) To assess the incidence of neurodevelopmental and/or behavioral problems at 24 months corrected age in our cohort. The incidence will be reported separately for MP and LP infants;
- 2) To provide an overview of neonatal MRI findings found within the BIMP-cohort and how these are distributed within a group of infants with a normal and suboptimal neurodevelopmental and/or behavioral outcome.

METHODS

1. *Study design*

Data used in this overview are part of the BIMP-study ('Brain Imaging in Moderate-late Preterm infants', The Netherlands trial register; NL6310). From August 2017 to November 2019, MLPT infants born between 32+0 and 35+6 weeks' gestation were recruited at the neonatal units (medium care, high care or intensive care unit) of Isala Women and children's Hospital, Zwolle, the Netherlands (21). Moderate preterm (MP) infants were defined as infants born between 32+0 - 33+6 weeks' gestation, and late preterm (LP) infants as infants born between 34+0 - 35+6 weeks' gestation. Infants with congenital anomalies of the central nervous system, inborn errors of metabolism, congenital infections and chromosomal disorders, or whose parents did not speak sufficient Dutch or English were excluded. Ethical approval was given by the Central Committee in Research Involving Human Subjects, The Hague, the Netherlands (NL52323.075.15).

Table 1. Previous studies reporting neurodevelopmental and behavioral outcome in MLPT infants at 24 months corrected age using ASQ, CBCL or M-Chat.

	Flamant 2011 N = 703	Pierrat 2017 N = 622	Mirzakhani 2020 N = 42	De Jong 2015 N = 94	Guy 2015 N = 1130	You 2019 N = 102
Country	France	France	USA	Netherlands	United Kingdom	China
Birth year cohort	2003 - 2006	2011	2009 - 2011	2010 - 2011	2009 - 2010	2011 - 2013
Corrected age in months	24	24.3	?	23.7	24.6	24 - 30
GA group	< 35 weeks	32+0 - 34+6 weeks	34+0 - 36+6 weeks	32+0 - 36+6 weeks	32+0 - 36+6 weeks	34+0 - 36+6 weeks
ASQ-version	ASQ-3	ASQ-2	ASQ-3	-	-	-
Cut-off point	< -2SD	< -2 SD	< -1 SD	-	-	-
Communication	26.2%	17.8%	14.3%	-	-	-
Gross motor	11.4%	5.1%	23.8%	-	-	-
Fine motor	9.0%	10.5%	19.0%	-	-	-
Problem solving	15.6%	10.9%	11.9%	-	-	-
Personal-social skills	18.3%	13.3%	26.2%	-	-	-
At least 1 domain < -2 SD	45.9%	36.2%	-	-	-	-
More than 1 domain < -2SD	20.8%	13.8%	-	-	-	-
CBCL version	-	-	-	CBCL	-	-
				1.5 - 5 years		
Cut-off point	-	-	-	< 60 (sub)clinical	-	-
Internalizing	-	-	-	5.4%	-	-
Externalizing	-	-	-	9.0%	-	-
Total	-	-	-	5.4%	-	-
M-Chat version	-	-	-	-	M-Chat (2001)	M-Chat (2001)
Initial positive screening	-	-	-	-	14.5%	8.8%
True positive after follow-up	-	-	-	-	2.4%	-

Percentages indicate the proportion of infants who scored below the cut-off point (ASQ3 and CBCL) or had a positive score after screening (M-chat). ASQ = ages and stages questionnaire; CBCL = child behavior checklist; GA = gestational age; M-Chat-(R-F) = Modified Checklist in Toddlers (Revised - with Follow-up).

1.1 Baseline characteristics

Perinatal characteristics such as GA at birth, birth weight, small for GA (SGA; birth weight $P < 10$ (22)), sex, ethnicity (Dutch/non-Dutch), multiple birth, Apgar score at 5 minutes and admission to the neonatal intensive care unit (NICU) were collected from the medical files. Type of feeding (formula, breastfeeding or a mix of both) was recorded at TEA. Parental educational level was provided by the parents at TEA and at 24 months corrected age. The most recently filled in educational level was used. Educational level was classified into three categories: low (no education, completed primary school, pre-vocational secondary education or general secondary education), middle (completed secondary vocational education or pre-university education) or high (completed higher professional education or university education).

1.2 Neuro-imaging

Infants underwent serial cUS and a single MRI examination in the neonatal period. Some parents did not give consent for the MRI or missed the appointment at TEA. For this descriptive overview, only the MRI findings were used.

2.2.1 MRI

Infants underwent MRI around TEA, preferably at a postmenstrual age between 38 and 44 weeks. They were swaddled and placed in a vacuum-bag immobilizer (MedVac® bag, CFI Medical Solutions/Contour Fabricators, Fenton, Michigan, USA). MRI was performed during natural sleep. Noise protection consisted of MiniMuffs (Natus Medical Incorporated, Foster City, California, USA), a headphone (EMS for kids, Hornchurch, UK) and a polystyrene noise-insulating coil cover. Infants were video-monitored and their heart rate and oxygen saturation were continuously measured (Invivo Precess MRI-compatible monitor, Invivo corporation, Orlando, FL, USA). A 3-Tesla Philips Ingenia MR system (Philips Medical Systems, Best, The Netherlands) was used. Three-dimensional T1- (slice thickness: 2mm), coronal and transverse T2- (slice thickness 2 mm) weighted images, diffusion weighted imaging (slice thickness: 3mm), and susceptibility weighted imaging (slice thickness: 1mm) were performed.

The assessment of the MRI scans is described in detail in a previous paper in this thesis (21). In summary, abnormal MRI findings were classified as mild or moderate-severe brain lesions.

- *Mild brain lesions:* (remnants of) IVH grade I-II, choroid plexus hemorrhage, punctate cerebellar hemorrhage(s), diffuse white matter signal changes, < 6 PWML, small focal lesion in the deep gray matter, choroid plexus cyst ≥ 6 mm, germinolytic or subependymal cyst ≥ 6 mm, mild ex-vacuo ventricular dilatation, irregular shape of the lateral ventricles, widened interhemispheric fissure, delayed myelination of the posterior limb of the internal capsule (PLIC), delayed gyration.
- *Moderate-severe brain lesions:* (remnants of) IVH grade III, post-hemorrhagic ventricular dilatation, limited or massive cerebellar hemorrhage, cystic white matter lesions, ≥ 6 PWML, periventricular hemorrhagic infarction, arterial infarction, moderate-severe ex-vacuo ventricular dilatation, moderate-severe deep gray matter lesions.

Parents were informed when brain lesions with likely clinical consequences were found. Although this may influence the results of our study, it was considered non-ethical not to inform parents about these findings. Infants with such lesions were closely monitored by a pediatrician-neonatologist and, if indicated, received additional check-ups and/or interventions by other disciplines (e.g. physiotherapist, ophthalmologist).

1.3 *Neurodevelopmental outcome*

Neurodevelopmental and behavioral outcome at 24 months corrected age were evaluated using three validated parent reported questionnaires, which are outlined below. Parents were asked to fill in and return the questionnaires between 22 and 26 months corrected age.

2.3.1 *Ages and Stages Questionnaire, third version (ASQ-3)*

The Ages and Stages Questionnaire, third version (ASQ-3)(23) contains 30 questions about five domains: communication, fine motor skills, gross motor skills, problem solving abilities, and personal-social skills. Parents could answer the questions with: yes (10 points), sometimes (5 points), or not yet (0 points). A score of 0-60 can be reached for each domain. The results were reported as normal or suboptimal for each domain, with a suboptimal score defined as a score $< -2SD$, using the 24 months reference group (23). We also reported how many infants had a suboptimal score in one of the domains and how many infants had suboptimal scores in two or more domains.

2.3.2 *Child Behavior Checklist (CBCL)*

The Dutch version of the Child Behavior Checklist (CBCL) for children aged 1,5 to 5 years consists of 99 items and is used to assess emotional and behavioral development (24). Seven scales are evaluated: emotionally reactive, anxious/depressed, somatic complaints, withdrawn, sleep problems, attention problems and aggressive behavior. The first five compose internalizing behavior and the last two externalizing behavior. Both scales together give a total problem score. The scores for internalizing, externalizing and total behavior problem subscales are given in T-scores. A score of < 60 is considered normal, a score of 60 – 64 subclinical and a score of ≥ 64 clinical. We dichotomized these scores into normal and suboptimal (subclinical and clinical) scores. We also indicated per subscale (internalizing, externalizing, total problems) the number of infants with a suboptimal score.

2.3.3 *Modified Checklist for Autism in Toddlers, Revised with Follow-Up (M-Chat-R/F)*

The Dutch version of the Modified Checklist for Autism in Toddlers, Revised with Follow-Up (M-Chat-R/F, 2009) is a tool for early identification of autism-associated behavior (25,26). The M-Chat-R/F consists of 23-items, which can be answered with yes or no. The total score indicates a low (0-2), moderate (3-7) or high (8-20) risk for the prediction of autism spectrum disorders (= initial positive screening). If the score indicated a moderate or high risk, we performed a follow-up by phone and reviewed the answers together with the parents (25). After follow-up, the total score was recalculated. If the total score after follow-up was still ≥ 3 , this was considered suboptimal (= true positive screening).

The results of the ASQ-3, CBCL and M-Chat-R/F were combined in a composite outcome score. If one of the test results was within the suboptimal range, the composite outcome was considered suboptimal.

To optimize the response rate of the questionnaires, parents of MLPT infants were contacted by phone and received information on the questionnaires. All parents, including those who could not be reached by phone, received the questionnaires by mail. We informed parents that if their infants' test results were within the normal range, they would not receive a phone call. Parents of infants with a suboptimal score received a phone call with a report of the test results and, if deemed necessary, recommendation on further evaluation.

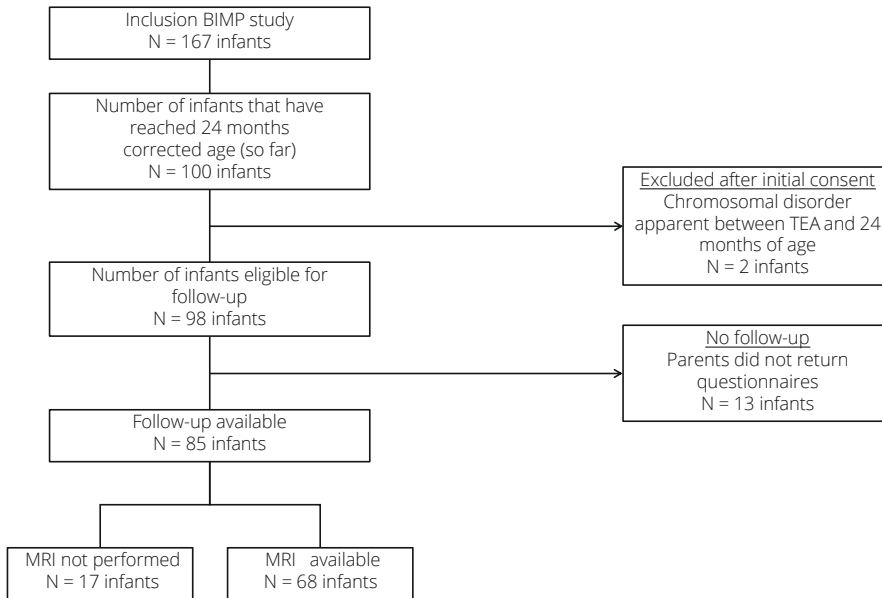
2. *Statistics*

As data collection of the follow-up at 24 months from all infants included in the BIMP study has not been completed yet, we only provide a descriptive overview of the results. No statistical analyses were performed at this time. Data were processed using the IBM SPSS Statistics software (version 26.0; SPSS inc, Chicago, Illinois, USA). Frequencies are reported in numbers and percentages. We report the incidence of a suboptimal result for each test separately (i.e. ASQ-3, CBCL, M-chat-R/F) and for the composite outcome. Incidence is presented for MP and LP infants and for the combined MLPT group. We describe the grading of abnormal MRI findings (none, mild, moderate-severe) for MP and LP infants and present the outcome per subgroup. In addition, we provide an overview of abnormal neonatal MRI findings (type and frequency) within the group of infants with normal and the group with a suboptimal outcome. Because of the small numbers this data is not presented for MP and LP infants separately.

RESULTS

1. *Participants BIMP-cohort*

Up to now, 100 of the 167 MLPT infants included in the BIMP-study have reached 24 months corrected age. Of these 100 MLPT infants, two were excluded after initial informed consent was given (chromosomal disorder apparent between TEA and 24 months of age). Parents of 13 infants did not return the questionnaires. Parents of 85 infants completed the questionnaires, all returned the questionnaires within the time window of 22 – 26 months. The response rate was 86.7% (85/98). The total group consisted of 30 MP infants and 55 LP infants (Figure 1). An overview of the participant characteristics is given in Table 2.

Figure 1. Inclusion flow-chart.**Table 2. Participant characteristics.**

Variable	Moderate preterm n = 30	Late preterm n = 55	Total group n = 85
Birth weight in grams, mean (SD)	1917 (396)	2422 (400)	2244 (465)
SGA (birth weight < P10), n (%)	10 (33.3)	12 (21.8)	22 (25.9)
Sex			
Male, n (%)	20 (66.7)	30 (54.5)	50 (58.8)
Female, n (%)	10 (33.3)	25 (45.5)	35 (41.2)
Multiple birth, n (%)	8 (26.7)	21 (38.2)	29 (34.1)
Apgar score at 5 min < 7, n (%)	1 (3.3)	3 (5.5)	4 (4.7)
NICU admission, n (%)	12 (40.0)	9 (16.4)	21 (24.7)
Formula or breast feeding at TEA			
Formula, n (%)	10 (33.3)	12 (21.8)	22 (25.9)
Breastfeeding, n (%)	14 (46.7)	29 (52.7)	43 (50.6)
Mix of the above, n (%)	6 (20.0)	14 (25.5)	20 (25.9)
Mother's educational level			
Low, n (%)	3 (10)	1 (1.8)	4 (4.7)
Middle, n (%)	17 (56.7)	26 (47.3)	43 (50.6)
High, n (%)	10 (33.3)	28 (50.9)	38 (44.7)
Unknown, n (%)	0 (0)	0 (0)	0 (0)
Father's educational level			
Low, n (%)	4 (13.3)	1 (1.8)	5 (6.8)
Middle, n (%)	17 (56.7)	31 (56.4)	48 (47.1)
High, n (%)	9 (30.0)	21 (38.2)	30 (35.3)
Unknown	0 (0)	2 (3.6)	2 (2.4)

2. Neurodevelopmental and behavioral outcomes BIMP-cohort

Mean corrected age at follow-up was 24.2 (SD 0.5) months (mean uncorrected age 25.5 (SD 0.5) months). In 72% of cases, parents completed the questionnaires together. In the other 28%, questionnaires were filled out by the mother.

The ASQ-3 was within the normal range in 67/85 (78.8%) infants. The domain 'communication' was reported suboptimal most frequently (9/85; 10.6%). The percentage of infants with at least one suboptimal ASQ-3 domain was respectively 20.0 and 21.8% for MP and LP infants (Table 3).

CBCL was within the normal range in 75/85 (88.2%) infants. Most infants with a suboptimal CBCL, showed externalizing problems (Table 3). The percentage of infants with a suboptimal score on the CBCL was higher in the MP group compared to the LP group (20.0% versus 5.6%). The CBCL was missing in one LP infant. This infant had a suboptimal score on one of the ASQ-3 domains and was therefore included in total number of infants with a suboptimal composite outcome.

None of the infants had a true positive score on the M-Chat-R/F after follow-up by phone. One LP infant had suboptimal scores on both the ASQ-3 and CBCL. In total, 12 (40%) MP infants and 14 (25.5%) LP infants had a suboptimal composite outcome score (Table 3).

Table 3. Overview of neurodevelopmental outcome at 24 months corrected age for moderate preterm and late preterm infants and for the total group.

	Moderate preterm N = 30	Late preterm N = 55	Total group N = 85
ASQ-3 domains			
Number of infants with a score < -2 SD			
Communication	4 (13.2)	5 (9.1)	9 (10.6)
Gross motor skills	1 (3.3)	6 (10.9)	7 (8.2)
Fine motor skills	2 (6.7)	1 (1.8)	3 (3.5)
Problem solving	0 (0)	0 (0)	0 (0)
Personal-social skills	3 (10.0)	2 (3.6)	5 (5.9)
At least one of the domains < -2 SD	6 (20.0)	12 (21.8)	18 (21.2)
More than 1 of the domains < -2 SD	3 (10.0)	2 (3.6)	5 (5.9)
CBCL*			
Number of infants with a T-score > 60			
Internalizing score	3 (10.0)	1 (1.9)	4 (4.8)
Externalizing score	5 (16.7)	2 (3.7)	7 (8.3)
Total problem score	3 (10.0)	2 (3.7)	5 (6.0)
At least one of the scores > 60	6 (20.0)	3 (5.6)	9 (10.7)
More than 1 of the scores > 60	3 (10.0)	1 (1.9)	4 (4.8)
M-Chat-R/F			
Number of infants with a score > 3			
Initial positive screening	1 (3.3)	0 (0)	1 (1.2)
True positive after follow-up	0 (0)	0 (0)	0 (0)
Composite outcome			
Number of infants with a suboptimal composite outcome	12 (40.0)	14 (25.5)	26 (30.6)

Numbers are reported as n (%). * The CBCL was missing in 1 infant.

3. Grading of MRI-findings and neurodevelopmental outcome in MP and LP infants

MRI was performed in 68/85 (80%) (23 MP and 45 LP infants). Of the infants with an MRI, twenty-one had a suboptimal composite outcome (21/68; 30.9%). MRI was missing in 17 infants, of whom 5/17 (29.4%) had a suboptimal composite outcome score.

Twenty-three infants had no abnormal MRI findings, of whom 10 (43.5%) had a suboptimal composite outcome. Forty-five infants had at least one abnormal MRI finding. Most infants had mild brain lesions (41/68; 60.3%). Of this group, 10 infants had a suboptimal composite outcome (10/41; 24%). Four infants had moderate-severe brain lesions (4/68; 5.9%). The encountered moderate-severe lesions were: periventricular hemorrhagic infarction ($n = 1$), ≥ 6 PWML ($n = 2$) and posterior cerebral arterial infarction combined with moderate-severe ex-vacuo dilatation ($n = 1$). Three infants with moderate-severe lesions were closely monitored by a pediatric-neonatologist based on likely clinical consequences. Only one infant with moderate-severe lesions had a suboptimal composite outcome (suboptimal CBCL). This was one of the infants with ≥ 6 PWMLs. Details for MP and LP infants are specified in Table 4.

Table 4. Results of the ASQ-3, CBCL and the composite outcome, subdivided for moderate and late preterm infants and for grading of MRI findings (none, mild or moderate-severe)

Moderate or late preterm	Grading of MRI findings	ASQ-3		CBCL		Composite outcome	
		normal	suboptimal	normal	suboptimal	normal	suboptimal
Moderate preterm	None $N = 5$	4 (80.0)	1 (20.0)	4 (80.0)	1 (20.0)	3 (60.0)	2 (40.0)
	Mild $N = 16$	13 (81.2)	3 (18.8)	14 (87.5)	2 (12.5)	11 (68.7)	5 (31.3)
$N = 23$	Moderate-severe $N = 2$	2 (100)	0 (0)	1 (50.0)	1 (50.0)	1 (50.0)	1 (50.0)
Late Preterm	None $N = 18^*$	12 (66.7)	6 (33.3)	14 (82.4)	3 (16.6)	10 (55.6)	8 (44.4)
	Mild $N = 25$	20 (80.0)	5 (20.0)	25 (100.0)	0 (0)	20 (80.0)	5 (20.0)
$N = 45$	Moderate-severe $N = 2$	2 (100.0)	0 (0)	2 (100.0)	0 (0)	2 (100.0)	0 (0)

Numbers are reported as n (%). The results of the M-chat-R/F are not displayed as none of the infants had a true positive screening after follow-up. * CBCL is missing in one LP infant with no abnormal MRI findings

4. Type of abnormal MRI findings and composite outcome

Fifteen different types of abnormal MRI findings were encountered (Table 5). The most frequently encountered type was mild ex-vacuo ventricular dilatation ($n = 22$). The majority of infants with mild ex-vacuo dilatation had a normal composite outcome (17/22 [77.3%]). Two out of three infants (66%) with delayed myelination of the PLIC had a suboptimal composite score (in one infant delayed myelination of the PLIC was the sole finding, the other infant also had a germinolytic cyst and diffuse white matter signal changes).

Table 5. Type of abnormal MRI- finding, frequency and distribution in infants with normal and suboptimal composite outcome

Type of abnormal MRI finding	Frequency of abnormal MRI finding		Normal		Composite outcome		Suboptimal composite outcome	
	n	n (%)	n	n (%)	n	n (%)	n	n (%)
<i>Hemorrhages</i>								
Remnants of low grade IVH	4	4 (100.0)			0		0	
Periventricular hemorrhagic infarction	1	1 (100.0)			0		0	
Choroid plexus hemorrhage	1	1 (100.0)			0		0	
Punctate cerebellar hemorrhages	6	4 (66.7)			2 (33.3)			
<i>White matter</i>								
Diffuse white matter signal changes [#]	20	14 (70.0)			6 (30.0)			
Punctate white matter lesions	10	8 (80.0)			2 (20.0)			
	2	1 (50.0)			1 (50.0)			
<i>Infarction</i>								
Arterial infarction	1	1 (100.0)			0			
Small focal lesion in the deep gray matter	1	1 (100.0)			0			
<i>Miscellaneous</i>								
Germinolytic or subependymal cyst ≥ 6 mm	6	5 (80.0)			1 (20.0)			
<i>Signs suggestive of brain atrophy</i>								
Ex-vacuo ventricular dilatation [§]	22	17 (77.3)			5 (22.7)			
	1	1 (100)			0			
Moderate-severe (> 15 mm)								
Irregular shape of the lateral ventricles	12	10 (83.3)			2 (16.7)			
Widened interhemispheric fissure [§]	12	10 (83.3)			2 (16.7)			
<i>Gyration and Myelination</i>								
Delayed Gyration	0	0			0			
Delayed myelination PLIC	5	5 (100.0)			0			
	3	1 (33.3)			2 (66.7)			
Both sides PLIC < 1/3								

[#] Not able to assess images in 7 infants due to movement artefacts (of whom 6 had a normal composite outcome); [§] Not able to assess images in 7 infants due to movement artefacts (of whom 4 had a normal composite outcome).

DISCUSSION

This chapter provides preliminary results of the neurodevelopmental and behavioral outcomes of MLPT infants who participated in the BIMP-study and describes MRI findings in infants with a normal and suboptimal (composite) outcome. Of the infants who have reached 24 months corrected age, almost a third had a suboptimal composite outcome. The incidence of a suboptimal composite outcome was higher in MP than in LP infants. Suboptimal outcomes were seen across almost all ASQ-3 domains, of which communication problems were most common. Externalizing behavior problems were more frequently reported than internalizing problems. None of the infants had a true positive M-Chat-R/F screening after follow-up by phone.

The incidence of neurodevelopmental and behavioral problems in our cohort are partially in agreement with previous studies. The studies by Flamant et al. (27) and Pierrat et al. (28) reported higher rates of suboptimal outcome than we did. However, these two studies included infants with different GA ranges (i.e. Flamant et al. included all infants with a GA below 35 weeks and Pierrat et al. included infants with a GA 32+0 to 34+6 weeks). Similar to these two studies, we most frequently encountered communication problems. Mirzakhani et al. (29) used a different cut-off value for normal/suboptimal outcome (-1 SD instead of -2 SD), making it difficult to compare their results with ours. The behavioral outcome based on the CBCL was comparable between the study of De Jong et al.(30) and ours. In our study, only one infant had an initial positive autism screening using the M-Chat-R/F, which was no longer positive after follow-up by phone. In the studies by Guy et al.(31) and You et al.(32) the rate of an initial and true positive autism screening was much higher. This might be partly explained by the used version of the M-Chat (version from 2009 in our study vs version from 2001 in the studies by Guy et al and You et al.).

Previous studies investigating the association between brain lesions and neurodevelopmental outcome in MLPT infants have been restricted to only a few types of brain lesions and gave conflicting results. Nakasone et al. found an association between IVH and neurodevelopment, while Cheong et al. did not find an association between brain lesions and brain maturation, and outcome (18,33). In our cohort, we encountered a variety of mostly mild abnormal MRI findings, including delayed brain development. At present, it is not possible to draw conclusions regarding a possible relationship between these MRI findings and neurodevelopmental outcome in MLPT infants. In contrast, the association between brain lesions and neurodevelopmental outcome has been frequently studied in very preterm infants (GA < 32 weeks). In this population, some mild abnormal MRI findings have been associated with neurodevelopmental delays and behavioural problems. Below we will discuss some of the associations and what they could mean for MLPT infants.

Mild ex-vacuo ventricular dilatation was the most frequently encountered abnormal MRI finding in our MLPT-cohort. Most MLPT infants with this MRI finding had a normal

composite outcome. In very preterm infants, ex-vacuo dilatation detected with cUS at TEA was associated with less optimal cognitive and motor outcome at 24 months corrected age (34). However, the results of this study cannot simply be compared to the results of our study, because different definitions were used to determine ex-vacuo dilatation. Brouwer et al. examined the ventricles on parasagittal cUS views, while we measured the ventricular index on a coronal MRI. Furthermore, diffuse white matter signal changes were frequently encountered in our cohort. It is suggested that these may represent mild white matter injury or delayed white matter maturation (35). Yet, consequences of this MRI finding are unclear as available studies on association with neurodevelopmental outcome are inconsistent (36).

Up to now, all MLPT infants with remnants of low grade IVH had a normal composite outcome in our study. This is in contrast to the previously mentioned study by Nakasone et al., in which all three infants (GA 34⁺⁰ – 35⁺⁶ weeks) with a low grade IVH had a suboptimal outcome at 18 months corrected age (18). Also studies in very preterm infants performed around 24 months show conflicting results (37-39). At school age, low grade IVH was not associated with intelligence and behavior, but an association was found for cerebral palsy (40,41).

Moderate-severe lesions were seen in four MLPT infants, of whom three were closely monitored by a pediatrician-neonatologist. Although PWMLs, periventricular hemorrhagic infarction and posterior cerebral arterial infarction are known to affect neurodevelopmental outcome (42-44), only one infant with moderate-severe findings in our study had a suboptimal composite outcome. This was one of the infants with ≥ 6 PWMLs. As we only used parent reported questionnaires, (subtle) neurological abnormalities or visual impairments, which could be expected after periventricular hemorrhagic infarction and posterior cerebral arterial infarction, are not captured in our study. The infants with moderate-severe lesions were intensively monitored and were offered early targeted assessments and interventions. They may have benefited from this resulting in a positive impact on their outcomes.

The relatively low incidence of a suboptimal outcome in MLPT infants with abnormal MRI findings may be explained the large number of parents with a high educational level in our study. Several studies have reported that a high parental educational level reduces the risk of a suboptimal outcome (8-11). In addition, most abnormal MRI findings found in our study were mild. These mild lesions may have a limited effect on neurodevelopmental outcome at very young age. Moreover, other factors such as high parental educational level may have a greater effect. Also in very preterm infants, mild lesions (such as diffuse white matter signal changes and low grade IVH) were less clearly associated with neurodevelopmental outcome (36-39). However, effects of mild brain lesions may still become apparent at school age (40,41).

Strengths of this preliminary overview are the high follow-up response rate, the use of three validated questionnaires, the use of a detailed 3-Tesla MRI protocol, thus assuring detection of also small and mild lesions, and the detailed information on different types of

abnormal MRI findings. However, some limitations need to be mentioned. Firstly, we did not include infants born between 36+0 – 36+6 weeks' gestation, although these infants account for a large part of the MLPT population. Secondly, we had no control group of full-term infants for comparison of MRI findings and follow-up. Thirdly, we used questionnaires to screen for neurodevelopmental and behavioral problems instead of more objective tests performed by pediatricians, physiotherapists and psychologists. However, detailed neurodevelopmental and behavioral evaluation of MLPT infants will require considerable time, money and trained personnel. Thus, parent reported questionnaires may be more appropriate, practical and applicable in large populations like the MLPT population and for well-baby clinics. Fourthly, not all infants underwent an MRI at TEA. Due to this and the relatively small number of infants per type of abnormal MRI finding, we were not (yet) able to analyze possible associations between type of abnormal MRI finding and the neurodevelopmental and behavioral outcome. In addition, infants with a brain lesion with likely clinical consequences, had closer follow-up and, if deemed necessary, received additional check-ups and/or interventions by other disciplines. These early interventions may have positively influenced their outcome. Finally, we did not (yet) correct outcome for several known factors associated with developmental outcome, such as sex, small for gestational age and parental educational level. When all infants who participated in the BIMP-study, have turned 24 months (corrected age) and completed follow-up, we will perform statistical analyses and will correct for possible confounders.

In the future, we strive to investigate the association between serial cUS, microstructural changes on MRI and neurodevelopmental outcome. As previously mentioned, Kelly et al. reported an association between white matter microstructural changes and cognitive and language outcome at 24 months corrected age in MLPT infants (23). A large number of MLPT infants included in the BIMP study had periventricular echogenicity or other mild lesions on early cUS, which disappeared at TEA. Although these lesions have disappeared macroscopically, they may have altered the microstructure of the brain and thus may affect neurodevelopment. If so, early cUS would be a much more patient-friendly and cheaper screening tool to detect those infants at risk of a suboptimal neurodevelopmental outcome than advanced MRI. Furthermore, even without visual lesions, other (perinatal) factors may have affected the microstructure of the brain, therefore further research into these factors and microstructural changes is needed as well.

To conclude, up to now 85 MLPT infants that participated in the BIMP-study, have turned 24 months corrected age and completed follow-up. Almost a third had a suboptimal composite outcome. The outcomes of the ASQ-3, CBCL and M-CHAT were largely in agreement with previous studies. Abnormal MRI findings were frequently encountered and were mostly mild. At present, it is not possible to draw conclusions regarding the relation between MRI findings and neurodevelopmental outcome. Statistical analyses will be performed once follow-up has been completed in all infants.

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REFERENCES

1. Chawanpaiboon S, Vogel J, Moller A, Lumbiganon P, Petzold M, Hogan D, et al. Global, regional, and national estimates of levels of preterm birth in 2014: a systematic review and modelling analysis. *Lancet Glob Health* 2019;7(1):e37-e46.
2. Voigt B, Pietz J, Pauen S, Kliegel M, Reuner G. Cognitive development in very vs. moderately to late preterm and full-term children: can effortful control account for group differences in toddlerhood? *Early Hum Dev* 2012;88(5):307-313.
3. Johnson S, Evans TA, Draper ES, Field DJ, Manktelow BN, Marlow N, et al. Neurodevelopmental outcomes following late and moderate prematurity: a population-based cohort study. *Arch Dis Child Fetal Neonatal Ed* 2015;100(4):F301-F308.
4. Hochstedler KA, Bell G, Park H, Ghassabian A, Bell EM, Sundaram R, et al. Gestational Age at Birth and Risk of Developmental Delay: The Upstate KIDS Study. *Am J Perinatol* 2020.
5. Hirvonen M, Ojala R, Korhonen P, Haataja P, Eriksson K, Rantanen K, et al. Intellectual disability in children aged less than seven years born moderately and late preterm compared with very preterm and term-born children - a nationwide birth cohort study. *J Intellect Disabil Res* 2017;61(11):1034-1054.
6. Townley Flores C, Gerstein A, Phibbs CS, Sanders LM. Short-Term and Long-Term Educational Outcomes of Infants Born Moderately and Late Preterm. *J Pediatr* 2021;232:31-37.e2.
7. Heinonen K, Eriksson JG, Kajantie E, Pesonen A, Barker DJ, Osmond C, et al. Late-Preterm Birth and Lifetime Socioeconomic Attainments: The Helsinki Birth Cohort Study. *Pediatrics* 2013;132(4):647-655.
8. Kerstjens JM, de Winter AF, Sollie KM, Bocca-Tjeertes IF, Potijk MR, Reijneveld SA, et al. Maternal and pregnancy-related factors associated with developmental delay in moderately preterm-born children. *Obstet Gynecol* 2013;121(4):727-733.
9. den Haan PJ, de Kroon M, L. A., van Dokkum NH, Kerstjens JM, Reijneveld SA, Bos AF. Risk factors for emotional and behavioral problems in moderately-late preterms. *PLoS One* 2019;14(5):e0216468.
10. Johnson S, Waheed G, Manktelow BN, Field DJ, Marlow N, Draper ES, et al. Differentiating the Preterm Phenotype: Distinct Profiles of Cognitive and Behavioral Development Following Late and Moderately Preterm Birth. *J Pediatr* 2018;193:85-92.e1.
11. Martínez-Nadal S, Demestre X, Schonhaut L, Muñoz SR, Sala P. Impact of neonatal morbidity on the risk of developmental delay in late preterm infants. *Early Hum Dev* 2018;116:40-46.
12. Kostović I, Sedmak G, Judaš M. Neural histology and neurogenesis of the human fetal and infant brain. *Neuroimage* 2019;188:743-773.
13. Budday S, Steinmann P, Kuhl E. Physical biology of human brain development. *Frontiers in cellular neuroscience* 2015;9:257.
14. Raybaud C, Ahmad T, Rastegar N, Shroff M, Al Nassar M. The premature brain: developmental and lesional anatomy. *Neuroradiology* 2013;55(2):23-40.
15. Guihard-Costa AM, Larroche JC. Growth velocity of some fetal parameters. I. Brain weight and brain dimensions. *Biol Neonate* 1992;62(5):309-16.
16. Hüppi PS, Warfield S, Kikinis R, Barnes PD, Zientara GP, Jolesz FA, et al. Quantitative magnetic resonance imaging of brain development in premature and mature newborns. *Ann Neurol* 1998;43(2):224-235.

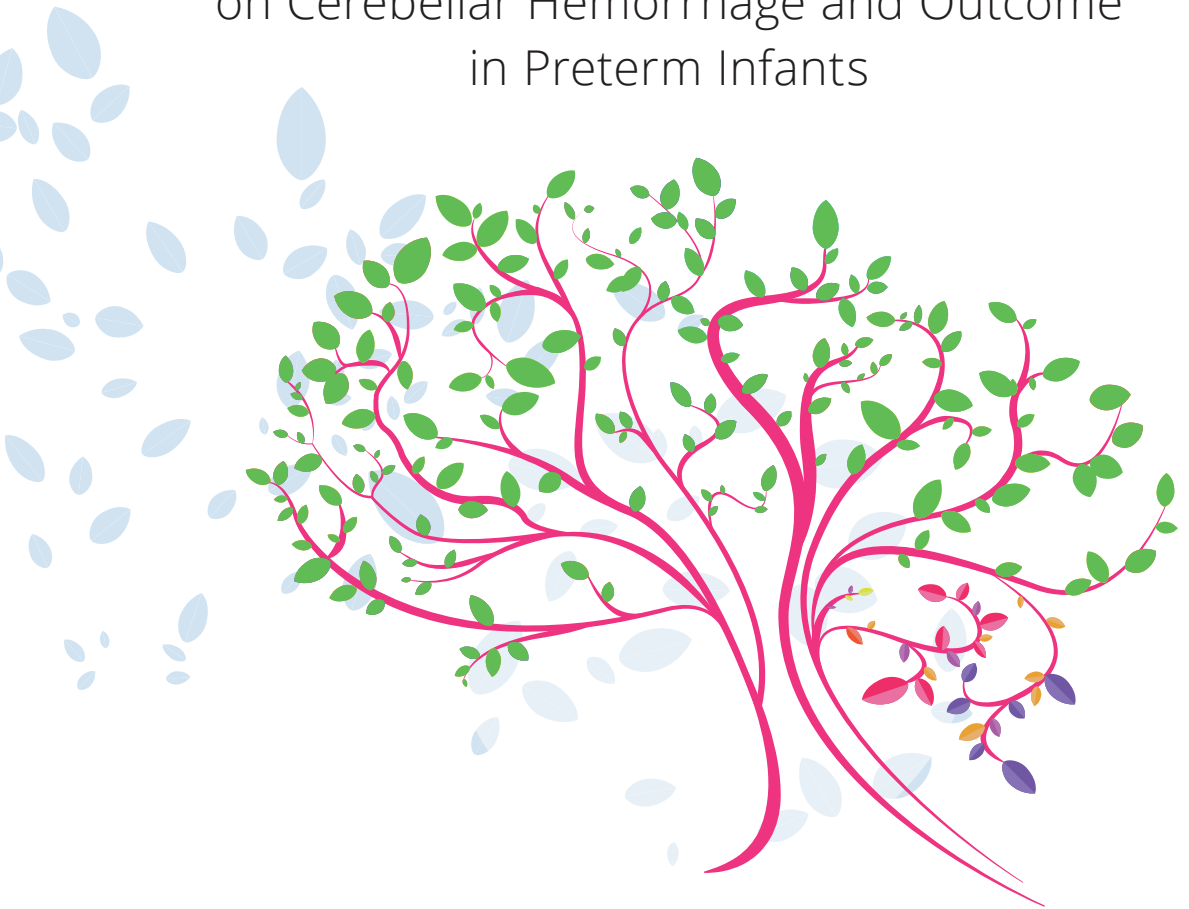
17. Kinney H. The near-term (late preterm) human brain and risk for periventricular leukomalacia: a review. *Semin Perinatol* 2006;30(2):81-88.
18. Nakasone R, Fujioka K, Kyono Y, Yoshida A, Kido T, Suga S, et al. Neurodevelopmental Outcomes at 18 Months of Corrected Age for Late Preterm Infants Born at 34 and 35 Gestational Weeks. *Int J Environ Res Public Health* 2021;18(2):640.
19. Cheong JLY, Thompson D, Spittle A, Potter C, Walsh J, Burnett A, et al. Brain Volumes at Term-Equivalent Age Are Associated with 2-Year Neurodevelopment in Moderate and Late Preterm Children. *J Pediatr* 2016;174:91-97.e1.
20. Kelly CE, Thompson DK, Spittle AJ, Chen J, Seal ML, Anderson PJ, et al. Regional brain volumes, microstructure and neurodevelopment in moderate-late preterm children. *Arch Dis Child Fetal Neonatal Ed* 2020;105(6):593-599.
21. Boswinkel V, Krüse-Ruijter MF, Nijboer - Oosterveld J, Nijholt IM, Edens MA, Mulder - de Tollenhaer SM., et al. Incidence of brain lesions in moderate-late preterm infants assessed by cranial ultrasound and MRI: The BIMP-study. *Eur J Radiol* 2021;136:109500.
22. Hoftiezer L, Hof MHP, Dijks-Elsinga J, Hogeveen M, Hukkelhoven, CWPM, van Lingen RA. From population reference to national standard: new and improved birthweight charts. *Obstet Gynecol* 2019;220(4):1-383.
23. Squires J, Bricker D. *Ages & Stages Questionnaires®, Third Edition (ASQ®-3): A Parent-Completed Child Monitoring System*. Baltimore: Paul H. Brookes Publishing Co., Inc; 2009.
24. Achenbach TM, Rescorla L. *Manual for the Child Behavior Checklist. Preschool Forms and Profiles*. : Burlington VT: University of Vermont Department of Psychiatry; 2000.
25. Robins DL, Fein D, Barton M. *The Modified Checklist for Autism in Toddlers, Revised with Follow-Up (M-CHAT-R/F)*. 2009.
26. Robins D, Casagrande K, Barton M, Chen C, Dumont Mathieu T, Fein D. Validation of the modified checklist for Autism in toddlers, revised with follow-up (M-CHAT-R/F). *Pediatrics* 2014;133(1):37-45.
27. Flamant C, Branger B, Nguyen The Tich S, de la Rochebrochard E, Savagner C, Berlie I, et al. Parent-completed developmental screening in premature children: a valid tool for follow-up programs. *PLoS One* 2011;6(5):e20004.
28. Pierrat V, Marchand-Martin L, Arnaud C, Kaminski M, Resche-Rigon M, Lebeaux C, et al. Neurodevelopmental outcome at 2 years for preterm children born at 22 to 34 weeks' gestation in France in 2011: EPIPAGE-2 cohort study. *BMJ* 2017;358:j3448.
29. Mirzakhani H, Kelly RS, Yadama AP, Chu SH, Lasky-Su JA, Litonjua AA, et al. Stability of developmental status and risk of impairment at 24 and 36 months in late preterm infants. *Infant Behav Dev* 2020;60:101462.
30. de Jong M, Verhoeven M, Lasham CA, Meijssen CB, van Baar AL. Behaviour and development in 24-month-old moderately preterm toddlers. *Arch Dis Child* 2015;100(6):548-553.
31. Guy A, Seaton SE, Boyle EM, Draper ES, Field DJ, Manktelow BN, et al. Infants born late/moderately preterm are at increased risk for a positive autism screen at 2 years of age. *J Pediatr* 2015;166(2):269-75.e3.
32. You J, Shamsi BH, Hao MC, Cao CH, Yang WY. A study on the neurodevelopment outcomes of late preterm infants. *BMC Neurol* 2019;19(1):108-0.

33. Cheong J, Doyle L, Burnett A, Lee K, Walsh J, Potter C, et al. Association Between Moderate and Late Preterm Birth and Neurodevelopment and Social-Emotional Development at Age 2 Years. *JAMA Pediatr* 2017;171(4):e164805.
34. Brouwer MJ, van Kooij BJM, van Haastert IC, Koopman Esseboom C, Groenendaal F, de Vries LS, et al. Sequential cranial ultrasound and cerebellar diffusion weighted imaging contribute to the early prognosis of neurodevelopmental outcome in preterm infants. *PLoS ONE* 2014;9(10):e109556.
35. Volpe JJ. Confusions in Nomenclature: "Periventricular Leukomalacia" and "White Matter Injury"-Identical, Distinct, or Overlapping? *Pediatr Neurol* 2017;73:3-6.
36. Rath CP, Desai S, Rao SC, Patole S. Diffuse excessive high signal intensity on term equivalent MRI does not predict disability: a systematic review and meta-analysis. *Arch Dis Child Fetal Neonatal Ed*. 2021;106(1):9-16.
37. Payne AH, Hintz SR, Hibbs AM, Walsh MC, Vohr BR, Bann CM, et al. Neurodevelopmental outcomes of extremely low-gestational-age neonates with low-grade periventricular-intraventricular hemorrhage. *JAMA Pediatr* 2013;167(5):451-459.
38. Bolisetty S, Dhawan A, Abdel-Latif M, Bajuk B, Stack J, Lui K, et al. Intraventricular hemorrhage and neurodevelopmental outcomes in extreme preterm infants. *Pediatrics* 2014;133(1):55-62.
39. Scott TE, Aboudi D, Kase JS. Low-Grade Intraventricular Hemorrhage and Neurodevelopmental Outcomes at 24-42 Months of Age. *J Child Neurol* 2020;35(9):578-584.
40. Ann Wy P, Rettiganti M, Li J, Yap V, Barrett K, Whiteside-Mansell L, et al. Impact of intraventricular hemorrhage on cognitive and behavioral outcomes at 18 years of age in low birth weight preterm infants. *J Perinatol* 2015;35(7):511-515.
41. Hollebrandse NL, Spittle AJ, Burnett AC, Anderson PJ, Roberts G, Doyle LW, et al. School-age outcomes following intraventricular haemorrhage in infants born extremely preterm. *Arch Dis Child Fetal Neonatal Ed* 2021;106(1):4-8.
42. Tusor N, Benders MJNL, Counsell SJ, Nongena P, Ederies MA, Falconer S, et al. Punctate White Matter Lesions Associated With Altered Brain Development And Adverse Motor Outcome In Preterm Infants. *Sci Rep* 2017;7(1):13250-x.
43. De Vries LS, Van Haastert IC, Rademaker KJ, Koopman C, Groenendaal F. Ultrasound abnormalities preceding cerebral palsy in high-risk preterm infants. *Journal of Pediatrics, The* 2004;144(6):815-20.
44. van der Aa NE, Dudink J, Benders MJNL, Govaert P, van Straaten HL, Porro GL, et al. Neonatal posterior cerebral artery stroke: clinical presentation, MRI findings, and outcome. *Dev Med Child Neurol* 2013;55(3):283-290.



CHAPTER 8

The CHOPIn Study: a multicenter study on Cerebellar Hemorrhage and Outcome in Preterm Infants



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ABSTRACT

Cerebellar hemorrhage (CBH) is a frequent complication of preterm birth and may play an important and under-recognized role in neurodevelopment outcome. Association between CBH size, location, and neurodevelopment is still unknown. The main objective of this study was to investigate neurodevelopmental outcome at 2 years of age in a large number of infants with different patterns of CBH. Of preterm infants (≤ 34 weeks) with known CBH, perinatal factors, neuro-imaging findings, and follow-up at 2 years of age were retrospectively collected. MRI scans were reassessed to determine the exact size, number, and location of CBH. CBH was divided into three groups: punctate (≤ 4 mm), limited (> 4 mm but $< 1/3$ of the cerebellar hemisphere), or massive ($\geq 1/3$ of the cerebellar hemisphere). Associations between pattern of CBH, perinatal factors, and (composite) neurodevelopmental outcome were assessed. Data of 218 preterm infants with CBH were analyzed. Of 177 infants, the composite outcome score could be obtained. Forty-eight out of 119 infants (40%) with punctate CBH, 18 out of 35 infants (51%) with limited CBH, and 18 out of 23 infants (78%) with massive CBH had an abnormal composite outcome score. No significant differences were found for the composite outcome between punctate and limited CBH ($p = 0.42$). The risk of an abnormal outcome increased with increasing size of CBH. Infants with limited CBH have a more favorable outcome than infants with massive CBH. It is therefore important to distinguish between limited and massive CBH.

INTRODUCTION

Cerebellar hemorrhage (CBH) is a common complication in very low birthweight infants. Reported incidences in infants born below 32 weeks' gestation, and/or weighing less than 1500 g at birth, range from 2.2 to 19.0% (1–3), depending on the population studied and the imaging techniques used. The etiology of CBH is multifactorial. Potential risk factors are traumatic delivery and circulatory events related to prematurity, such as impaired cerebrovascular autoregulation, large patent ductus arteriosus, and other parameters of a compromised cerebral circulation (4–7).

Until recently, it was thought that the cerebellum was mainly involved in motor system functions (8). However, in a retrospective, case-control design study, Limperopoulos et al. found cerebellar hemorrhagic injury in preterm infants to be associated with a high prevalence of long-term pervasive neurodevelopmental disabilities (2). Other studies have shown that injury to the cerebellum not only affects motor functions, but also non-motor functions, including cognition, learning, and behavioral abilities (9, 10). CBH may thus play an important and under-recognized role in the cognitive, learning, and behavioral problems known to affect survivors of extremely preterm birth (6, 11, 12).

In preterm infants with CBH, the size of the lesion may be of importance with respect to neurodevelopmental outcome. Three patterns of preterm CBH have been described (13, 14); the first being massive CBH, mainly seen in the sickest and smallest infants (< 28 weeks' gestation and/or < 750 g). These massive bleeds carry a high morbidity and mortality and are easily diagnosed with cranial ultrasonography (cUS), especially when the mastoid window is used (4, 15). In surviving infants, massive CBH leads to severe cerebellar destruction and subsequent atrophy, and is associated with long-term and severe neurodevelopmental disabilities, such as cerebral palsy (CP) and motor, language and/or cognitive delays (1, 2, 11). Associated supratentorial injury and/or cerebellar diaschisis may also play a role in this unfavorable outcome (5, 6).

Another pattern is small or punctate cerebellar hemorrhages. These are small (≤ 4 mm) lesions that are beyond the scope of cUS but are frequently encountered as a chance finding on magnetic resonance imaging (MRI), which is often performed in very preterm infants around term equivalent age (TEA) (14). These small CBH do not seem to lead to cerebellar atrophy and are associated with a more favorable prognosis. Tam et al. (16) found preterm infants with small CBH to have a 5-fold higher incidence of abnormal neurological examination at 3–6 years of age than those without CBH, but there was no significant difference in cognitive impairment. Steggerda et al. found no association between small CBH and neurodevelopmental outcome at 2 years of age (17).

The third, so far only rarely described pattern concerns “medium-sized” or limited hemorrhages that involve less than one third of the cerebellar hemisphere. These limited

hemorrhages are mostly located at the convexity of one of the cerebellar hemispheres and may be diagnosed with cUS, especially if the mastoid fontanel is used as an additional acoustic window (13). They occur in very preterm infants, do not seem to cause acute clinical symptoms and their possible influence on outcome is not yet known, in addition, it is not known whether these limited hemorrhages may lead to cerebellar atrophy (14, 18). As different parts of the cerebellum seem to be involved in different executive, affective, limbic, and sensorimotor functions, the relationship between location of the lesion(s) and neurodevelopmental and behavioral outcome is also worth investigating (11, 19–22).

Despite the increasing number of papers reporting about neonatal CBH, the number of included infants with CBH in these studies is limited. Therefore, drawing conclusions from associations between CBH size and location, and neurodevelopment is still a challenge. The main objective of this study was therefore to investigate and compare neurodevelopmental outcome at 2 years of age in a large number of infants with punctate, limited, and massive CBH. Other objectives were to investigate associations between:

- location of CBH and neurodevelopmental outcome;
- pattern of CBH and cerebellar atrophy;
- pattern of CBH and perinatal factors.

PATIENTS AND METHODS

In this retrospective, multi-center study, data from preterm infants ≤ 34 weeks' gestation with MRI diagnosed CBH who were admitted to one of 6 tertiary neonatal centers (4 Dutch and 2 Italian) were collected and analyzed. The participating centers were selected based on their neonatal neuro-imaging protocols with special attention for cerebellar injury. Infants were born between 2003 and 2016 and included if at least one neonatal MRI examination had been performed. Exclusion criteria were as follows: (suspected) brain malformation, dysmorphic features or congenital anomaly suggestive of a genetic syndrome, metabolic disorder, chromosomal abnormality, and/or proven central nervous system infection.

The participating centers were Isala Women and Children's hospital (IVKC), Zwolle, the Netherlands; Leiden University Medical Center (LUMC), Leiden, the Netherlands; University Medical Center Utrecht (UMCU), Utrecht, the Netherlands; Erasmus Medical Center (Erasmus MC), Rotterdam, the Netherlands; Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy; and Istituto Giannina Gaslini, Genova, Italy. Infants were selected from an institutional MRI register and from existing databases of infants known to have cerebellar abnormalities. As the study did not fall under the Medical Research Involving Human Subjects Act and clinically obtained anonymized data were used, the medical ethical committees of the participating centers waived an informed consent and ethical review procedure.

Patient Characteristics

Maternal, perinatal, and neonatal factors were retrieved from the medical records. Maternal factors included age, presence of pre-eclampsia, and use of antenatal steroids. Perinatal factors included gender, gestational age (GA), birth weight (BW), Z-score for BW according to Hoftiezer et al. (23), multiple birth, mode of delivery (i.e., breech extraction, instrumental delivery, cesarean section), umbilical cord pH, and Apgar score at 5 min. Neonatal factors were mechanical ventilation within the first postnatal week, high-frequency oscillation (HFO) ventilation within the first postnatal week, surfactant replacement therapy, hypotension (defined as need for inotropic support) within the first postnatal week, severe thrombocytopenia (defined as platelet count $< 50 \times 10^9/L$) within the first postnatal week, and in-hospital mortality.

Neuro-imaging

At IVKC, LUMC, UMCU, Fondazione IRCCS Ca' Granda and Gaslini, MRI was performed around term equivalent age (TEA) using a 1.5-T or 3.0-T MR system (Ingenia (IVKC), Achieva (LUMC, UMCU, Fondazione IRCCS Ca' Granda and Gaslini), Philips Medical Systems, Best, The Netherlands.

At Erasmus, MC MRI was obtained around the postmenstrual age (PMA) of 30 weeks, or, in infants born at GA > 30 weeks, as soon as possible after birth, using a 1.5-T GE EchoSpeed scanner (General Electric Healthcare Technologies, Waukesha, WI). In UMCU, MRI was routinely done in all infants < 28 weeks' gestation and in Fondazione IRCCS Ca' Granda in all infants < 32 weeks' gestation, while in the other hospitals MRI was performed if there was an indication, according to the local guidelines. T1-weighted, T2-weighted images (slice thickness 1.2 mm at Erasmus MC; 2 mm at IVKC, LUMC, UMCU, and Fondazione IRCCS Ca' Granda; 3 mm at Gaslini) and, if available, susceptibility weighted images (SWI) were used for detection and scoring CBH.

The SWI sequence was not available during the first years of the study and was only used since 2006 in some centers and since 2014 in all. Due to slice thickness of 3 mm for the T2-weighted images in Gaslini and therefore the limited ability to detect punctate CBH, especially before the SWI-era, only infants with limited or massive CBH were selected from this center.

All available MRI examinations were screened for the presence of CBH by one of the investigators (V.B.). Subsequently, all MRI examinations with CBH were reviewed by 2 investigators: V.B. along with a neonatologist of each center (G.M.; S.J.S.; L.S.V.; J.D.; A.P., or M.F.). These neonatologists are experts with at least 10 years of experience in neonatal neuro-imaging. In case of disagreement, an expert from one of the other centers was consulted.

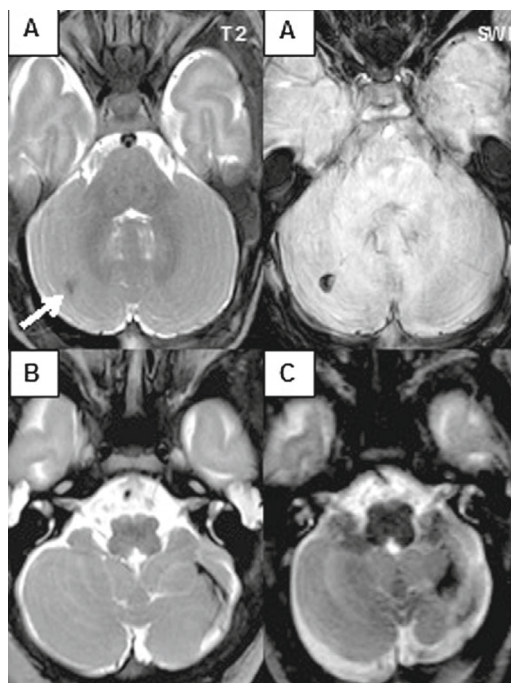
Pattern of CBH

The classification was modified from the cUS classification by Meijler and Steggerda (18) and previous work by others (13, 24) (Figure 1).

1. Punctate CBH: one or more lesions ≤ 4 mm; Infants with punctate CBH were subdivided into two groups: ≤ 6 lesions or > 6 lesions.
2. Limited CBH: the lesion(s) being > 4 mm, but involving $< 1/3$ of the cerebellar hemisphere;
3. Massive CBH: the lesion involving $\geq 1/3$ of the cerebellar hemisphere.

Figure 1. MR images performed around TEA.

A) T2-weighted MR image and SWI of a punctate CBH located in the right cerebellar hemisphere (arrow). B) Limited CBH at the convexity of the left cerebellar hemisphere. C) Massive CBH located in the left cerebellar hemisphere, also leading to destruction and atrophy of that hemisphere.



The pattern of CBH was determined based on the T2-W images (transverse planes). Foci of signal loss on T2-W images and/or SWI were considered to be hemosiderin deposits and thus (punctate) hemorrhages if there was no continuity with a vessel, suggesting a vascular structure.

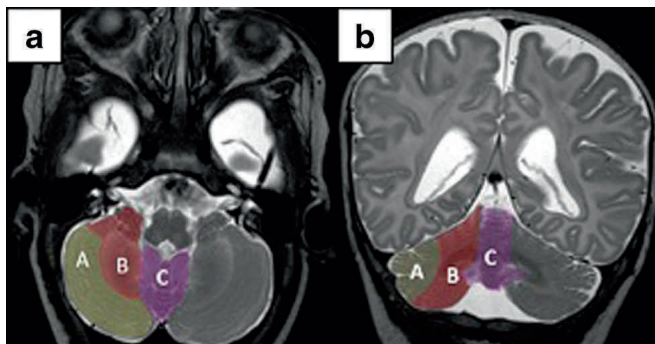
For the MRI scans performed around TEA (PMA 38–44 weeks), the transcerebellar diameter (TCD) was measured on a transverse T2-W plane, at the largest cerebellar diameter. Because the TCD increases with increasing PMA at MRI, we corrected the measured value according to the equation developed by Kidokoro et al. (25). Cerebellar atrophy was considered mild to moderate if the corrected TCD was < 50 mm, but ≥ 44 mm and severe if the corrected TCD was < 44 mm.

Location of CBH

We also categorized CBH according to location. Figure 2 shows a map of structural-function corresponding regions of the cerebellum on transverse and coronal planes. Region A comprises the convexity (lateral-posterior-inferior) hemispheric zones; region B, the anterior/medial hemispheric zones; and region C, the vermis. This map is based on previous works (19–21, 26).

Figure 2. Map of structural-function corresponding regions on transverse (a) and coronal (b) planes.

A. Convexity (lateral-posterior-inferior) hemispheric zones; B. Anterior/medial hemispheric zones; C. vermis



Supratentorial Injury

Intraventricular hemorrhage (IVH) (classified according to Volpe [27]) was recorded based on neonatal ultrasound reports. White matter injury was graded according to Kidokoro (3): grade 1: punctate lesions ≤ 3 mm in the periventricular white matter on one or both sides; grade 2: punctate lesions present within the corticospinal tract on both sides, or ≥ 3 lesions per hemisphere; grade 3: extensive lesions with high signal on T1-weighted images along the wall of the lateral ventricles; grade 4: cystic lesions in the periventricular white matter (this could be either cystic degeneration of periventricular hemorrhagic infarction or cystic periventricular leukomalacia). Ventricular dilatation (VD) was assessed on axial T2-weighted images at midventricular level. Bilateral ≥ 7.5 mm or one-sided ≥ 10 -mm VD were considered

severe. Based on these criteria, infants were categorized into presence or absence of severe supratentorial injury (i.e., IVH grades 3 and 4, WMI grades 3 and 4, and/or severe VD).

Follow-up

Results of the neurodevelopmental tests and neurological examinations performed between 18 and 36 months were retrieved from the medical files. In the four Dutch centers, a standard neurological examination and the Bayley scales of infant development (BSID-III American or Dutch edition) (28) were performed. As validation of the language scale of the BSID-III was not yet available during the entire study period in the Netherlands, only the cognitive and motor scales were tested. In addition, the Child Behavior Checklist (CBCL) - a questionnaire filled out by the parents - was used as a parameter of neurodevelopment (29). Italian infants were examined and tested by means of a standard neurological examination and the Griffiths Mental Development Scales Revised (GMDS) (30). The GMDS test comprises 5 subscales: locomotor, personal-social, hearing and speech, eye and hand coordination, and performance. The results of the neurological examination were considered abnormal when infants had cerebral palsy (hemi-, di-, or tetraplegia) and/or neurosensory hearing loss. Cerebral palsy was scored according to the Gross Motor Function Classification System (31), a score ≥ 1 was considered abnormal. Information on cerebral visual impairment could not be consistently retrieved from the medical records and was therefore not included. A score of < -1 SD for one of the subscales of the BSID-III or GMDS was considered abnormal, while a CBCL score above 60 was considered to be in the clinical range (29, 32, 33) and thus abnormal. If at least one of the outcome parameters (neurological examination, BSID-III or GMDS, CBCL) was abnormal, this was considered an abnormal composite outcome.

Firstly, each outcome parameter was compared between infants with punctate, limited, and massive CBH. Secondly, the composite outcome was compared between infants with punctate, limited, and massive CBH.

Statistical Analysis

Statistical analysis was performed using SPSS software (version 23.0, SPSS Inc., Chicago, IL, USA). Distribution of continuous variables was objectively assessed by means of the Shapiro-Wilk test. Continuous data are presented as median (range). Categorical variables are presented using frequency counts and percentages. Differences between variables were tested for significance using the Mann-Whitney U test when comparing two continuous variables, and the Kruskal-Wallis test when comparing three variables. For categorical variables, the Chi-square test or Fisher's exact test was used. The contribution of maternal, perinatal, and neonatal variables on the pattern of CBH was analyzed using logistic regression. Neurodevelopmental outcome was related to size of CBH using logistic regression; adjustments were made for GA, gender, and presence of severe supratentorial injury. Significance and odds ratio (OR) with 95% confidence interval (CI) were calculated and a P value < 0.05 was considered significant. Infants with missing data were not included in analyses.

RESULTS

Patients

Data of 218 infants with CBH were collected. The median GA at birth was 27.2 weeks (23.0–34.0 weeks) and median BW 958 g (400–2665 g). The median PMA at MRI was 41.3 weeks (27.7–64.1 weeks). One hundred forty-seven (67%) infants had punctate, 40 (18%) limited, and 31 (14%) massive CBH. Severe supratentorial injury was seen in 52% of the infants with punctate, 60% of the infants with limited, and 52% of the infants with massive CBH. There were no significant differences in incidences of severe IVH, WMI, or VD between the three groups (see Table 1). Ten infants died: two due to massive CBH and three due to severe supratentorial injury. The five other infants died due to systemic complications (i.e., sepsis, multi-organ failure, respiratory complications).

Perinatal Factors and Pattern of CBH

Table 1 shows perinatal variables for infants with punctate, limited, and massive CBH. Due to missing data p-values for mode of delivery and umbilical cord pH were not representative and are therefore not shown. Infants with limited and massive CBH had a lower GA than infants with punctate CBH. They also experienced more respiratory difficulties: more infants with limited and massive CBH needed mechanical ventilation and surfactant replacement therapy. Lower than 5 min Apgar score was more often seen in infants with massive CBH than in the other two groups. Infants with massive CBH more often needed high-frequency ventilation, had lower BW, and more often severe thrombocytopenia than infants with punctate CBH. Analyzing the contribution of these variables by logistic regression revealed GA, Apgar score, and severe thrombocytopenia as independent contributors to massive CBH, while mechanical ventilation remained an independent factor for limited CBH.

Cerebellar Atrophy

Severe atrophy (TCD < 44 mm) at MRI around TEA (N = 141) was seen in 1% (1/102) of infants with punctate CBH, 26% (6/23) of infants with limited CBH, and in 75% (12/16) of infants with massive CBH. Mild to moderate cerebellar atrophy (TCD < 50 mm and \geq 44 mm) was seen in 28%, in 48%, and in 19% of infants with respectively punctate, limited, and massive CBH. TCD was lower with increasingly larger CBH ($P < 0.01$; see Table 2).

Pattern of CBH and Neurodevelopmental Outcome

Follow-up was available for 177/208 (85%) of surviving infants (Table 3). The median corrected age at follow-up was 24.0 months (range 18.0–36.0 months). Infants with massive CBH had the highest percentage of abnormal results for neurological examination, BSID-III, GMDS, and CBCL (see Table 4). For all outcome parameters, except the BSID-III, there was a trend towards higher percentages of abnormal results with an increase in the size of CBH. As there was a striking difference between the test results of the GMDS and the Bayley-III, we analyzed the difference between Italian and Dutch children. Italian infants were significantly younger and had more respiratory difficulties, more often culture proven late onset sepsis and more surgery before TEA than Dutch infants (see Table 5).

Table 1. Maternal, perinatal and postnatal variables in relation to infants with punctate, limited and massive CBH.

	Punctate CBH N = 147	Limited CBH N = 40	Massive CBH N = 31	Punctate vs. Limited p-value	Punctate vs Massive p-value	Limited vs. Massive p-value
GA (weeks), median (range)	27.7 (23.7 – 34.0)	26.7 (24.0 – 32.4)	26.1 (23.0 – 33.0)	<0.05	<0.05	NS
BW in grams, median (range)	980 (530 – 2665)	980 (400 – 2350) ^a	760 (440 – 2168)	NS	<0.05	NS
Z-score BW, median (range)	-0.59 (-5.78 – 2.86)	0.03 (-3.90 – 2.85) ^a	-0.98 (-3.98 – 1.94)	NS	NS	NS
Male, n (%)	85 (58%)	24 (60%)	21 (68%)	NS	NS	NS
Age mother - years, median (range)	31 (18 – 45)	29 (19 – 41)	32 (23 – 46)	NS	NS	NS
Preeclampsia, n (%)	18 (13%) ^d	3 (8%) ^b	5 (18%) ^b	NS	NS	NS
Full course of antenatal steroids, n (%)	77 (56%) ^b	21 (54%) ^a	16 (53%) ^a	NS	NS	NS
C-section, n (%)	66 (45%)	22 (55%)	18 (58%)	NS	NS	NS
Multiple birth, n (%)	43 (29%)	10 (25%)	13 (42%)	NS	NS	NS
5 min Apgar, median (range)	8 (1 – 10)	8 (0 – 9)	7 (2 – 9)	NS	<0.05	<0.05
Severe thrombocytopenia (<50) within 5 days, n (%)	15 (10%) ⁱ	5 (13%) ^f	8 (27%) ^e	NS	<0.05	NS
Need for mechanical ventilation in first week, n (%)	110 (75%)	37 (97%) ^b	29 (94%)	<0.05	<0.05	NS
HFO, n (%)	53 (37%) ^b	20 (51%) ^a	17 (57%) ^b	NS	<0.05	NS
Surfactant, n (%)	88 (60%)	34 (87%) ^a	26 (84%)	<0.05	<0.05	NS
Inotropic support, n (%)	62 (43%) ^b	21 (54%) ^a	18 (60%) ^a	NS	NS	NS
Severe supratentorial injury, total, n (%)	77 (52%)	24 (60%)	16 (52%)	NS	NS	NS
Severe IVH, n (%)	36 (24%)	10 (25%)	8 (26%)	NS	NS	NS
Severe WMI, n (%)	17 (12%)	5 (13%)	3 (10%)	NS	NS	NS
Severe VD, n (%)	59 (40%)	22 (55%)	14 (45%)	NS	NS	NS

BW = birth weight; GA = gestational age; HFO = high frequency oscillation; Severe IVH = Intraventricular hemorrhage grade 3 – 4; Severe WMI = white matter injury grade 3 – 4; Severe VD = severe ventricular dilatation; a = 1 missing; b = 2 missing; c = 3 missing; d = 4 missing; e = 6 missing; f = 7 missing; g = 8 missing; h = 9 missing; i = 10 missing

Table 2. Median transcerebellar diameter (TCD) around TEA (38 – 44 weeks) per pattern of CBH.

CBH pattern	TCD (mm), median (range)
Punctate (N = 102)	51.9 (40.9 – 58.1)
Limited (N = 23)	46.5 (40.9 – 56.8)
Massive (N = 16)	38.1 (22.3 – 57.5)

Table 3. Follow- up: number of cases per type of test.

Outcome parameter		Cases (N)	Median age at test in months, (range)
Dutch infants N = 173	Died	8	
	No follow-up	29	
	Follow-up present	136	
	Type of follow-up		
	Neurological examination	132	25.3 (18.1 – 34.9)
	BSID III	114	25.5 (20.3 – 34.9)
	CBCL	110	25.4 (20.3 – 34.9)
Italian infants N = 45	Died	2	
	No follow-up	2	
	Follow-up present	41	
	Type of follow-up		
	Neurological examination	41	24.0 (18.0 – 36.0)
	GMDS	40	24.0 (18.0 – 36.0)

Table 4. Abnormal test results of infants with punctate, limited and massive CBH and infants.

Type of test	Punctate CBH		Limited CBH		Massive CBH		p-value*
	Total infants tested, N	% abnormal test result	Total infants tested, N	% abnormal test result	Total infants tested, N	% abnormal test result	
Neurological examination	116	15%	35	26%	22	36%	0.04
BSID-III	91	21%	15	20%	8	50%	0.16
GMDS	13	54%	16	69%	11	91%	0.14
CBCL	88	17%	14	21%	8	25%	0.81
Composite score**	119	40%	35	51%	23	78%	<0.01

* X²-test. ** If at least one of the separate tests was abnormal.

Table 5. Maternal, perinatal and postnatal variables of Dutch and Italian infants.

	Dutch infants N = 173	Italian infants N = 45	p-value
GA (weeks), <i>median (range)</i>	27.4 (24.0 – 34.0)	26.5 (23.0 – 32.4)	0.02
BW (g), <i>median (range)</i>	1000 (400 – 2665)	1087 (440 – 2350) ^a	0.03
Z-score for BW, <i>median (range)</i>	-0.48 (-5.78 – 2.86)	-0.78 (-3.75 – 2.85) ^a	0.62
Male, <i>n (%)</i>	101 (58%)	29 (64%)	0.46
Age mother - years, <i>median (range)</i>	31 (18 – 41)	32 (29 – 46)	0.01
Preeclampsia, <i>n (%)</i>	23 (14%) ^c	3 (8%) ^e	0.32
Full course of antenatal steroids <i>n (%)</i>	93 (56%) ^f	21 (51%) ^d	0.58
C-section, <i>n (%)</i>	74 (43%)	32 (71%)	< 0.01
Multiple birth, <i>n (%)</i>	48 (28%)	18 (40%)	0.11
5 min Apgar, <i>median (range)</i>	8 (1 – 10)	8 (0 – 10)	0.77
Severe thrombocytopenia (<50) within 5 days, <i>n (%)</i>	25 (16%) ⁱ	3 (8%) ^g	0.23
Need for mechanical ventilation in first week, <i>n (%)</i>	139 (80%)	37 (86%)	0.39
HFO, <i>n (%)</i>	67 (39%) ^a	23 (56%) ^d	0.05
Surfactant, <i>n (%)</i>	116 (67%)	32 (73%) ^a	0.47
Postnatal steroids, <i>n (%)</i>	44 (27%) ^a	23 (55%) ^c	<0.001
Inotropic support, <i>n (%)</i>	78 (45%) ^a	23 (55%) ^c	0.27
Severe Supratentorial Injury Total <i>n (%)</i>	97 (56%)	20(44%)	0.16
Punctate, <i>n (%)</i>	71 (54%)	6 (40%)	0.88
Limited, <i>n (%)</i>	16 (73%)	8 (44%)	0.07
Massive, <i>n (%)</i>	10 (53%)	6 (50%)	0.31
Surgery before TEA, <i>n (%)</i>	45 (26%)	19 (42%)	0.03

BW = birth weight; GA = gestational age; HFO = high frequency oscillation; TEA = term equivalent age; a = 1 missing; b = 2 missing; c = 3 missing; d = 4 missing; e = 6 missing; f = 7 missing; g = 8 missing; h = 9 missing; i = 10 missing j = 15 missing

A sub-analysis, performed in infants without severe supratentorial injury (N = 87), showed a trend towards higher percentages of abnormal results with increasingly larger CBH for an abnormal neurological examination and CBCL (Table 6). Overall, 33% of infants without severe supratentorial injury had an abnormal composite outcome. The composite outcome differed significantly between the three groups ($p < 0.01$) and again the percentages of infants with abnormal outcome increased with larger hemorrhages. Differences between the three groups remained significant ($p = 0.02$) in infants without severe supratentorial injury.

Comparing massive and punctate CBH, logistic regression analysis demonstrated an increased risk of an abnormal composite outcome in infants with massive CBH, with an adjusted OR of 5.52 (95%CI 1.75–17.43; $p < 0.01$). Comparing massive with limited CBH, the adjusted OR was 4.09 (95%CI 1.09–15.28; $p = 0.04$). No significant differences were found for the composite outcome between punctate and limited CBH ($p = 0.42$).

In a sub-analysis of infants with punctate CBH, we analyzed the neurodevelopmental outcome of infants with ≤ 6 punctate lesions as compared to infants with > 6 punctate lesions. Thirty-nine infants (40%) with ≤ 6 punctate lesions had an abnormal composite outcome score compared to eight infants (36%) of infants with more than six punctate lesions ($p = 0.79$).

Table 6. Abnormal test results of infants with punctate, limited and massive CBH without severe supratentorial injury.

Type of test	Punctate CBH		Limited CBH		Massive CBH		p-value*
	Total infants tested, N	% abnormal test result	Total infants tested, N	% abnormal test result	Total infants tested, N	% abnormal test result	
Neurological examination	59	5%	15	13%	11	18%	0.14
BSID-III	49	10%	4	0%	4	50%	0.14
GMDS	7	71%	9	55%	6	83%	0.63
CBCL	45	11%	4	25%	4	25%	0.28
Composite score**	60	25%	15	40%	12	67%	0.02

** If at least one of the separate tests was abnormal.

Location of CBH and Neurodevelopmental Outcome

Location A and location B were most frequently seen in infants with punctate and limited CBH. A combination of locations A and B, and of locations A, B, and C was most often seen in infants with massive CBH. Most infants ($N = 119$) had unilateral CBH. Although not significant, 67% of infants with bilateral limited CBH and 100% of infants with bilateral massive CBH had an abnormal composite outcome compared to respectively 48% and 74% of infants with unilateral limited and massive CBH (see Table 7). In none of the infants with limited CBH, location C (the vermis) was involved. For infants with punctate CBH, outcome did not differ between infants with and without vermis involvement. All infants with massive CBH and vermis involvement had an abnormal composite outcome (see Table 8). Due to the restricted number of infants per specific location, the relation between location and neurodevelopmental outcome could not be further investigated.

Table 7. Abnormal composite test results of infants with unilateral or bilateral CBH.

Composite score	Punctate CBH		Limited CBH		Massive CBH	
	Unilateral	Bilateral	Unilateral	Bilateral	Unilateral	Bilateral
Normal	40 (62%)	30 (64%)	15 (52%)	2 (33%)	5 (26%)	0 (0%)
Abnormal	24 (38%)	17 (36%)	14 (48%)	4 (67%)	14 (74%)	4 (100%)

Table 8. Abnormal composite test results of infants with or without vermis involvement.

Composite score	Punctate CBH		Limited CBH		Massive CBH	
	No vermis involvement	Vermis involvement	No vermis involvement	Vermis involvement	No vermis involvement	Vermis involvement
Normal	60 (61%)	12 (60%)	17 (49%)	0 (0%)	5 (33%)	0 (0%)
Abnormal	39 (39%)	8 (40%)	18 (51%)	0 (0%)	10 (67%)	8 (100%)

DISCUSSION

We analyzed associations between perinatal factors, cerebellar atrophy, neurodevelopmental outcome, and pattern of CBH determined according to a newly defined MRI classification of CBH that includes punctate, limited, and massive CBH. Several studies have demonstrated an increased risk of an impaired neurodevelopmental outcome for infants with CBH (2, 10, 16, 34). However, to the best of our knowledge, this is the first large sample study taking size of CBH into account. We demonstrated a higher risk of abnormal composite outcome with increasing size of CBH, infants with massive CBH having the highest chance of an unfavorable outcome. Even without severe supratentorial injury they still had a very high risk of an abnormal composite outcome. This is in accordance with the results of Limperopoulos et al. (2) and with data shown in the review of Hortensius et al. (12) who both demonstrated that cognitive, language and behavior sequelae occur frequently in infants with isolated CBH. However, in the systematic review of Hortensius et al. (12), an incidence of 43 to 75% for severe neurodevelopmental outcome in infants with isolated CBH was reported, which is much higher than the 33% found in this study. This may be explained by the number of infants with punctate CBH included in the analyses. In the systematic review, 15/126 infants with isolated punctate CBH were included, while in our study, 119/177 infants were included.

While the composite outcome did not differ significantly between infants with punctate and limited CBH, we found a significant difference in composite outcome between limited and massive CBH. These are important findings. So far, the outcome of infants with limited CBH has not been reported and previous studies, reporting on the neurodevelopmental outcome of infants with CBH, did not distinguish between limited and massive CBH. Combining these two patterns of CBH may have influenced the results in previous studies and may erroneously have suggested that limited CBH carries a similar high risk of an unfavorable

prognosis as massive CBH. While we found an abnormal composite outcome in 40% of the infants with limited CBH, this percentage was lower than in infants with massive CBH (67%).

Severe cerebellar atrophy, using the corrected TCD, was seen in 75% of infants with massive CBH and in a minority of infants with limited or punctate CBH. Due to major growth and development during the second half of gestation, the cerebellum is particularly vulnerable to developmental disruption (4). Also, without evident cerebellar injury, mild atrophy may develop in preterm born children. Cerebellar development may be disrupted by various factors, i.e., hemorrhage, toxic effects of hemosiderin deposition, or supratentorial injury (5, 6, 35). Not all infants with CBH developed cerebellar atrophy. It may be interesting and clinically relevant to investigate the pathogenesis of cerebellar atrophy in infants with CBH and the possible influence of timing of the CBH in a next, prospective study.

The location of CBH may be of clinical importance in infants with limited or massive CBH. Two studies described a negative effect of vermis involvement in global developmental outcome (2, 9), but did not distinguish between limited and massive CBH. While in our study all infants with massive CBH and vermis involvement had an abnormal composite score, in none of the infants with limited CBH the vermis was involved. In contrast to Hortensius et al. (12), we found a difference in composite outcome between infants with uni- or bilateral CBH, with a more favorable outcome in those with a unilateral CBH. In that study and ours the number of infants with bilateral CBH was small, therefore no conclusions can be drawn on the relation between laterality and outcome. In infants with punctate CBH, neither the number of lesions nor the location (uni- or bilateral or vermis involvement) had an influence on the composite score.

Several factors have been associated with CBH, such as low GA, low BW, HFO ventilation, inotropic support, and severe IVH (5, 6, 17, 34). We tried to identify factors that contributed to limited or massive CBH, when compared to punctate CBH. Mechanical ventilation was independently associated with limited CBH, while lower GA, lower Apgar score and severe thrombocytopenia were independently associated with massive CBH. Comparing limited and massive CBH, most factors were not significantly different between the two groups, suggesting that these may have a more similar pathogenesis.

We acknowledge several limitations of our study. Firstly, we compared infants with different patterns of CBH, but we did not compare them with a control group without CBH. Secondly, we retrospectively collected the data of infants with known CBH on MRI. Therefore, this is a selected group of preterm infants: except for the infants in Utrecht and Milan, MRI was performed when clinically indicated. Infants without or with only minor infra- and supratentorial lesions are therefore underrepresented. However, we have reached our primary aim to compare infants with small, limited, and massive CBH. Thirdly, the participating centers used different scan protocols. At Erasmus MC, infants were scanned around PMA 30 weeks, while at the other centers, infants were scanned around TEA. This

may have influenced the detection rate of small lesions: punctate white matter lesions often fade over time (36), the same may be true for punctate cerebellar lesions. However, we do not think this has influenced our results since in the majority of infants (68%), SWI was performed, enabling the detection of even tiny (remnants of) bleeds after a long period of time and we were still able to compare the different patterns of CBH. Furthermore, we examined cerebellar atrophy around TEA, while this may still develop after this age. Additionally, we only measured TCD and did not measure cerebellar volume and may thus have missed small alterations in cerebellar volumes. Finally, different neurodevelopmental tests were used. In Italy, this was GMDS, while the Dutch centers used BSID-III. Although Picciolini et al. (37) reported that the BSID-III had a higher agreement with GMDS than the BSID-II, there are still differences between the two tests. Italian infants were significantly younger and suffered more neonatal morbidity than Dutch infants; this may partially explain the less favorable outcome in the Italian infants. Moreover, the maternal education level could not be found for more than half of the included infants. This may have influenced test results. In the Dutch centers, the language scale of the BSID was not yet validated and could therefore not be used. It is however well known that language may be affected by cerebellar injury (2). This may be another explanation for the less favorable outcome of the Italian infants. All components of the composite outcome were allocated the same weight, but one could argue that for instance CP may impose a larger burden on the infant and its family than an abnormal CBCL score. Although we collected a large population of infants, distributed over 6 centers, the number of infants was still too small to investigate the relation between the specific location of CBH and neurodevelopmental outcome.

CONCLUSION

Limited and massive CBH are associated with the same perinatal factors. The risk of an abnormal composite outcome increases with increasing size of CBH. Infants with limited CBH have a more favorable outcome than infants with massive CBH. It is therefore important to distinguish between limited and massive CBH. Future studies should focus on the relation between location of CBH and neurodevelopmental outcome, and between size of CBH and neurodevelopmental outcome at school age. In addition, the influence of perinatal factors and timing of CBH on subsequent cerebellar atrophy should be evaluated.

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REFERENCES

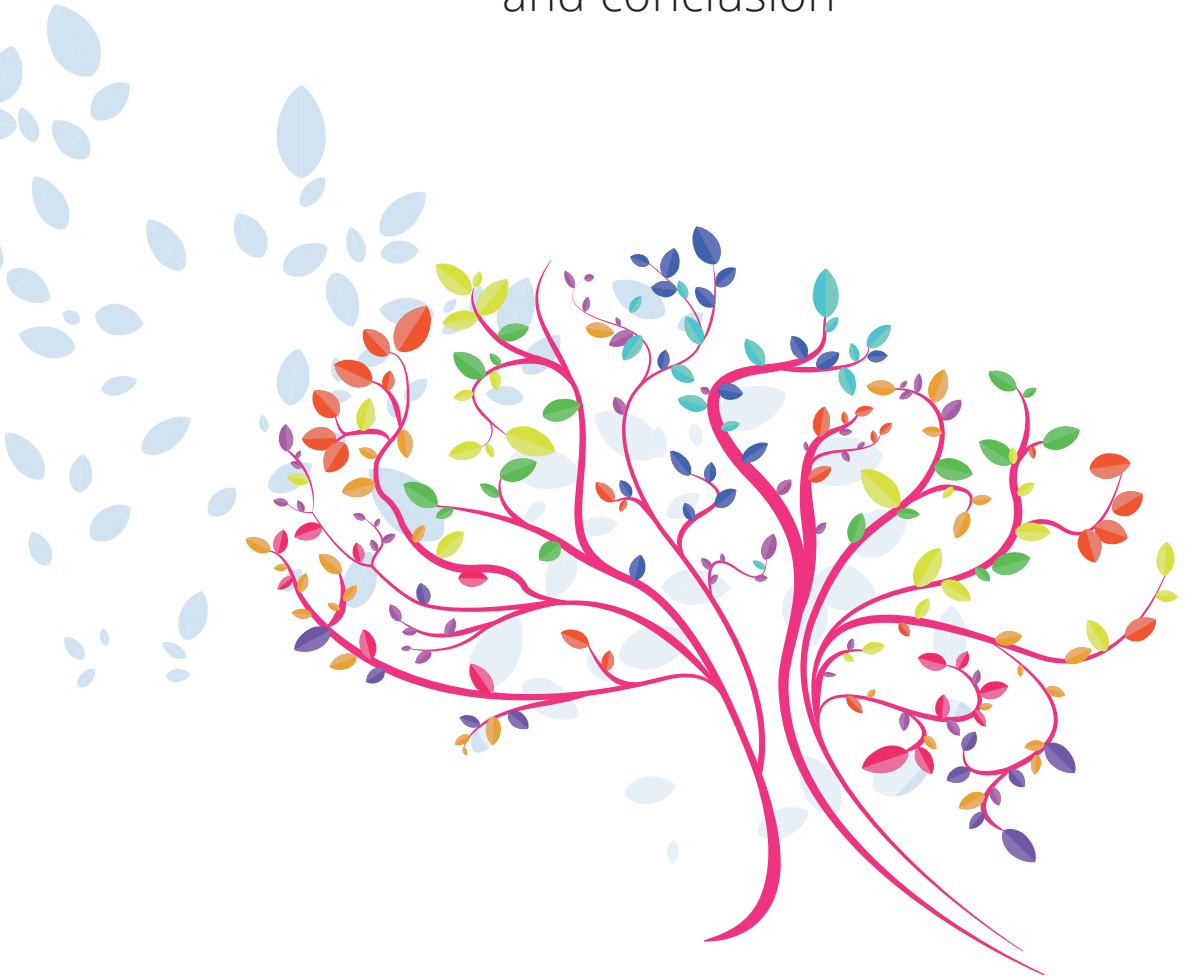
1. Steggerda SJ, Leijser LM, Wiggers-de Bruïne FT, van der Grond J, Walther FJ, van Wezel-Meijler G. Cerebellar injury in preterm infants: incidence and findings on US and MR images. *Radiology*. 2009;252(1):190–9.
2. Limperopoulos C, Bassan H, Gauvreau K, Robertson RLJ, Sullivan NR, Benson CB, Avery L, Stewart J, MDJSS, Ringer SA, Volpe JJ, duPlessis AJ. Does cerebellar injury in premature infants contribute to the high prevalence of long-term cognitive, learning, and behavioral disability in survivors? *Pediatrics*. 2007;120(3):584–593.
3. Kidokoro H, Anderson PJ, Doyle LW, Woodward LJ, Neil JJ, Inder TE. Brain injury and altered brain growth in preterm infants: predictors and prognosis. *Pediatrics*. 2014;134(2):444–53.
4. Volpe JJ. *Volpe's neurology of the newborn*. Sixth edition. ed. Philadelphia, PA: Elsevier; 2018.
5. Limperopoulos C, Soul J, Haidar H, Huppi P, Bassan H, Warfield S, et al. Impaired trophic interactions between the cerebellum and the cerebrum among preterm infants. *Pediatrics*. 2005;116(4):844–50.
6. Volpe JJ. Cerebellum of the premature infant: rapidly developing, vulnerable, clinically important. *J Child Neurol*. 2009;24(9):1085–104.
7. Tam EWY. Cerebellar injury in preterm infants. *Handb Clin Neurol*. 2018;155:49–59.
8. Schmahmann JD. Disorders of the cerebellum: ataxia, dysmetria of thought, and the cerebellar cognitive affective syndrome. *J Neuropsychiatry Clin Neurosci*. 2004;16(3):367–78.
9. Zayek MM, Benjamin JT, Maertens P, Trimm RF, Lal CV, Eyal FG. Cerebellar hemorrhage: a major morbidity in extremely preterm infants. *J Perinatol*. 2012;32(9):699–704.
10. Bednarek N, Akhavi A, Pietrement C, Mesmin F, Loron G, Morville P. Outcome of cerebellar injury in very low birth-weight infants: 6 case reports. *J Child Neurol*. 2008;23(8):906–11.
11. Brossard-Racine M, du Plessis AJ, Limperopoulos C. Developmental cerebellar cognitive affective syndrome in ex-preterm survivors following cerebellar injury. *The Cerebellum*. 2015;14(2):151–164.
12. Hortensius LM, Dijkshoorn ABC, Ecury Goossen GM, Steggerda SJ, Hoebeek FE, Benders MJNL, et al. Neurodevelopmental consequences of preterm isolated cerebellar hemorrhage: a systematic review. *Pediatrics*. 2018;142(5):e20180609.
13. Parodi A, Rossi A, Severino M, Morana G, Sannia A, Calevo MG et al. Accuracy of ultrasound in assessing cerebellar haemorrhages in very low birthweight babies. *Fetal and Neonatal*. 2015;100(4):289–92.
14. Steggerda SJ, van Wezel-Meijler G. Cranial ultrasonography of the immature cerebellum: role and limitations. *Semin Fetal Neonatal Med*. 2016;21:295–304.
15. Limperopoulos C, Benson CB, Bassan H, Disalvo DN, Kinnamon DD, Moore M, et al. Cerebellar hemorrhage in the preterm infant: ultrasonographic findings and risk factors. *Pediatrics*. 2005 Sep;116(3):717–24.
16. Tam EWY, Rosenbluth G, Rogers EE, Ferriero DM, Glidden D, Goldstein RB et al. Cerebellar hemorrhage on magnetic resonance imaging in preterm newborns associated with abnormal neurologic outcome. *J Pediatr*. 2011;158(2):245–50.
17. Steggerda SJ, De Bruïne FT, van den Berg-Huysmans AA, Rijken M, Leijser LM, Walther FJ, et al. Small cerebellar hemorrhage in preterm infants: perinatal and postnatal factors and outcome. *Cerebellum*. 2013;12(6):794–801.
18. Meijler G, Steggerda SJ. *Neonatal cranial ultrasonography*, third edition. Cham: Springer; 2019.
19. Schmahmann JD, Doyon J, McDonald D, Holmes C, Lavoie K, Hurwitz AS, et al. Three-dimensional MRI atlas of the human cerebellum in proportional stereotaxic space. *Neuroimage*. 1999;10(3): 233–60.

20. Koziol LF, Budding D, Andreasen N, D'Arrigo S, Bulgheroni S, Imamizu H, et al. Consensus paper: the cerebellum's role in movement and cognition. *The Cerebellum*. 2014;13(1):151–177.
21. Stoodley CJ, Limperopoulos C. Structure-function relationships in the developing cerebellum: evidence from early-life cerebellar injury and neurodevelopmental disorders. *Semin Fetal Neonatal Med*. 2016;21:356–64.
22. Klein AP, Ulmer JL, Quinet SA, Mathews V, Mark LP. Nonmotor functions of the cerebellum: an introduction. *Am J Neuroradiol*. 2016;37(6):1005–9.
23. Hoftiezer L, Hukkelhoven CWPM, Hogeveen M, Straatman HMPM, van Lingen RA. Defining small-for-gestational-age: prescriptive versus descriptive birthweight standards. *Eur J Pediatr*. 2016;175(8):1047–57.
24. Martin R, Roessmann U, Fanaroff A. Massive intracerebellar hemorrhage in low-birthweight infants. *J Pediatr*. 1976;89(2):290–3.
25. Kidokoro H, Neil J, Inder T. New MR imaging assessment tool to define brain abnormalities in very preterm infants at term. *Am J Neuroradiol* 2013;34(11):2208–14.
26. Bolduc M, du Plessis A, Sullivan N, Guizard N, Zhang X, Robertson R, et al. Regional cerebellar volumes predict functional outcome in children with cerebellar malformations. *Cerebellum*. 2012;11(2):531–42.
27. Volpe JJ. Intraventricular hemorrhage in the premature infant—current concepts. Part II. *Ann Neurol*. 1989;25(2):109–16.
28. Bayley N. Bayley scales of infant and toddler development. 3rd edition ed. San Antonio, TX: Harcourt Assessment; 2006.
29. Achenbach TM, Rescorla L. Manual for the child behavior checklist. Preschool forms and profiles: Burlington VT: University of Vermont Department of Psychiatry; 2000.
30. Griffiths R, Huntley M. The Griffiths mental development scales revised manual: from birth to 2 years. High Wycombe: Association for Research in Infant and Child Development; 1996.
31. Palisano R, Rosenbaum P, Walter S, Russell D, Wood E, Galuppi B. Development and reliability of a system to classify gross motor function in children with cerebral palsy. *Dev Med Child Neurol*. 1997;39(4):214–23.
32. Dekker MC, Koot HM, van der Ende J, Verhulst FC. Emotional and behavioral problems in children and adolescents with and without intellectual disability. *J Child Psychol Psychiatry*. 2002;43(8):1087–98.
33. van den Broek, AJ, Kok JH, Houtzager BA, Scherjon SA. Behavioural problems at the age of eleven years in preterm-born children with or without fetal brain sparing: a prospective cohort study. *Early Hum Dev*. 2010;86(6):379–84.
34. Dyet LE, Kennea N, Counsell SJ, Maalouf EF, Ajayi-Obe M, Duggan PJ et al. Natural history of brain lesions in extremely preterm infants studied with serial magnetic resonance imaging from birth and neurodevelopmental assessment. *Pediatrics*. 2006;118(2):536–48.
35. Limperopoulos C, Soul JS, Gauvreau K, Huppi PS, Warfield SK, Bassan H, et al. Late gestation cerebellar growth is rapid and impeded by premature birth. *Pediatrics*. 2005;115(3):688–95.
36. Kersbergen K, Benders MJNL, Groenendaal F, Koopman-Esseboom C, Nievelstein RAJ, van Haastert IC, et al. Different patterns of punctate white matter lesions in serially scanned preterm infants. *PLoS One*. 2014;9(10):e108904.
37. Picciolini O, Squarza C, Fontana C, Gianni M, Cortinovis I, Gangi S, et al. Neurodevelopmental outcome of extremely low birth weight infants at 24 months corrected age: a comparison between Griffiths and Bayley Scales. *BMC Pediatr*. 2015;15:139.



CHAPTER 9

General discussion, future directions
and conclusion



GENERAL DISCUSSION

Being born before 37 weeks of gestation may cause lifelong neurodevelopmental and behavioral problems(1). Although more than 80% of the total preterm population is born between 32 and 37 weeks, this population has so far received relatively little attention. These moderate-late preterm (MLPT) infants deserve more attention as a considerable number is affected by the impact of their preterm birth (2,3).

The focus in neonatal neuroimaging has traditionally been on brain injury in very preterm infants, born at a gestational age (GA) less than 32 weeks. As their brains are vulnerable to injury, very preterm infants undergo standard neuroimaging. This consists of serial cranial ultrasound (cUS) examinations from birth until term equivalent age (TEA) and, in specific cases, magnetic resonance imaging (MRI). The most common forms of acquired brain injury in this population are intraventricular hemorrhage (IVH), white matter injury (WMI) and cerebellar hemorrhage (CBH) (4-7). These types of brain injury are associated with several neurodevelopmental disabilities, such as motor and cognitive deficits and behavioral problems. (8-10). Furthermore, very preterm infants are enrolled in standardized follow-up programs after discharge. This enables early diagnosis of neurological impairment and thus early interventions (such as physiotherapy, speech therapy, hearing aid, physical and visual rehabilitation). Whether abnormal neuroimaging findings, similar to those found in VPT infants, are present in MLPT infants and affect neurodevelopmental outcome is not well known. Hitherto, MLPT infants do not undergo standard neuroimaging and follow-up programs.

Performing serial cUS and a single MRI examination in MLPT infants formed the basis for this thesis and made it possible to investigate the incidence of brain injury, the quantification of alterations in structural brain growth, and the association of brain injury and brain development with neurodevelopmental and behavioral outcome in MLPT infants. In addition, we focused on cerebellar hemorrhages, as this type of brain injury has also received relatively little attention.

PART I - CURRENT KNOWLEDGE AND PRACTICE REGARDING BRAIN INJURY IN MLPT INFANTS

In **Chapter 2** we give an overview of the current literature on brain injury and altered brain development in MLPT infants. We found that the most frequently studied types of brain injury were IVH and WMI (mainly cystic periventricular leukomalacia [PVL]). The incidence of these types of brain injury in MLPT infants varied widely between studies (range 0.0 – 23.5%), depending on the study sample, setting and imaging techniques used. None of the included studies performed serial cUS and MRI-TEA simultaneously. The incidences of other types of brain injury, including CBH, were sparsely reported. Several included studies

reported slightly higher incidences of IVH and WMI in very preterm infants. Only one study included full-term infants as a control group. We concluded that evidence regarding a higher or lower incidence of brain injury in MLPT compared to respectively very preterm or full-term infants, was weak due to moderate methodological quality of reported studies. Five studies investigated brain development and demonstrated several quantitative differences between MLPT and term infants. These results suggest that MLPT infants may have a delay or alteration in both gray and white matter development compared to their term born peers (11,12). The association between brain injury, altered development and neurodevelopmental outcome was not reviewed, as almost none of the studies included a neurodevelopmental assessment or follow-up. We identified several gaps in knowledge concerning brain injury and altered development in MLPT infants.

In **Chapter 3** we investigated current practice regarding neurological surveillance in MLPT infants. As (inter)national guidelines on this topic are limited, we suspected that several local policies were present, which might differ between and within countries. To investigate this, we sent a survey to Dutch and Canadian neonatal centers that provide care for MLPT infants. The results of this survey showed a large variety of neurological surveillance in MLPT infants in Dutch and Canadian neonatal centers. Our results are in line with a recent review by Braun et al., who concluded that large gaps in the understanding of the needs and optimal management of MLPT infants exist, which are reflected by large unexplained variations in care (13). Further research is needed to investigate the optimal strategy to identify MLPT infants at risk of developmental delay. Whether neuroimaging (cUS and/or MRI) can play a role in identifying these infants is currently unknown. In Part II and III we explored the various applications of neuroimaging in MLPT infants.

PART II - INCIDENCE OF BRAIN INJURY IN MLPT INFANTS

In **Chapter 2** we concluded that various studies on MLPT infants mainly reported the incidence of IVH and (cystic) PVL. In very preterm infants, the incidence of these types of brain injury has declined over the recent years (5). Therefore, as these preterm infants still experience several neurodevelopmental problems, focus has shifted towards more subtle, diffuse and milder forms of brain injury. These milder forms of brain injury are now frequently reported in very preterm infants and are, although to a lesser extent, also associated with neurodevelopmental delays and behavioral problems (14-16).

To investigate if these more subtle forms of brain injury were present in MLPT infants, we initiated the BIMP-study (Brain Imaging in Moderate-late Preterm infants), a prospective neuroimaging study. In **Chapter 4** we introduced the BIMP-cohort, consisting of MLPT infants admitted at Isala Women and Children's Hospital, Zwolle, the Netherlands. We performed serial cUS to follow brain development and brain lesions throughout the neonatal period, and MRI at TEA as the golden standard. We frequently encountered mild brain lesions

in MLPT infants, especially signs suggestive of white matter injury and small hemorrhages. Overall, mild and moderate-severe brain lesions were present in respectively 71.7% and 3.6% of the BIMP-cohort. Small hemorrhages such as remnants of low grade IVH, choroid plexus hemorrhages and punctate CBH were more frequently detected by MRI than by cUS. By investigating a large variety of abnormal neonatal neuroimaging findings on cUS and MRI, we contributed to the current understanding of brain injury and altered brain development in MLPT infants. In general, the types of abnormal neonatal neuroimaging findings in MLPT infants were comparable with the types seen in very preterm infants, but the incidence of abnormal neonatal neuroimaging findings was lower, as was to be expected (17,18).

PART III - QUANTIFICATION OF STRUCTURAL BRAIN GROWTH IN MLPT INFANTS

In addition to visual assessment of brain injury and brain development, quantitative measurements have been used to study possible alterations in brain growth. In very preterm infants without visual brain injury, smaller brain sizes at TEA were related to poorer neurodevelopmental outcome (9,19,20). This suggests that quantitative measurements can be used as an additional biomarker to identify infants with increased risk of a suboptimal neurodevelopmental outcome.

So far, only Walsh et al. performed linear brain measurements in MLPT infants. They performed these measurements on MRI and found smaller brain sizes in MLPT infants compared to full-term infants (21). Although MRI is the golden standard for detecting neonatal brain injury, cUS is the standard neuroimaging modality in neonates and is more practical. Therefore, in **Chapter 5** we explored if simple linear cUS measurements can be used to examine impaired brain growth in MLPT infants. We found no significant differences between moderate preterm (MP; GA 32+0 – 33+6 weeks) and late preterm (LP; GA 34+0 – 36+6 weeks) infants, but we demonstrated differences in the size of the biparietal diameter and basal ganglia width between MP and full-term infants and between LP and full-term infants. In addition, as these measurements were done in infants without or with only mild brain lesions, we considered measurements with a good to excellent (≥ 0.75) inter- and intrarater reliability as valuable reference values of brain measurements in MP and LP infants.

In addition to linear measurements, three-dimensional measurements can be used to calculate brain volumes. So far, only two research groups have studied brain volumes in MP and LP infants. Both Niwa et al. and Thompson et al. found no differences in brain volumes between MP and LP infants (22,23). While several studies performed in very preterm infants have demonstrated a negative effect of brain injury on brain volumes (20), this was not investigated by Niwa et al. nor by Thompson et al. In part II of this thesis, we found that mild brain lesions were frequently present in MLPT infants and were similar to the types seen

in very preterm infants. This generated the question if mild brain lesions affected brain volumes also in MLPT infants. In **Chapter 6**, we demonstrated that mild brain lesions did not affect brain volumes. In addition, we found no differences in brain volumes between MP and LP infants, thereby supporting the results of Niwa et al. and Thompson et al.

Currently, only one study investigated the association between brain volumes at TEA and neurodevelopment in MLPT infants. Cheong et al. (same research group as Thompson et al.) found an association between larger total brain volume, white matter and cerebellar volumes and a better neurodevelopmental outcome at two years of age (11). However, the predictive value of brain volumes (or other quantitative measurements) for neurodevelopmental outcome in MLPT infants was not investigated and remains to be determined.

In very preterm infants, studies addressing the predictive value of quantitative measurements are inconclusive. Skiöld et al. proposed a new scoring system for cUS-TEA combining five subjective assessments of brain injury with quantitative brain measurements (frontal ventricular horns, ventricular midbody, subarachnoidal spaces, interhemispheric distance, corpus callosum thickness) and categorized these into 4 groups: no, mild, moderate and severe abnormalities. They found a high negative predictive value (NPV), but a low positive predictive value (PPV) of moderate to severe abnormalities on cUS for cerebral palsy and severe cognitive delay (respectively NPV: 98 – 100% and PPV: 25 – 33%) (24). Hammerl et al. performed brain measurements on MRI (biparietal width, extracerebral space and interhemispheric distance) and reported a high NPV of 81.3 – 96.6% and a low to moderate PPV of 2.2 – 58.3% of enlarged extracerebral spaces and reduced biparietal width for neurodevelopmental outcome at 24 months corrected age (25). In contrast, Dewan et al. concluded that linear brain measurements (biparietal width, interhemispheric distance and transcerebellar diameter) and total brain abnormality score on MRI did not predict neurodevelopmental outcome at 24 months of age (26).

PART IV - NEURODEVELOPMENTAL AND BEHAVIORAL OUTCOME IN MLPT INFANTS AND IN INFANTS WITH CEREBELLAR INJURY

To date, only a small number of studies have been dedicated to neonatal neuroimaging and neurodevelopmental outcome in MLPT infants and they were all from the Australian research group (the important papers and names of the researchers have been frequently mentioned in this thesis, e.g. Walsh, Cheong, Kelly and Thompson et al.). To add a Dutch perspective on this topic, we are currently conducting a follow-up study at 24 months corrected age. In **Chapter 7**, we present preliminary data of the neurodevelopmental and behavioral outcome of the infants who participated in the BIMP study and have now reached 24 months corrected age. Parents completed three validated questionnaires regarding neurodevelopment, behavior and autism. The results of the three questionnaires were combined in a composite outcome. In our preliminary descriptive analysis of 85 infants,

almost a third (26/85; 30.5%) had a suboptimal composite outcome. This is in line with previous studies in MLPT infants, in whom the same type of follow-up – but no neuroimaging – was performed (27-32). Furthermore, we presented a detailed overview of abnormal MRI findings within infants with a normal and suboptimal composite outcome. As the BIMP-study follow-up is still ongoing, it was not yet possible to draw conclusions regarding these MRI findings and the neurodevelopmental outcome.

In a recent study, Arulkumaran et al. investigated the association between neonatal brain lesions on MRI and neurodevelopmental outcome at 20 months corrected age in preterm infants GA < 33 weeks (18). Forty-three percent of their cohort (205/477) had an abnormal neurodevelopmental outcome at 20 months of age. They found a high sensitivity (90%) and NPV (94%), but a low specificity (26%) and PPV (17%) for any lesion present and an abnormal motor outcome. Hence, a normal MRI at TEA was reassuring for the neurodevelopmental outcome. The classification of neonatal brain lesions used by Arulkumaran et al. was very similar to our study. When the follow-up of the BIMP-study is finalized, it will be very interesting to calculate predictive values for MRI (and cUS) in MLPT infants as well and to compare these with the study of Arulkumaran et al.

In **Chapter 8**, we focused specifically on CBH in preterm infants. Although CBH is a common complication in preterm infants and the number of studies on CBH has increased, the number of cases with CBH in these studies is limited. Therefore, we conducted a large retrospective Dutch/Italian multicenter study including 218 preterm infants (GA \leq 34 weeks) with different sizes of CBH to investigate and compare their outcomes around 24 months corrected age. We introduced a new MRI classification system for CBH size. Size of CBH was divided into punctate (\leq 4 mm), limited ($>$ 4 mm but $<$ 1/3 of the cerebellar hemisphere) and massive (\geq 1/3 of the cerebellar hemisphere). The outcome parameters included neurological examination (all infants), the Griffiths mental development scales test (Italian infants), or the child behavioral checklist and the Bayley scales of infant development test (Dutch infants). If one of the tests was abnormal, this was considered an abnormal composite outcome. Our results demonstrated that the risk of an abnormal composite outcome increased with increasing size of CBH. Although several studies have combined limited and massive CBH into one 'large CBH' group, we concluded that it is important to distinguish between these as infants with limited CBH have a more favorable outcome than infants with massive CBH. Infants with limited and massive CBH both had a lower GA and more respiratory difficulties than infants with punctate CBH. Furthermore, severe cerebellar atrophy (transcerebellar diameter $<$ 44mm), was seen in 75% of infants with massive CBH and in a minority of infants with limited or punctate CBH. In addition, we investigated the association between the location of CBH and neurodevelopmental outcome. CBH was most frequently unilateral, and seen in the convexity and anterior/medial hemispheric zones of the cerebellum. All infants with massive CBH and vermis involvement had an abnormal composite outcome.

In a recent study, Garfinkle et al. further specified the size of CBH by using manual volumetric segmentation on an early MRI (median postmenstrual age at scan: 32.0 weeks) in a cohort of 221 preterm infants (GA 24 - 32 weeks), of whom 36 infants had CBH (33). CBH total volume on early MRI was independently associated with motor scores, visual-motor integration and externalizing behavior at 4.5 years of age, but not with cognitive scores. Interestingly, they found no association between CBH size at TEA and any of the outcome parameters. In addition, they used voxel-based lesion-symptom mapping to determine the exact location of CBH. The region with the highest odds of an adverse outcomes was the inferior part of the cerebellum. The techniques used by Garfinkle et al. enabled a more precise description of CBH and contributed to the understanding of the pathophysiology of CBH.

FUTURE DIRECTIONS FOR RESEARCH

1. *Determining needs of MLPT population, risk stratification and cost-effectiveness*

Several studies demonstrated that MLPT infants have an increased risk of short- and long-term morbidities compared to full-term infants (3,34,35). At present, the best strategy to reduce this risk is not well known. Moreover, there are large gaps in our understanding of the needs and optimal management of MLPT infants (13). This is also apparent from our survey, as neonatal centers had different clinical practices of neurological surveillance in MLPT infants (**Chapter 3**). The MLPT population is large and heterogeneous, needing various levels of care. Since a large proportion of MLPT infants will have an uneventful neonatal course and neurodevelopment, it is obvious that not all MLPT infants need neonatal intensive care, neuroimaging and intensive follow-up. Future research should enable benchmarking to find the optimal strategy for detecting those MLPT infants at risk of morbidities. In addition, given the various possibilities in terms of interventions and the large size of the MLPT population, (society) costs can be considerably high (36-38). Therefore, studies should also investigate the cost-effectiveness of the offered interventions. Once there is more clear evidence on this, uniform guidelines can be implemented.

2. *Susceptibility of the brain, mild brain lesions and structural changes*

On a macroscopic level, we have seen that mild brain lesions are frequently present in MLPT infants (**Chapter 4**). Surprisingly, these mild brain lesions did not affect brain volumes (**Chapter 6**). However, we did find a difference in brain sizes between MP and LP, and full-term infants (**Chapter 5**). To further elucidate the vulnerability of the MLPT brain and the effect of mild brain lesions, it would be interesting to examine the microstructure of the brain using advanced imaging techniques. In very preterm infants, brain abnormalities were associated with lower fractional anisotropy and higher mean, axial and radial diffusivity in the white matter, suggesting microstructural alterations (39). Furthermore, Kelly et al. reported that microstructural alterations, but not brain volumes, were associated with poorer cognitive and language scores in MLPT infants at two years of age (40). We will further investigate the effect of mild brain lesions on microstructural alterations in the MLPT

brain as we also obtained diffusion tensor imaging (DTI) in our cohort. This will be reported separately. Furthermore, even without visible macroscopic lesions, other (perinatal) factors may affect the microstructure of the brain. Therefore, further research into these factors and microstructural changes will be performed as well. This will enable selection of infants with a high risk of abnormal brain development and neurodevelopmental problems. Ideally, MRI will be repeated at school age to assess the response of the brain to early lesions and other perinatal factors.

3. *Predictive value of neuroimaging and neurodevelopmental outcome at school age and beyond*

After the BIMP-study follow-up at 24 months is finalized, we strive to investigate the association between brain lesions and brain development, and long-term neurodevelopmental outcome. In addition, the predictive value of neonatal neuroimaging in MLPT infants, consisting of qualitative and quantitative assessment of brain injury and of microstructural brain development, for the neurodevelopmental outcome will be investigated. In addition to performing an MRI at school age, as mentioned above, it is of great interest and importance to evaluate neurodevelopmental outcome of MLPT infants at this time. If an association between neuroimaging, perinatal factors and neurodevelopmental outcome is found, this may lead to better care and intervention programs for targeted subgroups of MLPT infants and thus eventually to better outcomes and reduction of costs.

4. *Cerebellar hemorrhage and long-term neurodevelopmental outcome*

By classifying CBH into punctate, limited and massive lesions, we were able to demonstrate that infants with punctate and limited CBH had a more favorable outcome around 24 months corrected age than infants with massive CBH (**Chapter 8**). As we only explored the outcome around 24 months corrected age, the results of this study may not reflect the outcome at school age or beyond. At a later stage of life, higher cognitive functioning and behavioral disorders may become more apparent and may be mediated by CBH. Therefore, further research is warranted to gain insights into the long-term consequences of punctate, limited and massive CBH. In addition, more research is needed to investigate the relationship between location of the lesion(s) and outcome.

CONCLUSION

To conclude, in this thesis we have discussed two topics that previously received relatively little attention. We have highlighted the knowledge gaps within the neurological surveillance of MLPT infants. We have contributed to the current understanding of brain injury and altered brain development in MLPT infants by using visual and quantitative assessments on cUS and MRI. We found a high incidence of mild brain lesions in MLPT infants, and demonstrated differences in brain size between MLPT and full-term infants. In addition, we

have demonstrated that it is important to distinguish between limited and massive CBH as infants with limited CBH have a more favorable outcome.

Recommendations based on this thesis:

Although further research is still needed to provide strict recommendations, we advise the following based on our findings and the current literature:

- Standard neuroimaging in MLPT infants does not seem warranted at present.
- Use a low threshold for accepting MLPT infants in neurodevelopmental follow-up programs and interventions as they have a considerable risk of neurodevelopmental and behavioral problems.
- Assess CBH based on size (i.e. punctate [≤ 4 mm], limited [>4 mm but $< 1/3$ of the cerebellar hemisphere] and massive [$\geq 1/3$ of the cerebellar hemisphere]) and location. With a special note on vermal involvement.

REFERENCES

1. Blencowe H, Cousens S, Chou D, Oestergaard M, Say L, Moller A, et al. Born Too Soon: The global epidemiology of 15 million preterm births. *Reproductive Health* 2013;10(1):S2.
2. Natarajan G, Shankaran S. Short- and Long-Term Outcomes of Moderate and Late Preterm Infants. *Am J Perinatol* 2016;33(3):305-317.
3. Allotey J, Zamora J, Cheong-See F, Kalidindi M, Arroyo-Manzano D, Asztalos E, et al. Cognitive, motor, behavioural and academic performances of children born preterm: a meta-analysis and systematic review involving 64 061 children. *BJOG* 2018;125(1):16-25.
4. Papile LA, Burstein J, Burstein R, Koffler H. Incidence and evolution of subependymal and intraventricular hemorrhage: a study of infants with birth weights less than 1,500 gm. *J Pediatr* 1978;92(4):529-534.
5. Volpe JJ. Volpe's neurology of the newborn. Sixth edition. ed. Philadelphia, PA: Elsevier; 2018.
6. Martinez Biarge M, Groenendaal F, Kersbergen KJ, Benders MJNL, Foti F, Cowan FM et al. MRI based preterm white matter injury classification: the importance of sequential imaging in determining severity of injury. *PLoS One* 2016;11(6):e0156245.
7. Limperopoulos C, Bassan H, Gauvreau K, Robertson RLJ, Sullivan NR, Benson CB, et al. Does cerebellar injury in premature infants contribute to the high prevalence of long-term cognitive, learning, and behavioral disability in survivors? *Pediatrics* 2007;120(3):584-593.
8. De Vries LS, Van Haastert IC, Rademaker KJ, Koopman C, Groenendaal F. Ultrasound abnormalities preceding cerebral palsy in high-risk preterm infants. *Journal of Pediatrics*, The 2004;144(6):815-20.
9. Woodward LJ, Anderson PJ, Austin N, Howard K, Inder TE. Neonatal MRI to predict neurodevelopmental outcomes in preterm infants. *N Engl J Med* 2006;355(7):685-694.
10. Kidokoro H, Anderson PJ, Doyle LW, Woodward LJ, Neil JJ, Inder TE. Brain injury and altered brain growth in preterm infants: predictors and prognosis. *Pediatrics* 2014;134(2):444-53.
11. Cheong JLY, Thompson D, Spittle A, Potter C, Walsh J, Burnett A, et al. Brain Volumes at Term-Equivalent Age Are Associated with 2-Year Neurodevelopment in Moderate and Late Preterm Children. *J Pediatr* 2016;174:91-97.e1.
12. Munakata S, Okada T, Okahashi A, Yoshikawa K, Usukura Y, Makimoto M, et al. Gray matter volumetric MRI differences late-preterm and term infants. *Brain Dev* 2013;35(1):10-16.
13. Braun D, Edwards EM, Schulman J, Profit J, Pursley DM, Goodman DC. Choosing wisely for the other 80%: What we need to know about the more mature newborn and NICU care. *Semin Perinatol* 2021;45(3):151395.
14. Horsch S, Muentjes C, Franz A, Roll C. Ultrasound diagnosis of brain atrophy is related to neurodevelopmental outcome in preterm infants. *Acta Paediatr* 2005;94(12):1815-1821.
15. Nguyen The Tich S, Anderson PJ, Hunt RW, Lee KJ, Doyle LW, Inder TE. Neurodevelopmental and Perinatal Correlates of Simple Brain Metrics in Very Preterm Infants. *Archives of Pediatrics & Adolescent Medicine* 2011;165(3):216-222.
16. Kidokoro H, Neil J, Inder T. New MR imaging assessment tool to define brain abnormalities in very preterm infants at term. *Am J Neuroradiol* 2013;34(11):2208-14.

17. Leijser LM, de Bruïne FT, Steggerda SJ, van der Grond J, Walther FJ, van Wezel-Meijler G. Brain imaging findings in very preterm infants throughout the neonatal period: Part I. Incidences and evolution of lesions, comparison between ultrasound and MRI. *Early human development* 2009;85(2):101-109.
18. Arulkumaran S, Tusur N, Chew A, Falconer S, Kennea N, Nongena P, et al. MRI Findings at Term-Corrected Age and Neurodevelopmental Outcomes in a Large Cohort of Very Preterm Infants. *AJNR. American journal of neuroradiology* 2020;41(8):1509-1516.
19. Inder TE, Warfield SK, Wang H, Hüppi PS, Volpe JJ. Abnormal cerebral structure is present at term in premature infants. *Pediatrics*. 2005;115(2):286-94.
20. Keunen K, Kersbergen KJ, Groenendaal F, Isgum I, de Vries LS, Benders MJNL. Brain tissue volumes in preterm infants: prematurity, perinatal risk factors and neurodevelopmental outcome: a systematic review. *J Matern Fetal Neonatal Med* 2012;25 Suppl 1:89-100.
21. Walsh JM, Doyle LW, Anderson PJ, Lee KJ, Cheong JLY. Moderate and Late Preterm Birth: Effect on Brain Size and Maturation at Term-Equivalent Age. *Radiology* 2014;273(1):232-240.
22. Thompson D, Kelly C, Chen J, Beare R, Alexander B, Seal M, et al. Characterisation of brain volume and microstructure at term-equivalent age in infants born across the gestational age spectrum. *Neuroimage Clin* 2019;21:101630.
23. Niwa T, Suzuki K, Sugiyama N, Imai Y. Regional volumetric assessment of the brain in moderately preterm infants (30-35 gestational weeks) scanned at term-equivalent age on magnetic resonance imaging. *Early Hum Dev* 2017;111:36-41.
24. Skiöld B, Hallberg B, Vollmer B, Ådén U, Blennow M, Horsch S. A Novel Scoring System for Term-Equivalent-Age Cranial Ultrasound in Extremely Preterm Infants. *Ultrasound Med Biol* 2019;45(3):786-794.
25. Hammerl M, Zagler M, Zimmermann M, Griesmaier E, Janjic T, Gizewski ER, et al. Supratentorial Brain Metrics Predict Neurodevelopmental Outcome in Very Preterm Infants without Brain Injury at Age 2 Years. *Neonatology* 2020;117(3):287-293.
26. Dewan MV, Herrmann R, Schweiger B, Sirin S, Müller H, Storbeck T, et al. Are Simple Magnetic Resonance Imaging Biomarkers Predictive of Neurodevelopmental Outcome at Two Years in Very Preterm Infants? *Neonatology* 2019;116(4):331-340.
27. Flamant C, Branger B, Nguyen The Tich S, de la Rochebrochard E, Savagner C, Berlie I, et al. Parent-completed developmental screening in premature children: a valid tool for follow-up programs. *PLoS One* 2011;6(5):e20004.
28. Pierrat V, Marchand-Martin L, Arnaud C, Kaminski M, Resche-Rigon M, Lebeaux C, et al. Neurodevelopmental outcome at 2 years for preterm children born at 22 to 34 weeks' gestation in France in 2011: EPIPAGE-2 cohort study. *BMJ* 2017;358:j3448.
29. Mirzakhani H, Kelly RS, Yadama AP, Chu SH, Lasky-Su JA, Litonjua AA, et al. Stability of developmental status and risk of impairment at 24 and 36 months in late preterm infants. *Infant Behav Dev* 2020;60:101462.
30. de Jong M, Verhoeven M, Lasham CA, Meijssen CB, van Baar AL. Behaviour and development in 24-month-old moderately preterm toddlers. *Arch Dis Child* 2015;100(6):548-553.
31. Guy A, Seaton SE, Boyle EM, Draper ES, Field DJ, Manktelow BN, et al. Infants born late/moderately preterm are at increased risk for a positive autism screen at 2 years of age. *J Pediatr* 2015;166(2):269-75.e3.

32. You J, Shamsi BH, Hao MC, Cao CH, Yang WY. A study on the neurodevelopment outcomes of late preterm infants. *BMC Neurol* 2019;19(1):108-0.
33. Garfinkle J, Guo T, Synnes A, Chau V, Branson HM, Ufkes S, et al. Location and Size of Preterm Cerebellar Hemorrhage and Childhood Development. *Ann Neurol* 2020;88(6):1095-1108.
34. Kerstjens JM, de Winter AF, Bocca Tjeertes IF, ten Vergert EMJ, Reijneveld SA, Bos AF. Developmental delay in moderately preterm-born children at school entry. *J Pediatr* 2011;159(1):92-98.
35. de Jong M, Verhoeven M, van Baar AL. School outcome, cognitive functioning, and behaviour problems in moderate and late preterm children and adults: a review. *Semin Fetal Neonatal Med* 2012;17(3):163-169.
36. Khan KA, Petrou S, Dritsaki M, Johnson SJ, Manktelow B, Draper ES, et al. Economic costs associated with moderate and late preterm birth: a prospective population-based study. *BJOG* 2015;122(11):1495-1505.
37. Petrou S. Health economic aspects of late preterm and early term birth. *Semin Fetal Neonatal Med* 2019;24(1):18-26.
38. Speer RR, Schaefer EW, Aholoukpe M, Leslie DL, Gandhi CK. Trends in Costs of Birth Hospitalization and Readmissions for Late Preterm Infants. *Children (Basel)* 2021 ;8(2):127.
39. Thompson D, Lee K, Egan G, Warfield S, Doyle L, Anderson P, et al. Regional white matter microstructure in very preterm infants: predictors and 7 year outcomes. *Cortex* 2014;52:60-74.
40. Kelly CE, Thompson DK, Spittle AJ, Chen J, Seal ML, Anderson PJ, et al. Regional brain volumes, microstructure and neurodevelopment in moderate-late preterm children. *Arch Dis Child Fetal Neonatal Ed* 2020;105(6):593-599.



CHAPTER 10

Summary in English and Dutch
(Samenvatting in het Engels
en Nederlands)



SUMMARY IN ENGLISH

BORN JUST A FEW WEEKS EARLY... IS THIS RELEVANT?

Insights into brain lesions, brain growth, and outcome in moderate-late preterm infants

This thesis gives insights into neonatal brain injury, brain growth and neurodevelopmental outcome at two years of age in moderate-late preterm (MLPT) infants, born at 32 – 36 weeks' gestation. In addition, we describe the neurodevelopmental outcome of preterm infants with cerebellar hemorrhage (CBH).

In **Chapter 2** we give an overview of the current literature regarding brain injury and brain development in MLPT infants. We found that the most frequently studied types of brain injury were intraventricular hemorrhage (IVH) and periventricular leukomalacia (PVL). The incidences of other forms of brain injury, such as CBH, were sparsely reported. In addition, there is a lack of knowledge regarding the effect of brain injury on neurodevelopmental outcome in MLPT infants.

In **Chapter 3** we investigate current practice regarding neurological surveillance with special attention for neuroimaging and follow-up in MLPT infants in the Netherlands and Canada. We found a large variation in local practices regarding admission criteria, laboratory testing, neuroimaging, neurological examination, and follow-up. This variation may partially be explained by the lack of (international) guidelines regarding the care of MLPT infants, in contrast to the situation in very preterm infants (gestational age (GA) < 32 weeks).

The above indicates that considerable knowledge about this large and vulnerable, but relatively understudied population is still lacking. By conducting a prospective cohort study, we sought to fill some of the gaps in knowledge. This study, entitled "Brain Imaging in Moderate-late Preterm infants" (BIMP study), was performed between 2017 - 2019 at the Isala Woman and children's Center in Zwolle, the Netherlands, in collaboration with the Department of Radiology. The MLPT infants who participated in the BIMP study underwent serial cranial ultrasound (cUS) during the neonatal period until term equivalent age (TEA) and a single magnetic resonance imaging (MRI) around TEA. The results of the cUS and MRI examinations are presented in **Chapters 4, 5, and 6**.

In the BIMP cohort, which consisted of 166 MLPT infants, we found a high incidence of mild brain lesions (71.7%). The most frequent findings were signs suggestive of white matter injury, and small hemorrhages, including punctate CBH. Moderate-severe brain lesions were seen in 3.6% of infants (**Chapter 4**). In **Chapter 5**, we compared the linear measurements of different brain structures between MLPT and full-term infants. The biparietal diameter and basal ganglia width were significantly smaller in MLPT infants than in full-term infants. We found no differences in brain structures between moderate preterm infants (MP, gestational age 32+0 - 33+6 weeks) and late preterm infants (LP, gestational age 34+0 - 35+6 weeks).

We also found no differences in the volumes of the measured brain structures between MP and LP infants (**Chapter 6**). Furthermore, the previously described mild brain lesions had no effect on the calculated volumes of different brain structures on MRI (**Chapter 6**).

In **Chapter 7** a preliminary overview is given of the follow-up of the first 85 MLPT infants who participated in the BIMP study and have now reached 24 months corrected age (adjusted for prematurity). The follow-up consists of three parent-completed questionnaires related to development and behavior. Almost one in three MLPT infants (26/85; 30.6%) had an abnormal score on one of the three questionnaires. Of the 85 participants, 68 underwent an MRI around TEA. Abnormal neonatal MRI findings were seen in infants with and without abnormal scores. Given the descriptive nature of this overview and the fact that follow-up is still ongoing, we could not yet draw any further conclusions.

CBH is frequently seen in very preterm infants and - as was recently shown in **Chapter 4** - also in MLPT infants. In **Chapter 8**, we further explore the association between the size and location of CBH in preterm infants and neurological outcome around two years corrected age. For this study, titled “Cerebellar Hemorrhage and Outcome in Preterm infants (CHOPiN study),” the data of 218 Dutch and Italian infants (GA \leq 34 weeks) with CBH were collected. Based on the neonatal MRI, the location of CBH was described and classified into three categories based on CBH size: punctate (≤ 4 mm), limited (>4 mm but $< 1/3$ of the cerebellar hemisphere), and massive ($\geq 1/3$ of the cerebellar hemisphere). Neurological outcome at two years of age was defined by neurological examination and neurodevelopmental tests. CBH was most frequently unilateral, and seen in the convexity and anterior/medial hemispheric zones of the cerebellum. All infants with massive CBH and involvement of the vermis had a less favorable outcome. Based on the CHOPiN study, we concluded that it is important to distinguish between limited and massive CBH as infants with limited CBH have a more favorable outcome than infants with massive CBH. Until recently generally only distinguishment was done between small and larger (both limited and massive) CBH.

Although much research is still needed to provide strict recommendations, we advise the following based on our findings and the current literature:

- Standard neuroimaging in MLPT infants does not seem warranted at present.
- Use a low threshold for accepting MLPT infants in neurodevelopmental follow-up programs and interventions as they have an increased risk of neurodevelopmental and behavioral problems compared to full-term counterparts.
- Assess CBH based on size (i.e. punctate [≤ 4 mm], limited [>4 mm but $< 1/3$ of the cerebellar hemisphere] and massive [$\geq 1/3$ of the cerebellar hemisphere]) and location. With a special note on vermian involvement.

SUMMARY IN DUTCH/SAMENVATTING IN HET NEDERLANDS

SLECHTS EEN PAAR WEKEN TE VROEG GEBOREN... IS DAT RELEVANT?

Inzicht in hersenletsel, hersengroei en ontwikkeling van matig-laag prematuur geboren kinderen

In dit proefschrift beschrijven we de inzichten in neonataal hersenletsel, hersengroei en de ontwikkeling op de leeftijd van 24 maanden van matig-laag te vroeg geboren (MLPT) kinderen, geboren na een zwangerschapsduur van 32 - 36 weken. Daarnaast beschrijven we de neurologische uitkomst van te vroeggeboren kinderen met een cerebellaire bloeding (CBH).

In **Hoofdstuk 2** geven we een overzicht van de huidige literatuur met betrekking tot hersenletsel en hersenontwikkeling in MLPT-kinderen. Hieruit blijkt dat voornamelijk de incidentie van intraventriculaire bloedingen (IVH) en periventriculaire leukomalacie (PVL) bij MLPT kinderen beschreven is. De incidentie van andere vormen van hersenletsel, zoals CBH, is nauwelijks beschreven. Daarnaast ontbreekt kennis over het effect van hersenletsel op de ontwikkeling.

In **Hoofdstuk 3** onderzoeken we het huidige beleid met betrekking tot het monitoren van de hersenen en de neurologische ontwikkeling van MLPT-kinderen in Nederland en Canada. We vonden een grote variatie in het beleid met betrekking tot opname criteria, laboratoriumdiagnostiek, beeldvorming van de hersenen, neurologisch onderzoek en het vervolgen van de ontwikkeling van de kinderen op latere leeftijd. Deze variatie is waarschijnlijk deels te verklaren door het ontbreken van (internationale) richtlijnen met betrekking tot de zorg voor MLPT-kinderen, dit in tegenstelling tot de situatie bij zeer vroeggeboren kinderen (zwangerschapsduur < 32 weken).

Uit bovenstaande blijkt dat nog veel kennis over deze grote en kwetsbare, maar relatief onderbestudeerde populatie ontbreekt. Door het opzetten van een prospectief cohortonderzoek trachtten wij enkele van de hiaten in kennis op te vullen. Dit onderzoek, getiteld "Brain Imaging in Moderate-late Preterm infants" (BIMP-studie), werd van 2017 tot 2019 uitgevoerd in het Isala Vrouw-kind centrum te Zwolle, in samenwerking met de afdeling Radiologie. De MLPT-kinderen die deelnamen aan de BIMP-studie ondergingen seriële schedelechografie gedurende de neonatale periode en één magneetveldonderzoek (MRI) rond de uitgerekende datum. De bevindingen van deze onderzoeken zijn gepresenteerd in **Hoofdstuk 4, 5, en 6**.

In het BIMP-cohort, bestaande uit 166 MLPT-kinderen, vonden we een hoge incidentie van milde hersenlaesies (71.7%). De meest voorkomende bevindingen waren tekenen suggestief voor witte stof schade en kleine bloedingen, waaronder puntvormige CBH. Matig-ernstig hersenletsel werd bij 3.6% van de kinderen gezien (**Hoofdstuk 4**). In **Hoofdstuk 5** vergelijken we de afmetingen van verschillende hersenstructuren tussen MLPT en voldragen kinderen. We zagen dat de bipariëtale diameter en basale kernen bij MLPT-kinderen

kleiner waren dan bij voldragen kinderen. Tussen matig prematuur geboren kinderen (MP, zwangerschapsduur tussen 32+0 en 33+6 weken) en laat prematuur geboren kinderen (LP, zwangerschapsduur tussen 34+0 en 35+6 weken) zagen we geen significante verschillen in de afmetingen van hersenstructuren. Daarnaast vonden we geen verschillen in het volume van de hersenstructuren tussen MP en LP kinderen (**Hoofdstuk 6**). Ook hadden de eerder beschreven milde hersenlaesies geen effect op het volume van verschillende hersenstructuren (**Hoofdstuk 6**).

In **Hoofdstuk 7** beschrijven we het vervolgonderzoek van de BIMP-studie, dat op de gecorrigeerde leeftijd (gecorrigeerd voor prematuriteit) van 24 maanden wordt uitgevoerd. Het onderzoek bestaat uit drie door ouders ingevulde vragenlijsten met betrekking tot de ontwikkeling en het gedrag. In het hoofdstuk wordt een voorlopig overzicht gegeven waarin de uitkomsten van de eerste 85 deelnemers worden beschreven. Bijna één op de drie MLPT-kinderen (26/85; 30.6%) had een afwijkende score op een van de drie vragenlijsten. Van de 85 deelnemers was bij 68 een MRI rond de uitgerekenende datum verricht. In het beschrijvend overzicht van de MRI-bevindingen en de uitkomst op de leeftijd van 24 maanden zagen we dat lang niet alle kinderen met afwijkende MRI bevindingen een afwijkende score op een van de vragenlijsten hadden. Gezien het beschrijvende karakter van dit overzicht en het feit dat het vervolgonderzoek nog gaande is, konden wij hier nog geen verdere conclusies aan verbinden.

Hoewel CBH voornamelijk bij zeer te vroeggeboren kinderen onderzocht is, zagen we in **Hoofdstuk 4** dat puntvormige CBH ook regelmatig voorkwam bij MLPT-kinderen. In **Hoofdstuk 8** gaan we verder in op de associatie tussen de grootte en locatie van CBH bij te vroeg geboren en de neurologische uitkomst rond de gecorrigeerde leeftijd van twee jaar. Voor dit onderzoek, getiteld "Cerebellar Hemorrhage and Outcome in Preterm infants (CHOPiN-studie)" werden de gegevens van 218 Nederlandse en Italiaanse kinderen (zwangerschapsduur ≤ 34 weken) met CBH verzameld. Op basis van het neonatale MRI-onderzoek werd de locatie van CBH beschreven en op basis van CBH-grootte ingedeeld in drie categorieën: puntvormig (≤ 4 mm), middelmatig (>4 mm maar $< 1/3$ van de cerebellaire hemisfeer) en groot ($\geq 1/3$ van de cerebellaire hemisfeer). De neurologische uitkomst rond 24 maanden werd gedefinieerd aan de hand van een neurologisch onderzoek en ontwikkelingsonderzoeken. CBH werd vooral unilateraal gezien en kwam vaker voor in de convexiteit en anterieure/mediale zones van de cerebellaire hemisfeer dan in de vermis. Alle kinderen met een grote bloeding en betrokkenheid van de vermis hadden een minder gunstige uitkomst. Uit de CHOPiN-studie komt naar voren dat het belangrijk is om een onderscheid te maken tussen middelmatige en grote bloedingen omdat kinderen met een middelgrote bloeding een gunstigere uitkomst hebben dan kinderen met een grote bloeding.

Hoewel er nog veel onderzoek nodig is, kunnen we op basis van dit proefschrift en de huidige literatuur momenteel het volgende aanbevelen:

- Op dit moment wordt het niet aangeraden om standaard beeldvorming van de hersenen bij MLPT-kinderen te verrichten.
- MLPT-kinderen moeten laagdrempelig in aanmerking komen voor (neurologische) screeningsprogramma's en interventies, aangezien zij een wezenlijk risico hebben op neurologische ontwikkelings- en gedragsproblemen.
- Beoordeel CBH op basis van grootte (d.w.z. puntvormig [≤ 4 mm], middelmatig [>4 mm maar $< 1/3$ van de cerebellaire hemisfeer] en groot [$\geq 1/3$ van de cerebellaire hemisfeer]) en locatie, besteed daarbij specifiek aandacht aan de vermis.



CHAPTER 11

Appendices



List of abbreviations
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LIST OF ABBREVIATIONS

ASQ	Ages and Stages Questionnaire
BET	Brain extraction tool
BIMP	Brain Imaging in Moderate-late Preterm infants
BSID	Bayley Scales of Infant Development
BW	Birth weight
CBCL	Child Behavior Checklist
CBH	Cerebellar hemorrhage
cGM	Cortical gray matter
CHOPiN	Cerebellar Hemorrhages and Outcome in Preterm infants
CI	Confidence interval
(c)PVL	(cystic) Periventricular leukomalacia
CSF	Cerebrospinal fluid
CMV	Cytomegalovirus
CP	Cerebral Palsy
CUS	Cranial ultrasound
dGM	Deep gray matter
DTI	Diffusion tensor imaging
DWI	Diffusion weighted imaging
FSL	FMRIB Software library
GA	Gestational age
GMDS	Griffiths Mental Development Scales
HFO	High frequency oscillation
IVH	Intraventricular hemorrhage
IQ	Intelligence quotient
JBH	Johanna Briggs Institute
LP	Late preterm
LSV	Lenticulostriate vasculopathy
MANTiS	Morphologically Adaptive Neonatal Tissue Segmentation
M-Chat-(R-F)	Modified Checklist for Autism in Toddlers (Revised – with Follow-up)
MLPT	Moderate-late preterm
MP	Moderate preterm
MRI	Magnetic resonance imaging
NA	Not assessable / Not applicable
NICU	Neonatal intensive care unit
PLIC	Posterior limb of the internal capsule
SD	Standard deviation
SGA	Small for gestational age
SSI	Severe Supratentorial Injury
SWI	Susceptibility weighted imaging
TEA	Term equivalent age

VD	Ventricular dilatation
WM	White matter
WMI	White matter injury

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LIST OF PUBLICATIONS

1. **Boswinkel V**, Steggerda SJ, Fumagalli M, Parodi A, Ramenghi LA, Groenendaal F, et al. The CHOPIn Study: a Multicenter Study on Cerebellar Hemorrhage and Outcome in Preterm Infants. *Cerebellum*. 2019 Dec;18(6):989-998. doi: 10.1007/s12311-019-01053-1.
2. **Boswinkel V**, Nijboer-Oosterveld J, Nijholt IM, Edens MA, Mulder-de Tollenaer SM, Boomsma MF, et al. A systematic review on brain injury and altered brain development in moderate-late preterm infants. *Early Hum Dev*. 2020 Sep;148:105094. doi: 10.1016/j.earlhumdev.2020.105094. Epub 2020 May 28. Erratum in: *Early Hum Dev*. 2020 Oct 17;105226.
3. **Boswinkel V**, Krüse-Ruijter MF, Nijboer-Oosterveld J, Nijholt IM, Edens MA, Mulder-de Tollenaer SM, et al. Incidence of brain lesions in moderate-late preterm infants assessed by cranial ultrasound and MRI: The BIMP-study, *Eur J Radiol*. 2021 Mar;136:109500. doi: 10.1016/j.ejrad.2020.109500. Epub 2020 Dec 24.
4. **Boswinkel V**, Sok FI, Krüse-Ruijter MF, Nijholt IM, Jansen FAR, Haak MC, et al. Ultrasound measurements of brain structures differ between moderate-late preterm infants and full-term infants at term equivalent age. *Early Hum Dev*. 2021 Sep;160:105424. doi: 10.1016/j.earlhumdev.2021.105424. Epub 2021 Jul 14.
5. **Boswinkel V**, Verschuur AS, Nijholt IM, van Osch JAC, Nijboer-Oosterveld J, Beare RJ, Slump CH, de Vries LS, Boomsma MF, van Wezel-Meijler G. Mild brain lesions do not affect brain volumes in moderate-late preterm infants. *Eur J Paediatr Neurol*. 2021 Aug 17;34:91-98. doi: 10.1016/j.ejpn.2021.08.003. Epub ahead of print.
6. Verschuur AS, **Boswinkel V**, Tax CMW, van Osch JAC, Nijholt IM, Slump CH, et al. Improved segmentation of neonatal brain MRI scans by addressing motion artifacts with data interpolation, *Submitted*.

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Vivian Boswinkel was born on November 27th, 1991 in Deventer, the Netherlands. She grew up in a nearby village named Olst, together with her older sister. After graduating from high school (VWO, Etty Hillesum Lyceum, Deventer) in 2010, she started studying Medicine at the University of Groningen. During her studies, Vivian was involved in several research projects, and she was member of various student committees. In 2016, she conducted an internship in Global Health in Malawi.

Vivian performed her master thesis at the department of neonatology under the supervision of dr. Gerda Meijler and dr. Sylke J. Steggerda. She completed her final internship at the departments of neonatology and pediatrics at Isala, Zwolle. After obtaining her medical degree, Vivian was given the opportunity to start a PhD-trajectory at the University of Utrecht and start-up and coordinate the BIMP-study 'Brain Imaging in Moderate-late Preterm infants' at Isala Zwolle, under the supervision of dr. Gerda Meijler, prof. dr. Linda S. de Vries and dr. Martijn F. Boomsma. Her research focuses on neonatal brain injury, brain growth and neurodevelopmental outcome in moderate-late preterm (MLPT) infants, born at 32 – 36 weeks' gestation. During her PhD-trajectory, Vivian successfully supervised three master student theses and presented her work at several national and international conferences. Vivian completed her PhD-thesis in the summer of 2021. Since then, she started working as a resident (ANIOS) at the department of pediatrics at Isala Zwolle, and continued working on the results of the BIMP-study.

During her free time, she likes to sport (running, cycling, swimming and yoga) and enjoys spending time with her friends and family. Her greatest wish is to obtain a traineeship in pediatrics and eventually to become a pediatrician.





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