Advanced MR brain imaging in preterm infants

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Introduction

General Introduction

Each year around 180.000 infants are born according to the Perinatal Registry in the Netherlands. Eight percent of them are born prematurely (gestational age <37 weeks) and around 2% are born very prematurely (gestational age <32 weeks) (Stichting PRN, 2011).

Despite improvements in neonatal care, very preterm infants who survive the neonatal period are still at risk of neurodevelopmental disabilities as a result of injury to the brain. Neurodevelopmental delay is seen twice as often in infants born between 30 – 33 weeks as compared to term born infants and spasticity can even be seen seven times as often.[1]

Therefore neurodevelopmental delay is an important problem not only for the surviving infants themselves, but also for their parents or caretakers and for health care and society in general. Hence there is an urgent need for clinicians to provide parents and caretakers with predictive information on the neurological development of their preterm born infant and brain imaging has become standard care of very preterm infants.

Cognitive and motor impairment are associated with peri- and intraventricular hemorrhage (P/IVH) and white matter injury.[2,3] Over the last years there has been a gradual change from cystic periventricular white matter injury, readily depicted by cranial ultrasound, to a more diffuse form of white matter injury.[4,5,6] Apart from P/IVH and cystic periventricular leucomalacia, diffuse white matter injury is thought to be the main determinant for a poorer outcome in these very preterm infants.[3] Cranial ultrasound is an easily accessible and reliable tool to detect P/IVH and cystic periventricular leucomalacia.[7] MRI potentially detects more subtle white matter damage, but is less accessible, due to the necessity to transport the infant to the radiology department. The use of MRI compatible incubators has largely overcome this problem.

Cranial ultrasound seems to underestimate diffuse white matter injury and as 25 – 50% of very preterm infants with diffuse white matter injury develop cognitive problems,[3] this may prompt the use of MRI. Advanced MRI techniques such as diffusion tensor imaging (DTI) allow assessment of brain microstructure and quantification of brain growth and development and potentially detect brain injury; however it is still uncertain if MRI should be used on a routine basis in a clinical setting.

AIM AND OUTLINE OF THE THESIS

The aim of the thesis is to investigate the diagnostic value of MRI performed around term equivalent age in evaluating brain injury and predicting neurodevelopmental outcome at two years corrected age in very preterm infants (gestational age <32 weeks).

*Chapter*2 is a review on the radiological assessment of white matter injury in very preterm infants.

In *chapter 3* we investigate the association between DTI values of white matter tracts and age, white matter injury and clinical factors.

In *chapter 4* we assess whether DTI tractography performed around term equivalent age can independently predict neurodevelopmental outcome of very preterm infants at two years.

In *chapter 5* we examine the reliability of a classification system for white matter injury on sequential cranial ultrasound performed in the perinatal period to detect diffuse white matter injury, using MRI performed around term equivalent age as reference standard.

In *chapter 6* we evaluate the practical implications of ultrasound detection of white matter injury in very preterm neonates. The predictive values of ultrasound abnormalities for white matter injury on MRI and for neurological outcome are assessed and recommendations are proposed for neuro-imaging in very preterm infants.

In *chapter 7* we evaluate the clinical implications of MR imaging findings in the white matter in very preterm infants in relation to clinical follow up at two years.

In *chapter 8* we investigate the incidence and findings of cerebellar injury in very preterm infants on cranial ultrasound and MRI.

In *chapter 9* we study the clinical value of gradient echo MRI for brain imaging in very preterm infants.

In *chapter 10* the diagnostic value of MR brain imaging in very preterm infants at term equivalent age is summarized and discussed. Concluding remarks are made and future perspectives discussed.

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Radiological assessment of white matter injury in very preterm infants

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Gerda van Wezel-Meijler

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Abstract

Despite improvement of neonatal care, infants born very prematurely who survive the neonatal period are still at risk for neurodevelopmental disabilities. One of the main determinants for a poorer outcome seems to be damage to the cerebral white matter which frequently occurs during the perinatal period. This article summarizes the radiological assessment of white matter injury in very preterm infants, striving to aid clinicians who provide parents and caretakers with predictive information on the development of their preterm born infants. As the expertise of radiologists in assessing neonatal brain MRI may vary widely amongst centers, we also strive to provide radiologists with information on imaging findings of white matter injury.

Introduction

In infants born very prematurely (gestational age <32 weeks), germinal matrix and intraventricular hemorrhage, and white matter injury are frequently encountered,[1,2,3] while cerebellar injury is increasingly recognized.[4,5,6] These injuries are associated with later cognitive and motor impairment.[1,7,8,9,10,11,12,13]

White matter injury, also called periventricular leucomalacia (PVL) is one of the most frequently occurring forms of brain injury in infants born very prematurely.

Over the last years there has been a gradual change in incidence from cystic white matter injury to more diffuse white matter injury, where the majority of very preterm infants now show more subtle abnormalities of the developing white matter.[3,7,14,15]

Diffuse white matter injury is generally held responsible for the high incidences of cognitive and behavioural disorders in very preterm born infants.[1,2,8]

The two neuro-imaging modalities generally used in the neonatal period are cranial ultrasonography (CUS) and magnetic resonance imaging (MRI). CUS is safe, easily accessible, can be used on a serial basis and is reliable for detection of most forms of neonatal brain injury.[16] MRI is a safe and valuable tool to assess development and pathology of the very preterm infant's brain and gives detailed information on the exact location and extension of injury.[9,17,18,19,20] Advanced MR techniques such as diffusion tensor imaging (DTI) or volumetric analyses can detect axonal disturbances and volume loss resulting from diffuse white matter injury.

CT should only be used for specific limited indications in the neonate as it involves considerable radiation and generally will not provide more information than CUS and/or MRI.[16]

The aim of this article is to describe, in detail, the role and limitations of both widely accepted neonatal neuro-imaging modalities (CUS and MRI), with a specific focus on preterm white matter injury, the findings that can be encountered and the predictive significance of these findings.

White matter injury

The main pathogenic mechanisms for white matter injury in the very preterm neonate are ischemia and infection. These often coexist and may lead to focal or diffuse white matter injury and/or hemorrhages in the perinatal period due to the vulnerability of

Figure 1a. Cystic degeneration of white matter in the centrum semi-ovale (arrows) on axial T2-w MR image.

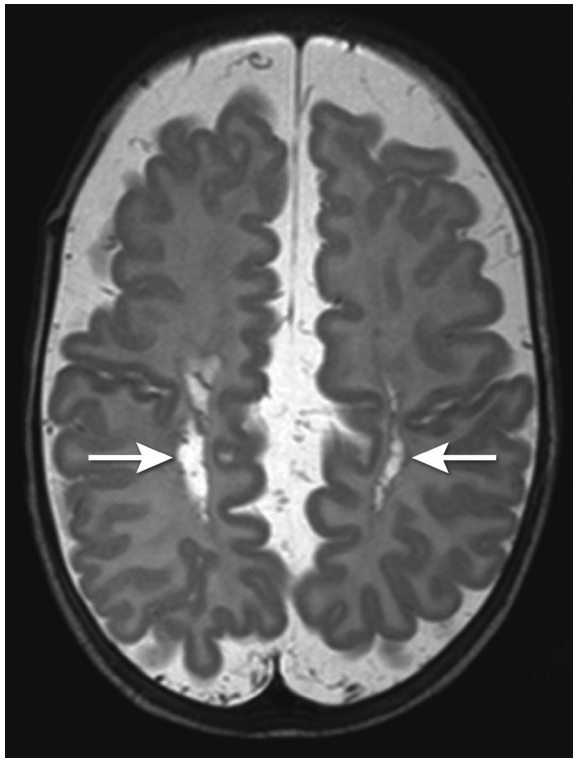


Figure 1b. Cystic periventricular leucomalacia (PVL) readily diagnosed (arrows) on a sagittal ultrasonography image.

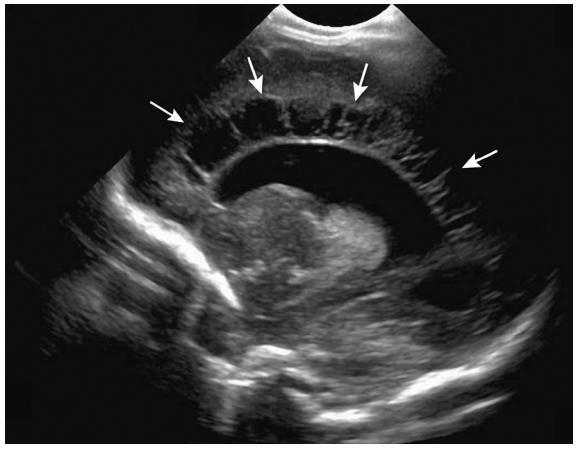
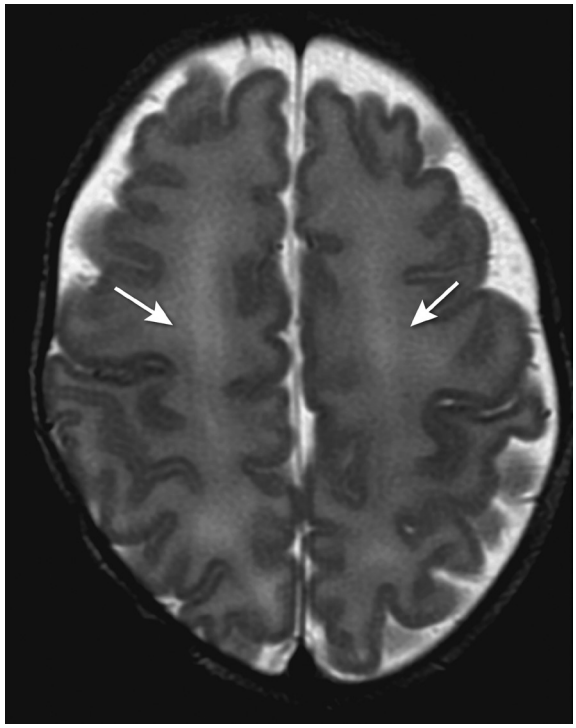


Figure 2. Diffuse white matter injury leading to hypomyelination and volume loss, resulting in widening of the pericerebral space. The arrows indicate the absence of myelination in the centrum semi-ovale in a very preterm infant imaged around term.



the developing white matter, immature vasculature and impaired cerebrovascular auto regulation of the immature brain.[2,21,22]

In focal white matter injury or cystic periventricular leucomalacia (Figure 1a and 1b) there is localized necrosis with loss of cellular elements that evolves over several weeks into macroscopic cystic lesions, readily visualized by both CUS and MRI. More commonly, the necrosis is microscopic in size and evolves into glial scars over several weeks. This more diffuse white matter injury accounts for the vast majority of cases.[2] The glial scars are characterized by astrogliosis and microgliosis. Damage to and significant decrease in premyelinating oligodendrocytes occurs.[2,21,23] Subsequently this leads to hypomyelination and cerebral white matter loss (Figure 2), resulting in decreased volumes of commissures, such as the corpus callosum.[24] The white matter injury will eventually also lead to grey matter loss and decreased volumes of the thalamus, basal ganglia, cerebral cortex, and cerebellum as early as term equivalent age, as a result of neuronal and axonal loss and abnormal connectivity.[2,7,23,25,26] Dif-

fuse non cystic white matter injury in itself is not readily depicted by neuro-imaging. The resulting volume loss can be identified by measuring the ventricular dilatation or by volumetric analysis of white and grey matter structures.[27,28] DTI studies have suggested axonal loss in the white matter of preterm infants at term equivalent age.[2,26,29,30,31,32,33]

Figure 3a. High echogenicity (arrows) of non physiological periventricular echo densities (PVE), as shown on a coronal ultrasonography image at the level of choroid plexus in the lateral ventricles in preterm infant with a gestational age of 31 weeks.

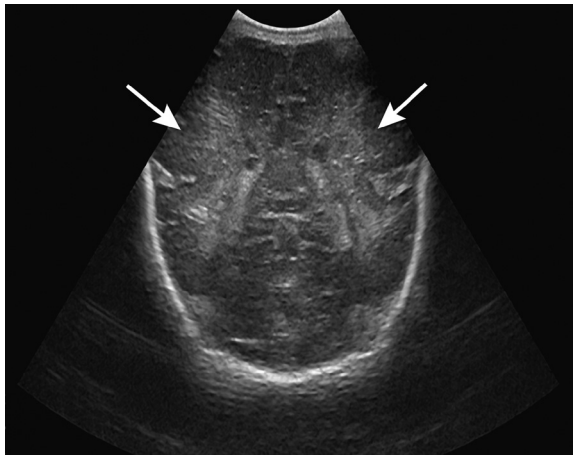
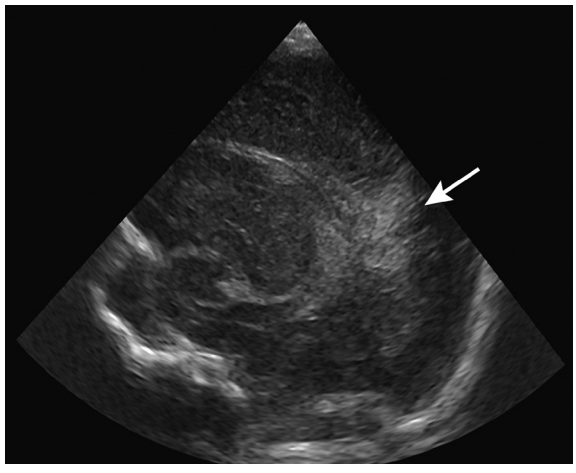


Figure 3b. Sagittal view in the same infant showing inhomogeneous PVE in the parietal white matter (arrow).



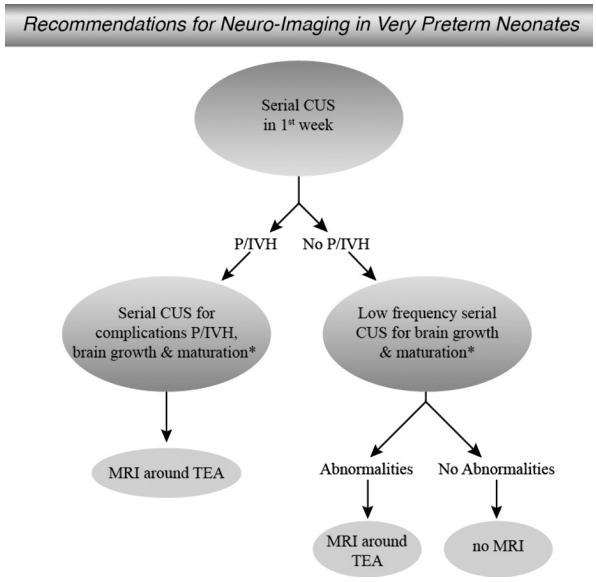
Imaging findings of white matter injury on CUS and MRI

Periventricular echo densities

On CUS, non-physiological periventricular echo densities (PVE) (Figures 3a and 3b) of the white matter are thought to reflect white matter injury. Their appearance is classified as homogeneous or inhomogeneous, and the echogenicity staged as grade one or two.[34] The more inhomogeneous and echogenic the PVE and the longer their duration, the more likely they present white matter injury. Presence of non-physiological PVE on CUS is predictive of abnormal white matter on MRI at term. However, absence of PVE does not predict normal white matter on MRI at term, but does predict a favorable outcome.[35] Presence of non-physiological PVE in itself is not associated with unfavorable short term outcomes.[35,36,37]

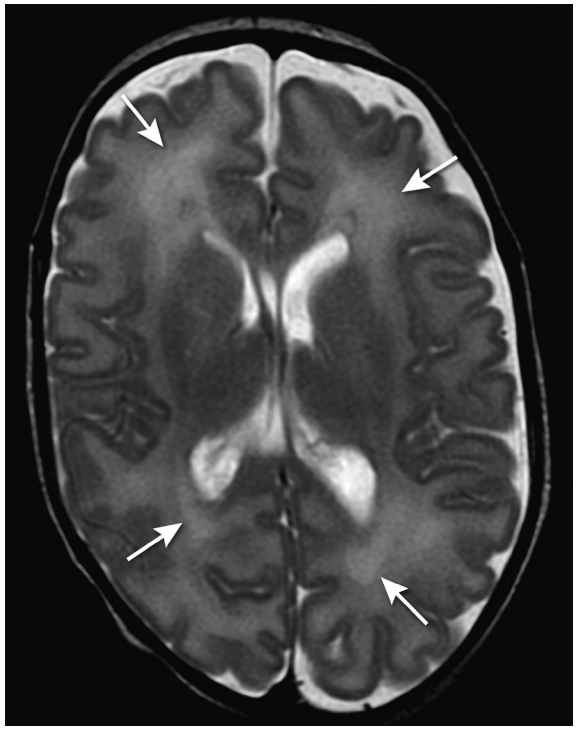
In a retrospective study, inhomogeneous PVE showed no association with punctate white matter lesions (PWML) on MRI.[38] The MRI or histological equivalent of inhomogeneous PVE remains unknown. The retrospective study by Leijser *et al*. showed that the performance of a MRI study before term equivalent age besides sequential CUS did not seem warranted in infants with mild to moderate abnormal white matter. Additional MRI only slightly increased the predictive value of CUS in severe white matter changes.[38] In our recent study on ultrasound detection of white matter injury and its practical implications we provided recommendations on performing serial CUS in all very preterm neonates during the perinatal period and a MRI at term equivalent age in some (Figure 4).[35]

Figure 4: Recommendations for neuro-imaging in very preterm neonates.



*Intensify if complications occur.
CUS = cranial ultrasonography,
P/IVH=peri/intraventricular hemorrhage,
TEA=term equivalent age.

Figure 5. Periventricular DEHSI (arrows) is now thought to be a developmental phenomenon rather than white matter injury.



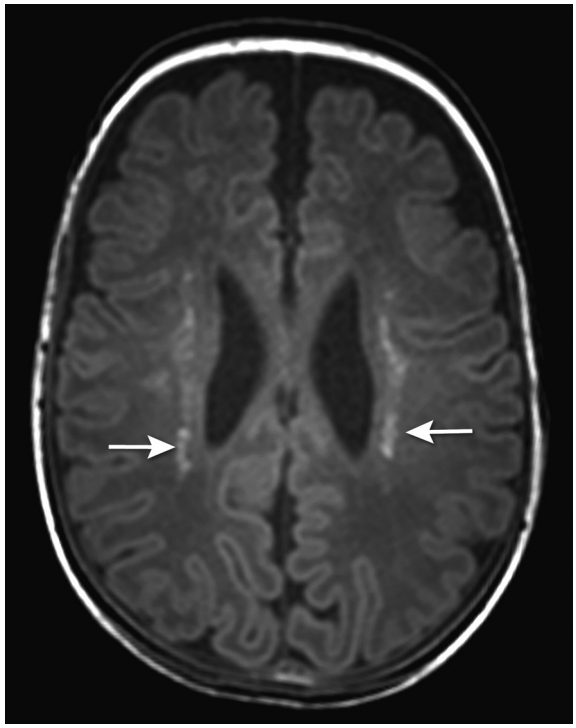
Diffuse excessive high signal intensity

Diffuse excessive high signal intensity (DEHSI) (Figure 5) on conventional T2-weighted (w) MRI has been described in the periventricular white matter in premature infants and is seen in the majority of these infants.[39,40,41] For a long time, it was thought to represent diffuse white matter injury on account of altered apparent diffusion coefficient (ADC) and fractional anisotropy (FA) values compared with normal term born neonates.[31] However, this has recently been questioned by several authors.[41,42,43] It is now assumed that DEHSI represents a developmental phenomenon rather than white matter injury, because of its high incidence and the lack of association with short-term neurodevelopmental outcome.[41,44,45] So far, no histological equivalent of DEHSI has been found.

Punctate white matter lesions

Focal small punctate white matter lesions (PWML) (Figure 6) have been described as small areas with high signal intensity on T1-w MRI images and a less pronounced low signal intensity on T2-w MRI images.[46,47] These lesions can be differentiated from small

Figure 6. PWML (arrows) located in the deep white matter around the lateral ventricles are thought to represent more focal white matter injury.



hemorrhages by using gradient echo MRI techniques, which are susceptible to hemorrhages and blood break down products such as hemosiderin.[48]

PWML are thought to represent more focal white matter injury. There is no known histological correlate and the pathogenesis is not completely understood, although they may be the MR equivalent of astrogliosis.[40] Some PWML are hemorrhagic. If so, these lesions probably occur due to increased pressure in the medullary veins draining towards the ventricles, and represent small hemorrhagic venous infarctions.[48] In the acute phase, some of these lesions show diffusion restriction on diffusion weighted imaging (DWI) sequences compatible with small venous infarcts. In the perinatal period when these lesions occur, they can easily be missed on CUS.

Since PWML occur during the perinatal period and tend to fade and decrease in number over time, it is likely that the exact incidence of these lesions is underestimated at term equivalent age, the preferred age of MR imaging for most preterm infants to investigate the extent of white matter injury.[40] These focal PWML are associated with a poorer neurodevelopmental outcome.[41,46]

Neuro-imaging modalities used to depict white matter injury

Cranial Ultrasonography (CUS)

Serial CUS is very reliable for the detection of peri- and intraventricular hemorrhage and its complications (post-hemorrhagic ventricular dilatation and periventricular hemorrhagic infarction).[3,49] In addition, it is used to evaluate ventricular size, and the status of the basal ganglia and the white matter in very preterm neonates during the perinatal period.[16] Recent studies have shown that ultrasonography can reliably detect severe (cystic) white matter injury, but it is less reliable for the detection of mild or moderate white matter abnormalities.[50,51] Moreover, it has been shown that PVE of the white matter on ultrasonography can predict abnormal white matter on MRI at term equivalent age, but absence of PVE did not predict absence of white matter changes. Germinal matrix and intraventricular hemorrhages, on the other hand, were predictive of abnormal white matter on MRI and together with abnormal ventricular size or shape, were reasonably predictive of unfavorable outcome.[35]

Optimization of CUS to increase its accuracy and reliability, has been extensively described by our group.[16,49,52] However, even while using optimal protocols and a modern ultrasound system operated by an experienced ultrasonographist, CUS seems to underestimate diffuse white matter injury. As 25 - 50% of very preterm infants with diffuse white matter injury develop cognitive problems,[2] this may prompt the use of MRI around term equivalent age in these infants.[35]

Magnetic resonance imaging

MRI is becoming more widely available and increasingly important for neonatal brain imaging. It is safe and reliable, but poses challenges regarding patient preparation, safety and sequence optimization in neonates.[19] Compared to ultrasonography, it has the disadvantage of the necessity to transport the neonate from the neonatal intensive care unit to the radiology department. The development of MRI compatible incubators has largely overcome this disadvantage as patient preparation can now be performed in the neonatal intensive care unit and after transportation, the entire incubator can be placed into the MR scanner.[9]

In our hospital, all neonatal MRI examinations are performed using a 3T MRI system (Philips Medical Systems, Best, the Netherlands) according to a standard protocol for imaging the newborn infant's brain.[19] The infants are sedated using chloral hydrate (55mg/kg), lay supine and are swaddled during the scanning procedure. Ear protection consists of neonatal earmuffs (Natus Mini Muffs; Natus Medical Inc., San Carlos, CA, USA) covered by a headphone. All MRI examinations include a 3D T1-Turbo Field Echo

sequence (TR 9.7 ms, TE 4.6 ms, FOV 180 mm, matrix size 192x152, flip angle 8º, TFE factor 128, slice thickness 1 mm), a T2-Turbo Spin Echo sequence (TR 6269 ms, TE 120 ms, FOV 180 mm, matrix size 336x234, TSE factor 18, slice thickness 2 mm), a T2* Fast Field Echo sequence (TR 735 ms, TE 16 ms, FOV 230 mm, matrix size 256x163, flip angle 18º, slice thickness 4 mm) and a DWI sequence (SE-EPI in 3 directions, *b*-value of 1000 s/mm^2, TR 2406 ms, TE 64 ms, EPI factor 37, FOV 180 mm, matrix size 96x69, slice thickness 4 mm).

Frequently used MRI techniques

Most MRI sequences are performed to assess the development or injury of the brain in preterm infants. Specifically myelination can be assessed on T1-w and T2-w sequences. MRI can easily detect germinal matrix/intraventricular hemorrhages, periventricular hemorrhagic infarctions, cystic white matter lesions and PWML using T1-w, T2-w, T2*-w gradient echo and/or DWI sequences. White matter volume loss, resulting in increased pericerebral spaces, ventricular dilatation and thinning of the corpus callosum, can reliably be evaluated on T1-w and T2-w sequences. The grey matter volume loss, resulting from white matter injury can be recognized as a less complicated gyral pattern and lower volumes of the basal ganglia and/or thalami.

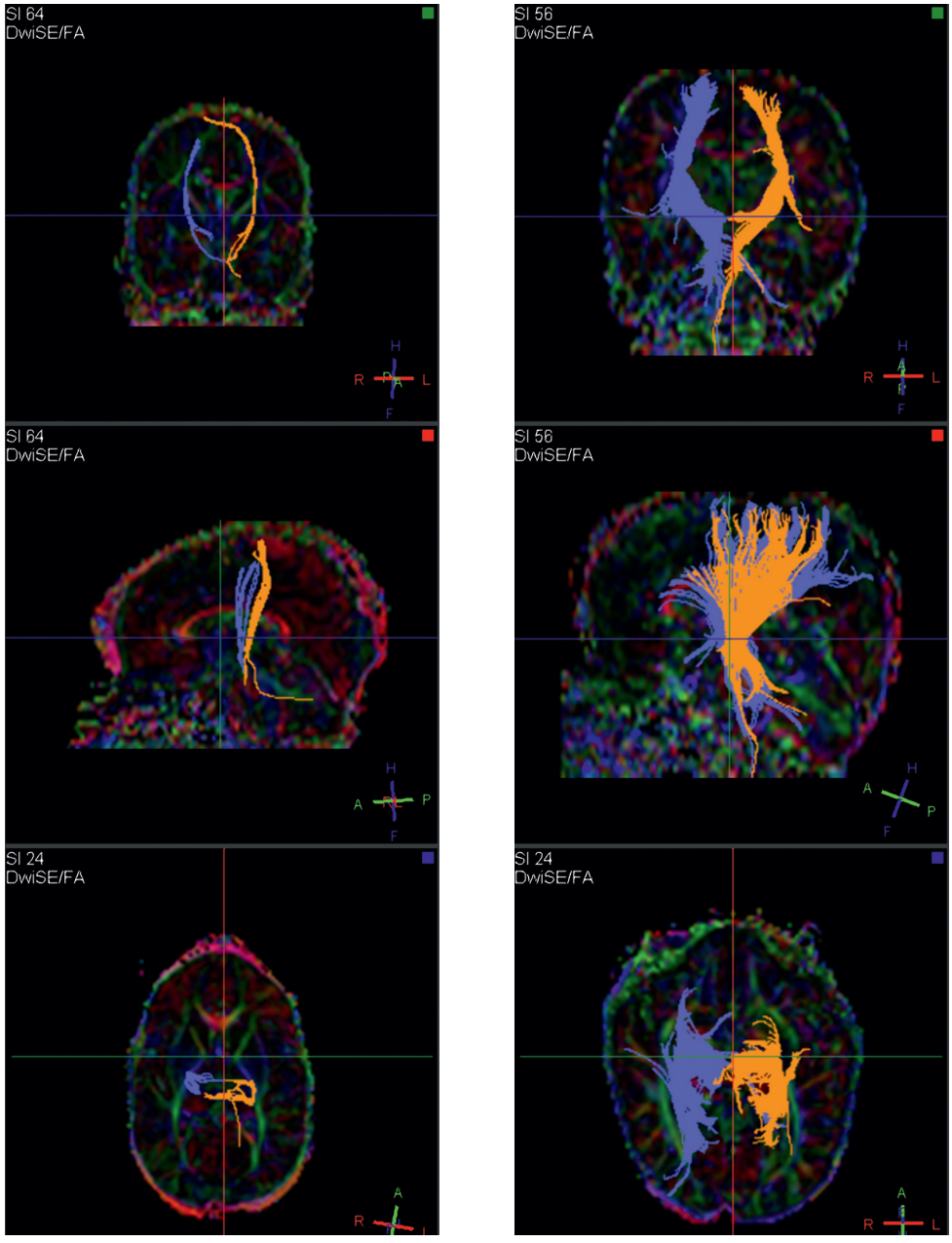
MRI obtained at term equivalent age in preterm infants has predictive significance, as parenchymal lesions such as hemorrhages, changes consistent with white matter injury, infarctions, hypomyelination and reduction of white matter volumes have been shown to be predictive of cognitive and motor delay and cerebral palsy at two years of age.[41,44,53] The combination of these different parenchymal lesions adds up to predict an adverse outcome in most preterm infants with severe white matter lesions, but prognosis is less certain in infants with mild or moderate white matter lesions, which occurs in the majority.[54]

Advanced MRI techniques

DTI has been proposed as an additional tool in the assessment of white matter injury in preterm infants and may provide more adequate diagnostic and predictive information in relation to neurological outcome than other MR techniques.[55,56] DTI describes the diffusion of water molecules in tissues and reflects the direction of the underlying microstructure. In DTI, diffusion is measured in at least six diffusional directions, while in DWI, diffusion is measured in only three perpendicular directions. Contrast is based on the Brownian motion of water molecules, which is influenced by various factors including fibre orientation, integrity of the cell membranes and the degree of myelination. DTI can be used to assess cerebral development and connectivity by calculating diffusivity values.[55,57]

Figure 7. (left) Fibers passing through the posterior limb of the internal capsule in a preterm infant imaged at a postmenstrual age of 40 weeks.

Figure 8. (right) Preterm infant imaged at a postmenstrual age of 62 weeks shows an increase in length and number of fibers passing through the posterior limb of the internal capsule as a result of brain maturation and development.



The physical constant characterizing water molecule displacement is called the apparent diffusion coefficient (ADC) or mean diffusivity (MD). In very preterm infants the ADC of the white matter is high due to the high water content of the immature brain. When the brain further matures the ADC will decrease.[55] ADC values may be abnormal in infants with brain injury or abnormal brain development.[58]

While the axons in the developing brain organise and myelinate the displacement of water molecules, as described by the fractional anisotropy (FA) value, is most restricted in the perpendicular direction and least restricted parallel to the myelinating fibres. The maturation of white matter is accompanied by an increase in anisotropic diffusion and thus in FA.

Fibre tractography offers insight into developing white matter by visualisation of the white matter tracts (Figure 7 and 8).[55,59,60,61,62,63]

Diffusion parameters at term equivalent age have only been scarcely studied in relation to neurodevelopmental outcome and have shown an association between lower FA values in the posterior limb of the internal capsule and higher ADC values in the splenium of the corpus callosum at term, and motor delay around two years of age.[64,65] DTI values at term equivalent age may help further prediction of neurodevelopmental outcome at two years. In combination with clinical parameters and white matter injury seen on T1-w and T2-w MRI, specificity further increases.

Over the last decade, numerous MRI techniques have been proposed to measure brain volumes in the very preterm infant as a measure of brain development and injury. Segmentation techniques for grey matter, unmyelinated and myelinated white matter, and cerebrospinal fluid have been developed.[28,66] However, in daily clinical practice, their use is not feasible and the relation with neurodevelopmental outcome has not been studied extensively. Linear measurements have been developed and validated in the preterm infants' brain and can be applied manually to 2D and 3D datasets.[67]

The utility of MR spectroscopy for risk-stratifying preterm infants in relation to long term adverse outcome is not well established. There are difficulties concerning the use of this technique, such as age related differences in metabolites, as measured by MR spectroscopy in the perinatal and early childhood period.[68] MR spectroscopy has not been found to be a good predictor of outcome in preterm infants at the age of 18 to 24 months.[69]

Future perspectives

Development of brain functions and the structural-functional correlates of brain injury remain difficult to evaluate in preterm infants. MRI at term equivalent age better depicts diffuse white matter injury in very preterm infants than ultrasonography. Combined grading of white matter injury and advanced (quantitative) MRI techniques, such as DTI, help to predict adverse neurodevelopmental outcome.[54] However, most very preterm infants show mild to moderate diffuse white matter injury, and in this group, prediction of outcome remains uncertain. Whole brain statistical methods developed for neonatal DTI analysis, such as optimized tract-based spatial statistics[70] and atlas-based analysis,[71] might have the potential to detect mild to moderate white matter injury related to the neurological outcome. Another quantitative MR technique to evaluate brain development and possibly brain injury in preterm infants is magnetization transfer imaging, which can be used to evaluate myelination.[72] Magnetization transfer is a MR imaging phenomenon based on the interaction between immobile protons in macromolecules and free water protons of tissue. A magnetization transfer ratio is obtained by calculating the percentage difference between two images, one with and one without an off-resonance radio frequency pulse.[73] Magnetization transfer ratio provides a reproducible measurement sensitive to myelination and thus an index to brain maturation.[74]

Functional resting state MRI may be a new non invasive technique to assist evaluating early life brain function and its recovery from injury.[75,76] This technique is based on data analysis applied to functional MRI, revealing patterns of interconnections between neural networks. Resting state networks have been identified in preterm infants.[77,78,79] Additional research is necessary to determine the clinical utility of resting state functional connectivity analyses and the potential for the method to reveal the anatomical substrate for cognitive deficits in preterm infants who do not appear to have abnormalities on other imaging techniques.[68]

Conclusion

Long term clinical follow up remains necessary to further evaluate the predictive values of certain neuro-imaging findings and quantitative values around term equivalent age, especially for cognitive neurodevelopmental outcome in very preterm neonates.

Advanced techniques, such as DTI, magnetization transfer imaging, functional

resting state MRI and volumetric methods are still under active investigation. Serial MRI and the application of these newer analysis techniques will provide insights into the trajectories of brain development and the impact of injury on the development.[9]

Executive summary

White matter injury

- White matter injury occurs frequently in very preterm neonates.
- White matter injury seems to be one of the main determinants for a poorer neurodevelopmental outcome in very preterm infants.
- White matter injury results in hypomyelination, underdevelopment of white matter tracts, grey matter and commissures.

Imaging findings of white matter injury on CUS and MRI

- Diffuse white matter injury is a common finding on MRI in preterm infants but is not reliably detected by ultrasonography.
- It is now assumed that DEHSI represents a developmental phenomenon rather than white matter injury, as there is no association with short-term neurodevelopmental outcome.

Advanced MRI techniques

- DTI quantifies development and injury to the white matter.
- Abnormal DTI values around term equivalent age in preterm infants predict psychomotor delay.

Future perspectives

- Long term follow up is necessary to further evaluate the predictive value of MRI findings at term equivalent age for especially cognitive neurodevelopmental outcome.
- Serial MRI and the application of newer analysis techniques will provide further insights into the trajectories of the developing and injured brain in the very preterm infant.

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3

Tractography of developing white matter of the internal capsule and corpus callosum in very preterm infants

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Abstract

Objectives: To investigate in preterm infants associations between Diffusion Tensor Imaging (DTI) parameters of the posterior limb of the internal capsule (PLIC) and corpus callosum (CC) and age, white matter (WM) injury and clinical factors.
Methods: In 84 preterm infants DTI was performed between 40 - 62 weeks postmenstrual age on 3T MR. Fractional anisotropy (FA), apparent diffusion coefficient (ADC) values and fibre lengths through the PLIC and the genu and splenium were determined. WM injury was categorised as normal/mildly, moderately and severely abnormal. Associations between DTI parameters and age, WM injury and clinical factors were analysed.
Results: A positive association existed between FA and age at imaging for fibres through the PLIC ($r=0.48$ $p<0.001$) and splenium ($r=0.24$ $p<0.01$). A negative association existed between ADC and age at imaging for fibres through the PLIC ($r= -0.65$ $p<0.001$), splenium ($r= -0.35$ $p<0.001$) and genu ($r= -0.53$ $p<0.001$). No association was found between DTI parameters and gestational age, degree of WM injury or categorical clinical factors.
Conclusions: These results indicate that in our cohort of very preterm infants, at this young age, the development of the PLIC and CC is ongoing and independent of the degree of prematurity or WM injury.

Introduction

Infants born at a gestational age (GA) below 32 weeks are prone to diffuse white matter (WM) injury.[1,2] This may eventually result in damage, underdevelopment and atrophy of the internal capsule and the corpus callosum (CC).[3-7] Conventional MRI techniques detect WM injury and depict features of brain maturation.[2,8,11-15] Diffusion Tensor Imaging (DTI) has been proposed as an additional tool in the assessment of WM injury and may provide more adequate diagnostic and prognostic information in relation to neurological outcome than conventional MR imaging.[8-13] DTI enables quantitative assessment of maturation, tract organisation and injury by calculating fractional anisotropy (FA) and Apparent Diffusion Coefficient (ADC) values. Fibre tractography offers insight into developing WM by visualisation of the WM tracts.[9-11,14-17] The non-invasive nature of DTI permits longitudinal and diagnostic clinical studies.[18-21,26-29]

Until now, the clinical use of DTI fibre tractography in very preterm infants (VPTI) has been restricted by the lack of normal reference values for FA and ADC.[8] Only a few studies have used DTI in VPTI with a GA below 32 weeks.[8,10,15-17] In most of these studies MRI was performed before term equivalent age (TEA). Little is known about the changes in FA or ADC in the developing brain around TEA.[10,22-25] Data on the influence of diffuse WM injury on tract maturation and organisation around TEA are scarce.[23,26,27]

We studied DTI parameters (FA and ADC values and lengths of fibres) of WM tracts passing through the posterior limb of the internal capsule (PLIC) and the CC in VPTI undergoing MRI at or within the first months of TEA. The aims of this exploratory study were to establish the association between DTI parameters and:

- GA and postmenstrual age (PMA) at the time of imaging
- The degree of WM injury
- Categorical clinical factors that may influence brain maturation

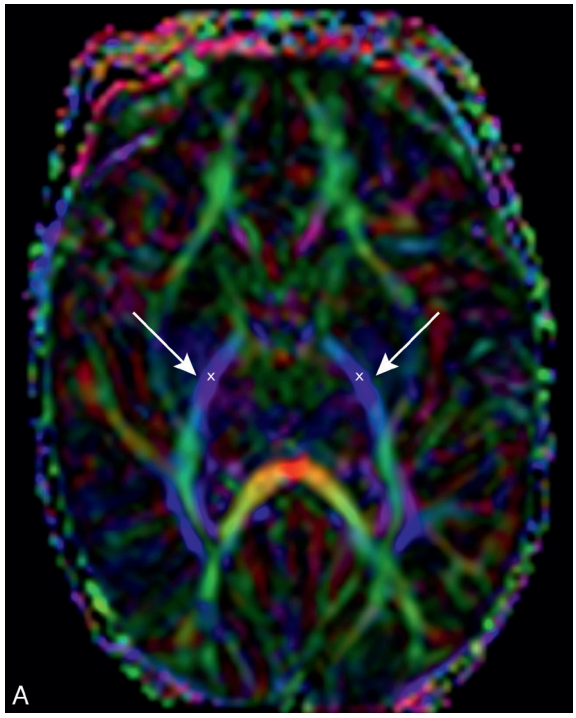
Materials and Methods

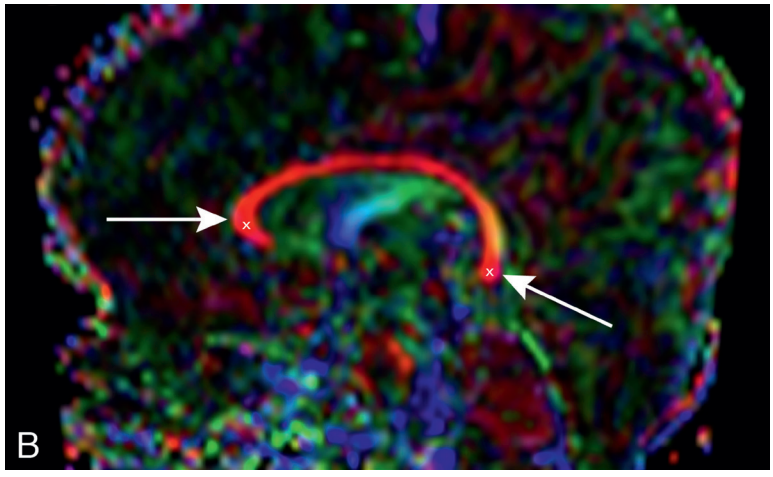
Preterm infants

As part of an ongoing prospective study of VPTI with a GA ≤ 32 weeks, admitted to the neonatal intensive care unit of our institution, 113 infants underwent MRI (3 Tesla) between May 2006 and October 2007.

MRI was preferably performed at TEA (PMA 40 – 44 weeks). For infants who were unstable and/or ventilator dependent around that age, MRI was postponed (PMA range 40 – 62 weeks). Ethical approval for the study was given by the institutional review

Figure 1 A-B. (A) Axial colour-coded DTI map with right (→) and left (←) PLIC in blue and (B) sagittal colour-coded DTI map with CC in red, the regions of interest of genu (→) and splenium (←) are defined. The "x" marks the place where a single seed point was placed in order to generate the fibre tracts.





board and informed parental consent was obtained for each infant. In 102 infants DTI was performed. Three children with congenital brain abnormalities were excluded. In 15 cases, fibre tractography was not possible due to motion artefacts. In the remaining 84 infants a complete DTI dataset was acquired. Clinical parameters were retrospectively collected from the patient's medical records (Table 1).

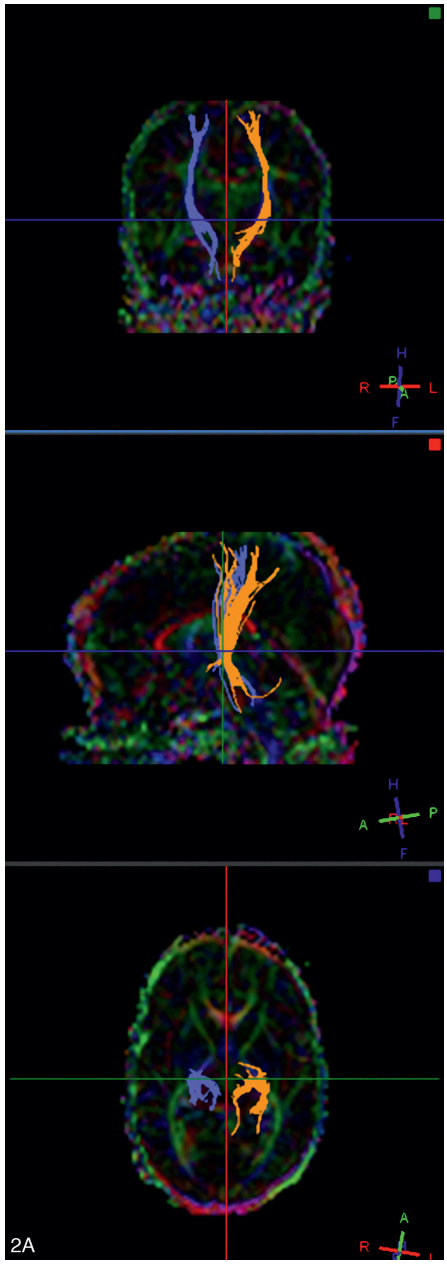
Image and data acquisition

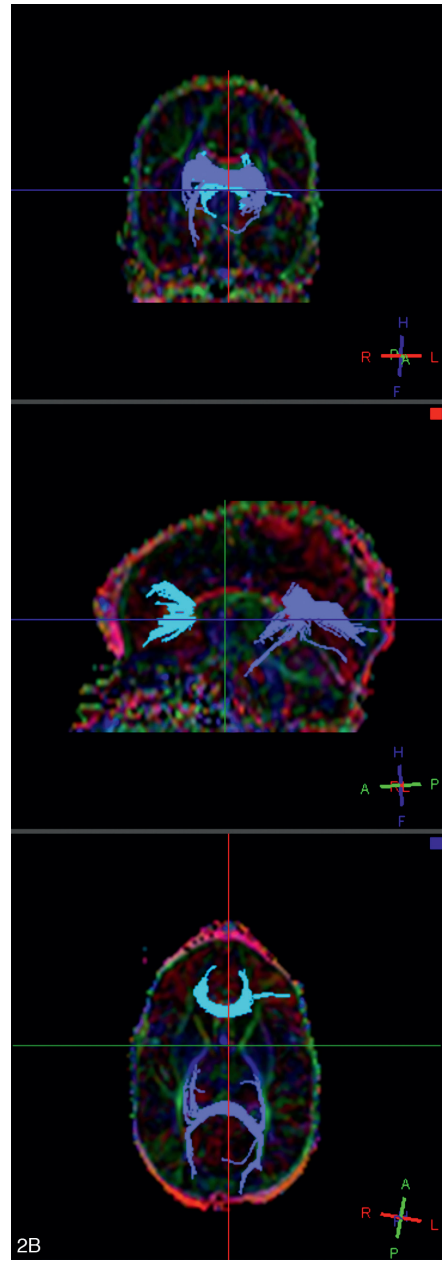
All MRI examinations were performed on a 3 Tesla MR system (Philips Medical Systems, Best, the Netherlands) according to a standard protocol.[28] The infants were sedated using chloral hydrate (55mg/kg), laid supine and snugly swaddled during the imaging procedure. Ear protection consisted of neonatal earmuffs (Natus Mini Muffs; Natus Medical Inc., San Carlos, CA, USA) covered by a headphone. All MRI examinations included a 3D T1-TFE sequence (TR 9.7 ms, TE 4.6 ms, flip angle 8, TFE factor 128, slice thickness 1 mm) and a T2-TSE sequence (TR 6269 ms, TE 120 ms, turbo factor 18, slice thickness 2 mm). In addition a DTI sequence (SE-EPI, TR 7456 ms, TE 54 ms, slice thickness 2 mm, voxel size 1,4 x 1,4 x 2 mm with diffusion acquisitions in 32 directions and a *b*-value of 1000 s/mm2, EPI factor 56) with an imaging time of 5 min and 34 sec was acquired.

Fibre tractography of the PLIC and CC

DTI datasets were analysed on an off-line workstation using commercially available processing software as provided by the manufacturer (FiberTrak, by Philips Medical Systems, Best, the Netherlands). DTI colour-coded maps were automatically computed, red representing a right-left, green an anterior-posterior and blue a superior-inferior orientation. These colour-coded DTI maps were used to place a single seed to perform fibre tracking using an automated 3D seeded algorithm. First, for the PLIC in the axial plane at the level of the lateral ventricles and subsequently for the genu and splenium of the CC in the mid-sagittal plane regions of interest were defined. The seed was placed as is shown in Fig. 1. Position changes of the seed- point in the structure of interest resulted in identical fibre tracts, indicating the robustness of the method used. Two investigators (FTdB and AvS, blinded to subject's age and degree of WM injury) identified the regions of interest and manually placed the individual seeds in consensus for each region of interest. Subsequently, fibre tracts were generated through the PLIC and the callosal genu and splenium resulting in visualisation of fibres (Fig.2). The quality of all tracts was visually assessed in order to minimise erroneous pathways, which were erased and not used in further analyses. In all analyses, default settings were used consisting of a minimum FA of 0.15, a maximum angle change of 27.0 degrees and a minimum fibre length

Figure 2 A-B. Fibre tracts in an infant undergoing imaging at PMA of 41 weeks (A) through the PLIC and (B) through the genu and splenium of the CC using an automated 3D seeded technique according to default settings (FiberTrak, Philips Medical Systems, Best the Netherlands; minimum FA 0.15, maximum angle change 27.0 degrees, minimum fibre length 10.0 mm).





of 10.0 mm. We used the manufacturer default settings, since (small) changes of these settings, to optimize the performance of the fibre tracking, did not have any influence on the fibres picked by the tracking routine. Finally FA and ADC values of these fibre bundles were obtained and the length of the fibres was calculated.

WM injury

All T1-w and T2-w sequences were analysed by two investigators (FTdB, paediatric neuroradiologist with more than 15 years of experience, LML, with more than 4 years' experience) by consensus to assess WM injury. We categorised the WM injury into.[29]

- Normal/mildly abnormal WM: normal appearing WM or homogeneous diffuse and excessive high signal intensity (DEHSI) as seen on T2-w images, or few (≤6) punctate white matter lesions (PWML)
- Moderately abnormal WM: multiple (>6) PWML and/or small localised cystic lesions and/or heterogeneous DEHSI
- Severely abnormal WM: extensive or diffuse heterogeneous signal intensity changes and/or haemorrhagic or cystic lesions involving the periventricular and/or subcortical WM

Accordingly, 18 infants were classified as having normal/mildly, 51 infants moderately and 15 infants severely abnormal WM (Table 1).

Statistical analysis

Data were analysed using SPSS 16.0.2 for Windows. Frequency counts and percentages were used to summarise categorical variables. For continuous variables, the mean, standard deviation of the mean and range are reported (Table 1).

Differences between DTI values of left and right PLIC and between the genu and splenium of the CC were determined by using Wilcoxon signed rank tests for related samples.

To establish the association between DTI parameters and age, weight and head circumference (HC) Spearman Rank correlation coefficients were calculated.

Stepwise linear regression analysis was used to assess the relative importance of the various study population characteristics that were associated with the DTI parameters through the PLIC and CC. Applying this model we corrected all other parameters for PMA at MRI and HC. Variables, for which the p value by univariate analyses tests was ≤ 0.1, were included in the subsequent linear stepwise regression analysis.

To determine the association between DTI parameters and WM injury and categorical clinical factors, Mann-Whitney U analyses or Kruskal-Wallis tests were performed where appropriate. One-way ANOVA with post-hoc Scheffe tests were used to compare

Table 1. Distribution of categorical clinical factors, white matter injury and continuous clinical parameters in the study population (n=84).

Categorical clinical factors	**Number (N)**	**Percentage (%)**
Male	55	65.5
Female	29	34.5
Plurality	23	27.4
IUGR	10	11.9
Antenatal corticosteroids	43	51.2
Perinatal infection	30	35.7
RDS	44	52.4
PDA	23	27.4
Hypotension	29	34.5
BPD	41	48.8
Use of dexamethasone for BPD	11	13.1
Degree of WM injury		
Normal/mildly abnormal WM	18	21.4
Moderately abnormal WM	51	60.7
Severely abnormal WM	15	17.8
Continuous clinical parameters	*Mean (SD)*	*Range*
GA (weeks)	29.0 (2.0)	25.6-31.9
Birth weight (g)	1199 (365)	585-1960
Head circumference at birth (cm)	25.8 (4.0)	22.0-31.2
PMA at MRI (weeks)	45.0 (4.2)	40.0-62.1
Weight at MRI (gram)	4002 (867)	2010-7005
Head circumference at MRI (cm)	37.7 (1.8)	32.7-41.5

IUGR= intrauterine growth retardation, RDS=respiratory distress syndrome, PDA=patent ductus arteriosus, BPD=bronchopulmonary dysplasia, WM=white matter, GA=gestational age, PMA=postmenstrual age

DTI parameters between groups categorised as normal/mildly, moderately or severely abnormal WM.

A p value ≤ 0.05 was considered statistically significant for all analyses.

RESULTS

General characteristics of the study population

Clinical characteristics for all 84 infants are shown in Table 1.

Table 2. Average FA, ADC values and lengths of fibres through the PLIC (posterior limb internal capsule) and genu and splenium of the CC (corpus callosum) (n=84).

	Mean (SD)	**Range**
PLIC		
FA	0.37 (0.02)	0.34-0.43
ADC (10^{-3} mm^2/s)	1.06 (0.05)	0.95-1.23
Length (mm)	59.8 (8.2)	38.5-80.6
CC genu		
FA	0.37 (0.04) ***	0.31-0.55
ADC (10^{-3} mm^2/s)	1.32 (0.10) ***	1.10-1.56
Length (mm)	46.7 (10.1) ***	26.8-79.6
CC splenium		
FA	0.40 (0.04)	0.34-0.51
ADC (10^{-3} mm^2/s)	1.36 (0.11)	1.10-1.69
Length (mm)	59.3 (13.1)	27.5-80.2

FA=fractional anisotropy, ADC=apparent diffusion coefficient
***: significant difference ($p<0.001$) between genu and splenium of the CC

Quantitative DTI values

The FA and ADC values and length of fibres (mean, SD and range) through the PLIC and genu and splenium of the CC are shown in Table 2. As we found no statistical differences or any laterality between left and right DTI values in the PLIC, these DTI values were averaged. In the genu lower FA ($p<0.001$), lower ADC values ($p<0.001$) and shorter fibres ($p<0.001$) were found compared with the splenium of the CC.

Association between DTI parameters and continuous clinical variables

Table 3 shows the associations between DTI parameters for the PLIC and genu and splenium and continuous clinical variables. DTI parameters in the PLIC ($p<0.001$) and CC ($p<0.001$ and $p<0.01$) correlated strongly with PMA at MRI, except for the FA in the genu of the CC. The FA ($p<0.01$), ADC ($p<0.001$) and fibre length ($p<0.01$) through the PLIC and fibre length through the genu and splenium of the CC ($p<0.001$, $p<0.01$ and $p<0.001$, $p<0.001$ respectively) correlated with weight and HC at MRI. No or only weak associations were found between FA, ADC and fibre length through the PLIC or CC and GA or birth weight.

Because of the wide age range, we re-analysed data for a subgroup of infants (69/84)

Table 3. Spearman Rank correlation coefficients between DTI parameters for PLIC and CC and age, weight and head circumference at birth and MRI.

	GA	Birth weight	HC at birth	PMA at MRI	Weight at MRI	HC at MRI
PLIC						
FA	0.02	-0.07	-0.02	0.48 ***	0.30 **	0.29 **
ADC	0.06	0.12	0.05	-0.65 ***	-0.48 ***	-0.42 ***
Length	-0.13	-0.09	-0.03	0.42 ***	0.30 **	0.34 **
CC genu						
FA	-0.03	-0.15	-0.35 **	0.17	0.07	0.10
ADC	0.13	0.23 *	0.20	-0.53 ***	-0.22 *	-0.14
Length	-0.14	-0.10	-0.04	0.46 ***	0.35 ***	0.34 **
CC splenium						
FA	-0.001	-0.08	-0.16	0.24 **	0.17	0.10
ADC	-0.06	0.05	-0.02	-0.35 ***	-0.19	-0.21
Length	0.09	0.05	0.33 *	0.43 ***	0.42 ***	0.52 ***

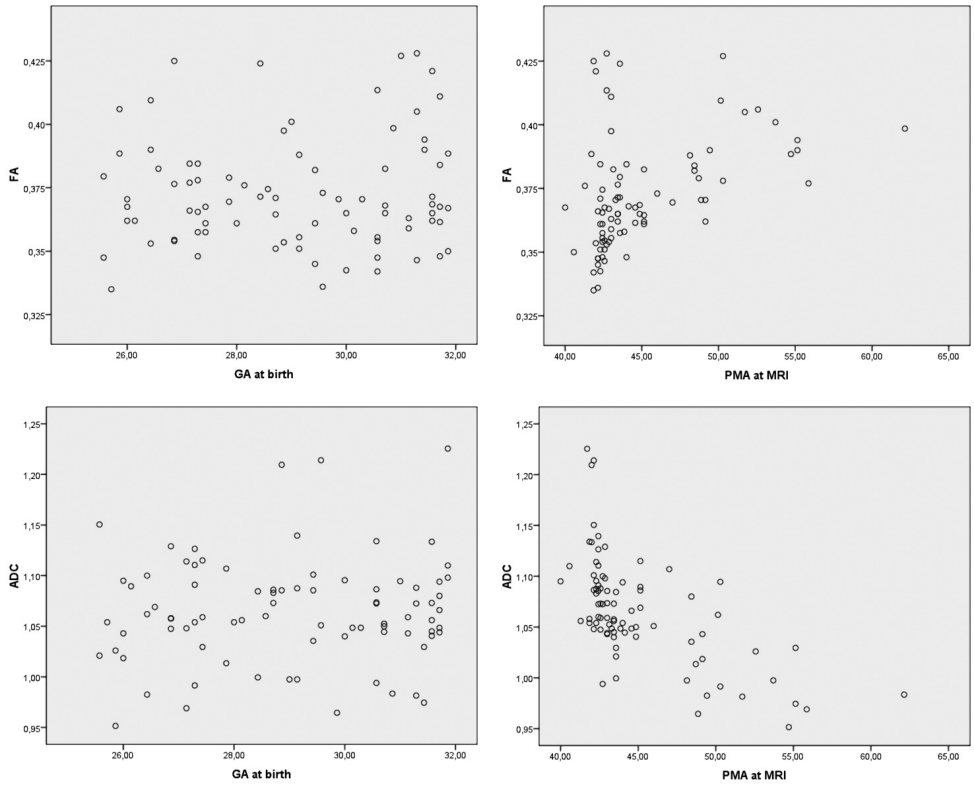
HC= head circumference, GA=gestational age, PMA=postmenstrual age,
* significant association: $p<0.05$, ** significant association: $p<0.01$, *** significant association: $p<0.001$

who underwent imaging between 40 – 46 weeks PMA. For this subgroup, we still found a significant association between FA ($r=0.252$ $p<0.05$), ADC ($r=-0.470$ $p<0.01$) and fibre length in the PLIC ($r=0.410$ $p<0.01$) and PMA at MRI. The association between FA, ADC values or fibre length in the PLIC and weight and HC at MRI no longer existed. For the genu, no association between FA and any continuous clinical variable was found, while the association between ADC ($r=-0.268$ $p<0.05$) or fibre length ($r=0.402$ $p<0.01$) and PMA at MRI remained. For the splenium we found an association between fibre length and weight and HC at MRI (respectively $r=0.337$ $p<0.01$and $r=0.332$ $p<0.05$). The association with PMA at MRI was no longer present, still the association between fibre length and HC at birth remained ($r=0.417$ $p<0.05$).

Figure 3 shows the individual data depicting the association between the FA and ADC of fibres through the PLIC and GA as well as PMA at MRI. This Figure illustrates the association between FA and ADC values with PMA at imaging, whereas no such association exists with GA.

Results of the stepwise regression analyses are shown in Table 4, delineating the strongest contributing factor of clinical parameters. In all but three cases, PMA at MRI was the strongest contributing factor to most of the DTI parameters (FA and ADC of the fibres through the PLIC, ADC and fibre length of the fibres through the genu and sple-

Figure 3. Scatter plots of the individual data of the association between the FA and ADC of fibres through the PLIC and GA and PMA at MRI, showing no association with GA, a positive association between FA and PMA and a negative association between ADC and PMA.



nium, and FA of the splenium). For two of the remaining three parameters (FA in the fibres through the genu and fibre length in the fibres through the splenium) HC at birth contributed most. For the length of fibres through the PLIC, the HC at MRI contributed most.

Association between DTI parameters and WM injury and categorical clinical factors

We found no association between DTI parameters for the PLIC or CC and degree of WM injury. When comparing the groups with different degrees of WM injury, we also found no significant differences in DTI parameters (Table 5). These results remained, even after correction for PMA at MRI.

No associations existed between DTI parameters and categorical clinical factors.

Table 4. Stepwise linear regression models for DTI parameters (PLIC and CC), including the strongest contributing factors.

	R	p	Predictors	β
PLIC				
FA	0.51	<0.001	PMA at MRI	0.002
ADC	0.63	<0.001	PMA at MRI	-0.008
Length	0.31	<0.01	HC at MRI	1.27
CC genu				
FA	0.37	0.02	HC at birth	-0.008
ADC	0.55	<0.001	PMA at MRI	-0.013
Length	0.33	<0.01	PMA at MRI	0.80
CC splenium				
FA	0.23	0.04	PMA at MRI	0.002
ADC	0.41	<0.001	PMA at MRI	-0.01
Length	0.69	<0.001	PMA at MRI	1.52
			HC at birth	3.02

PMA=postmenstrual age, HC=head circumference

Table 5. DTI parameters for PLIC and CC and categorised WM injury (n=84).

	Normal-mild (n=18)	**Moderate (n=51)**	**Severe (n=15)**	**Overall**	**Normal-Mild vs. Severe**	**Normal-Mild vs. Moderate**	**Moderate vs. Severe**
	Mean(SD)			p value			
PLIC							
FA	0.38 (0.02)	0.37(0.02)	0.37 (0.03)	0.27	1.00	0.34	1.00
ADC	1.05 (0.05)	1.07 (0.05)	1.07 (0.04)	0.64	1.00	0.73	1.00
length	59.4 (8.0)	60.0 (7.0)	59.6 (11.9)	0.96	1.00	0.76	1.00
CC genu							
FA	0.38 (0.05)	0.37 (0.04)	0.38 (0.04)	0.58	1.00	1.00	1.00
ADC	1.30 (0.11)	1.32 (0.09)	1.33 (0.10)	0.65	1.00	1.00	1.00
Length	45.6 (11.1)	47.4 (9.6)	45.6 (11.1)	0.73	1.00	1.00	1.00
CCsplenium							
FA	0.40 (0.04)	0.39 (0.03)	0.41 (0.03)	0.54	1.00	1.00	0.83
ADC	1.32 (0.09)	1.36 (0.12)	1.39 (0.11)	0.16	0.17	0.60	0.84
length	62.7 (12.2)	59.3 (12.7)	55.2 (15.2)	0.27	0.31	1.00	0.84

PLIC=posterior limb of the internal capsule, CC=corpus callosum, WM=white matter.
WM injury was categorised: normal/mildly abnormal, moderately abnormal and severely abnormal.

Discussion

In this study, we determined FA and ADC values and the length of WM fibres passing through the PLIC and CC in a cohort of 84 VPTI imaged between 40 and 62 weeks PMA. We assessed the association between DTI parameters and age, diffuse WM injury and clinical factors.

All DTI parameters of the PLIC and most DTI parameters of the CC were strongly associated with PMA at imaging. We found no associations between DTI parameters and GA, the degree of WM injury or any categorical clinical factor.

In a previous study by Dudink *et al*. of very low birth weight infants undergoing imaging within 4 days of birth an association was found between FA of the PLIC and GA. They found no association between ADC values and GA.[8] Although we did not find an association between FA or ADC of the PLIC and GA, we found a positive association between FA and PMA at MRI and a negative association between ADC and PMA at MRI. Increase in FA and decrease in ADC have been described before and reflect the (pre) myelination and parallel organisation of fibres, such as expected in the development of the PLIC.[9,15,16] As we found no association with GA, the maturation of the PLIC seems to progress with age, independent of the degree of prematurity. Similar to our findings in the PLIC, no associations were found between DTI parameters of fibres passing through the CC and GA, while DTI parameters of the CC showed a significant association with PMA at MRI, except for the FA values of the genu. This indicates ongoing maturation of the splenium of the CC around TEA, again independent of GA, while for the genu this seems less clear. These findings are in agreement with the results of Partridge *et al*. who found no significant maturational trends of the genu of the CC in their serial study of 14 premature neonates with a GA between 25 and 34 weeks, of whom eight underwent a second MRI around TEA.[16]

We found no associations between DTI values for the PLIC or CC and the degree of WM injury. These findings are only partly in agreement with the results of Hüppi *et al*., who at TEA found no difference in ADC values of WM between preterm infants with and without WM lesions. They found a 20% lower regional anisotropy in fibres descending from the internal capsule in children with WM injury compared with those without.[10] Our data do not confirm this latter finding. This may be explained by the fact that we studied a prospective cohort of unselected VPTI, with relatively small numbers of children with normal or severely abnormal WM. Hüppi *et al*. selected ten infants with WM injury and matched these with ten infants of the same GA and neonatal course but with normal MRI. Our results also differ from those of Counsell *et al*., who demonstrated in preterm infants who underwent imaging around TEA (38.86 – 43.86 weeks),

that radial diffusivity in the PLIC and the splenium of the CC and axial and radial diffusivity in the WM were significantly elevated in infants with DEHSI compared with those with normal appearing WM and term control infants.[25] Counsell *et al*. randomly recruited 38 preterm infants and compared these with 8 healthy term born controls. The preterm infants were divided into a group with normal appearing WM and a group with DEHSI. Infants with overt WM injury were excluded from their study. The difference with our results may again be explained by the fact that we studied a group of unselected VPTI. It may also be hypothesised that the wide age range at imaging in our study group plays a role. However, as the results in the subgroup of infants undergoing imaging before 46 weeks were comparable to those in the whole group, we feel this will not have influenced our results. Possibly the unequal distribution of infants with normal/mildly, moderately or severely abnormal WM has played a more important role. However, when comparing the 18 subjects with normal/mildly abnormal WM with the 15 with severe WM injury we found no difference between these two groups. In a recent study Cheong *et al*.[27] found a difference in FA in the PLIC in VPTI with extensive WM injury compared with VPTI with normal or focal WM injury using DTI region of interest measurements. In their study 39 infants had no WM signal intensity abnormalities, 59 infants had focal and 13 infants extensive WM abnormalities. The categorisation of WM injury we used is very similar to that of Cheong *et al*., but they included a large number of infants with normal appearing WM whereas that group was relatively small in our study.

Furthermore, in accordance with the study of Counsell *et al*.[25] we did not identify any categorical clinical factors associated with DTI parameters.

For the CC we found significantly different DTI values between the genu and splenium. In the genu FA and ADC values were significantly lower, indicating that at this young age the genu is less developed than the splenium. In terms of development similar findings have been reported by Barkovich, who pointed out that the myelination of the splenium precedes that of the genu on conventional MRI.[30]

One of the main limitations of our study is that, due to clinical circumstances not all VPTI were imaged around TEA. Therefore, although most were imaged before 46 weeks PMA (69/84), a wide age range existed. On the other hand, this enabled us to study the relation between PMA and DTI values. Another limitation of the present study is that we did not perform DTI in normal term infants. Therefore we were unable to compare the DTI values of the VPTI with those of a healthy control group.

We performed fibre tractography with commercially available software and with given default settings. Still, one of the limitations of fibre tracking is that in other studies different parameters or different tracking programs may be used, complicating the di-

rect comparison of fibre tracking values between studies. Finally, as we performed fibre tractography of fibres passing through PLIC and CC, we only calculated DTI values in the WM tracts passing through these structures. We may therefore have missed a possible association between DTI parameters and either normal or injured WM.

In conclusion, our data show in VPTI imaged around or within 5 months of TEA, a strong association between DTI parameters of the PLIC and CC and PMA at imaging, but not between these values and GA. No association was found between DTI parameters of the PLIC and CC and the degree of WM injury or categorical clinical factors.

Although it has been reported that WM injury may result in atrophy of the PLIC and CC later on in life,[3-7] our results indicate that in our cohort of VPTI, at this young age, the development of the PLIC and CC is ongoing and independent of the degree of prematurity or diffuse WM injury.

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4

Tractography of white matter tracts in very preterm infants; a two-year follow up study

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Abstract

Aim: The aim of this study was to determine whether tractography of white-matter tracts can independently predict neurodevelopmental outcome in very preterm infants.
Method: Out of 84 very preterm infants, 64 [41 (64%) male, median gestational age 29.1 (25.6-31.9) weeks, birth weight 1163 (585-1960) grams] underwent follow up at 2 years. DTI values obtained around term were associated with a neurological examination and a mental and psychomotor developmental index score at 2 years based on the Bayley Scales (BSID-III). Univariate and logistic regression analyses tested for associations between DTI values and follow up parameters. Cut-off values predicting motor delay and cerebral palsy were determined for fractional anisotropy (FA), apparent diffusion coefficient (ADC) and fibre lengths.
Results: Infants with psychomotor delay and cerebral palsy had significantly lower FA values ($p=0.002$, $p=0.04$) and shorter fibre lengths ($p=0.02$, $p=0.02$) of the posterior limb of the internal capsule (PLIC). Infants with psychomotor delay also had significantly higher ADC values ($p=0.03$) and shorter fibre lengths ($p=0.002$) of the callosal splenium. FA values of the PLIC independently predicted motor delay and cerebral palsy with sensitivity between 100 - 80% and specificity 69 - 66%. ADC values of the splenium independently predicted motor delay with sensitivity 100% and specificity 65%.
Interpretation: DTI tractography at term equivalent age independently predicts psychomotor delay at 2 years of age in preterm infants.

Introduction

White matter injury is a common finding on MR imaging performed around term equivalent age in very preterm infants.[1-5] White matter injury may eventually result in damage, underdevelopment and atrophy of the internal capsule and the corpus callosum.[6-9] Recently it has been demonstrated that fibre tractography offers additional insight in the developmental status of the white matter by visualisation and characterization of the white matter tracts.[10-15] Moreover, diffusion tensor imaging (DTI) has been proposed as an additional tool to provide more adequate prognostic information, around term equivalent age in relation to cognitive and psychomotor neurodevelopmental outcome than conventional MR imaging in preterm and low birth weight infants.[16-20]

Although these studies clearly indicate the potential of DTI to predict neurodevelopmental damage at a later age, its independent value is not well established; well-known individual predictors of neurodevelopmental outcome such as gestational age, gender, perinatal infections, oxygen and mechanical ventilation dependency, intrauterine growth retardation, and white matter injury and ventricular dilatation on MR imaging, were not taken into consideration in most studies. Previously, we have demonstrated that in very preterm infants around term equivalent age, especially the white matter tracts passing through the posterior limb of the internal capsule (PLIC) or through the corpus callosum are associated with the developmental status at term equivalent age, independent of the degree of prematurity and the classification of white matter injury.[13] The aim of the present study was to establish the independent predictive value of DTI tractography performed around term equivalent age, especially of fibres passing through the PLIC and corpus callosum, on neurodevelopmental outcome at the age of 2 years.

Method

Preterm infants

As part of a continuing prospective neuro-imaging study of very preterm infants (gestational age <32 weeks), admitted to the neonatal intensive care unit of the Leiden University Medical Centre between May 2006 and October 2007, 113 infants underwent MRI. MRI was performed at a median postmenstrual age of 43.4 weeks, within a range of 40 to 62 weeks. Ethical approval for the study was given by the institutional review board and informed parental consent was obtained for each infant. In 102 infants DTI

was performed. Three infants with congenital brain abnormalities were excluded. In 15 additional infants fibre tractography was not possible due to motion artefacts. In the remaining 84 infants a complete DTI dataset was acquired. Clinical parameters were collected from the patients' files. Baseline characteristics and baseline DTI parameters of the entire cohort have been published previously.[13,21]

Image and data acquisition

All MRI examinations were performed on a 3 Tesla MR system (Philips Medical Systems, Best, the Netherlands) according to a standardized protocol.[22] The infants were sedated using chloral hydrate (55mg/kg), lay supine and were swaddled during the imaging procedure. Ear protection consisted of neonatal earmuffs (Natus Mini Muffs; Natus Medical Inc., San Carlos, CA, USA) covered by a headphone. The MRI examination included a DTI sequence (SE-EPI, TR 7456 ms, TE 54 ms, slice thickness 2 mm, gap 0 mm, voxel size 1,4 x 1,4 x 2 mm with diffusion acquisitions in 32 directions and a *b*-value of 1000 s/mm2, EPI factor 56) with an image time of 5 min and 34 s.

White matter injury and ventricular dilatation

To assess white matter injury, and ventricular dilatation, all T1-weighted (w), T2-w and T2*-w gradient echo sequences were analyzed by two investigators (FTdB and LML or SJS) together by consensus. Presence and location of more than 6 punctate white matter lesions (PWML) and/or cystic and/or hemorrhagic white matter lesions were scored[5] as well as the level of myelination of the PLIC in comparison to the lentiform nucleus. PWML were defined as punctate respectively high and low SI lesions, more pronounced on T1-w than on T2-w images, not visible on T2*-w gradient echo sequences.[23-25] Cystic white matter lesions were appreciated on T1-w and T2-w sequences, whereas hemorrhagic lesions were scored on T2*-w gradient echo sequences. On coronal reconstructions from the three-dimensional T1-w images, the ventricular index was measured as the total width of the lateral ventricles, divided by 2, in analogy to ventricular index measurements on ultrasound. A ventricular index between 12 and 16 mm was considered moderate dilatation and a ventricular index larger than 16 mm was considered severe dilatation.[5]

Fibre tractography of the PLIC and corpus callosum

DTI datasets were analysed on an off-line workstation using commercially available processing software as provided by the manufacturer (FiberTrak, by Philips Medical Systems, Best, the Netherlands). DTI colour-coded maps were automatically computed, red representing a right-left, green an anterior-posterior and blue a superior-inferior orientation. These colour-coded DTI maps were used to place a single seed to perform

fibre tracking using an automated 3D seeded algorithm. First, for the PLIC in the axial plane at the level of the lateral ventricles and subsequently for the genu and splenium of the corpus callosum in the mid-sagittal plane regions of interest were defined. Position changes of the seed-point in the structure of interest resulted in identical fibre tracts, indicating the robustness of the method used. Two investigators (FTdB and AvS, blinded to subject's age and degree of WM injury) identified the regions of interest and manually placed the individual seeds in consensus for each region of interest (Figure 1). Subsequently, fibre tracts were generated through the PLIC and the callosal genu and splenium resulting in visualisation of fibres (Figure 2). The quality of all tracts was visually assessed in order to minimise erroneous pathways, which were erased and not used in further analyses. In all analyses, default settings were used consisting of a minimum FA of 0.15, a maximum angle change of 27.0 degrees and a minimum fibre length of 10.0 mm. We used the manufacturer default settings, because (small) changes of these settings, to optimize the performance of the fibre tracking, did not have any influence on the fibres picked by the tracking routine. Finally FA and ADC values of these fibre bundles were obtained and the length of the fibres was calculated.

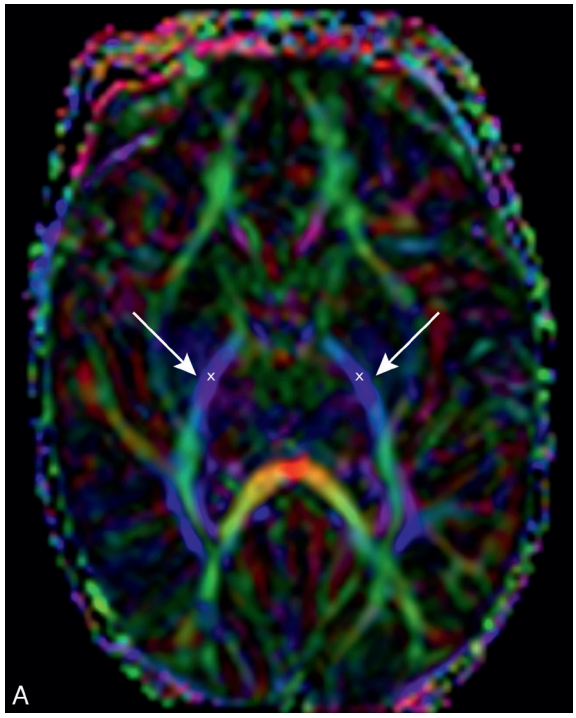
Follow up

Around 2 years corrected age, infants were seen for clinical follow up by an experienced neonatologist, unaware of the MRI findings. Each child underwent a standardized neurologic examination to assess the presence of cerebral palsy or abnormal muscular tone. In all infants a Gross Motor Function Classification System (GMFCS) level was assigned.[26] A GMFCS level of more than 2 was considered cerebral palsy.

Cognitive and psychomotor development was assessed using the Dutch version of the Bayley Scales of Infant Development (BSID-III). A mental developmental index score (MDI) and a psychomotor developmental index score (PDI) were calculated for the corrected age of the child. A score of 1 standard deviation or more below the normative mean was defined as a delay in development. Three infants, diagnosed with cerebral palsy, in whom testing of the gross and fine motor function with the BSID-III was not feasible, were assigned a PDI score of 50.

To evaluate child behavior, the Dutch version of the Child Behavior Checklist (CBCL)[27] was sent to the child's address prior to the follow up visit, to be completed by either parent. The questionnaire consists of 99 items rated on a 3-point scale. By summing the scores an internalizing, externalizing, total and other problem score can be computed. Dichotomized T scores between the 84th percentile and 90th percentile were assigned borderline range, whereas T scores above 90th percentile were assigned abnormal range and defined as behavioral problems.[27]

Figure 1. 1A Axial color-coded DTI map with right (→) and left (←) PLIC in blue and 1B sagittal color-coded DTI map with corpus callosum in red, the regions of interest of genu (→) and splenium (←) are defined. The "x" marks the place where a single seed point was placed in order to generate the fibre tracts.



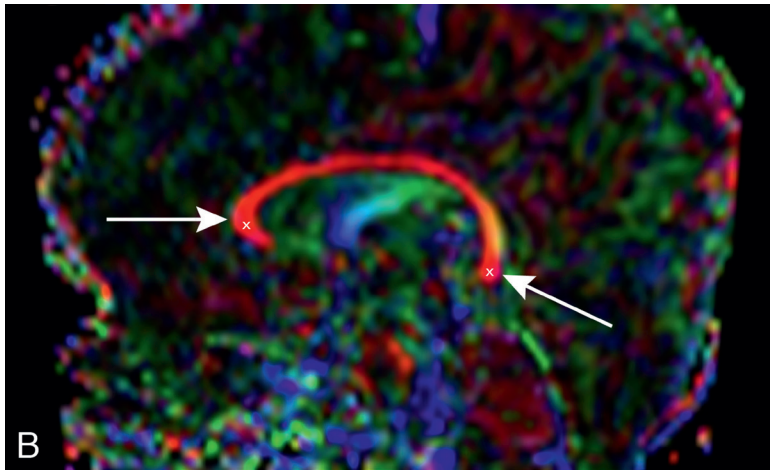
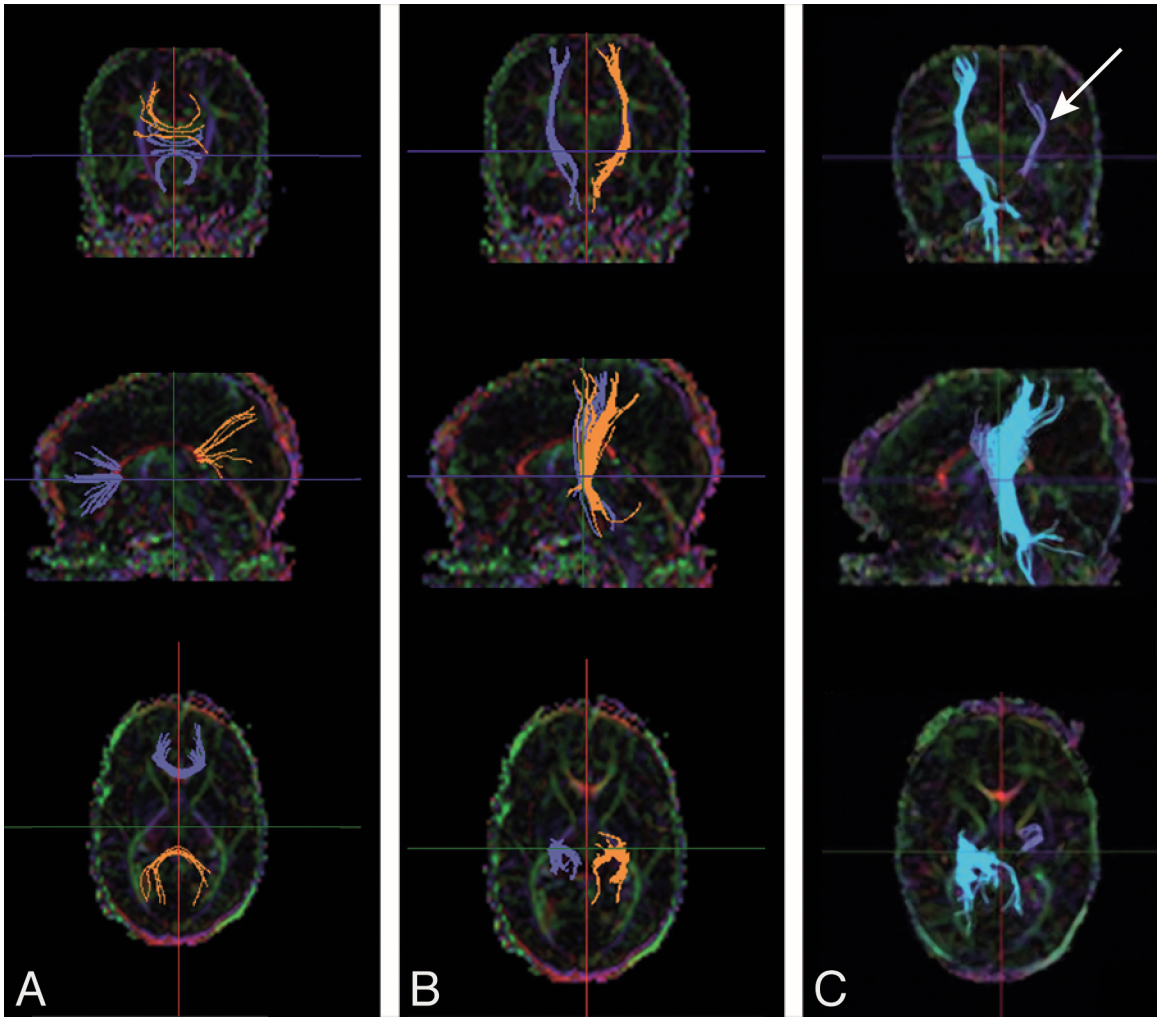


Figure 2. Coronal, sagittal and axial views of fibres passing through the genu and splenium of the corpus callosum (A) and the PLIC (B) in an infant with normal outcome and fibres of the PLIC in an infant with unfavorable outcome (C). Note the less well developed PLIC (←) on the left as compared to the right sided PLIC.



Statistical analysis

Data were analyzed using SPSS 18.0 for Windows. Continuous variables were expressed as mean with standard deviation and range, and categorical variables as count with percentage. DTI parameters of infants with and without neurodevelopmental delay (BSID III), cerebral palsy and behavioral problems (CBCL) were compared using Student's *t*-test or Mann-Whitney U test where appropriate. Mean differences were reported.

Backward logistic regression was used to adjust for potentially confounding clinical and MRI parameters related to outcome and/or DTI parameters. Subsequently, using MedCalc software (www.medcalc.org) version 11.6 for Windows, receiver operating characteristic curves (ROC) and corresponding areas under the curve (AUC) were generated for the DTI parameters that were independent predictors for neurodevelopmental outcome according to logistic regression. Cut-off values and predictive accuracy of these individual DTI parameters were assessed.

Two-sided tests were used throughout and a p-value <0.05 was considered statistically significant.

Results

Follow up

Follow up was obtained in 64 of the 84 (76.2%) infants. Mean age at follow up was 31.8 months (SD 4.4, range 23-44).

Twenty infants were lost to follow up due to miscellaneous reasons such as rejection of participation or practical problems, including travel distance to the hospital. At term equivalent age there were no differences in clinical or DTI parameters between infants with or without follow up.

Clinical parameters, white matter injury and DTI parameters.

The baseline clinical and DTI parameters in this cohort (n=64) are shown in Table 1. Median gestational age was 29.1 weeks (range 25.6 – 31.9), 41 out of the 64 (64.1%) infants were male. Table 1 also shows the number and percentage of infants with white matter injury, consisting of more than 6 PWML and moderate and severe ventricular dilatation. The location of the PWML was mainly in the periventricular white matter of the centrum semi ovale, at the level of the trigonum or optic radiation. There were only a few infants with cystic and/or hemorrhagic lesions.

Table 2 shows the mean differences in baseline DTI parameters between infants with normal development and infants with abnormal cognitive development, abnormal motor development or presence of cerebral palsy after 2 years. Three infants (3.6%) showed mental delay and 5 (6.0%) infants psychomotor delay according to the BSID. According to the GMFCS, 5 (6.0%) infants had cerebral palsy. Infants with psychomotor delay according to the BSID had significantly lower FA values and shorter fibre lengths of the PLIC ($p=0.002$ and $p=0.02$), and higher ADC values

Table 1. Distribution of categorical clinical parameters (N (%)), continuous clinical parameters (median (range)), white matter injury (N (%)) and DTI parameters (mean (SD)).

Categorical clinical parameter	*N (%)*
Male	41 (64.1)
Female	23 (35.9)
Gestational age < 28 weeks	23 (35.9)
Birth weight < 1000 gram	21 (32.8)
IUGR	8 (12.5)
Continuous clinical parameters	*Median (range)*
Gestational age (weeks)	29.1 (25.6-31.9)
Birth weight (gram)	1163 (585-1960)
Postmenstrual age at MRI (weeks)	43.4 (40-62)
Weight at MRI (gram)	4010 (2010-7005)
White matter injury	*N (%)*
> 6 PWML	11 (17.2)
Moderate ventricular dilatation	35 (54.7)
Severe ventricular dilatation	12 (18.8)
Cysts	4 (6.3)
Hemorrhagic lesions	3 (4.7)
DTI values	*Mean (SD)*
PLIC	
FA	0.37 (0.02)
ADC (10^{-3} mm²/s)	1.06 (0.05)
Length (mm)	58.7 (7.8)
Corpus callosum	
CCA FA	0.37 (0.04)
CCA ADC (10^{-3} mm²/s)	1.32 (0.10)
CCA length (mm)	46.9 (10.5)
CCP FA	0.40 (0.04)
CCP ADC (10^{-3} mm²/s)	1.36 (0.12)
CCP length (mm)	58.7 (12.8)

SD=standard deviation, IUGR=intrauterine growth retardation, PWML=punctate white matter lesion, PLIC=posterior limb of the internal capsule, CCA=genu, CCP=splenium

and shorter fibre lengths of the callosal splenium (p=0.03 and p=0.002). Infants with cerebral palsy according to the GMFCS also had significantly lower FA values and shorter fibre lengths of the PLIC (p=0.04 and p=0.02). The box plot in Figure 3 shows the FA of the PLIC in infants without and with motor delay.

We found a significant relation between myelination of the PLIC and FA (p<0.001)

Table 2. Mean difference (95% CI) in DTI parameters of the PLIC and corpus callosum for neuro-developmental outcome (BSID-III, GMFCS) in 64 infants. Between brackets the number (n) of abnormal infants.

BSID-III or GMFCS	Normal development vs mental delay BSID (n=3)	p-value	Normal development vs psychomotor delay BSID (n=5)	p-value	Normal development vs cerebral palsy GMFCS (n=5)	p-value
PLIC parameters						
FA	0.027 (-0.016 – 0.070)	0.05	0.027 (0.013 – 0.041)	0.002*	0.020 (0.0004 – 0.0408)	0.04*
ADC	0.013 (-0.047 – 0.072)	0.81	-0.020 (-0.059 – 0.018)	0.28	-0.004 (-0.049 – 0.042)	0.90
length	5.76 (-3.78 – 15.30)	0.18	7.90 (4.10 – 11.70)	0.02*	7.39 (2.99 – 11.79)	0.02*
CC parameters						
CCA FA	0.021(-0.030 – 0.073)	0.45	-0.002 (-0.046 – 0.041)	0.66	0.0007 (-0.041 – 0.042)	0.53
CCA ADC	0.005 (-0.112 – 0.121)	0.79	-0.034 (-0.125 – 0.056)	0.40	-0.016 (-0.110 – 0.078)	0.77
CCA length	-0.224 (-12.84 – 12.39)	0.89	6.29 (-3.33 – 15.91)	0.19	1.55 (-8.29 – 11.39)	0.87
CCP FA	0.007 (-0.036 – 0.049)	0.96	0.001 (-0.032 – 0.035)	0.95	-0.006 (-0.039 – 0.028)	0.70
CCP ADC	-0.058 (-0.202 – 0.086)	0.58	-0.148 (-0.250 - -0.045)	0.03*	-0.086 (-0.194 – 0.023)	0.07
CCP length	2.61 (-12.90 – 18.11)	0.62	19.32 (8.23 – 30.42)	0.002*	13.06 (-6.13 – 32.26)	0.05

*statistically significant
BSID=Bayley Scales of Infant Development, GMFCS=Gross Motor Function Classification System
PLIC=posterior limb of the internal capsule, CC=corpus callosum,
CCA= genu of the corpus callosum, CCP=splenium of the corpus callosum

Figure 3. FA of the PLIC in children without and with motor delay.

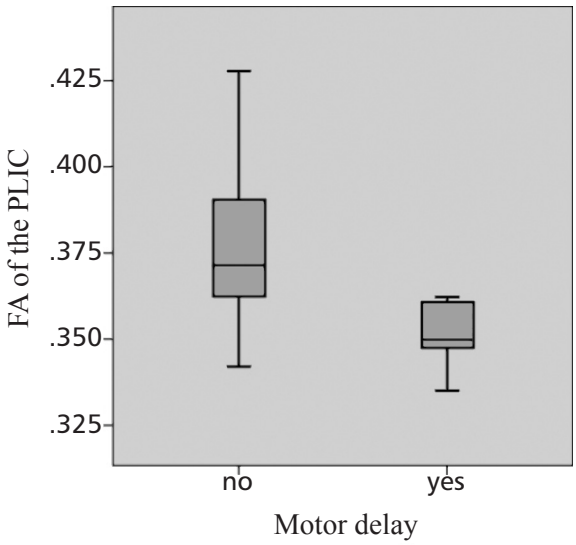


Table 3. Summary statistics of ROC curves for FA and length of the PLIC and ADC of the splenium.

	AUC (p-value)	Cut-off value	Sensitivity % (95%CI)	Specificity % (95%CI)
FA of the PLIC				
Motor delay	0.89 (<0.001)	0.36	100 (48-100)	69 (55-82)
Cerebral palsy	0.79 (<0.001)	0.36	80 (28-100)	66 (53-78)
Length of the PLIC				
Motor delay	0.80 (0.03)	53.9	80 (28-100)	79 (66-90)
ADC of the splenium				
Motor delay	0.80 (<0.001)	1.38	100 (48-100)	65 (50-78)

ROC=receiver operating characteristic curve, AUC=area under the curve

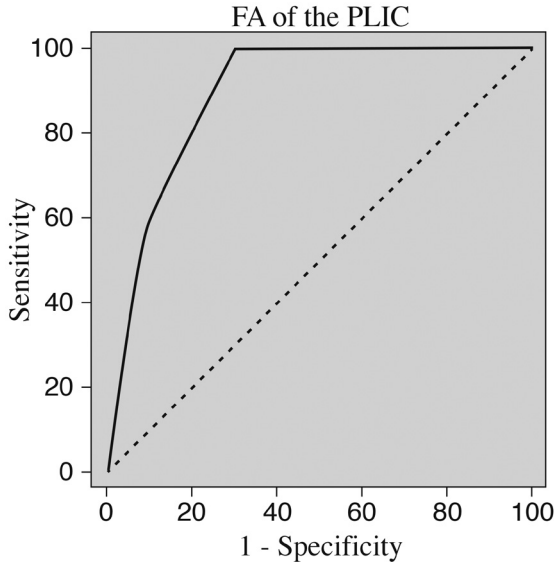
and ADC ($p<0.001$) of the PLIC. All infants with psychomotor delay according to the BSID and cerebral palsy according to the GMFCS showed incomplete myelination of the PLIC; however this finding was not significant as, respectively 77.6% and 79.3% of the infants without psychomotor delay or cerebral palsy also showed an incompletely myelinated PLIC. Therefore (incomplete) myelination in the PLIC was not directly related to outcome ($p=0.57$, both for psychomotor delay and for cerebral palsy).

We found no correlations between DTI parameters at term equivalent age and behavioral problems based on CBCL scores after 2 years.

Backward logistic regression analyses, correcting for potentially confounding clinical parameters (gestational age, gender, intrauterine growth retardation, perinatal infections, number of days of oxygen requirement, number of days of mechanical ventilation) and white matter injury and ventricular dilatation on MRI showed that a lower FA value and a shorter fibre length of the PLIC were independent predictors for psychomotor delay ($p=0.004$, OR 0.27(95% CI 0.09-0.80)) and ($p=0.001$, OR 0.99 (95% CI 0.98-0.99)) and FA of the PLIC also to a lesser extent for cerebral palsy ($p=0.09$, OR 0.46 (95% CI 0.21-1.03)). A higher ADC value of the callosal splenium ($p=0.02$, OR 1.11 (95% CI 1.02-1.21)) was an independent predictor for psychomotor delay.

Table 3 shows the summary statistics of the ROC curves for the relevant DTI parameters predicting motor delay and cerebral palsy. Since DTI values are age dependent, ROC curves were only based on 59 infants imaged at tern equivalent age (between 40 – 44 weeks). Follow up was available in 44/59 (74.6%) infants.

Figure 4 ROC curve for motor delay of the FA of the PLIC.



A cut-off value of the FA in the PLIC of 0.36 predicted motor delay with a sensitivity of 100% and a specificity of 69% (illustrated in Figure 4) and cerebral palsy with a sensitivity of 80% and a specificity of 66%. A fibre length shorter than 53.9 mm through the PLIC predicted motor delay with a sensitivity of 80% and a corresponding specificity of 79%.

A cut-off value for the ADC of the callosal splenium of 1.38 (10^{-3} mm^2/s) predicted motor delay with a sensitivity of 100% and a specificity of 65%.

Discussion

In this study we correlated DTI tractography values of white matter tracts acquired around term equivalent age in very preterm infants with neurodevelopmental follow up at the age of 2 years. Low FA values and decreased fibre lengths of the PLIC at term equivalent age are associated with psychomotor delay and cerebral palsy, and high ADC values and short fibre lengths of the callosal splenium with psychomotor delay.

These associations were independent from other types of brain damage at term equivalent age such as white matter injury, ventricular dilatation and/or clinical parameters, and can predict motor delay and/or cerebral palsy with a high sensitivity and reasonable specificity.

The main pathogenic mechanisms for white matter injury in the very preterm neonate are ischemia and infection. These often co-exist and may lead to focal or diffuse white matter injury and/or hemorrhages in the perinatal period due to the vulnerability of the developing white matter, immature vasculature and impaired cerebrovascular auto regulation of the immature brain.[2] DTI studies have suggested axonal loss in the white matter of preterm infants at term equivalent age and have shown to be predictive for neurodevelopmental outcome.[16,18-20]

Our data partly confirm the results of the study by Arzoumanian and coworkers, showing a correlation between a decreased FA of the PLIC near term equivalent age in low birth weight preterm infants and neurologically abnormal infants, including cerebral palsy at the age of 18 and 24 months.[16] Also our data substantiate the findings of Rose *et al*., in which a reduction in FA and higher ADC values in the callosal splenium and right-sided PLIC at term equivalent age were correlated with abnormal neurological outcome at 18 months.[18] In our study, we corrected for all clinical and MRI factors separately in one regression model, whereas Rose and coworkers used one overall MRI score. In a recent study by Van Kooij *et al*., it was demonstrated that tract based spatial statistics of FA and axial and radial diffusivity of DTI data at term equivalent age are a potential biomarker for subsequent neurodevelopment. After correction for gestational age and postmenstrual age at scan, gross motor scores were associated with radial diffusivity in the corpus callosum and internal and external capsule.[20] In an earlier fibre tractography study, Van Kooij and coworkers found gender differences regarding associations between fibre tracking parameters and cognitive and motor outcome at 2 years of age in preterm infants after correcting for gestational age, birth weight, intraventricular hemorrhage, white matter injury and maternal education.[19] In our study we did not find gender differences, but our data show that lower FA values of the PLIC, shorter fibre lengths of the PLIC and higher ADC values of the callosal splenium, after correcting for clinical parameters, white matter injury and ventricular dilatation, are independent predictors for psychomotor delay and/or cerebral palsy at the age of 2 years.

The predictive value of MRI findings for motor and cognitive development in preterm infants is an area of continuing research.[28-30] Prediction of neurodevelopmental outcome in very preterm born infants is considered to be highly important,[29] as early intervention may be beneficial for neurodevelopmental outcome.[31-33] So far, most studies have used qualitative assessment of white matter injury combined with ventricular dilatation or delay in myelination to predict neurodevelopmental outcome.[25,34-37] In a study by Woodward *et al*. qualitative assessment of white matter injury showed reasonable sensitivity (65%) and specificity (85%) to predict motor delay.[34] Spittle and co-workers

demonstrated that white matter abnormalities predicted a delay in motor development at 12 months' corrected age with a specificity of more than 90%, however the sensitivity was very low.[38] Qualitative assessment of diffuse high signal intensity of the white matter in itself did not correlate with abnormal neurodevelopmental outcome at 2 years, whereas ventricular dilatation and the existence of more than 6 punctate white matter lesions did.[37,39,40] By generating ROC curves we determined cut-off values for the FA of the PLIC, the fibre length of the PLIC and the ADC of the splenium of the corpus callosum, predicting motor delay with a high sensitivity and reasonable specificity.

A limitation of our study is that follow up was unavailable in 20 out of 84 (23.8%) of the very preterm infants. Although it cannot be excluded that inclusion bias affected our data, at term equivalent age there were no differences in clinical parameters, white matter injury or DTI values between infants with and without follow up. Also the low number of infants with an unfavorable outcome reduces the statistical power of the study. Although our results show that DTI fibre tracking of the PLIC and corpus callosum around term equivalent age offers valuable data to assess an individual prognosis after 2 years, it should be kept in mind that, combining conventional MRI with quantitative MRI techniques overall improves the performance of MRI for prognosis.[3,31] The set up of our study with relatively short term follow up at 2 years of age limits confident prognosis for especially cognitive impairment. Long term follow up at school age is needed to further evaluate the prognostic values of certain MRI findings and quantitative values around term equivalent age for cognitive neurodevelopmental outcome.

We conclude that DTI tractography values obtained around term equivalent age are of additional help in predicting neurodevelopmental outcome at 2 years corrected age in very preterm infants. Prediction of motor delay and cerebral palsy is possible by determining the FA value and fibre length of the PLIC and the ADC value of the splenium of the corpus callosum.

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Is sequential cranial ultrasound reliable for detection of white matter injury in very preterm infants?

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Abstract

Introduction: Cranial ultrasound (cUS) may not be reliable for detection of diffuse white matter (WM) injury. Our aim was to assess in very preterm infants the reliability of a classification system for WM injury on sequential cUS throughout the neonatal period, using magnetic resonance imaging (MRI) as reference standard.

Methods: In 110 very preterm infants (gestational age <32 weeks), serial cUS during admission (median 8, range 4-22) and again around term equivalent age (TEA) and a single MRI around TEA were performed. cUS during admission were assessed for presence of WM changes, and contemporaneous cUS and MRI around TEA additionally for abnormality of lateral ventricles. Sequential cUS (from birth up to TEA) and MRI were classified as normal/mildly abnormal, moderately abnormal or severely abnormal, based on a combination of findings of the WM and lateral ventricles. Predictive values of the cUS classification were calculated.

Results: Sequential cUS were classified as normal/mildly abnormal, moderately abnormal and severely abnormal in respectively 22%, 65% and 13% of infants, and MRI in respectively 30%, 52% and 18%. The positive predictive value of the cUS classification for the MRI classification was high for severely abnormal WM (0.79) but lower for normal/mildly abnormal (0.67) and moderately abnormal (0.64) WM.

Conclusion: Sequential cUS during the neonatal period detects severely abnormal WM in very preterm infants, but is less reliable for mildly and moderately abnormal WM. MRI around TEA seems needed to reliably detect WM injury in very preterm infants.

Introduction

Diffuse white matter injury is frequently encountered in very preterm infants.[1-15] It is reflected by signal changes in the white matter (WM) on magnetic resonance imaging (MRI).[2-14] Concerns have been raised that cranial ultrasound (cUS) is not sensitive enough to detect diffuse WM injury, as the abnormalities may be subtle.[2-10] Preterm infants with diffuse WM injury are at risk for motor and cognitive impairment.[11,15] Several authors have therefore suggested a standard MRI examination in all infants born very prematurely (gestational age (GA) <32 weeks).[6-9]

In preterm infants WM injury has been associated with reduced WM and deep (i.e. basal ganglia and thalami) and cortical grey matter volumes and increased cerebrospinal fluid volumes around term equivalent age (TEA).[16-18] However, previous cUS studies on WM injury in very preterm infants have only assessed changes within the WM but did not assess associated changes such as ventricular dilatation.[3,4,6-8]

In this prospective study, we assess the reliability of a classification system for WM injury in very preterm infants, based on a combination of findings of the WM and lateral ventricles, on frequent, sequential high-quality cUS throughout the neonatal period, using a MRI classification system as reference standard.

Materials and Methods

Patients

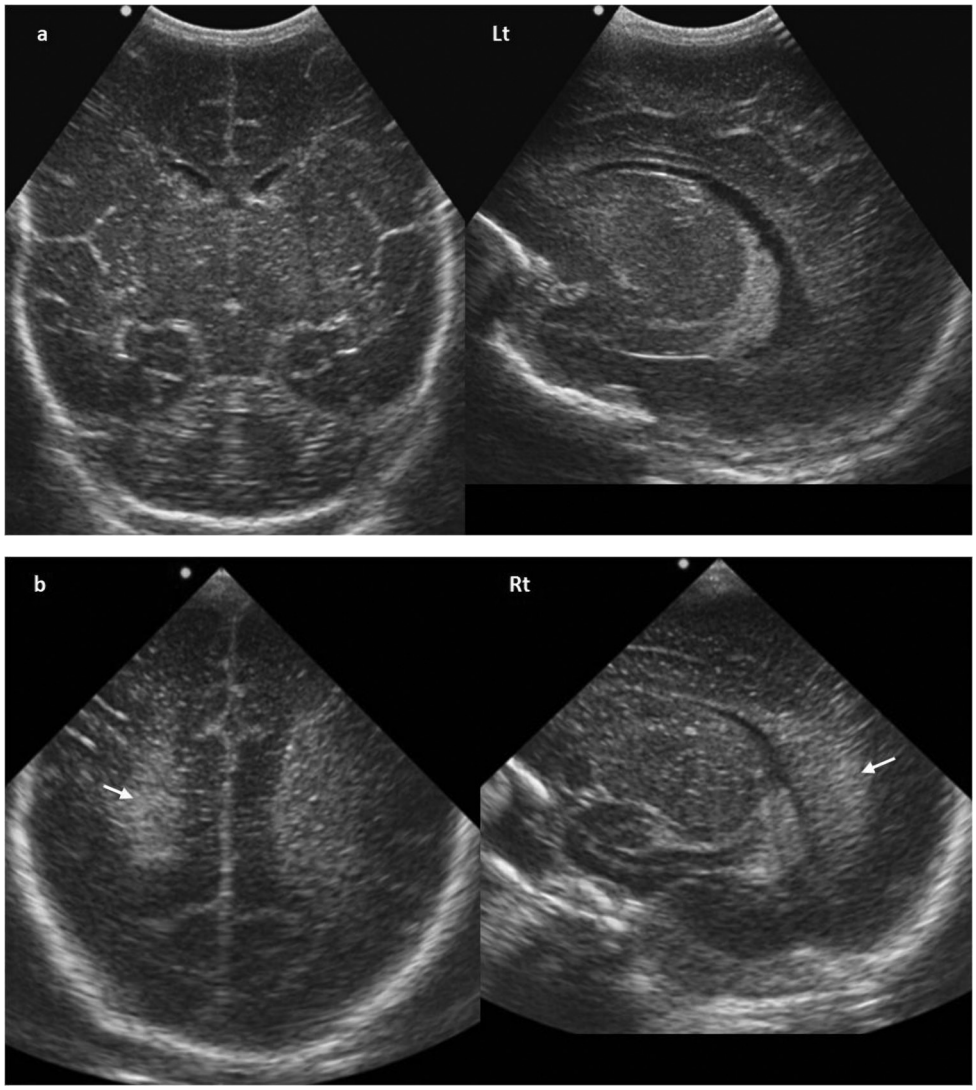
Very preterm infants (GA <32 weeks), admitted to the tertiary neonatal intensive care unit of the Leiden University Medical Center between May 2006 and October 2007, were eligible for a prospective neuro-imaging study, assessing normal brain maturation and brain injury by cUS and MRI. The study was approved by the Medical Ethics Committee, and informed consent was obtained from the parents. Exclusion criteria were congenital anomalies of the central nervous system, severe other congenital anomalies, chromosomal and metabolic disorders, and neonatal meningitis.

Cranial ultrasound

1. Image acquisition

Serial cUS scans were performed by a team of experienced examiners, using an Aloka α10 scanner (Biomedic Nederland B.V., Almere, the Netherlands) with a special standardized preset and according to a standard protocol; scanning with a transducer frequency of 7.5MHz within 24 hours of birth, at least weekly from the day of birth or

Figure 1. A, Coronal (left) and parasagittal (right) cUS of preterm infant (GA 27.9 weeks), scanned at PMA 31.1 weeks, showing normal echogenicity of periventricular WM (WM score of serial cUS during admission: normal/mildly abnormal).
B, Coronal (left) and parasagittal (right) cUS of preterm infant (GA 28.0 weeks), scanned at PMA 32.3 weeks, showing homogeneous PVE in parieto-occipital WM on the left and inhomogeneous PVE in parieto-occipital WM on the right (arrows) (WM score of serial cUS during admission: normal/mildly abnormal).



admission until discharge or transfer to another hospital, and again on the day of the MRI examination around TEA.[19,20]

2. Assessment

All cUS examinations were assessed by two experienced investigators (LML with SJS (both >5 years of experience) or LML with GvWM (>15 years of experience)).

For this part of the study, special attention was paid to the WM. On the serial cUS scans performed during admission periventricular echodensities (PVE) were defined and classified according to van Wezel-Meijler *et al*., relating the echogenicity of the WM to that of the choroid plexus, and their appearance (homogenous or inhomogeneous) was noted (Figure 1).[2,21] As, based on previous experience, PVE may still be seen at discharge and consequently the duration of PVE cannot be reliably assessed, we decided not to include the total duration of PVE in our WM classification. Subtle, symmetric echogenic areas in the frontal WM or adjacent and parallel to the atrium of the lateral ventricles were considered normal phenomena and not scored as PVE.[21-22]

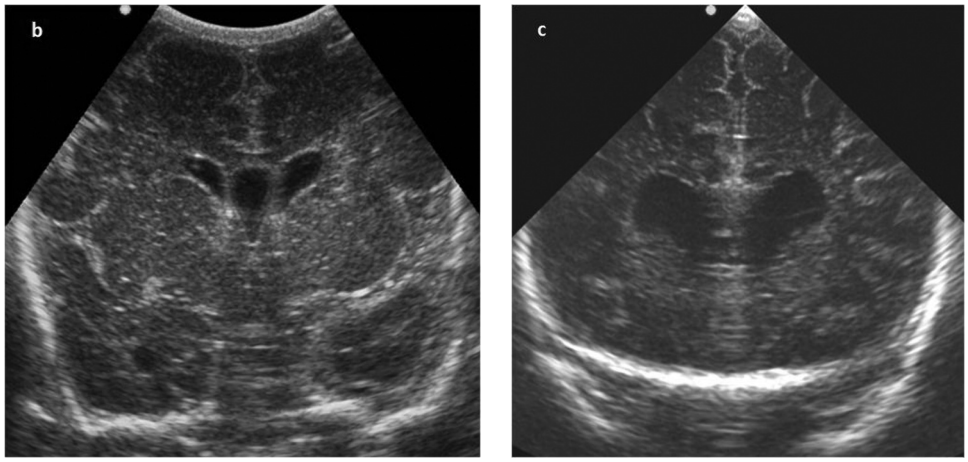
Unilateral or asymmetric, localized areas of high echogenicity within the WM were scored as focal WM echodensities. If co-existing with an intraventricular hemorrhage on the ipsilateral side, these mostly represent periventricular hemorrhagic infarction.[23] When in doubt whether an echodensity in the WM represented PVE or a focal echodensity, it was scored as inconclusive. Porencephalic cyst was defined as a large cystic lesion, communicating with the lateral ventricle.[23]

On the cUS performed within several hours of the MRI (i.e. TEA-cUS), besides assessment of WM changes, the ventricular index (VI) was measured according to Levene, by an independent investigator who was not involved in patient care and unaware of the imaging findings.[24] A VI of <13 mm was considered as normal/mildly abnormal, between 13-15 mm as moderately abnormal, and of >15 mm as severely abnormal. In addition, on TEA-cUS, the shape of the lateral ventricles was visually assessed and graded as normal/mildly abnormal (i.e. normal or mildly abnormal shape), moderately abnormal (i.e. moderately abnormal shape, including irregular, plump or square-shaped ventricles) (Figure 2a) or severely abnormal (i.e. severely abnormal shape) (Figure 2b). In case of discordance consensus was reached.

3. WM classification

The WM classification was scored by the same investigators (LML with SJS or LML with GvWM). In case of discordance consensus was reached.

Figure 2. Left: coronal cUS of preterm infant (GA 28.0 weeks), scanned at PMA 42.0 weeks, showing moderately dilated lateral ventricles (TEA-cUS WM score: moderately abnormal). Right: coronal cUS preterm infant (GA 30.3 weeks), scanned at PMA 41.9 weeks, showing severe lateral ventricular dilatation (TEA-cUS WM score: severely abnormal). Poor image quality is due to small size of fontanel.



WM classification for serial cUS during admission:

- Normal/mildly abnormal: no PVE or homogeneous grade 1 PVE (Figure 1a).[21]
- Moderately abnormal: inhomogeneous grade 1 PVE (regardless of duration), grade 2 PVE (regardless of appearance and duration), and/or small, localized cystic lesions (Figure 1b).[21]
- Severely abnormal: multicystic lesions, focal WM echodensity, and/or porencephalic cyst.[23]

WM classification for TEA-cUS:

- Normal/mildly abnormal: homogeneous WM and normal/mildly abnormal lateral ventricles, the VI being less than 13 mm with the shape normal or at the most mildly abnormal.
- Moderately abnormal: inhomogeneous WM and/or small, localized cystic lesions and/or moderately abnormal lateral ventricles, the VI being 13-15 mm and/or the shape moderately abnormal (Figure 2a).
- Severely abnormal: multicystic lesions and/or focal WM echodensity and/or porencephalic cyst, and/or severely abnormal lateral ventricles, the VI being over 15 mm and/or the shape severely abnormal (Figure 2b).

The WM score of the TEA-cUS was based on the most severe changes.

The WM classification for the serial cUS during admission combined with the TEA-cUS (i.e. sequential cUS) was based on the most severe changes over time.

MRI

1. Image acquisition

MRI examinations were performed in all very preterm infants according to a standard protocol for imaging the newborn infant's brain, using a 3.0 T Philips MR system (Philips Medical Systems, Best, the Netherlands) as recently described.[25] In summary, scans included at least T_1-weighted three dimensional images (TR/TE: 9.7/4.6 ms), allowing reconstruction in every desired orientation, T_2-weighted images (TR/TE: 6269/120 ms), diffusion-weighted images (TR/TE: 2406/64 ms) and susceptibility weighted images (TR/TE: 735/16 ms) in the transverse plane. The MRI examinations were performed around TEA, preferably between 40 and 44 weeks' postmenstrual age (PMA) (i.e. TEA-MRI). For infants who were still unstable and/or ventilator dependent around that age, MRI was postponed. For this part of the study the T_1- and T_2-weighted sequences were analyzed.

2. Visual assessment

All MRI examinations were assessed by two experienced investigators (LML with FTdB or SJS with FTdB, the latter being a pediatric neuroradiologist with >15 years of experience), who were blinded to the cUS findings.

Special attention was paid to the brain WM. Punctate white matter lesions (PWML) were defined as small areas of high signal on T_1-weighted images, with mostly low signal on T_2-weighted images (Figure 3).[3,20] Diffuse and excessive high signal intensity (DEHSI) was defined as areas of excessive high signal intensity diffusely distributed within the periventricular and/or subcortical WM on T_2-weighted images (Figure 4).[3-5,11,13,20]

From the T_1-weighted three dimensional images, coronal reconstructions were obtained and the VI was measured in the coronal plane showing the third ventricle. The total width of the lateral ventricles was measured and divided by 2, in analogy to measurements of the VI on cUS. The shape of the lateral ventricles was assessed visually and graded as normal/mildly abnormal (Figure 5a), moderately abnormal (Figure 5b) or severely abnormal (Figure 5c) as described for TEA-cUS. In case of discordance consensus was reached.

3. WM classification

The WM classification was adapted from Sie *et al*. and scored by the same investigators. This was based on the most severe changes.[2] In case of discordance consensus was reached.

Figure 3. Transverse T_1- (left) and T_2-weighted (right) MRI at high-ventricular level of preterm infant (GA 26.9 weeks), scanned at PMA 42.7 weeks, showing bilateral, multiple PWML (arrows) in a linear distribution parallel to the LV. Also showing dilated, irregularly shaped lateral ventricles and widening of extracerebral spaces (TEA-MRI WM score: moderately abnormal).

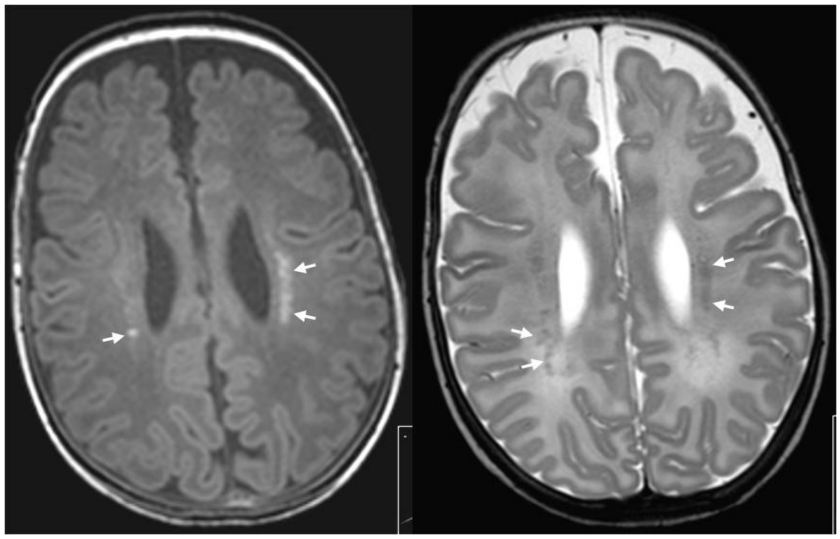


Figure 4. Transverse T_2-weighted MRI at mid-ventricular level of preterm infant (GA 30.6 weeks), scanned at PMA 42.3 weeks, showing homogeneous SI increase (DEHSI) in periventricular and subcortical WM (TEA-MRI WM score: normal/mildly abnormal).

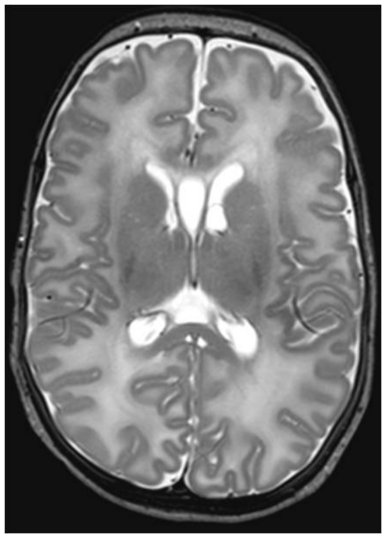
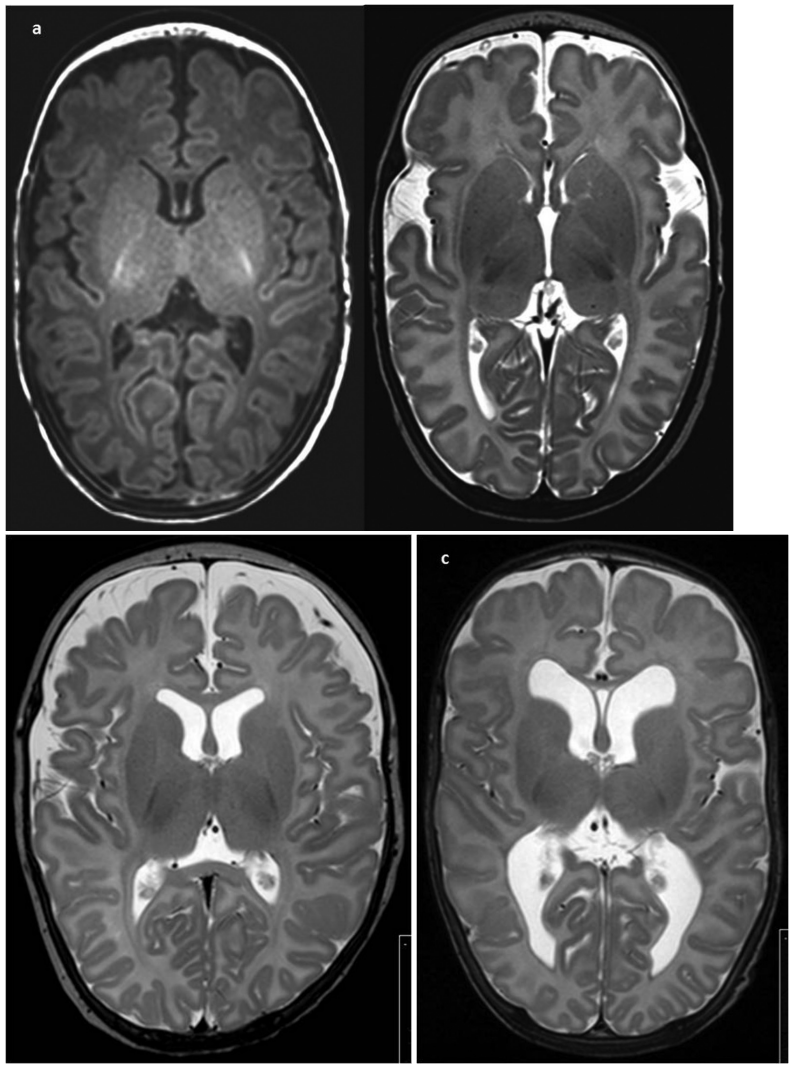


Figure 5. A, Transverse T_1- (left) and T_2-weighted (right) MRI at mid-ventricular level of preterm infant (GA 27.1 weeks), scanned at PMA 42.1 weeks, showing normal signal in WM. Also showing normal size and shape of lateral ventricles (TEA-MRI WM score: normal/ mildly abnormal).

Figure 5. B, Transverse T_2-weighted MRI at mid-ventricular level of preterm infant (GA 27.9 weeks), scanned at PMA 43.1 weeks, showing moderately dilated and abnormally square-shaped lateral ventricles (TEA-MRI WM score: moderately abnormal).

Figure 5. C, Transverse T_2-weighted MRI at mid-ventricular level of preterm infant (GA 31.6 weeks), scanned at PMA 43.4 weeks, showing severely dilated and abnormally shaped lateral ventricles due to volume loss of the WM (TEA-MRI WM score: severely abnormal).



- Normal/mildly abnormal: normal appearing WM, homogeneous DEHSI or few (≤6) PWML and normal/mildly abnormal lateral ventricles, the VI being less than 13 mm with the shape normal or at the most mildly abnormal (Figures 4 and 5a).
- Moderately abnormal: multiple (>6) PWML and/or small localized cystic lesions and/or inhomogeneous DEHSI and/or moderately abnormal lateral ventricles, the VI being 13-15 mm and/or the shape moderately abnormal (Figures 3 and 5b).
- Severely abnormal: extensive or diffuse inhomogeneous SI changes and/or hemorrhagic or cystic lesions involving the periventricular and/or subcortical WM and/or severely abnormal lateral ventricles, the VI being over 15 mm and/or the shape severely abnormal (Figure 5c).

Data analysis

Statistical analyses were performed using SPSS (version 14.0; SPSS Inc., Chicago, IL, USA). Predictive values of the WM classification of TEA-cUS and of sequential cUS for the WM classification of TEA-MRI were calculated. As the signal of the WM and of WM lesions on MRI changes with increasing PMA and, therefore, may be different around TEA (PMA 40-44 weeks) than beyond TEA (PMA >44 weeks), we first calculated the predictive values for the total group of very preterm infants and subsequently only for the group of infants with TEA-cUS and TEA-MRI between 40 and 44 weeks' PMA.

RESULTS

Patients

During the study period, 182 very preterm infants were eligible for the study, of which 130 infants (80 male) were included (Figure 6). Fifty-two infants were excluded from the study; three because of structural brain abnormalities and 49 because informed parental consent was not obtained. Reasons for not obtaining consent included transfer to another hospital or death within a very short period of birth, rejection of participation, and practical problems such as language barrier and travel distance to the hospital. Median GA and birth weight of included infants were 29.0 (range 25.6-31.9) weeks and 1141 (520-1960) grams. There were no significant differences in GA and birth weight between infants with and without informed consent.

In all 130 infants, serial cUS scans were performed during admission (median 8, range 4-22). In 20 infants no or inadequate TEA-cUS and TEA-MRI were obtained. In the remaining 110 infants (68 male) contemporaneous cUS and MRI were obtained at

Figure 6. Flow diagram showing the number of infants eligible for the study, the number of infants included and not included in the study, and the final number of infants with sequential cUS and MRI between 40 and 44 weeks' PMA (n, number of infants).

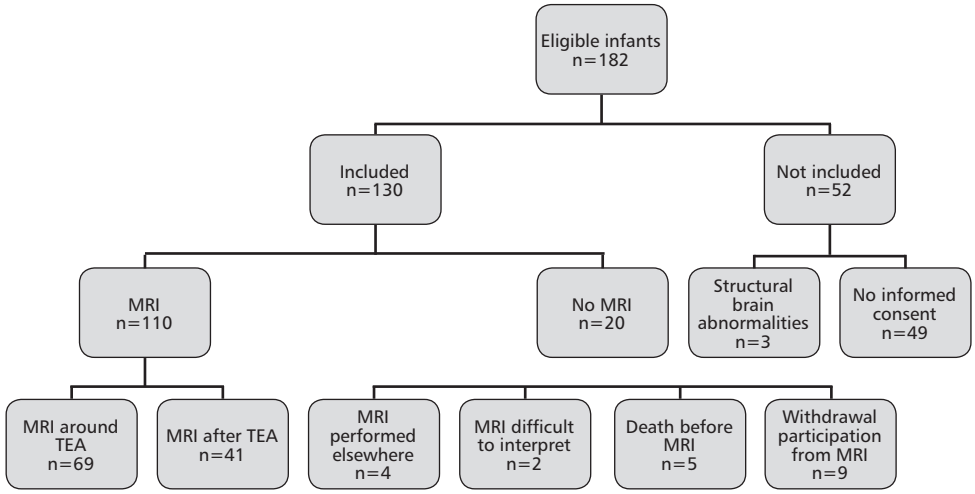


Table 1. Distribution of the WM classifications for serial cUS during admission (adm-cUS), TEA-cUS, sequential cUS and TEA-MRI (n, number of infants).

WM classification	**Imaging technique**, *n* (% of total number)			
	Adm-cUS	**TEA-cUS**	**Sequential cUS**	**TEA-MRI**
Normal/mild	27 (24.5)	56 (51)	24 (22)	33 (30)
Moderate	75 (68.2)	40 (36)	72 (65)	57 (52)
Severe	8 (7.3)	14 (13)	14 (13)	20 (18)

a median PMA of 43.4 (40.1-55.9) weeks. In 69 infants this was around TEA (PMA 40-44 weeks), and in 41 infants between 44.0-55.9 weeks' PMA.

Cranial ultrasound

The distribution of the WM classifications for serial cUS during admission (based on WM changes), TEA-cUS (based on WM changes and size and shape of lateral ventricles) and sequential cUS (based on the most severe classification for serial cUS during admission and TEA-cUS) is shown in Table 1. Table 2 shows the distribution of WM findings that determined the WM classifications.

Table 2. Distribution of WM changes and abnormalities of lateral ventricles determining the WM classification of serial cUS during admission (adm-cUS), TEA-cUS and TEA-MRI (DEHSI, diffuse and excessive high signal intensity; LV, lateral ventricles; PVE, periventricular echodensities; PVHI, periventricular hemorrhagic infarction; PWML, punctate white matter lesions).

WM classification		Findings determining WM classification	Number of infants
Adm-cUS	*Normal/mild*	No PVE	17
		Homogeneous grade 1 PVE	10
	Moderate	Inhomogeneous grade 1 PVE	57
		Grade 2 PVE	12
		Small, localized cystic lesions	6
	Severe	Multicystic lesions	1
		PVHI	7
TEA-cUS	*Normal/mild*	Homogeneous WM and normal/mildly abnormal LV	56
	Moderate	Inhomogeneous WM	3
		Moderately abnormal LV	31
		Inhomogeneous WM and moderately abnormal LV	6
	Severe	Severely abnormal LV	14
TEA-MRI	*Normal/mild*	Normal WM/homogeneous DEHSI and normal/mildly abnormal LV	30
		Few PWML and normal/mildly abnormal LV	3
	Moderate	Multiple PWML	4
		Moderately abnormal LV	45
		Multiple PWML and moderately abnormal LV	8
	Severe	Severely abnormal LV	20

MRI

The distribution of the WM classification for TEA-MRI (based on WM changes and size and shape of lateral ventricles) is shown in Table 1. Table 2 shows the distribution of WM findings that determined the WM classification.

Relation between cUS and MRI

Predictive values of the WM classifications of TEA-cUS and of sequential cUS for the WM classification of TEA-MRI for the total group of preterm infants (n=110) are presented in Table 3. This table shows high positive predictive values of cUS for MRI in infants with severely abnormal WM, but lower positive predictive values in infants with normal/mildly abnormal and moderately abnormal WM on cUS.

Predictive values of the WM classifications of TEA-cUS and of sequential cUS for the WM classification of TEA-MRI for the subgroup of infants with TEA-cUS and TEA-MRI

Table 3. Predictive values of the WM classifications of TEA-cUS and of sequential cUS for the WM classification of TEA-MRI for the total group of preterm infants (NPV, negative predictive value; PPV, positive predictive value).

cUS WM classification		Predictive values for TEA-MRI WM classification			
		Sensitivity	**Specificity**	**PPV**	**NPV**
TEA-cUS	*Normal/mild*	0.94	0.68	0.55	0.96
	Moderate	0.51	0.79	0.73	0.60
	Severe	0.55	0.97	0.79	0.91
Sequential cUS	*Normal/mild*	0.48	0.90	0.67	0.80
	Moderate	0.81	0.51	0.64	0.71
	Severe	0.55	0.97	0.79	0.93

Table 4. Predictive values of the WM classifications of TEA-cUS and sequential cUS for the WM classification of TEA-MRI for the subgroup of infants with TEA-cUS and TEA-MRI between 40 and 44 weeks' PMA (NPV, negative predictive value; PPV, positive predictive value).

cUS WM classification		Predictive values for TEA-MRI WM classification			
		Sensitivity	**Specificity**	**PPV**	**NPV**
TEA-cUS	*Normal/mild*	0.95	0.60	0.50	0.97
	Moderate	0.46	0.79	0.71	0.55
	Severe	0.40	0.98	0.80	0.91
Sequential cUS	*Normal/mild*	0.44	0.90	0.67	0.76
	Moderate	0.83	0.42	0.62	0.68
	Severe	0.40	0.98	0.80	0.91

between 40 and 44 weeks' PMA (n=69) are presented in Table 4. These predictive values do not differ from those for the total group of infants.

Discussion

To our knowledge, this is the first prospective study comparing WM injury on cUS and MRI (reference standard) in very preterm infants based on newly designed classification systems, not only including changes within the WM but also scoring other changes in the brain thought to be related to WM injury.

The most important finding of this study is that the positive predictive value of both the TEA-cUS and the sequential cUS WM classification for the TEA-MRI WM classifica-

tion was high for severely abnormal WM; nearly all infants classified as severely abnormal for cUS were also classified as severely abnormal for MRI. However, cUS was less predictive for the moderately abnormal and normal/mildly abnormal groups.

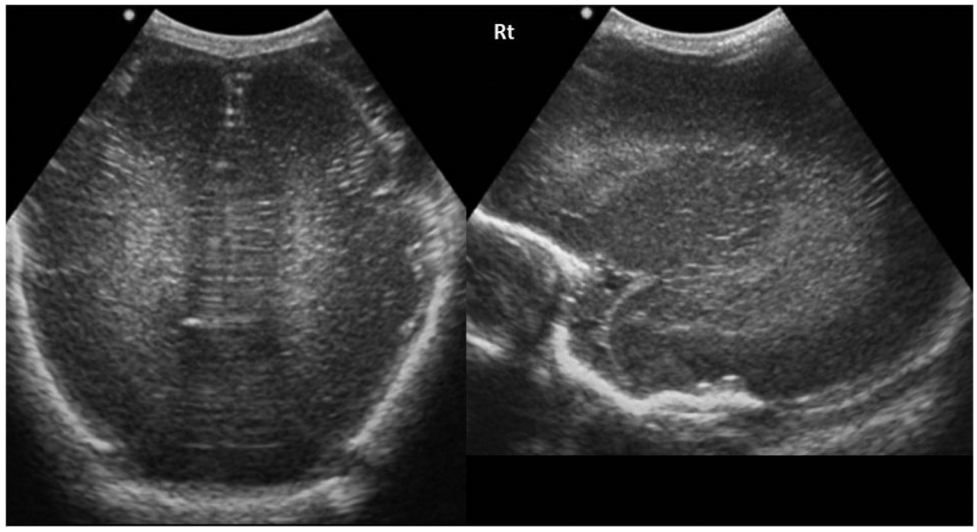
There are several possible explanations for these results. MRI was performed with a 3.0 T MR system, providing a high resolution that cannot be obtained with ultrasonography. Using this high field strength and a special neonatal imaging protocol, small lesions, including very small punctate WM lesions (PWML), are depicted.[20] In addition, in accordance with Sie *et al*., we used 6 as cut-off number for 'few' (mildly abnormal WM) or 'multiple' (moderately abnormal WM) PWML on MRI, which is rather arbitrary.[2] In 10/12 infants whose MRI classification was based on 'multiple' PWML, just over 6 lesions were detected, mostly isolated in organization and located in the periventricular WM at the level of the centrum semiovale and/or adjacent to the optic radiation. Only in two infants, the number of PWML was considerably higher and lesions were more widely distributed in the WM and organized linearly and/or in clusters. In this respect, we may have overrated the severity of WM changes on MRI in several infants. Finally, when only the anterior fontanel is used as acoustic window, the occipital region is not easy to visualize with cUS. For this study we did not additionally use the posterior fontanel, while this could have improved visualization of WM changes in the occipital region.[19]

cUS not only underestimated WM injury (Figure 7), but seemed to overestimate WM injury in some cases. This can be explained by the transient nature of some WM changes and the fact that the WM classification of sequential cUS was based on the most severe changes over time.[20]

Combining the WM classification of serial cUS during admission with that of TEA-cUS (i.e. sequential cUS) improved the predictive value of cUS in case of normal/mildly abnormal WM but decreased it for moderately abnormal WM.

The results of this study may arouse the erroneous suggestion that frequent, serial cUS performed during admission insufficiently contributes to detection of WM injury in very preterm infants. However, our classification systems are used to grade the severity of WM injury and do not provide information on separate imaging findings or the evolution of lesions. By substantially limiting the number of cUS examinations during admission, details on (transient) WM changes will be lost, the evolution of lesions cannot be followed, and distinction between several forms of WM injury (i.e. focal or more diffuse) may not be possible, as only the end stages of WM injury are visualized and, for instance, focal cysts may have resolved by TEA. By restricting cUS to the early neonatal period and TEA, as has been suggested, no information will be available on the evolution of lesions, lesions may remain undetected, and their severity underestimated.[26]

Figure 7. Coronal (left) and parasagittal (right) cUS of preterm infant (GA 26.9 weeks), scanned at PMA 29.1 weeks, showing only mild, homogeneous PVE bilaterally in the parietal WM; the cUS of this infant performed around TEA (PMA 42.7 weeks) showed normal WM and mildly abnormal size and shape of lateral ventricles (sequential cUS WM score: normal/mildly abnormal). However, the MRI of the same infant (see Figure 3) showed bilateral, multiple PWML and dilated, irregularly shaped lateral ventricles (TEA-MRI WM score: moderately abnormal).



Our results are in agreement with those of others comparing WM findings on cUS and MRI, showing that MRI is more sensitive for detecting (particularly subtle) WM changes than cUS.[2-10, 6-27] In addition, in most cases with WM injury that was detected by cUS, MRI demonstrated the exact site and extent of lesions more precisely, which is in agreement with previous findings.[14]

We appreciate some limitations of our study. Firstly, the most stable infants with (nearly) normal cUS findings were discharged sooner than those being less stable and/or having more severe findings. We may therefore have missed progression towards more severe changes on cUS in some infants with minor changes or changes developing after discharge. This may have biased our results and negatively influenced the reliability of cUS as cUS was not very reliable in infants with normal/mildly abnormal WM. Secondly, as in half (44/89) of the infants with PVE on cUS these were seen on the last cUS performed before discharge but no longer on TEA-cUS, we were not able to reliably assess the total duration of PVE. The total duration of PVE was therefore not included in the WM classification. We only performed a single MRI examination around

TEA. Therefore, while cUS also reflected early and/or transient WM changes and the evolution of changes, MRI reflected only the later stages of WM injury. In more than one third of the infants, MRI was performed after TEA, at a time when the signal of the WM may have changed. However, the predictive values of the WM classification of cUS for that of MRI calculated for the subgroup of preterm infants with TEA-cUS and TEA-MRI between 40 and 44 weeks' PMA did not differ from those for the total group of infants. Besides, due to ongoing brain maturation, the signal of the WM increases on T_1- and decreases on T_2-weighted images, while it generally decreases on T_1- and increases on T_2-weighted images due to diffuse injury. We therefore feel that including infants scanned beyond TEA has not influenced our data. We only measured the width of frontal horns of the lateral ventricles, while in some cases widening of the lateral ventricles may be more prominent posteriorly. However, by also visually assessing the shape of the lateral ventricles, we probably did not overlook other changes to the lateral ventricles. As reference values for VI in preterm infants around TEA are unavailable, the grouping of VI in the WM classification was based on VI values obtained by us in low-risk (near) term neonates (unpublished data). However, as the same grouping was used for TEA-cUS and TEA-MRI, we feel that this will probably not have influenced our results. We chose not exclude infants with intraventricular hemorrhage, as these may well have concomitant WM injury. Nine infants with intraventricular hemorrhage complicated by post hemorrhagic ventricular dilatation were included. Due to the ventricular dilatation, these nine infants may have been allocated to a more severe WM classification group. As the VI was measured both on TEA-cUS and TEA-MRI, we feel this will probably not have influenced our results. In addition, in our WM classification, focal echodensities, mostly diagnosed as PVHI, and porencephalic cysts, were classified as severely abnormal, regardless of their size, while bilateral, inhomogeneous PVE not evolving into multicystic lesions were classified as moderately abnormal. One may question this classification, as widespread, inhomogeneous PVE may be more serious than a small focal echodensity. However, also for the MRI classification porencephalic cysts were classified as severely abnormal. We therefore feel this will not significantly have influenced our results. Brain imaging findings (cUS and MRI) were scored by two experienced investigators. This was done in consensus and no interobserver agreement was calculated. Finally, we are not yet informed on the clinical significance of our WM classifications and the implications of the milder and more subtle WM changes, including PVE without cystic evolution on cUS and PWML and more diffuse SI changes on MRI, for neurological outcome. Follow up data are currently obtained.

In conclusion, sequential cUS, even when performed frequently and being of good quality, may underestimate WM injury in very preterm infants. Therefore, while await-

ing clinical follow up data on our and other study-populations, routine MRI around TEA in addition to serial cUS during admission seems needed in infants born very prematurely.

Future studies on WM injury in preterm infants should focus on the clinical significance of the WM classifications and of the separate changes, both on cUS and MRI, indicative of WM injury.

Acknowledgements

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6

Ultrasound detection of white matter injury in very preterm neonates: practical implications

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Abstract

Aim: Diffuse white matter injury is not well detected by cranial ultrasonography (CUS). The aim of the study was twofold:

- To assess in very preterm neonates the predictive values of individual CUS abnormalities for white matter injury on MRI and neurological outcome
- To develop a strategy optimizing CUS detection of white matter injury.

Method: Very preterm neonates (n=67; 44 males) underwent serial CUS and single MRI. Predictive values of CUS findings for a white matter classification on MRI, individual MRI findings and neurological outcome at 2 years corrected age were calculated. The effects of timing and frequency of CUS were evaluated.

Results: Periventricular echo densities (PVE) predicted abnormal white matter on MRI, but absence of PVE did not predict absence of white matter changes. Peri- and intraventricular hemorrhage (P/IVH) was highly predictive of abnormal white matter on MRI. Frequency and timing of CUS did not influence predictive values.

P/IVH and abnormal ventricular size/shape were reasonably predictive of unfavorable outcome, whereas absence of CUS abnormalities predicted a favorable outcome.

Interpretation:

1. If PVE are present, there is a significant chance of abnormal white matter on MRI.
2. Increasing frequency of CUS does not increase its diagnostic performance for white matter injury.
3. P/IVH is highly predictive of abnormal white matter on MRI and reasonably predictive of unfavorable outcome.
4. Absence of PVE or P/IVH on CUS does not guarantee normal white matter, but predicts a favorable outcome.

INTRODUCTION

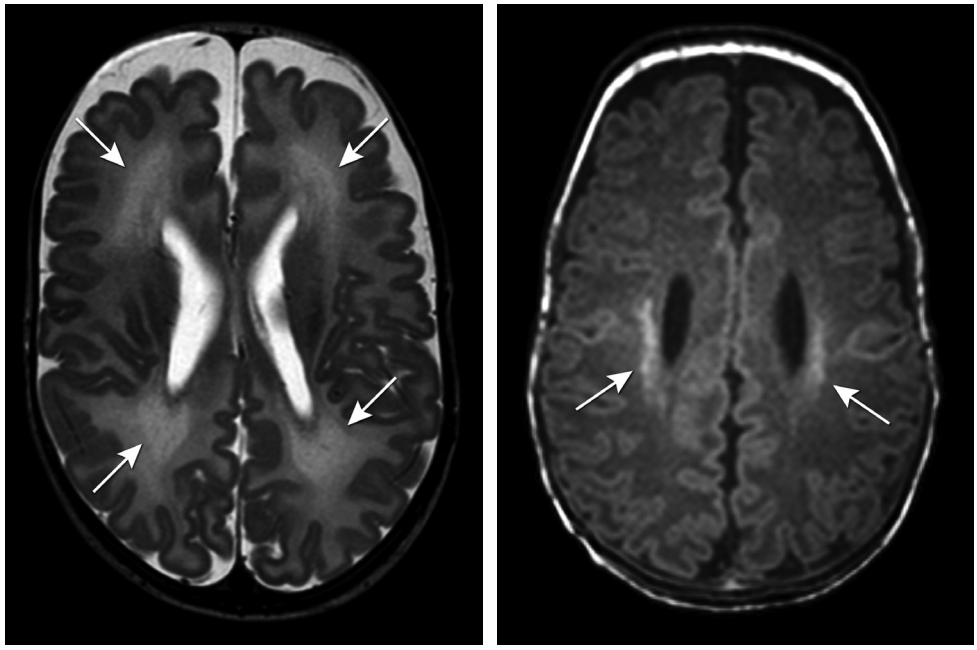
White matter (WM) injury is one of the most frequently occurring forms of brain injury in infants born very prematurely (gestational age <32 weeks).

During recent decades, the character of WM injury has shifted from "classic periventricular leukomalacia" to more subtle or diffuse WM injury. The latter, occurring in more than 80% of infants born very preterm, cannot be reliably diagnosed by ultrasonography.[1-4] On T2-weighted MRI, it is assumed to be represented by areas of altered signal intensity throughout the WM, so-called diffuse excessive high signal intensity (DEHSI) (Figure 1) and by focal signal intensity changes, so-called punctate white matter lesions (PWML).[4-6] PWML are better visualized on T1-weighted MRI (Figure 2).

Although MRI is increasingly used in neonates and has proved safe and reliable to detect various forms of neonatal brain injury, it is not suitable for repetitive examina-

Figure 1. (left) T2-weighted MR image in a very preterm infant, scanned at TEA, showing DEHSI in the frontal and occipital WM (arrows). The figure also shows mildly dilated and abnormally shaped lateral ventricles.

Figure 2. (right) T1-weighted MR image in a very preterm infant, imaged around TEA, showing bilateral, multiple, confluent PWML (arrows) in the central WM.



tions.[7,8] Therefore, cranial ultrasonography (CUS) is still the preferred modality for serial neuro-imaging during the neonatal period.[9] In this perspective it is important to develop a CUS screening system, enabling optimal detection of WM injury and selection of neonates needing MRI. A recent study, considering MRI as the criterion standard, showed a good reliability of CUS in very preterm infants with severely abnormal WM. However, CUS was less reliable in demonstrating mild and moderate WM abnormalities.[4] In that study, CUS and MRI classifications of WM injury were introduced, based on respectively echogenicity- and signal intensity changes in the WM and on loss of WM volume. However, we did not test the performance of individual CUS findings for predicting WM injury and did not analyse the influence of frequency and timing of CUS examinations on the reliability of CUS.

Early detection of brain injury may be important for timely intervention in high-risk neonates. With the present study we aim to develop a strategy to optimize CUS detection of WM injury, focusing on specific CUS findings and on the number and timing of CUS examinations. We hypothesized that specific CUS findings, including inhomogeneous and grade 2 periventricular echo densities (PVE), are predictive of WM injury on MRI.[10] In addition we hypothesized, based on the results of former studies that PVE on CUS predict DEHSI on MRI and that increasing the number of CUS examinations increases the reliability of CUS for detecting WM injury.[10,11] The specific aims were to assess the predictive values of individual CUS findings for:

1) The WM classification on MRI
2) Individual MRI findings
3) Neurological outcome at 2 years corrected age

We also assessed whether:

4) Increasing the number of CUS examinations increases the reliability of CUS for detecting WM injury and
5) Timing of CUS examinations influences the reliability of CUS for detecting WM injury

METHOD

Patients

Between May 2006 and October 2007 eligible infants, born very preterm and admitted to our tertiary neonatal unit were included in a neuro-imaging study, comprising serial CUS, according to the standard of care for these neonates[9] and a single MRI, preferably performed around term equivalent age (TEA). The study was approved by the Medi-

cal Ethics Committee and informed consent was obtained from the parents. Results on prevalence of CUS and MRI abnormalities and on prediction of WM injury by CUS, based on the WM classification, were published elsewhere.[1,4,12]

Cranial Ultrasonography

CUS examinations were performed within 24 hours after birth, thereafter at least weekly until discharge or transfer to another hospital and again around TEA, on the same day as the MRI, according to a standard protocol.[9]

All CUS examinations were assessed by LML and GvW-M or LML and SJS, focusing on individual CUS findings. The following echogenicity changes in the WM were recorded: non-physiological PVE, cystic lesions (small and localized or more extensive), and focal WM echodensity presenting periventricular hemorrhagic infarction. The appearance of PVE (homogeneous, inhomogeneous and grade 1 or grade 2) was noted.[11] In addition, peri-and intraventricular hemorrhages (P/IVH) grade 1 to 3 according to the classification of Volpe,[13] and the size and shape of the lateral ventricles were recorded. The latter was only done for the CUS examination performed around TEA. The size and shape of the ventricles were visually scored as normal/mildly abnormal, moderately abnormal and severely abnormal.

MRI

MRI examinations were performed according to a standard protocol for imaging the newborn infant's brain, using a 3.0 T MR system (Philips Medical Systems, Best, the Netherlands) as recently described.[7] In summary, scans included at least 3D T_1-weighted gradient echo MRI (TR/TE: 9.7/4.6 ms, flip angle 8), T_2-weighted TSE MRI (TR/TE: 6269/120 ms, turbofactor 18), diffusion-weighted images in three directions (TR/TE: 2406/64 ms) and T2* susceptibility-weighted MRI (TR/TE: 735/16 ms) in the transverse plane. All MRI examinations were assessed by LML and FTdB or by SJS and FTdB, who were blinded to the CUS findings. For this part of the study the T_1- and T_2-weighted images were assessed. Particular attention was paid to the WM by using a recently described classification to score the grade of WM injury. In summary, the WM was scored as normal or mildly abnormal if no signal intensity changes or only homogeneous DEHSI and/or few (≤6) PWML were seen and if the shape and size of the lateral ventricles were normal or only mildly abnormal. A moderately abnormal WM score was applied if multiple (>6) PWML and/or small localized cystic lesions and/or inhomogeneous DEHSI and/or moderately abnormal lateral ventricles were seen. The WM was scored as severely abnormal in the case of more serious abnormalities.[4]

Follow up

Around 2 years of age, the infants were seen by an experienced neonatologist for clinical follow up. The children underwent a standardized neurologic examination to assess the presence of cerebral palsy or abnormal muscular tone.

Cognitive and psychomotor development was assessed using the Dutch version of the Bayley Scales of Infant Development (BSID-III). A mental developmental index score and psychomotor developmental index score were calculated for the corrected age. A score of ≥ 1 SD below the normative mean was defined as a developmental delay.

Statistical methods

Firstly, sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) of individual CUS findings (presence, aspect, duration and grade of PVE; focal WM echodensity; P/IVH; and shape and size of the lateral ventricles on the CUS performed around TEA) for the MRI WM classification were calculated. Secondly, predictive values of PVE on CUS for DEHSI on MRI and of inhomogeneous PVE on CUS for PWML on MRI were calculated.

In addition, to explore whether increasing the number of CUS examinations improved the predictive value of CUS for MRI, the predictive values of PVE present on the first CUS examination and also respectively on any CUS performed during the first week, first 2 weeks or first 4 weeks of life were calculated. Furthermore, to assess the influence of timing of CUS examinations on the predictive value of CUS, predictive values of PVE, seen in a certain period (first, second, third, or fourth week after birth) were calculated. For statistical analysis, WM on MRI was divided into two groups: normal and mildly abnormal versus moderately and severely abnormal WM. The aspect of the lateral ventricles on CUS and MRI was also divided in two groups: normal versus abnormal shape and/or size of the lateral ventricles.

Finally, the predictive values of individual CUS findings for unfavorable neurological outcome at 2 years corrected age, defined as either a developmental delay and/or cerebral palsy, were calculated.

Results

Patients

A total of 130 very preterm infants were included in the study of which 108 underwent MRI. In 67 of the 108 infants, MRI was performed before the postmenstrual age of

Table 1. General characteristics and ultrasound findings of total group included (N=108) and group with MRI at PMA < 44 weeks (N=67).

General characteristics	Total group	PMA at MRI < 44 wks
Patients included	108	67
Male (%)	67 (62.0)	44 (65.7)
Mean weight at birth (range)	1205.7 (585-1960)	1228.2 (585-1960)
Mean GA in weeks (range)	29.0 (25.6-31.9)	28.9 (25.6-31.2)
Mean PMA at MRI in weeks (range)	44.9 (39.1-62.1)	42.5 (39.1-44.0)
MRI findings		
Normal/mildly abnormal MRI (%)	26 (24.1)	18 (26.9)
Moderately/severely abnormal MRI (%)	82 (75.9)	49 (73.1)
DESHI on MRI (%)	76 (70.4)	59 (88.1)
>6 PWML on MRI (%)	18 (16.7)	13 (19.4)
CUS findings		
PVE (%)	87 (80.6)	55 (82.1)
Grade 2 PVE (%)	11 (10.2)	8 (11.9)
Inhomogeneous PVE (%)	72 (66.7)	46 (68.7)
Duration PVE > 14 days (%)	43 (39.8)	29 (43.3)
P/IVH grade 1-2 (%)	23 (21.3)	15 (22.4)
P/IVH grade 3 (%)	8 (7.4)	4 (6.0)
Abnormal size and/or shape ventricles (%)	56 (51.9)	28 (41.8)

44 weeks. In the other 41 infants, MRI was postponed owing to their instable condition. As DEHSI is currently only described in children scanned around TEA, statistical analysis was confined to the 67 infants with MRI around TEA.

Table 1 shows the general characteristics and CUS findings of the whole study group and the subgroup of 67 infants scanned around TEA.

Predictive value of CUS for MRI

The predictive values of the CUS findings for the MRI WM classification are listed in Table 2. This table shows that presence of PVE, regardless of characteristics (aspect, grade and duration) is predictive of abnormal WM on MRI. However, absence of PVE does not predict normal WM.

In addition, presence of P/IVH is highly predictive of abnormal WM on MRI, but again with a low NPV. Repeating our analysis for the 48 children without P/IVH, we found comparable predictive values of PVE for abnormal WM. Abnormal size and/or shape of the lateral ventricles, as seen around TEA, was also highly predictive of abnormal WM, whereas normal size and shape of the ventricles did not predict normal WM.

Table 2. Predictive values of individual ultrasound findings for WM classification on MRI, of PVE for DESHI[§] and of inhomogeneous PVE for PWML[§§§] for patient group with MRI at PMA < 44 weeks (N=67),

	WM classification on MRI			
CUS finding	**Sensitivity**	**Specificity**	**PPV**	**NPV**
PVE	81.6 (40/49)	16.7 (3/18)	72.7 (40/55)	25.0 (3/12)
Inhomogeneous PVE	67.3 (33/49)	27.8 (5/18)	71.7 (33/46)	23.8 (5/21)
Grade 2 PVE	14.3 (7/49)	94.4 (17/18)	87.5 (7/8)	28.8 (17/59)
Duration > 14 days	46.9 (23/49)	66.7 (12/18)	79.3 (23/29)	31.6 (12/38)
P/IVH grade 3	8.2 (4/49)	100.0 (18/18)	100.0 (4/4)	28.6 (18/63)
P/IVH grade 1-3	34.7 (17/49)	88.9 (16/18)	89.5 (17/19)	33.3 (16/48)
Size and shape ventricles	55.1 (27/49)	94.4 (17/18)	96.4 (27/28)	43.6 (17/39)
	DESHI and PWML on MRI			
CUS finding	**Sensitivity**	**Specificity**	**PPV**	**NPV**
PVE[§]	81.4 (48/59)	12.5 (1/8)	87.3 (48/55)	8.3 (1/12)
Inhomogeneous PVE[§§§]	69.2 (9/13)	31.5 (17/54)	19.6 (9/46)	81.0 (17/21)

We found no obvious improvement of predictive values, increasing the number of CUS examinations: if PVE were seen in the first week, this predicted abnormal MRI in 73% of infants, with a sensitivity of 78%, specificity of 31% and NPV of 42%. While if PVE were also seen in 4 consecutive weeks after the first week, the PPV remained 73%, and the sensitivity and NPV increased slightly towards respectively 87% and 50%, but with a specificity decreasing to 25%. In addition, there was no influence of age at CUS examination on the predictive values of CUS: predictive values of PVE seen in the first week were in the same range as those seen at later age.

Calculating predictive values of individual CUS findings for individual MRI findings, we found high PPV and sensitivity of PVE for DEHSI, the NPV again being low. Inhomogeneous PVE did not predict PWML, but the NPV was high (Table 2).

Follow up

Of the 67 infants who underwent MRI before the postmenstrual age of 44 weeks, follow up was available for 50 (75%). A total of 7 children (14%) had an unfavorable outcome at 2 years corrected age. Two children had a mental developmental index score ≥ 1 SD below the standard mean, six had a psychomotor developmental index score ≥ 1 SD below the standard mean and four had cerebral palsy. All the children with cerebral palsy had a psychomotor delay and one also a mental delay.

Predictive value of CUS for outcome

P/IVH and abnormal size and/or shape of the ventricles were predictive of outcome at 2 years corrected age (PPV respectively 34 and 31%, negative predictive values 94%). The PPV of the other CUS findings were 14 to 17%, with NPV 88 to 93%, indicating a high chance of a normal outcome when the CUS finding was absent.

Discussion

WM injury is probably responsible for the most disabilities in very preterm neonates.[14] As CUS is the most frequently used imaging modality for detecting brain injury in high-risk neonates, its detection of WM injury may be useful in targeting interventional therapy. This study assessed the predictive values of individual CUS findings for WM injury on MRI and the influence of timing and frequency of CUS, aiming to optimize CUS protocols for detecting WM injury. In addition, we assessed the predictive values of individual CUS findings for neurologic outcome at 2 years corrected age. We found that presence of PVE on CUS was predictive of abnormal WM on MRI. However, absence of PVE was not predictive of normal WM. In other words, absence of changes in the WM on CUS is no guarantee of a normal MRI. In addition, increasing the number of CUS examinations and varying the timing of CUS did not influence the reliability of CUS for detecting WM injury.

To our surprise P/IVH were more predictive of abnormal WM on MRI than PVE. This may be for several reasons: First, P/IVH is reliably detected by CUS, therefore false positive and false negative diagnoses of P/IVH are rare.[1] Second, P/IVH originates from and might damage the germinal matrix. This might have consequences for further development of glial cell precursors and astrocytes, originating from the germinal matrix, possibly contributing to WM injury.[15] In addition, elevated free iron in the cerebrospinal fluid resulting from intraventricular hemorrhage might catalyse radical formation and WM injury may ensue. Furthermore, even mild ventricular dilatation may influence WM development and P/IVH may cause microglial activation.[16]

We additionally found that presence of PVE on CUS predicted DEHSI on MRI. In recent literature there is doubt whether DEHSI indeed presents WM injury.[17-19] We therefore feel it is not justified to conclude that PVE are the CUS representative of diffuse WM injury. We found no association between inhomogeneous PVE and PWML. This seems to be in conflict with results of an older study, in which a fair association between inhomogeneous PVE on CUS and PWML on MRI was found. However, the latter study was retrospective, fewer infants were included and the MRI was performed at variable ages, often during the preterm period.[10]

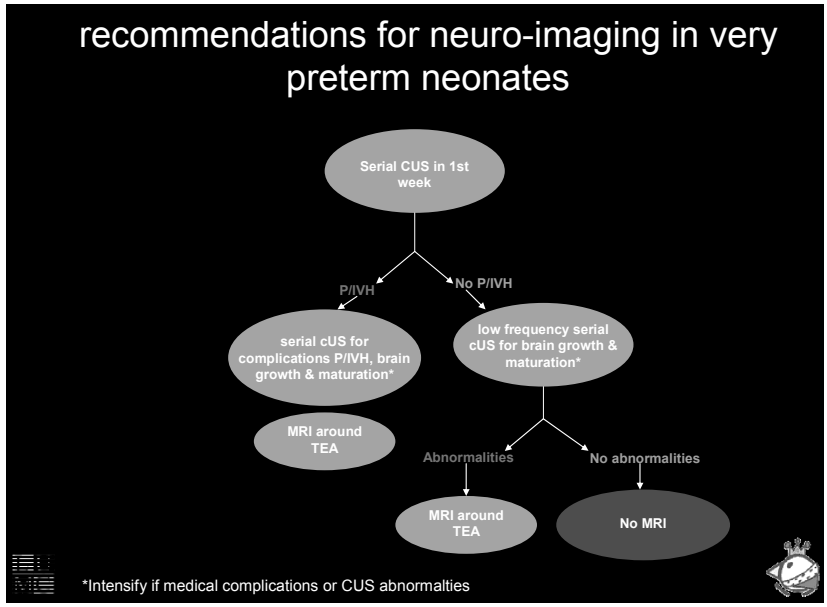
P/IVH and abnormal lateral ventricles were reasonably predictive of unfavorable outcome at 2 years corrected age, whereas all the other individual CUS findings did not predict unfavorable outcome. This is partly in agreement with another study, finding a higher incidence of neurological abnormality during the first year of life in very preterm infants with prolonged PVE, P/IVH grade 2 or 3, and ventricular dilation than in infants without these CUS abnormalities.[20] Amess *et al*.[21] assessed the predictive value of CUS for neurological outcome at 12 months corrected age and found high risk CUS findings to be predictive of abnormal neurological outcome (sensitivity 83%), but their high risk CUS findings included more serious abnormalities than the individual CUS findings we assessed. Rademaker *et al*.[22] found significant differences in motor and mental outcome at school age between very preterm infants with normal/mild CUS findings and severely abnormal CUS findings. They included more infants and their follow up period was much longer. De Vries *et al*.[23] in a large prospective study among preterm infants found major CUS abnormalities to be highly predictive of cerebral palsy. Again, their major CUS abnormalities were more severe and differed from the individual CUS findings we assessed. Comparison of our study with the aforementioned studies is difficult, because of the changes in the character and definition of WM injury over recent years. We found high negative predictive values of CUS for outcome around 2 years corrected age, indicating a high chance of a normal outcome when CUS abnormalities were absent. This is in accordance with the study by de Vries *et al*.[23] showing high negative predictive values of absence of major CUS abnormalities for cerebral palsy around 2 years of age.

We acknowledge the limitations of this study: First, MRI could not be performed around TEA in all patients. We could therefore only analyse the data in a subgroup of our infants, implying a limited number of patients per WM group and with individual CUS and MRI findings. Therefore the number of infants with some individual CUS findings, especially P/IVH grade 3 and PVE grade 2, was too low to draw final conclusions about the prediction of these CUS findings for unfavorable outcome. Secondly, it is uncertain whether DEHSI represents WM injury. Finally, our follow up period is short. More subtle cognitive deficits, with possible consequences on school performance, may still develop.

With respect to these limitations, we make the following conclusions:

- If PVE is present on CUS, regardless of timing, duration, and appearance, there is a significant chance of abnormal WM on MRI.
- If PVE is seen any time during the neonatal period, additional CUS examinations do not increase the diagnostic performance of CUS for detecting WM abnormality. Therefore, increasing the number of CUS examinations in these cases is of limited clinical importance.

Figure 3.



- P/IVH is highly predictive of abnormal WM on MRI.
- Absence of PVE and P/IVH on CUS does not guarantee normal WM on MRI.
- P/IVH and ventricular dilatation, but not PVE, seems to be reasonably predictive of abnormal neurological outcome at 2 years corrected age.
- Absence of any CUS abnormality in the WM or lateral ventricles is highly predictive of a normal outcome at 2 years corrected age.

The practical consequences of these conclusions are as follows (Figure 3):

1. In very preterm neonates, CUS examinations in the first week of life are necessary to detect P/IVH.
2. If P/IVH is seen during the first week, frequent follow up CUS is indicated for detecting complications that may need intervention (24).
3. If no P/IVH is seen in the first week, low frequency CUS examinations throughout the neonatal period are indicated to follow the brain growth and maturation and to detect changes related to clinical instability.
4. If medical complications or instability occur, CUS examinations should be intensified.
5. For reliable detection of WM injury in very preterm infants an MRI examination, performed around TEA is needed. This is, however, of little clinical relevance in infants without any CUS abnormality.

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7

Clinical implications of MR imaging findings in the white matter in very preterm infants; a two-year follow up study

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Abstract

Purpose: To explore the association between diffuse excessive high signal intensity (DEHSI), punctate white matter lesions (PWML) and ventricular dilatation around term equivalent age (TEA) and clinical follow up at 2 years in very preterm infants.

Material and Methods: Ethical approval for this prospective study was given by the institutional review board and informed parental consent was obtained. An unselected cohort of 110 preterm infants (gestational age <32 weeks) were imaged around or after TEA. Clinical follow up was performed at a corrected age of 2 years and consisted of a neurological examination and a mental and developmental assessment (Bayley Scales of Infant Development). Univariate analyses and logistic and linear regression were performed to examine relationships between variables.

Results: DEHSI was found in 58 of 65 (89%) infants imaged around TEA. DEHSI was never detected in infants imaged after postmenstrual age of 50 weeks and showed no association with neurodevelopmental outcome. PWML and ventricular dilatation were significantly associated with mental ($p=0.02$ for PWML) and psychomotor developmental delay ($p<0.001$ and $p=0.03$, respectively), motor delay ($p=0.002$ and $p=0.02$, respectively) and cerebral palsy ($p=0.01$ and $p=0.03$, respectively).

Conclusion: Because of its high incidence in preterm infants around TEA, its absence after a postmenstrual age of 50 weeks, and its association with normal neurological outcome at a corrected age of 2 years, DEHSI should not be considered part of the spectrum of WM injury, but rather a prematurity related developmental phenomenon.

Introduction

Cystic periventricular leucomalacia and periventricular venous infarction are severe forms of white matter (WM) injury in preterm infants and are associated with abnormal neurological outcome.[1-5] Over the last years, there has been a gradual change in incidence, from cystic periventricular leucomalacia to more diffuse WM injury, where the majority of very preterm infants now show more subtle abnormalities of the developing WM.[6-9] Magnetic resonance (MR) imaging findings such as diffuse excessive high signal intensity (DEHSI) and punctate white matter lesions (PWML) have been assumed to represent a diffuse and subtle form of WM injury in preterm infants.[5,7,10-14] Diffuse WM injury is generally held responsible for the high incidences of mental and behavioural disorders in very preterm born infants.[3,4,14-17] Due to the lack of histological proof and an indistinct association with neurological outcome, it remains uncertain whether cystic periventricular leucomalacia, DEHSI and PWML are a continuum of disorders resulting from an injurious process of the developing WM.[3,4,18] Several classifications of WM injury have been proposed.[6,14,19] Some of these have included DEHSI.[19-21] However, on the basis of the very high incidence of DEHSI found in very preterm infants who are imaged around term equivalent age (TEA).[6,7,14] We hypothesize that DEHSI reflects a prematurity related developmental phenomenon. PWML seem to be associated with microstructural changes in the WM and impaired brain development and neurological function around TEA.[22-24] In addition, alterations in brain volumes and ventricular dilatation are thought to reflect diffuse WM injury and may be associated with early adverse neurodevelopmental outcome.[11,25,26]

Since the impact of DEHSI, PWML and ventricular dilatation on neurodevelopment is still unclear, we aimed to explore the association between DEHSI, PWML and ventricular dilatation around TEA and clinical follow up at the corrected age of 2 years in an unselected cohort of very preterm infants (gestational age <32 weeks).

Patients and Methods

Preterm infants

As part of a prospective neuro-imaging study performed in an unselected group of very preterm infants (gestational age <32 weeks), admitted to the tertiary neonatal unit of our hospital between May 2006 and October 2007, 113 infants were included for MR imaging around or within four months of TEA. Ethical approval for the study was given by the institutional review board and informed parental consent was obtained for each infant.

MR imaging was preferably performed between 40 and 44 weeks postmenstrual age, but was postponed in infants who were still clinically unstable and could not be transported to the MR imaging unit at that age.

Three infants were excluded from this part of the study because of congenital nervous system abnormalities, diagnosed on their MR images, resulting in 110 very preterm infants. The mean age of the whole group was 29.4 weeks ±2.0 (standard deviation), with a range of 25.6-31.9 weeks. There were 68 male (mean age 28.8 weeks ±2.0, range 25.6-31.9 weeks) and 42 female (mean age 29.6 weeks ±2.0, range 25.7-31.9 weeks) infants.

Baseline data on WM injury and cerebellar injury and incidences of abnormalities in this cohort have been published previously.[7,24,25]

Perinatal characteristics

To adjust for other factors that may influence neurodevelopmental outcome, relevant clinical parameters[3,24,26] were collected from the patient files (Table 1).

Image and data acquisition

All MR imaging examinations were performed with a 3 Tesla MRI system (Philips Medical Systems, Best, the Netherlands) according to a standard protocol for imaging the newborn infant's brain.[27] The infants were sedated by using chloral hydrate (55mg per kilogram of body weight), laid supine and snugly swaddled during the scanning procedure. Ear protection consisted of neonatal earmuffs (Natus Mini Muffs; Natus Medical Inc., San Carlos, CA, USA) covered by a headphone. All MR examinations included a 3D T1-TFE sequence (TR 9.7 ms, TE 4.6 ms, FOV 180 mm, matrix size 192 x 152, flip angle 8, TFE factor 128, slice thickness 1 mm) and a T2-TSE sequence (TR 6269 ms, TE 120 ms, FOV 180 mm, matrix size 336 x 234, turbo factor 18, slice thickness 2 mm).

DEHSI, PWML and ventricular dilatation

All T1-W and T2-W images were analyzed at the same time in one consensus reading by two investigators (FTdB, pediatric neuroradiologist with more than 15 years of experience and LML, research physician with more than 4 years of experience) for presence of DEHSI, PWML and ventricular dilatation.

DEHSI was defined as regions of high signal intensity in the periventricular frontal and parieto-occipital area on T2-W images, corresponding to a low signal intensity on the T1-W images and approaching the signal intensity of cerebrospinal fluid[5,7] (Figure 1).

Table 1. Clinical characteristics and MRI findings (n=110).

Categorical clinical parameters	
	n (%)
Male	68 (61.8)
Female	42 (38.2)
GA < 28 wk	39 (35.5)
Birth weight < 1000 g	35 (31.8)
Plurity	33 (30.9)
IUGR	12 (10.9)
Antenatal corticosteroids	70 (63.6)
Perinatal infection	48 (43.6)
RDS	59 (53.6)
PDA	28 (25.5)
Hypotension	32 (29.1)
BPD	49 (44.5)
Dexamethasone treatment	18 (16.4)
Continuous clinical parameters	
	Mean, SD
GA (weeks)	29.0 ± 2.0
Birth weight (gram)	1214.5 ± 369.0
Head circumference at birth (cm)	25.8 ± 2.3
GA at MRI (weeks)	44.9 ± 3.9
Weight at MRI (gram)	4035.6 ± 839.2
Head circumference at MRI (cm)	37.6 ± 1.7
MRI Findings	
	n (%)
DESHI	76 (69.1)
PWML	29 (26.4)
Normal-mildly dilated ventricles	29 (26.4)
Moderately dilated ventricles	57 (51.8)
Severely dilated ventricles	22 (20.0)

GA=gestational age, IUGR=intra uterine growth retardation, RDS=respiratory distress syndrome, PDA=patent ductus arteriosus, BPD=bronchopulmonary dysplsia, SD=standard deviation, DEHSI=diffuse excessive high signal intensity, PWML=punctate white matter lesions.

PWML were assessed on T1-W and T2-W images as punctate respectively high and low signal intensity lesions, more pronounced on T1-W than on T2-W images. For differentiation from small hemorrhagic lesions, they were only defined as PWML if they were not visible on T2*- W gradient echo sequences (Figure 2).[5,12,14,23,28]

Figure 1. T2-W MR image in a very preterm neonate at around term equivalent age shows diffuse excessive high signal intensity (DEHSI) in the frontal and occipital areas (arrows).

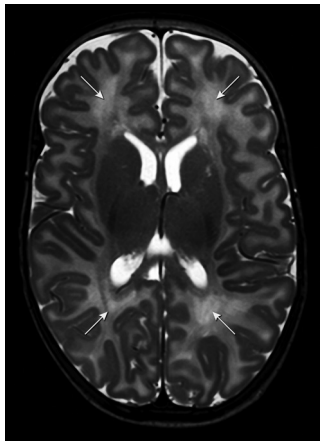
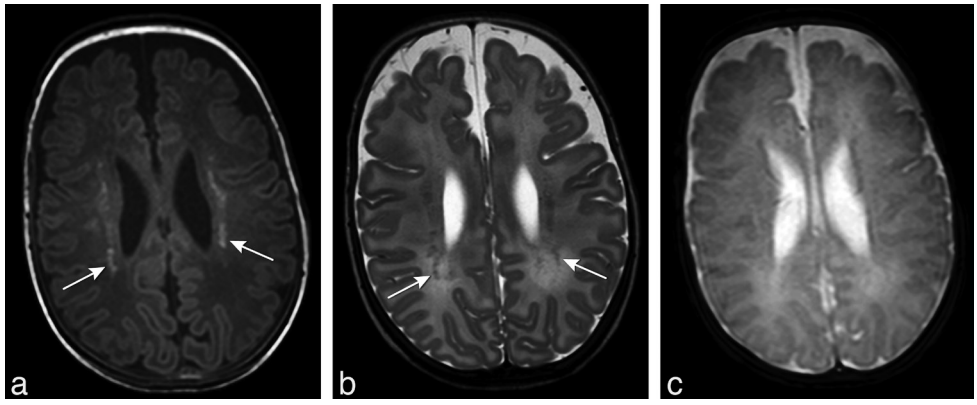


Figure 2. Punctate white matter lesions (PWML) (arrows) appear as high signal intensity lesions on (a) T1-W MR image and as less pronounced low signal intensity lesions on (b) T2-W MR image , but are not visible on (c) T2-W gradient echo MR image, which is susceptible to hemorrhages or calcification.*



Infants with PWML were categorized in two groups: those with 6 or fewer PWML and those with more than 6 PWML, adapted from the WM abnormality grading by Miller *et al*.[10]

Ventricular size was measured on coronal reconstructions of T1-W three dimensional images.[29] A ventricular size of less than 12 mm was considered normal or mild dilata-

tion, a ventricular size between 12 and 16 mm was considered moderate dilatation and a ventricular size greater than 16 mm was considered severe dilatation.[21]

Clinical follow up of very preterm infants

Around 2 years of age corrected for prematurity, the infants were seen by an experienced neonatologist, who was unaware of the neuro-imaging findings. Each child underwent a standardized neurological examination to assess presence of cerebral palsy or abnormal muscular tone. Gross Motor Function Classification System (GMFCS) level was assigned.[30] A level of 2 – 3 was considered moderate cerebral palsy and a score of 4 – 5 was considered severe cerebral palsy.

The mental and psychomotor development was assessed by a psychological assistant, who was unaware of the neuro-imaging findings, using the Dutch version of the Bayley Scales of Infant Development (BSID, version 3). A mental developmental index score (MDI) and a psychomotor developmental index score (PDI) were calculated for the corrected age of the child. A score of at least 1 standard deviation below the normative mean was defined as a moderate delay in development, and a score of 2 or more standard deviation below the normative mean was defined as a severe delay in development. Five infants, with a diagnosis of cerebral palsy, in whom testing of the gross and fine motor function with the BSID was not feasible, were assigned a PDI score of 50.

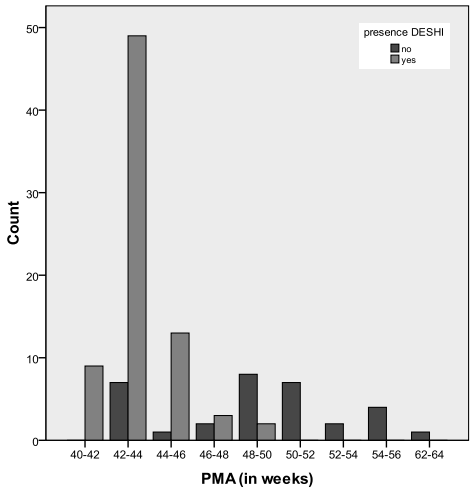
To evaluate behavior, the Dutch version of the Child Behavior Checklist (CBCL)[31] was sent to the child's address prior to the follow up visit, to be completed by either parent or care giver. The questionnaire consists of 99 items rated on a three-point scale and yields three summary indexes (internalizing, externalizing and total problems) with associated, age-standardized T-scores (mean=50, standard deviation =10). Higher scores indicate increasing behavioral problems.

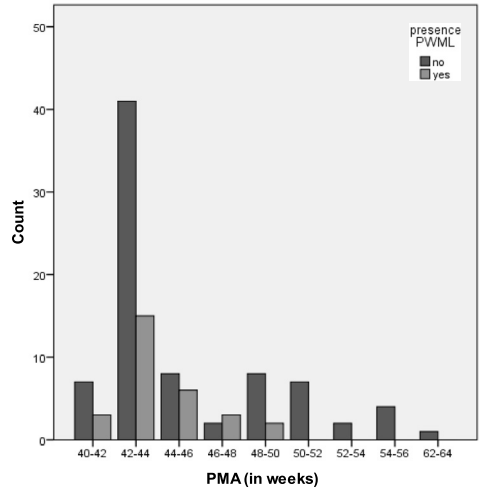
Statistical analyses

Data were analyzed by using SPSS 17.0 software for Windows (SPSS, Chicago, Illinois). Frequency counts and percentages were used to summarize categorical variables. For continuous variables, mean and standard deviation were reported.

Baseline characteristics of infants with and those without follow up at a corrected age of 2 years were compared to assess if selective loss to follow up occurred. Categorical variables were compared by chi-square or Fisher-exact tests, where appropriate. For categorical variables in ordered categories, chi-square for linear trend was used. Differences between groups for continuous variables were evaluated using Student t-test and one-way analysis of variance (ANOVA) or Mann-Whitney U test, if data were skewed.

Figure 3. (left) Number of children with DESHI on MRI per age group.
Figure 4. (right) Number of children with PWML on MRI per age group.





To estimate the effect of the separate MR imaging findings on neurodevelopmental outcome, odds ratios (OR) and 95 percent confidence interval (95% CI), derived from chi-square analyses, were reported. Logistic regression was applied to correct for confounding clinical characteristics (characteristics that reached a p-value <0.05 in the univariate analysis), resulting in adjusted OR. For continuous variables, linear regression was used to assess predictive characteristics.

Two-sided tests were used throughout and a p-value below <0.05 was considered to indicate a statistically significant difference.

RESULTS

Table 1 summarizes the clinical characteristics and MR imaging findings (DEHSI, PWML and ventricular dilatation) in 110 very preterm infants. DEHSI was encountered in nearly 70% (76 of 110) of the total group of infants, and in 89% (58 of 65) of infants imaged around TEA (postmenstrual age 40 – 44 weeks). PWML were seen in 26% (29 of 110) of infants. More than half (57 of 110) of infants had moderately dilated ventricles and 20% (22 of 110) had severely dilated ventricles. The distribution of DEHSI and PWML per age group is shown in Figures 3 and 4.

Table 2. Neurodevelopmental outcome at a corrected age of 2 years (n=86)

Loss to follow up	24/110 (22.0%)
MDI (mean, sd)	96.1 ± 12.3
Any mental delay	5/81 (6.2%)
Moderate mental delay	2/81 (2.5%)
Severe mental delay	3/81 (3.7%)
PDI (mean, sd)	94.3 ± 14.4
Any motor delay	7/72 (9.7%)
Moderate motor delay	2/72 (2.8%)
Severe motor delay	5/72 (6.9%)
Cerebral Palsy	6/84 (7.1%)
Severe cerebral palsy	1/84 (1.2%)
Abnormal muscular tone	6/84 (7.1%)
Severely abnormal muscular tone	2/84 (2.4%)
CBCL	
Internalizing problems (mean, sd)	48.1 ± 9.6
Externalizing problems (mean, sd)	50.4 ± 9.5
Total problems (mean, sd)	49.1 ± 9.2

MDI=mental developmental index score, PDI=psychomotor developmental index score, CBCL=child behavior checklist, sd=standard deviation

Neurodevelopmental outcome

At a corrected age of 2 years 86 of 110 (78%) children were seen for follow up. The educational level of the infants' mothers was representative of the Dutch population, whereby 13 of 77 (17%) had completed primary school, 30 (39%) had completed high school and 34 (44%) had completed college or university. Twenty seven of 86 children (31.4%) were twins or triplets. Twenty four infants were lost to follow up (22%). No differences in clinical parameters or occurrence of DEHSI, PWML and ventricular dilatation existed between infants with and those without clinical follow up. Mean MDI was 96.1 (standard deviation 12.3) and mean PDI 94.3 (standard deviation 14.4). Mental delay was seen in five of 81 (6.2%), motor delay was seen in seven of 72 (9.7%) infants, and cerebral palsy and abnormal muscular tone were seen in six of 84 (7.1%) infants (Table 2). For the CBCL there was a significant difference between singletons and twins or triplets. The latter showed significant fewer behavioral problems ($p<0.001$ for externalizing problems and $p<0.02$ for total problems) than did singletons.

Table 3. Presence of DESHI , PWML, and ventricular dilatation on MR images and Neurodevelopmental outcome at a corrected age of 2 years (n=86)

Outcome Measure	DEHSI			PWML			Ventricular dilatation			
	no (n=26)	yes (n=59)	p-value	no or ≤ 6 (n=67)	> 6 (n=18)	p-value	Normal-mild (n=21)	Moderate (n=45)	Severe (n=18)	p-value
MDI	93.7 ± 10.6	97.2 ± 13.0	0.23	97.5 ± 12.1	89.3 ± 11.1	0.02*	97.2 ± 7.6	96.1 ± 14.3	94.4 ± 12.5	0.79
Any mental delay	2 (7.7)	3 (5.5)	0.65	3 (3.5)	2 (14.3)	0.20	0 (0)	3 (7.1)	2 (12.5)	0.12
Moderate mental delay	1 (3.8)	1 (1.8)	0.54	1 (1.5)	1 (7.1)	0.32	0 (0)	1 (2.4)	1 (6.2)	0.24
Severe mental delay	1 (3.8)	2 (3.6)	1.00	2 (3.0)	1 (7.1)	0.44	0 (0)	2 (4.8)	1 (6.2)	0.31
PDI	94.8 ± 12.9	93.1 ± 16.3	0.66	97.2 ± 11.2	78.9 ± 20.5	< 0.001*	95.2 ± 9.5	96.7 ± 13.0	85.2 ± 21.3	0.03*
Any motor delay	1 (4.3)	6 (12.2)	0.42	2 (3.4)	5 (35.7)	0.002*	0 (0)	3 (8.3)	4 (23.8)	0.02*
Moderate motor delay	0 (0)	2 (4.2)	1.00	1 (1.7)	1 (7.1)	0.35	0 (0)	2 (5.6)	0 (0)	0.98
Severe motor delay	1 (4.3)	4 (8.2)	1.00	1 (1.7)	4 (28.6)	0.004*	0 (0)	1 (2.8)	4 (23.8)	0.008*
Cerebral palsy	1 (3.8)	5 (8.8)	0.66	2 (3.0)	4 (25.0)	0.01*	1 (4.8)	1 (2.2)	4 (25.0)	0.03*
Severe CP	1 (3.8)	0 (0)	0.31	0 (0)	1 (6.3)	0.19	0 (0)	0 (0)	1 (6.2)	0.11
CBCL										
Internalizing problems	48.2 ± 10.2	48.1 ± 9.5	0.96	47.0 ± 8.8	53.5 ± 11.8	0.05	45.5 ± 8.6	50.1 ± 9.8	49.4 ± 9.9	0.22
Externalizing problems	50.1 ± 9.9	50.6 ± 9.4	0.84	49.2 ± 9.2	56.5 ± 9.1	0.01*	49.4 ± 9.9	51.4 ± 10.1	50.1 ± 9.6	0.75
Total problems	49.0 ± 10.1	49.1 ± 8.8	0.98	47.6 ± 8.5	56.4 ± 9.3	0.003*	47.3 ± 8.3	50.7 ± 9.6	48.7 ± 8.7	0.37

Note: Data are means ± standard deviations; otherwise data are number of infants and data in parentheses are percentages. *indicates significant p-value.
DEHSI=diffuse excessive high signal intensity, PWML=punctate white matter lesions
MDI=mental developmental index score, PDI=psychomotor developmental index score, CP= cerebral palsy, CBCL=child behaviour checklist

Relation between MR findings and neurodevelopmental outcome

Table 3 shows the presence of DEHSI, PWML and ventricular dilatation in relation to neurodevelopmental outcome.

Presence of DEHSI was not associated with lower mental (MDI) or psychomotor (PDI) development, or with cerebral palsy. The presence of 6 or more PWML was significantly associated with lower MDI ($p=0.02$) and PDI ($p<0.001$), and more severe motor delay ($p=0.002$) and cerebral palsy ($p=0.01$). These infants also had more total and externalizing behavioural problems according to the CBCL ($p=0.003$ and $p=0.01$ respectively). Ventricular dilatation was associated with lower PDI ($p=0.03$), more severe motor delay ($p=0.02$) and cerebral palsy ($p=0.03$).

Logistic regression, corrected for sex, gestational age, birth weight, bronchopulmonary dysplasia and postmenstrual age at MR imaging, revealed that DEHSI was not predictive of neurological outcome, whereas PWML and ventricular dilatation remained predictive for motor delay (OR 18.38, CI [2.06-164.34] and OR 4.57, CI [1.13-18.47] respectively for PWML and ventricular dilatation). Linear regression showed that 6 or more PWML remained predicative for MDI (adjusted R square = 0.11), externalizing behavioural problems (adjusted R square = 0.22) and total behavioural problems (adjusted R square = 0.17), after adjusting for possible confounding factors.

Discussion

We studied in very preterm infants the association between MR imaging findings in the WM and ventricular dilatation, and neurodevelopmental outcome at a corrected age of 2 years. Our most important finding is that DEHSI, detected in nearly 90% of our very preterm infants around TEA, was not associated with abnormal neurodevelopmental outcome. The fact that DEHSI only occurs during a specific age period, is found in the vast majority of infants imaged around TEA, and in contrast to PWML and ventricular dilatation, is unrelated to neurological outcome, supports our hypothesis that DEHSI is a developmental, prematurity related phenomenon and does not reflect WM injury. In addition, in an earlier study we did not find any independent clinical risk factors for the occurrence of DEHSI.[24]

Authors of previous cross-sectional studies, either using qualitative or quantitative MR imaging, suggested that DEHSI in preterm infants reflects WM injury.[13,18,32,33] However, in recent literature, there is considerable doubt about the clinical significance of DEHSI and whether DEHSI indeed reflects WM injury.[3,5,17,34,36] We studied the direct associa-

tion between DEHSI and neurodevelopmental outcome at 2 years. Our results confirm the doubts of aforementioned authors and indicate that DEHSI does not reflect WM injury.[3,5,17,34]

In a neurodevelopmental follow up study of preterm infants assessed at a corrected age of 18 months, Dyet and colleagues[16] concluded that diffuse WM abnormalities and post hemorrhagic ventricular dilatation correlate with reduced developmental quotients. However, the number of infants included with only DEHSI, which in their study correlated with a significantly reduced developmental quotient, was very small. Krishnan and colleagues,[35] using diffusion weighted imaging, found an association between higher apparent diffusion coefficients (ADC) in WM and poorer developmental performance at a corrected age of 2 years in 38 preterm infants imaged at TEA. However, ADC values were determined in the WM of the centrum semi-ovale and specific periventricular frontal and parieto-occipital WM regions where DEHSI mostly occurs were not assessed.

The process of injury to the developing WM in preterm infants is still not fully understood, partly because of the lack of histological correlates.[4,17,37] Therefore, the exact origin of DEHSI remains unclear. The same is true for PWML, of which, although they are thought to represent isolated clusters of microglia, the exact histological or pathological process is, to our knowledge, so far unknown.[18] However, the focal appearance of PWML, in combination with the lower incidence (29 of 110, 26%) and the significant relation with abnormal neurological outcome, suggests that, in contrast to DEHSI, this finding should be regarded as part of the spectrum of WM injury. Other authors have shown that PWML influence brain development and may cause microstructural changes in the WM of the cortical spinal tracts and that they are associated with impaired visual function around TEA.[38,39] In some other studies, that included smaller numbers of infants with PWML, in contrast to our results, no correlations were found between PWML and neurodevelopmental outcome.[14,16]

Because injury to the brain occurs around premature birth[23,40] and especially PWML seem to fade or disappear,[5,16] imaging around or even after TEA may be a limitation to our study. However, it is currently accepted to perform MR imaging in very preterm infants around TEA for assessment of brain injury or impaired brain development.[3,5,6] Another limitation of our study is that nearly one-quarter of our cohort was lost to follow up. However, at baseline, there were no differences in clinical parameters or occurrence of DEHSI, PWML, and ventricular dilatation between infants with and infants without clinical follow up. In addition, there were a small number of neurologically

impaired children, which reduced statistical power. Finally, mental delay or handicap is especially difficult to test at this young age. Subtle influences on neurodevelopmental outcome can therefore not be excluded. Long term follow up at school age is needed to assess the exact influence of MR imaging findings on neurodevelopmental outcome in children born very prematurely.

In summary DEHSI was only seen during a specific age period and its incidence was very high in infants imaged around TEA. DESHI was not related to abnormal neurological outcome at a corrected age of 2 years, while PWML and ventricular dilatation were. These results indicate that DEHSI should not be considered part of the spectrum of WM injury, but rather a prematurity related developmental phenomenon.

Advances in knowledge

Diffuse excessive high signal intensity (DEHSI) of the white matter represents a prematurity related developmental phenomenon rather than white matter injury, given its high incidence in preterm infants around term equivalent age, its absence after a postmenstrual age of 50 weeks, and its association with normal neurological outcome at a corrected age of 2 years.

Implications for patient care

Diffuse excessive high signal intensity (DEHSI) of the white matter in very preterm infants around term equivalent age is not correlated with an adverse neurological outcome at 2 years.

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8

Cerebellar injury in preterm infants: incidence and findings on ultrasound and MRI

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Abstract

Purpose: To investigate the incidence and characteristics of cerebellar injury in a cohort of very preterm infants, using the mastoid fontanelle (MF) and posterior fontanelle (PF) approach in addition to routine cranial ultrasound (CUS) through the anterior fontanelle (AF), using MRI as the reference standard.

Materials and methods: The institutional review board approved this prospective study and informed consent was obtained. A cohort of 77 preterm infants (<32 weeks) was studied with serial CUS throughout the neonatal period, using the AF, PF, and MF. MRI was performed around term equivalent age in 59/77 infants. Sensitivity, specificity, positive predictive value and negative predictive value of routine CUS and CUS with additional views were calculated.

Results: Seven of 77 infants (9%) were identified with posterior fossa hemorrhage, using the MF CUS approach. In only 2/7 infants the lesions were seen on routine AF views. The PF approach did not increase the detection rate of posterior fossa hemorrhage. MRI confirmed CUS findings in all cases. MRI showed punctate hemorrhages in the cerebellum in 6 infants with normal CUS findings. Among the 59 infants studied with both CUS and MRI, cerebellar injury was diagnosed in 11 (19%).

Conclusion: Cerebellar injury is a frequent finding in very preterm infants. CUS through the MF can demonstrate injury missed by the routine AF approach. Punctate hemorrhagic lesions may remain undetected even when the MF is used; the prognostic implications of these smaller lesions need further attention.

Introduction

Brain injury can be a major complication of preterm birth, posing survivors at risk for developmental disorders, cognitive dysfunction, and behavioral difficulties. Until recently, supratentorial brain injury, such as white matter injury and intraventricular hemorrhage (IVH), was considered to be responsible for the impaired neurological outcome of children born prematurely. More recently, injury to the developing cerebellum has been described as additional complication of prematurity, with major impact on neurodevelopmental outcome.[1-3]

The exact incidence of cerebellar injury related to preterm birth is unknown. Posterior fossa hemorrhages can occur in association with supratentorial hemorrhage, but they can also be an isolated finding and be clinically silent.[4] Autopsy studies reported cerebellar hemorrhage in 10-25% of preterm infants.[5-7] Ultrasound studies in surviving very preterm infants (gestational age <32 weeks and/or birth weight <1500g) have shown that, when imaging is specifically focused on the posterior fossa, cerebellar lesions are not rare and that the incidence may range from 2.3-3% in preterm infants <1500g up to 19% in infants <750 g.[4,8,9] In addition, cerebellar growth and development can be impeded by preterm birth and both supra- and infratentorial brain injury.[10-13]

Cranial ultrasonography (CUS) is the best tool for serial imaging of the newborn brain. It is routinely performed through the anterior fontanelle (AF). This provides an excellent view of supratentorial structures, but visualization of infratentorial structures located further away from the transducer is less optimal.[14] Decreasing transducer frequency and directing focus on the posterior fossa improves visualization, but the echogenic tentorium and vermis still impede the detection of cerebellar injury. When using the mastoid fontanelles (MF) and the posterior fontanelle (PF) as additional windows, the transducer is closer to the posterior fossa structures and they are approached at a different angle. This provides a better detection of cerebellar injury.[4,15-17] Despite the advantages of the MF and PF windows and the implications of cerebellar injury for neurodevelopmental outcome, these windows are generally not included in the ultrasound examination of the brain of preterm infants.

Magnetic Resonance Imaging (MRI) shows brain maturation in detail and demonstrates the size and extent of injury more precisely.[18-24] The posterior fossa is well depicted by MRI. However, compared to CUS, MRI is a burdensome procedure for the very preterm infant. Therefore, unlike serial CUS examinations, serial MR examinations are undesirable and most neonatal centers only perform MRI to confirm CUS abnormalities and to demonstrate the localization and extent of lesions more precisely.[14,25]

To our knowledge, cerebellar injury has not been studied systematically by combining serial CUS (including scanning via MF and PF) and MRI in preterm infants.

The aims of our study were to investigate the incidence and characteristics of cerebellar injury in a cohort of very preterm infants, using the MF and PF approach in addition to routine CUS through the AF, using MRI as the reference standard.

Patients and Methods

Preterm infants

Very preterm infants (gestational age [GA] <32 weeks), admitted to the neonatal unit of the Leiden University Medical Center (tertiary neonatal referral center) between April and October 2007, were eligible for participation in an ongoing neuro-imaging study, including serial CUS examinations throughout the neonatal period, and cerebral MRI examination around term equivalent age (TEA). The institutional review board approved this prospective study and parental consent was obtained. Exclusion criteria were congenital abnormalities of the central nervous system, chromosomal and metabolic disorders. Gestational age was estimated from the date of the mother's last menstrual period and early prenatal ultrasound. Birthweight, gender and mode of delivery were recorded.

Patient characteristics are shown in Table 1. During the study period, 77 preterm infants <32 weeks were admitted to our neonatal unit and eligible for inclusion in the neuro-imaging study. All infants underwent serial CUS, including views through the MF and PF. CUS findings in all 77 infants were analyzed. Parental consent for performing MRI around TEA was obtained in 63/77 infants (82%). Four infants died during the neonatal period, after consent was obtained, but before MRI could be performed. Consequently, in 59/77 infants (77%) results of both serial CUS and MRI around TEA were available (Figure 1).

Cranial ultrasound

CUS was performed using an Aloka α10 ultrasound system with multifrequency (5–10 MHz) transducers (Biomedic Nederland B.V., Almere, The Netherlands). The standard CUS protocol in very preterm infants (<32 weeks) includes frequent examinations performed by the attending neonatologist, from the day of birth until discharge or transfer to another hospital. The AF is used as acoustic window and images are recorded in at least 6 coronal and 5 sagittal planes.[14] Transducer frequency is set at 7.5 MHz. To assess deeper structures, including the posterior fossa, a frequency of 5 MHz can be applied.

Table 1. Patient characteristics.

Parameter	All infants	Infants with CUS and MRI data at TEA	Infants with consent died before MRI	Infants without consent for MRI (only CUS data)	P value*
No.	77	59	4	14	
GA at birth , median (range), weeks + days	29 +1 (25-31+ 6)	29+ 2 (25-31+ 6)	27+ 2 (26-29+ 4)	29+ 5 (27-31+ 6)	0.85
Birth weight, median (range), grams	1214 (585-1960)	1232 (585-1960)	868 (698-1040)	1235 (869-1635)	0.12
Male, no. (%)	41 (53%)	33 (56%)	1 (25%)	7 (50%)	0.47
Grade II IVH, no. (%)	5 (6%)	5 (8%)	0	0	0.20
Grade III IVH, no. (%)	6 (8%)	5 (8%)	1 (25%)	0	0.55
PHVD, no. (%)	2 (3%)	2 (3%)	0	0	0.43
Cystic PVL, no. (%)	2 (3%)	2 (3%)	n.a.	0	0.43

Note: gestational age (GA), intraventricular hemorrhage (IVH), post hemorrhagic ventricular dilatation (PHVD), periventricular leukomalacia (PVL)

* P-values calculated for differences between infants with MRI around TEA and those without MRI, level of significance < 0.05

Figure 1. Flow diagram showing the number infants eligible for inclusion, the number of infants with serial CUS and the final number of included infants with both serial CUS and MRI around TEA.

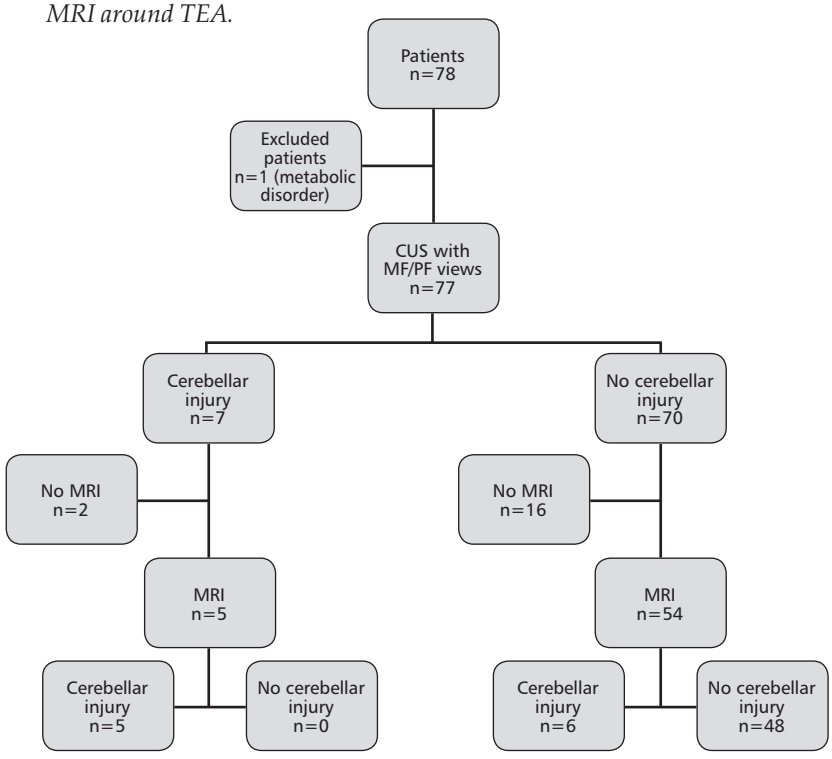
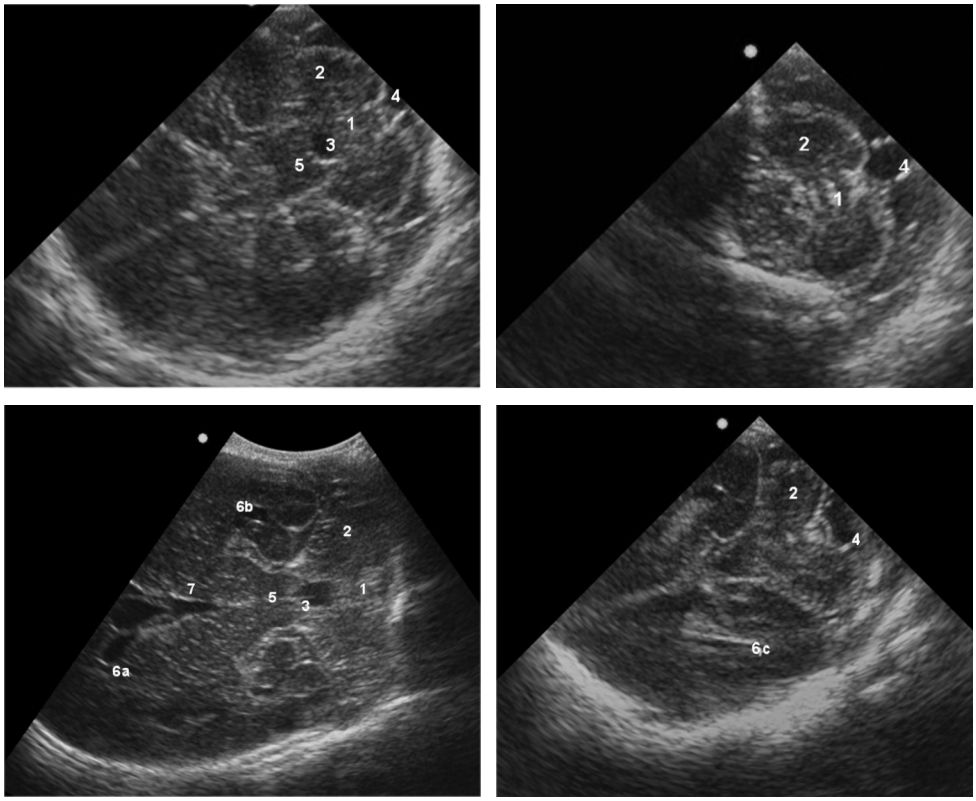


Figure 2. Normal ultrasound scan using the MF as acoustic window (male infant, GA 27 weeks)
A: axial view through the cerebellum, fourth ventricle and pons
B: inferior axial view through cerebellar hemispheres
C: coronal view through the cerebellum, fourth ventricle and pons
D: posterior-inferior coronal view through both cerebellar hemispheres



Vermis (1), cerebellar hemisphere (2), fourth ventricle (3), cisterna magna (4), pons (5), lateral ventricle (6a anterior horn, 6b temporal horn, 6c trigone), 3rd ventricle (7)

During the study period, the CUS examinations with additional MF and PF views were performed biweekly, starting between the 3rd and 7th day of life. When abnormalities were suspected, these additional views were performed more frequently. All CUS scans that included MF and PF views were performed by SJS, having five years of experience in CUS imaging. CUS was repeated (by SJS) around TEA, on the same day as the MRI, including images obtained through the AF, PF, and MF. The mean number of CUS with additional MF and PF views was 2.7/ patient (range 1-6).

Scanning through the PF included 5 sagittal and 3 coronal views, as described by

Correa *et al*.[16] Scanning through the MF was performed as described by van Wezel-Meijler[14] and Enriquez *et al*.[17] Scans were performed in axial and coronal planes with views of the ventricular system, brain stem, cerebellar peduncles, vermis, and hemispheres (Figure 2).

All CUS scans were reviewed by at least two investigators (GvWM, SJS and LML with 20, 5 and 4 years of experience in CUS imaging, respectively) for abnormalities of the posterior fossa, including echogenicity changes in the cerebellar parenchyma (hemispheres and/or vermis), abnormalities in echogenicity, size and shape of the 4th ventricle, and abnormalities of the structures surrounding the cerebellum. In addition, the cerebellar parenchyma was evaluated for signs of abnormal development or atrophy. This was done at least 3 months after the scans were performed. The investigators, who were blinded to the patient names, reviewed the scans together and any discrepancies in interpretation were solved by means of consensus. While evaluating CUS scans, the presence of major supratentorial injury such as cystic periventricular leukomalacia (PVL), periventricular hemorrhagic infarction (PVHI), post hemorrhagic ventricular dilatation (PHVD), and supratentorial hemorrhage (i.e. germinal matrix/ intraventricular hemorrhage, [GMH/IVH]) was also recorded. GMH/IVH was classified according to Volpe.[26] PHVD was defined according to Levene.[27] PVL was classified according to de Vries *et al*.[28]

MRI

MRI examination of the brain was performed around TEA, using a 3 Tesla MRI system (Philips Achieva 3T, Philips Medical Systems, Best, The Netherlands). Infants were sedated with chloral hydrate (50 mg/kg) 30–45 minutes prior to the procedure and ear protection (Natus MiniMuffs, Natus Medical Inc, San Carlos, CA) was applied. The MRI protocol included T_2 turbo spin echo (TSE), $T_1$3D turbo field echo (TFE), T_2* fast field echo (FFE), diffusion-weighted spin echo (DwSE) and diffusion-tensor imaging (DTI) sequences in transverse planes. Slice thickness was 1-4 mm without interslice gap and field of view 180-230 mm. All scans were reviewed, by at least 2 experienced investigators (FTdB and GvWM, having 15 years, and SJS and LML having 4 years experience in neonatal MR imaging). To avoid recall bias this was done at least 6 weeks after reviewing the CUS scans. The investigators reviewed the scans together and any discrepancies in interpretation were solved by means of consensus. The pediatric neuroradiologist (FTdB), who was unaware of the CUS findings, was always present. The other investigators (GvWM, SJS, and LML), who were involved in patient care during admission, were blinded to the patient names. Scans were reviewed for presence of posterior fossa hemorrhage, and the cerebellar parenchyma was examined for signs of disruption, in-

farction, or atrophy. If lesions were present, the location, extent, and laterality were noted and whether the lesions were visible on the T_1- and/or T_2-weighted images or only on the susceptibility scan.

Data analysis

The incidence of cerebellar injury was calculated. Infants with and without MRI around TEA were compared for general characteristics and supratentorial CUS findings, using a t-test for numerical data and a Pearson Chi-Square or Fisher's Exact Test where appropriate for categorical data. P values less than .05 were considered to indicate a significant difference. The diagnostic competence of serial CUS throughout the neonatal period until TEA, using additional windows for the detection of cerebellar injury, and the diagnostic competence of the AF view alone were compared with the results of MRI, which was used as the reference standard. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated.

RESULTS

Preterm infants

We found no significant differences in general characteristics and supratentorial CUS findings between patients with and without MRI (Table 1).

CUS

Among the total group of 77 very preterm infants, seven (9%) had abnormalities in the infratentorial region detected by CUS (Table 2). In 5/7 infants, these occurred in combination with supratentorial hemorrhage, whereas hemorrhage was isolated to the cerebellum in two infants. Two of the seven infants with cerebellar lesions died during the neonatal period. Of the five surviving infants, two developed cerebellar atrophy on follow up CUS scans.

In 5/7 infants (71%), the abnormalities in the posterior fossa were only detected using the MF, but not on AF or PF views. Unilateral focal echogenic areas in one of the cerebellar hemispheres were seen in three infants. Two of these infants also showed echodensities in the 4th ventricle (probably presenting a hemorrhagic clot) and in one infant the cerebellar vermis was also involved (Figure 3). Two other infants showed echodensities in the 4th ventricle (probably presenting a hemorrhagic clot), co-existing with infratentorial extra-axial hemorrhage. On follow up ultrasound scans both infants

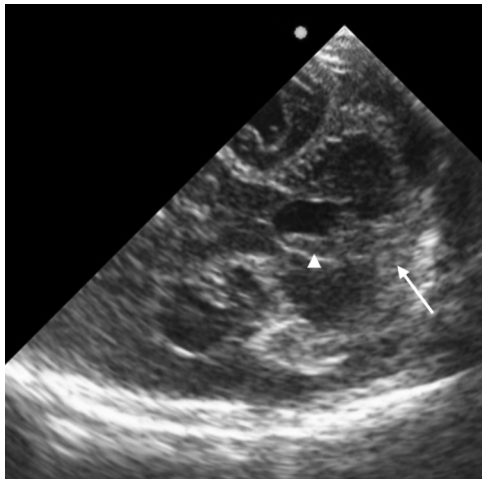
Table 2. Imaging findings in infants with infratentorial CUS and/or MRI abnormalities.

Infant no.	GA weeks	*Age at CUS	Cranial ultrasonography, mastoid fontanelle	MRI	Supratentorial hemorrhage
1	26	Day 3	Lentiform shaped echodense lesion right cerebellar hemisphere	Died, no MRI	None
2	26	Day 4	Circular echodense lesion right cerebellar hemisphere	Died, no MRI	IVH grade 3
3	26	Day 3 Day 14	Bilateral hemorrhages cerebellar hemispheres. Subsequent loss of parenchymal tissue and atrophy.	Hemorrhage both cerebellar hemispheres, severe atrophy	None
4	26	Day 3	Echodense lesion in 4th ventricle, vermis, and left cerebellar hemisphere	Hemosiderin residue in 4th ventricle and vermis, small hemorrhagic lesion left hemisphere	IVH grade 3, PHVD
5	27	Day 3	Echodense lesion 4th ventricle and left hemisphere	Hemosiderin residue in 4th ventricle, small hemorrhagic lesion left hemisphere	IVH grade 2, PVHI
6	26	Day 5	Echodense lesion 4th ventricle and surrounding cerebellar parenchyma. Dilatation 4th ventricle	Hemosiderin residue in 4th ventricle and subarachnoid space surrounding cerebellum	IVH grade 3
7	30	Day 4 Day 14	Echodense lesion 4th ventricle and around cerebellum, dilatation 4th ventricle. Persistent dilatation and deformity 4th ventricle, cystic abnormality posterior fossa and cerebellar atrophy	Hemorrhage and dilatation of 4th ventricle, arachnoid cyst posterior fossa, atrophy left hemisphere	IVH grade 3, PHVD, PVHI
8	27		Normal	Single small hemorrhagic lesion vermis	None
9	29		Normal	Single punctate hemorrhage hemisphere	None
10	27		Normal	Multiple punctate hemorrhages both hemispheres	None
11	29		Normal	Single punctate hemorrhage hemisphere	IVH grade 2
12	28		Normal	Multiple punctate hemorrhages left hemisphere	IVH grade 2
13	26		Normal	Punctate hemorrhage and hemosiderin in 4th ventricle	IVH grade 3

Note: intraventricular hemorrhage (IVH), post hemorrhagic ventricular dilatation (PHVD), periventricular hemorrhagic infarction (PHVI)

* indicates the postnatal age at which the abnormalities in the posterior fossa were detected

Figure 3. Coronal ultrasound scan in preterm infant (female, GA 26 weeks, with bilateral IVH grade 3) on 3rd day of life, using the left MF as acoustic window, demonstrating hemorrhage and post hemorrhagic dilatation of the 4th ventricle (arrowhead) with extension of the hemorrhage in the vermis and right cerebellar hemisphere (arrow).



developed dilatation and deformity of the 4th ventricle, in one of them combined with a cystic abnormality in the posterior fossa (Figure 4).

In 2/7 infants cerebellar injury was seen on the AF, PF and MF views. AF sonography showed an echogenic area in the right cerebellar hemisphere in one infant and bilateral echodensities in the cerebellum in the other, probably presenting hemorrhages. MF imaging improved visualization of these lesions in both infants (Figure 5). There were no patients in whom addition of PF views increased the detection rate of cerebellar injury.

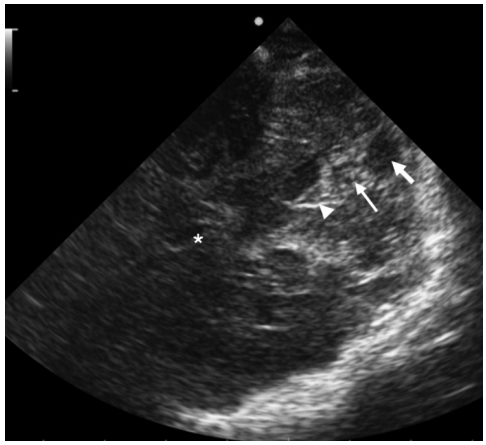
MRI

Forty-eight/fifty-nine infants (81%) had normal findings of the infratentorial region. In 11 infants (19%) MRI showed hemorrhagic injury of the cerebellum (Table 2).

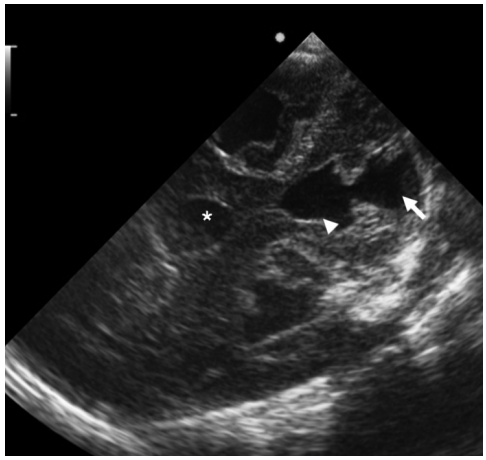
MRI in infants with infratentorial CUS abnormalities

In all five surviving infants with abnormalities on CUS in the infratentorial region, these lesions were confirmed by MRI. These five infants had hemorrhagic lesions in the cerebellar hemispheres and/or the vermis, and/or the 4th ventricle (Figure 5). In the infant with a cystic abnormality in the posterior fossa on CUS, a fluid collection thought to be an arachnoid or leptomeningeal cyst was diagnosed by MRI (Figure 4).

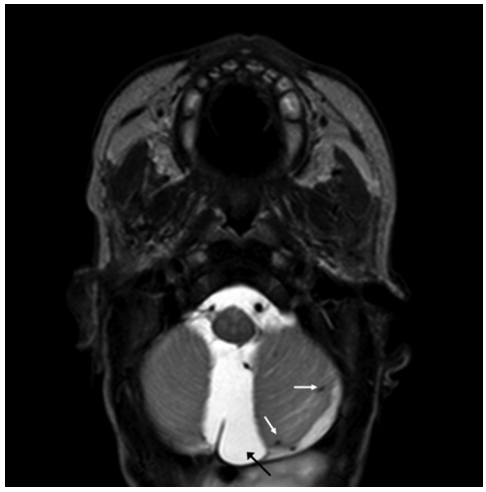
Figure 4. Ultrasound scan in preterm infant (male, GA 30 weeks, with bilateral IVH grade 3, first seen on 3rd day of life)



A: Coronal view using the MF as acoustic window, shows extension of hemorrhage in the 4th ventricle (arrowhead) and possible extension in the vermis (arrow). The 3rd ventricle is mildly dilated (asterisk). Note the normal shape of the vermis and cisterna magna (bold arrow).

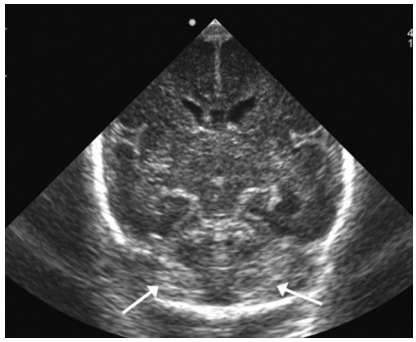


B: Two weeks after birth CUS shows deformity and dilatation of the 4th ventricle (arrow head) and cystic dilatation of the cisterna magna (arrow). There is post hemorrhagic dilatation of the 3rd ventricle (asterisk) and temporal horns of the lateral ventricles.



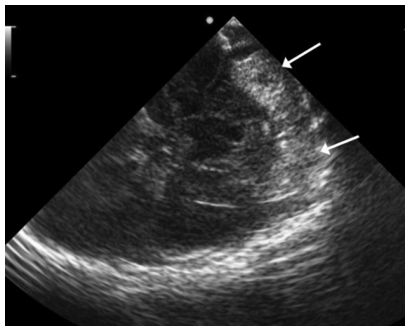
C: T2-weighted image performed on day of life 81, illustrating fluid collection in posterior fossa (black arrow) and small hemosiderin deposits in left cerebellar hemisphere (white arrows).

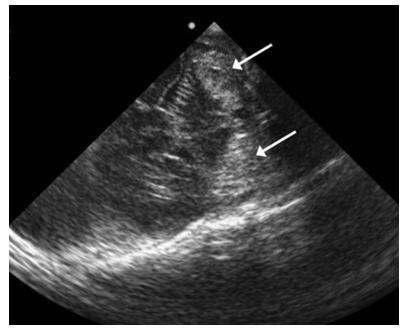
Figure 5. CUS scan in preterm neonate (male, GA 26 weeks), on 3rd day of life.



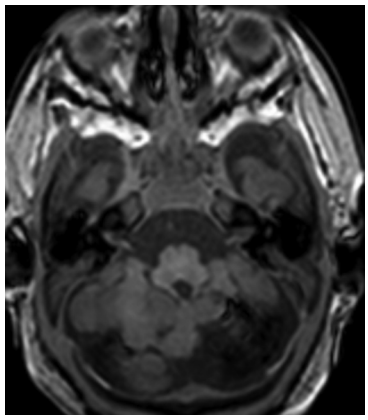
A: Coronal view through the cerebellum using the AF as acoustic window and transducer frequency set at 5 MHz, showing a suspect echogenic area in both cerebellar hemispheres (arrows). No signs of supratentorial hemorrhage.

B: (left) Axial and C: Coronal views through the cerebellum, using the left MF as acoustic window, clearly demonstrating the hemorrhage in both hemispheres (arrows).





D: (left) T1-weighted image performed on day of life 109 illustrates destructive lesions in both cerebellar hemispheres.
E: Susceptibility scan, showing bilateral extensive cerebellar hemorrhages.



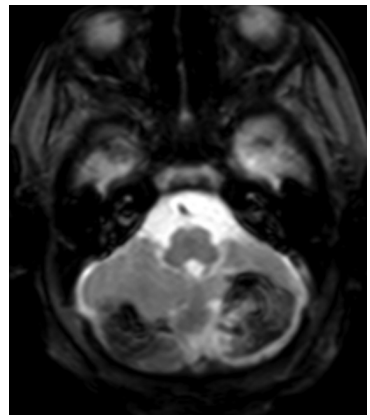
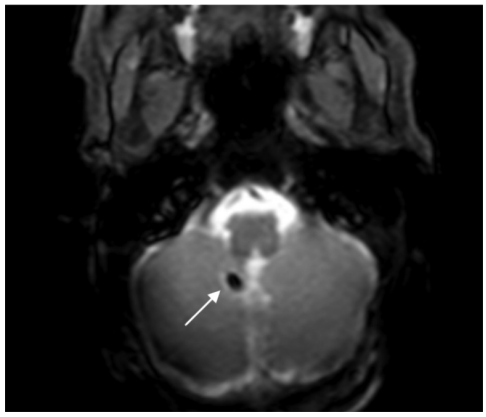


Figure 6. Susceptibility MRI scan performed around TEA in a preterm infant (female, GA 27 weeks) with normal findings on CUS showing hemosiderin residue in cerebellum (arrow).



Two infants with cerebellar injury developed cerebellar atrophy as shown by the MRI around TEA, confirming CUS findings.

MRI in infants without infratentorial CUS abnormalities.
In six out of 54 infants who underwent MRI and had normal CUS findings of the infratentorial region on both AF and MF views, MRI showed punctate hemorrhages in the cerebellar hemispheres and/or vermis (Figure 6). These small hemorrhagic lesions were most prominently seen on the susceptibility scan, but were also detected on T_{1}- or T_{2}-weighted images in all infants. Three infants had a single, unilateral punctate hemorrhage; the other three had multiple small hemorrhagic lesions. In three infants these lesions were associated with supratentorial IVH. None of these six infants developed cerebellar atrophy.

The sensitivity and specificity of routine CUS for detection of posterior fossa abnormalities as seen on MRI, were 18% and 100%, and of additional CUS 45% and 100%, respectively. The PPV and NPV of routine CUS were 100% and 84%, and of additional CUS 100% and 89%, respectively.

DISCUSSION

The developing cerebellum is extremely vulnerable to injury. Several factors can lead to destruction of immature cerebellar structures and developmental arrest.[10-13;29] This may have great impact on neurological development.[1,2] Therefore, detection of cerebellar

injury is of importance in high-risk preterm infants. Among a group of 59 very preterm infants, studied with both CUS and MRI, we found an incidence of hemorrhage in the cerebellum and/or 4th ventricle of 19%.

Of the total group of 77 infants, seven (9%) had abnormalities in the infratentorial region that were detected by CUS. This percentage is high compared to previous literature reports that describe an overall incidence of 2.3-3% in preterm infants <1500g[4,8,9] and is probably related to the fact that we performed serial CUS, including the MF approach, and paid special attention to cerebellar injury. In a recent study, Müller *et al*.[9] reported cerebellar hemorrhage in six out of 260 (2.3%) very preterm infants (GA <32 weeks), using AF sonography, focusing on the cerebellum. The detection rate in their study-population would probably have been higher if the MF had also been used. In our study cerebellar injury was missed using only the AF in 5/7 (71%) infants with abnormalities in the posterior fossa, despite adapting transducer frequency and focusing on the fossa posterior. In these infants cerebellar injury was only detected using the MF. Merill *et al*.[4] also showed a better detection of posterior fossa hemorrhage by using the MF. In a prospective study over a 2-year period, they identified 13/525 (3%) infants (including 250 infants weighing <1500 g) with posterior fossa hemorrhage, whereas only two cases were identified over a retrospective 3-year period, using only AF views. The lower incidence they found may be related to the fact that they only reported large cerebellar hemispheric hemorrhages, whereas we also included other types and localizations of posterior fossa hemorrhage. Apart from the five infants with echogenic areas in the cerebellar hemispheres, we found two infants with 4th ventricular hemorrhage who, on follow up CUS, developed obvious abnormalities in the cerebellar region. In one of these infants a retrocerebellar fluid collection, thought to be an arachnoid or leptomeningeal cyst, was seen by US and MRI. Most arachnoid cysts are congenital, developmental abnormalities. However, a small number are acquired due to adhesions following hemorrhage, meningitis, or surgery.[30] In this patient the fluid collection in the cisterna magna was only visualized on late CUS scans and MRI around term. This has been described before in a patient with intraventricular hemorrhage.[31] We hypothesize that entrapment of spinal fluid within arachnoid adhesions, following hemorrhage may be the cause of the cyst formation.

In a large retrospective ultrasound study, Limperopoulos *et al*.[8] detected cerebellar injury in 35/1242 (3%) infants weighing <1500 grams. During the last part of their study they found cerebellar hemorrhage in 19% of infants <750 gram. An increasing expertise with the MF approach and a decrease in mortality may have played a role in the increased detection of cerebellar lesions. In our study population, the majority of infants with cerebellar injury had a GA <28 weeks.

In our study, MRI confirmed presence and location of lesions in the five infants with infratentorial abnormalities on CUS. MRI demonstrated small hemorrhagic lesions in the cerebellar region in six additional infants without CUS abnormalities. Dyet *et al*.[3] performed serial MRI in preterm infants born between 23-30 weeks gestation and found cerebellar hemorrhagic lesions on early MRI scans in 8/119 preterm infants (7%). They used a 1.0 T MRI system in the majority of infants and assessed T_1- and T_2-weighted images. For our study, a 3.0 T system was used, slice thickness 1-2 mm for T_{1-} and T_2-weighted images, without interslice gap, allowing detailed imaging. This may have been an advantage for the detection of small lesions. Furthermore, we performed susceptibility-weighted imaging, being more sensitive for small hemorrhagic foci than conventional T_1- and T_2-weighted imaging.[32] Although small hemorrhagic lesions were detected on T_1- and/or T_2-weighted images, they were most prominently seen on the susceptibility-weighted images. The long-term consequences of small hemorrhagic lesions remain unclear and need further investigation. Hemosiderin deposits on the cerebellar surface can cause damage to underlying structures and may impair further growth and development. In infants and children with superficial cerebellar siderosis, subsequent cerebellar atrophy has been described.[33-35] The toxic effects of hemosiderin may especially be pronounced in the immature and rapidly developing cerebellum. In a large group of preterm infants with different patterns of cerebellar atrophy on MRI, performed at 2 months to 6 years of life, Messerschmidt *et al*.[11] found hemosiderin deposits in the majority of infants, even in the absence of primary cerebellar hemorrhage. We detected cerebellar atrophy in 2/7 infants with overt cerebellar hemorrhage, but did not see cerebellar atrophy in the six infants with only punctate cerebellar lesions. As these were early MRI examinations, performed around TEA, atrophy may still develop. In addition, we did not perform volumetric analysis, so small alterations in cerebellar volumes may have been overlooked. The effect of small punctate hemorrhages and hemosiderin deposits on cerebellar volume and on neurodevelopmental outcome should be the subject of further study.

Our study had limitations. For several reasons, including early neonatal death and lack of parental consent for MRI, we did not perform MRI in all infants. However, as there were no significant differences between the infants with and without MRI for GA at birth, birth weight, male gender, and supratentorial abnormalities, this probably did not cause a bias in our study population. Another limitation is that some of the investigators were involved in patient care and interpreted CUS examinations during admission, may have recalled CUS images when reviewing the MRI scans. However, there was a 6 week interval between reviewing the CUS examinations and the MRI scans.

Combining the results of high-quality CUS, including the use of supplemental acoustic windows and MRI, we found cerebellar lesions in 11/59 (19%) of very preterm infants. CUS, focusing on the posterior fossa and including MF views detected most lesions, but small punctate hemorrhages remained beyond the scope of CUS. The PF approach did not contribute to detection of cerebellar injury. The high incidence of cerebellar injury warrants routine CUS examinations including MF views in very preterm infants. Perinatal risk factors, the effect of cerebellar hemorrhage on neurological outcome, and the prognostic implications of punctate lesions need further investigation.

ADVANCES IN KNOWLEDGE

1) Cerebellar injury occurs frequently in very preterm infants.
2) Addition of mastoid fontanelle views in cranial ultrasonography leads to improved detection of cerebellar injury compared with anterior fontanelle sonography alone.
3) Small hemorrhagic cerebellar lesions remain beyond the scope of sonography and can only be detected with MRI.

IMPLICATIONS FOR PATIENT CARE

The high incidence of cerebellar injury warrants serial cranial ultrasound examinations, including mastoid fontanelle views, in very preterm infants (<32 weeks gestation).

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9

Clinical value of gradient echo MRI for brain imaging in preterm infants

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Abstract

Purpose: Gradient echo techniques are the most sensitive MRI sequences for detecting hemorrhages in the brain. Still, reports on its use in neonates are scarce. The aim of this study is to correlate presence of hemosiderin deposits in the brain of very preterm infants (gestational age <32 weeks) detected by T2*-w gradient echo MRI to white matter (WM) injury and neurodevelopmental outcome at two years.
Methods: In 101 preterm infants presence and location of hemosiderin were assessed on T2*-w gradient echo MRI performed around term equivalent age. White matter injury was defined as the presence of more than 6 punctate white matter lesions (PWML), cysts and/or ventricular dilatation. Six infants with post hemorrhagic ventricular dilatation detected by ultrasound in the neonatal period were excluded. Infants were seen for follow up at two years. Univariate and regression analysis assessed the relation between presence and location of hemosiderin, WM injury and neurodevelopmental outcome.
Results: In 38/95 (40%) infants hemosiderin was detected. Twenty percent (19/95) of the infants were lost to follow up. There was a correlation between hemosiderin in the ventricular wall with more than 6 PWML ($p<0.001$) and cysts ($p<0.001$) at term equivalent age, and with a lower psychomotor development index (PDI) ($p=0.02$) at 2 years. After correcting for known confounders (gestational age, gender, intrauterine growth retardation and WM injury) the correlation with PDI was no longer significant.
Conclusion: The clinical importance of detecting small hemosiderin deposits is limited as there is no independent association with neurodevelopmental outcome.

Introduction

Magnetic resonance imaging (MRI) is a safe and valuable tool to assess development and pathology of the preterm infant's brain.[1-4] In infants born very prematurely, germinal matrix and intraventricular hemorrhage (GMH/IVH) and white matter injury are frequently encountered,[5-7] while cerebellar hemorrhage is increasingly recognized.[8-11] All are associated with later cognitive and motor impairment.[5,12-15]

Elevation of free radicals and iron in cerebrospinal fluid is associated with both hemorrhages and white matter injury in very preterm and low birth weight infants.[16,17] Therefore an independent influence of hemorrhages on neurodevelopmental outcome can not be excluded.

Although the T2*-w gradient echo technique has a much higher sensitivity for detection of (small) hemorrhages, in particular hemosiderin deposits, than T1-w and T2-w MR techniques[10,18,19] its added clinical value for brain imaging in preterm infants has not been determined yet.

In the present study, we used a T2*-w gradient echo sequence for detection and location of small hemosiderin deposits in an unselected cohort of very preterm infants who underwent MRI within 3 months after term equivalent age. We investigated the clinical significance of these hemosiderin deposits by evaluating the association with white matter injury and neurodevelopmental outcome around the corrected age of two years.

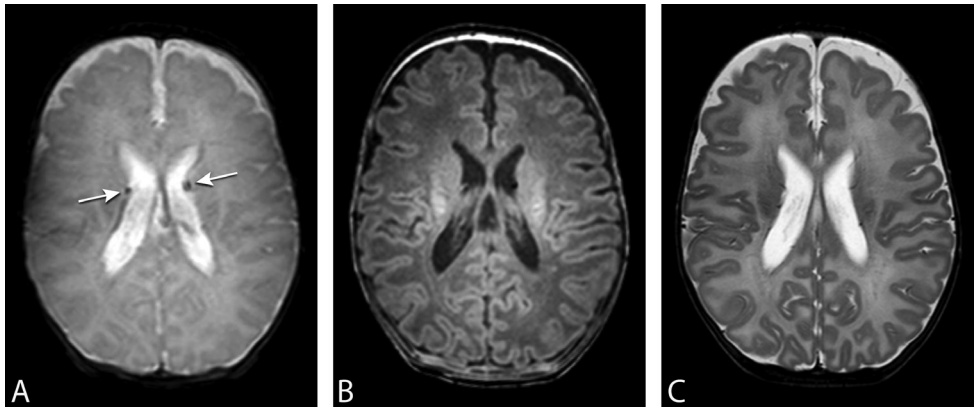
Patients and Methods

Very preterm infants

As part of a prospective neuro-imaging study performed in an unselected group of very preterm infants (gestational age <32 weeks), admitted to the tertiary neonatal unit of our hospital, 113 infants underwent MRI, preferably around term at a postmenstrual age of 40 – 44 weeks. For infants who were unstable around that age, MRI was postponed, resulting in an age range of 40 – 60 postmenstrual weeks at imaging. Ethical approval for the study was given by the institutional review board and informed parental consent was obtained for each infant.

In three infants congenital malformations of the central nervous system were found on MRI and they were excluded from the study. In nine infants a T2*-w gradient echo sequence was not performed. Six infants diagnosed with a grade III-IV intraventricular hemorrhage and post hemorrhagic ventricular dilatation (PHVD) on brain ultrasound

Figure 1. Hemosiderin deposits detected in the germinal matrix / lateral ventricular walls (arrows) on T2-w gradient echo images (A), but not noted on T1-w and T2-w MR images (B, C).*



in the neonatal period were excluded from further analysis to ascertain that ventricular dilatation on the term MRI's resulted ex-vacuo from white matter injury.

Therefore, for this part of the study, data of 95 infants were included. Clinical parameters were collected from the patients' files.

Image and data acquisition

All MRI examinations were performed on a 3T MRI system (Philips Medical Systems, Best, the Netherlands) according to a standard protocol for imaging the newborn infant's brain.[3] The infants were sedated using chloral hydrate (55mg/kg), lay supine and were swaddled during the scanning procedure. Ear protection consisted of neonatal earmuffs (Natus Mini Muffs; Natus Medical Inc., San Carlos, CA, USA) covered by a headphone. All MRI examinations included a 3D T1-Turbo Field Echo sequence (TR 9.7 ms, TE 4.6 ms, FOV 180 mm, matrix size 192x152, flip angle 8º, TFE factor 128, slice thickness 1 mm), a T2-Turbo Spin Echo sequence (TR 6269 ms, TE 120 ms, FOV 180 mm, matrix size 336x234, TSE factor 18, slice thickness 2 mm), and a T2* Fast Field Echo sequence (TR 735 ms, TE 16 ms, FOV 230 mm, matrix size 256x163, flip angle 18º, slice thickness 4 mm).

Hemosiderin deposits

To assess the presence of hemosiderin, two investigators (FTdB and SJS) examined the T2*-w gradient echo images together by consensus.

Hemosiderin deposits, originating from hemorrhages occurring in the perinatal period were defined as hypointense signal intensity lesions or blooming effects (Figure 1). The location of hemosiderin was noted in anatomic regions as shown in Table 2.

White matter injury and ventricular size

Two investigators (FTdB and LML) analyzed all T1-w and T2-w sequences together by consensus for presence of white matter injury and ventricular dilatation. White matter injury was defined as more than 6 (non-hemorrhagic) punctate white matter lesions (PWML) and/or cystic white matter lesions and/or ventricular dilatation with the exclusion of PHVD. PWML were defined as punctate hyperintense lesions on T1-w sequences, slightly hypointense on T2-w sequences, not visible on T2*-w gradient echo sequences, in order to differentiate these from small hemorrhages. On coronal reconstructions from the three-dimensional T1-w images the ventricular index was measured as the total width of the lateral ventricles, divided by 2, in analogy to ventricular index measurements on ultrasound.[20] A ventricular index between 12 – 16 mm was considered moderate dilatation and a ventricular index of more than 16 mm severe dilatation.[21] Diffuse Excessive High Signal Intensity (DEHSI) was not considered part of the spectrum of white matter injury.[22]

Follow up

Around two years of age, the infants were seen by an experienced neonatologist, who was unaware of the neuro-imaging findings. Each child underwent a standardized neurologic examination to assess the presence of cerebral palsy or abnormal muscular tone. A Gross Motor Function Classification System (GMFCS) level was assigned.[23] A GMFCS level of 2 or more was considered cerebral palsy.

The cognitive and psychomotor development was assessed by a psychology assistant, who was unaware of the neuro-imaging findings, using the Dutch version of the Bayley Scales of Infant Development (BSID-III). A mental developmental index score (MDI) and a psychomotor developmental index score (PDI) were calculated for the corrected age of the child. Five infants diagnosed with cerebral palsy, in whom testing the gross and fine motor function with the BSID-III was not feasible, were assigned a PDI score of 50.

To evaluate child behavior, the Dutch version of the Child Behavior Checklist 1½ to 5 (CBCL)[24] was sent to the child's home address prior to the follow up visit, to be completed by either parent or caretaker. The questionnaire consists of 99 items rated on a 3-point scale. By summing the scores an internalizing, externalizing, total and other problem score can be computed. These raw scores were transformed into normalized T-scores and used as secondary outcome parameters in the analyses, where a higher score represented more severe behavioral problems.

Statistical analyses

Data were analyzed using SPSS 17.0.1. Frequency counts and percentages were used

Table 1. Distribution of categorical clinical parameters (N (%)), continuous clinical parameters (mean (SD)) and white matter injury (N (%)) N=95.

Categorical clinical parameters	**N (%)**
Male	58 (61.1)
Female	37 (38.9)
GA < 28 wk	33 (34.7)
Birth weight < 1000 gram	32 (33.7)
IUGR	12 (12.6)
Continuous clinical parameters	**Mean (SD)**
GA (weeks)	29.0 (2.0)
Birth weight (gram)	1199 (372.8)
GA at MRI (weeks)	45.1 (4.0)
Weight at MRI (gram)	4078.0 (805.5)
White matter injury	**N (%)**
> 6 PWML	16 (16.8)
Moderate ventricular dilatation	49 (51.6)
Severe ventricular dilatation	17 (17.9)
Cysts	6 (6.3)

GA=gestational age, IUGR=intrauterine growth retardation, PWML=punctate white matter lesions

Table 2. Frequency of hemosiderin deposits on MRI per location (N (%))

Supratentorial	25 (26.3)
Germinal matrix/caudo thalamic groove	13 (13.7)
Ventricular wall	21 (22.1)
Intraventricular	16 (16.8)
Periventricular	9 (9.5)
Deep white matter	1 (1.1)
Infratentorial	23 (24.2)
4^{th} ventricular wall	4 (4.2)
Cerebellar vermis	5 (5.3)
Cerebellar hemispheres	19 (20.0)
Total	38 (40.0)

to summarize categorical parameters. For continuous parameters, mean and standard deviations are reported.

The clinical parameters of the infants with and without two year follow up were compared with chi-square, Fisher-exact and Mann-Whitney U tests where appropriate.

The association between presence of hemosiderin per location and WM injury (more than 6 PWML, cystic lesions and ventricular dilatation) and outcome parameters was evaluated using chi-square, Fisher-exact and Mann-Whitney U tests. For the relation with outcome backward linear and logistic regression were subsequently used to adjust for known potentially confounding neonatal characteristics (gestational age, gender, intra uterine growth retardation) and white matter injury on MRI. Statistical significance was defined by two-tailed p value <0.05.

Results

Clinical parameters

The clinical parameters of infants (N=95) are shown in Table 1. The numbers of infants with white matter injury (more than 6 PWML, cystic white matter lesions, ventricular dilatation) are mentioned separately.

Hemosiderin deposits

Table 2 shows the number of infants with hemosiderin deposits and the location as seen on T2*-w gradient echo. Hemosiderin deposits were detected in 38/95 (40%) infants. These were supratentorially located in 25/95 (26.3%) and infratentorially located in 23/95 (24.2%) infants (Figure 2). In 10/23 infants with infratentorial hemosiderin deposits, supratentorial hemosiderin deposits co-existed.

Association between hemosiderin deposits per location and white matter injury

Table 3 shows the associations per location between the presence of hemosiderin deposits on T2*-w gradient echo sequences and white matter injury on MRI. The presence of hemosiderin in the wall of the lateral ventricles was strongly associated with more than 6 PWML ($p<0.001$). Furthermore there were associations between hemosiderin deposits in and around the ventricle and more than 6 PWML ($p= 0.03$ and $p=0.04$) and between intraventricular hemosiderin deposits and severe ventricular dilatation ($p=0.02$). There were significant associations between hemosiderin deposits at most locations and cystic white matter lesions. There were no associations between infratentorial cerebellar hemosiderin deposits and supratentorial white matter injury.

Figure 2. (A) T2-w gradient echo image showing hemosiderin in the cerebellum (arrow), not visible on the T2-w image (B).*

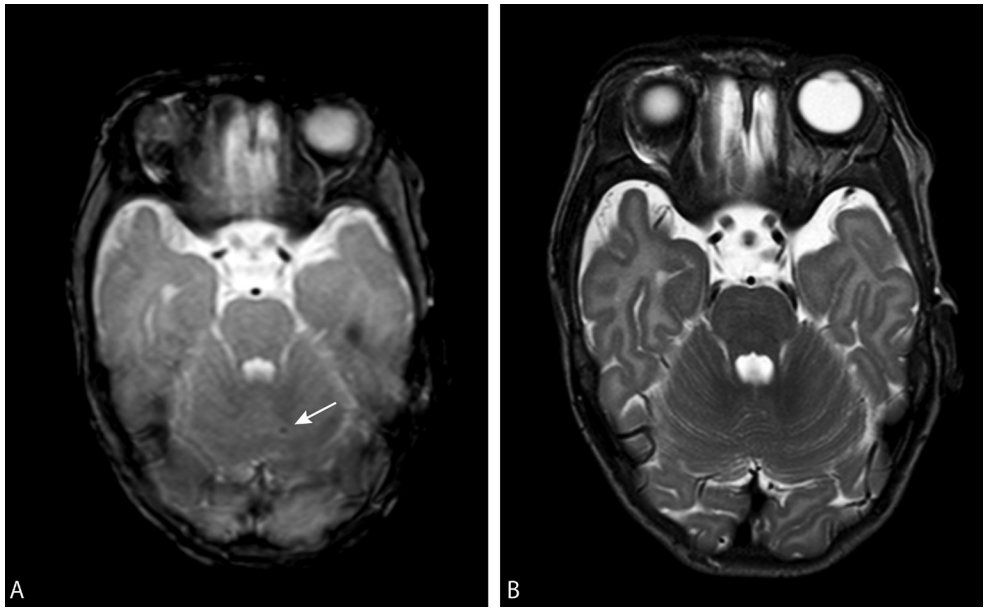


Table 3. Association between hemosiderin and white matter injury (> 6 PWML/ex-vacuo ventricular dilatation/cysts) on MRI.

	> 6 PWML			**Ex-vacuo ventricular dilatation**				**Cysts**		
	No	*Yes*		*No*	*Moderate*	*Severe*		*No*	*Yes*	
	N=79	*N=16*	*p-value*	*N=27*	*N=49*	*N=17*	*p-value*	*N=89*	*N=6*	*p-value*
Supratentorial	15	10	0.001*	6	13	6	0.36	19	6	<0.001*
Germinal matrix	9	4	0.22	2	7	4	0.14	9	4	0.003*
Ventricular wall	11	10	<0.001*	5	10	6	0.24	15	6	<0.001*
Intraventricular	10	6	0.03*	1	10	5	0.02*	10	6	<0.001*
Periventricular	5	4	0.04*	1	6	2	0.31	4	5	<0.001*
Deep white matter	1	0	1.0	0	0	1	0.10	0	1	0.06
Infratentorial	18	5	0.53	5	14	3	0.90	18	5	0.003*
4^{th} ventricular wall	2	2	0.13	1	2	1	0.75	1	3	0.001*
Cerebellar vermis	3	2	0.20	2	0	2	0.75	3	2	0.03*
Cerebellar hemispheres	16	3	1.0	4	14	1	0.72	16	3	0.09
Total	28	10	0.04*	10	21	6	1.0	32	6	0.003*

Table 4. Regression analysis: significant relations (p< 0.05) between presence of hemosiderin deposits on MRI and neurodevelopmental outcome at two years of age.

	Psychomotor developmental index (BSID-III)			
	Unadjusted beta coefficient (SE)	*p-value*	*Adjusted beta coefficient (SE)* [¥]	*p-value*
Supratentorial	-8.0 (3.9)	0.04	1.4 (4.2)	0.75
Ventricular wall	-9.8 (3.9)	0.02	0.8 (4.6)	0.86

[¥] adjusted for gestational age, gender, intra uterine growth retardation and white matter injury on MRI (> 6 PWML/ ventricular dilatation /cysts)
SE = standard error

Follow up

Follow up was available for 76 (80%) infants. Infants were lost to follow up due to miscellaneous reasons such as rejection of participation or practical problems, including travel distance to the hospital. Mean corrected age at follow up was 29.7 months (range 20.1 – 42.1 months, SD 4.5) No difference in baseline clinical parameters or presence of hemosiderin deposits was found between infants with and without follow up.

Table 4 shows the unadjusted and adjusted associations between hemosiderin deposits per location and the PDI at two years. In infants with hemosiderin deposits supratentorially and/or in the ventricular wall, we found a lower PDI (respectively p=0.04 and p=0.02). However, after correcting for clinical parameters (gestational age, gender and intrauterine growth retardation) and white matter injury, the association was no longer significant. Backward linear regression analyses showed that the presence of more than 6 PWML (p=0.002) and cystic white matter lesions (p=0.02) were independent predictors of a lower PDI. No differences in cognitive or motor delay, cerebral palsy or CBCL scores were found between infants with (N=32) and without (N=44) hemosiderin deposits.

DISCUSSION

In this study we assessed presence of hemosiderin deposits, as detected by T2*-w gradient echo MRI, in an unselected cohort of very preterm infants and correlated presence and location of hemosiderin deposits with white matter injury and neurodevelopmental outcome at two years of corrected age. Our most important finding is that especially hemosiderin deposits located in the ventricular wall are correlated with white matter injury and a less favorable neurodevelopmental outcome. However, after adjusting for

clinical parameters and white matter injury the association between these hemosiderin deposits and a less favorable neurodevelopmental outcome was no longer significant. The presence of more than 6 PWML and cystic white matter lesions were independent predictors for psychomotor delay.

The clinical use of gradient echo techniques, including susceptibility-weighted imaging (SWI), to detect microbleeds or hemosiderin deposits in the elderly is widely accepted.[19,25,26] Yet reports on the use of gradient echo techniques in infants are scarce.[27,28] In a recent study the use of SWI to distinguish hemorrhagic from non hemorrhagic punctate white matter lesions in neonates was reported.[29] However, in this paper the clinical relevance of detecting hemorrhagic lesions in relation to neurodevelopmental follow up was not discussed.

From large cohort studies[30] it is known that amongst other pathologies, intraventricular hemorrhage is predictive of a less favorable neurodevelopmental outcome in very preterm neonates. This is especially true for grade III – IV intraventricular hemorrhages detected by ultrasound in the neonatal period.[31,32] Ultrasound studies have also reported poorer neurodevelopmental outcomes at 20 months in extremely low birth weight infants with grade I - II intraventricular hemorrhages. However, ultrasound could have missed additional injury associated with intraventricular hemorrhages explaining the poorer outcomes.[33] As we excluded infants with PHVD diagnosed by ultrasound, the ventricular dilatation in our cohort most likely resulted from white matter injury. We found that especially hemosiderin deposits in and around the ventricular walls, without parenchymal involvement are associated with PWML and cystic white matter lesions, and that these hemosiderin deposits are correlated with a less favorable neurodevelopmental outcome. In a recent study it was found that GMH/IVH was associated with hemorrhagic punctate white matter lesions, possibly as a result of impaired venous drainage and increased pressure in the medullary veins resulting in periventricular white matter damage.[29] Our data showed that after correction for clinical parameters and white matter injury, the association between hemosiderin deposits in the ventricular wall and a less favorable neurodevelopmental outcome was no longer significant, while the presence of more than 6 PWML and cystic white matter lesions were independent predictors for a lower PDI. Therefore our data show that the presence of white matter injury is a better predictor for a less favorable neurodevelopmental outcome than small ventricular wall hemorrhages.

A limitation of our study is that we used a T2*-w gradient echo technique, while SWI is increasingly being used in clinical practice.[27,29] However, the clinical significance of de-

tecting more and/or even smaller hemosiderin deposits with SWI as compared to T2*-w gradient echo MRI[26] seems to be limited.

In this cohort of preterm infants we defined ventricular dilatation as ex-vacuo ventricular dilatation from white matter injury resulting in volume loss.[34,35] By excluding infants with PHVD diagnosed by ultrasound during the neonatal period, we intended to differentiate between ex-vacuo ventricular dilatation and PHVD. However, both forms of ventricular dilatation may co-exist and are interrelated. Moreover, we may have missed hemosiderin deposits, as the age range (40 – 60 weeks postmenstrual age) at which the infants were imaged may be of influence on the detectability of hemosiderin originating from hemorrhages occurring in the perinatal period.

Another limitation of our study is that a substantial number of infants (20%) were lost to follow up. However, at baseline, there were no differences in clinical parameters or presence of hemosiderin deposits between infants with or without clinical follow up. As especially cognitive delay or handicap is difficult to test at this young age, long term follow up at school age may still demonstrate differences in cognitive and behavioral performance.

In conclusion, we demonstrated that T2*-w gradient echo sequences detect and localize small hemosiderin deposits in very preterm infants, especially in and around the ventricular system. The clinical importance of detecting these small hemosiderin deposits is however limited as there is no independent association with neurodevelopmental outcome.

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10

Summary

The general aim of this thesis is to assess the diagnostic and predictive value of MRI in imaging the preterm infant's brain.

In May 2006 a prospective study was started including very preterm infants with a gestational age <32 weeks, admitted to the neonatal intensive care unit of the Leiden University Medical Center. Bedside cranial ultrasound examinations were performed following a standard routine and 113 of these infants underwent an MRI examination preferably performed at term equivalent age. Ethical approval for the study was given by the institutional review board and informed consent was obtained for each infant. All MRI examinations were performed using a standard protocol for imaging the newborn infant's brain, using a 3.0 T MRI system (Philips Medical Systems, Best, the Netherlands).

Chapter 2

In the second chapter we review the literature regarding radiological assessment of white matter injury in very preterm infants. We discuss the imaging findings on cranial ultrasound and MRI, and the imaging modalities and techniques used to depict white matter injury. The use and utility of advanced techniques such as diffusion tensor imaging, volumetric and segmentation techniques, magnetization transfer imaging, functional resting state MRI, as well as MR spectroscopy are discussed.

Chapter 3

This chapter focuses on diffusion tensor imaging and fibre tractography. We performed these techniques to study the developing white matter tracts of the internal capsule and corpus callosum around term equivalent age in our cohort of very preterm infants. The aim of the study was to establish the association between DTI parameters and age, white matter injury and clinical factors. We found associations between FA and ADC values and postmenstrual age at imaging, indicative of developing white matter. However, we did not find associations between DTI parameters and gestational age, white matter injury categorized as mild, moderate or severe, or clinical factors.

Chapter 4

The aim of the study described in this chapter was to investigate whether tractography of white matter tracts performed at term equivalent age independently predicts neurodevelopmental outcome at two years of age in very preterm infants. We found associations between lower FA values in the posterior limb of the internal capsule and psychomotor delay and cerebral palsy, and also an association between higher ADC values in the splenium of the corpus callosum and psychomotor delay, independent of white

matter injury, ventricular dilatation and clinical factors. These observations confirm that DTI tractography at term equivalent age can independently predict psychomotor delay and cerebral palsy at two years of age.

Chapter 5

The research question in this study was whether cranial ultrasound performed in the perinatal period can reliably predict diffuse white matter injury as seen on MRI performed around term equivalent age. A classification for white matter injury was used for both imaging modalities. The predictive value of the cranial ultrasound classification for white matter injury on MRI was calculated. There was a reasonably high positive predictive value for detection of severe white matter on MRI, but to a lesser extent for mild or moderate white matter injury, prompting the indication for an MRI examination at term equivalent age to detect diffuse white matter injury.

Chapter 6

As diffuse white matter injury is not well detected by cranial ultrasound, our aim was to assess the predictive value of individual abnormalities on cranial ultrasound for white matter injury on MRI around term equivalent age and neurological outcome. Periventricular echo densities in the white matter on cranial ultrasound reasonably predict mild, moderate and severe white matter injury observed on term equivalent age MRI. They also predict the occurrence of diffuse excessive high signal intensity (DEHSI) on MRI. However, absence of periventricular echo densities does not predict absence of white matter injury or DEHSI on MRI. No associations existed between inhomogeneous periventricular echo densities and focal punctate white matter lesions on MRI. Peri and intraventricular hemorrhages were highly predictive of abnormal white matter on MRI and also, together with ventricular dilatation, reasonably predictive of an unfavorable outcome at two years. Absence of cranial ultrasound abnormalities in the white matter and normal ventricular size and shape are highly predictive of a normal outcome at two years.

Chapter 7

In the study described in this chapter we investigate the clinical implications of individual MR imaging findings in the white matter on MRI at term equivalent age in terms of neurodevelopmental outcome at two years. The most important finding was that DEHSI, which occurred in the majority of infants imaged at term equivalent age showed no association with an abnormal neurologic outcome. We therefore postulated that DEHSI represents a prematurity related developmental phenomenon rather than white matter

injury. Furthermore punctate white matter lesions and ventricular dilatation were associated with mental and psychomotor developmental delay and cerebral palsy.

Chapter 8

The study described in this chapter demonstrated that cerebellar hemorrhage in the very preterm infant is a frequent finding and, using ultrasound, it is better diagnosed using the mastoid fontanelle approach in addition to routine cranial ultrasound through the anterior fontanelle. Gradient echo MRI, used as a reference standard demonstrated more (punctate) hemosiderin deposits not diagnosed on cranial ultrasound. We concluded that the predictive implications of these smaller lesions needed further attention.

Chapter 9

In this chapter we studied the clinical value of gradient echo MRI detecting small punctate hemosiderin deposits in relation to neurodevelopmental outcome at two years. Presence of small hemosiderin deposits in the ventricular wall correlated with white matter injury on MRI around term equivalent age and with a lower psychomotor developmental index at two years. However, after correction for gestational age, white matter injury and clinical factors, this correlation was no longer significant. We concluded that the importance of detecting small hemosiderin deposits using gradient echo MRI is limited as there is no independent association with neurodevelopmental outcome.

Concluding remarks and future perspectives

The aim of this thesis was to investigate the diagnostic value of MRI performed at term equivalent age in terms of detecting brain injury and predicting neurodevelopmental outcome in very preterm infants (gestational age <32 weeks).

In these patients, MRI is a powerful tool to diagnose all types of white matter injury, while cranial ultrasound can detect hemorrhages, ventricular dilatation and cystic white matter lesions. MRI is sensitive in detecting punctate white matter lesions, which are associated with mental and psychomotor delay and cerebral palsy at follow up. White matter echo densities on ultrasound in the neonatal period do not correlate with these punctate white matter lesions on MRI around term equivalent age. Although MRI is a sensitive technique for detecting white matter lesions in general, the positive predictive value of specific MRI findings such as punctate white matter lesions, and also cystic lesions and ventricular dilatation for cognitive and motor delay is low.

Currently conventional MRI can predict that a very preterm infant will have a nor-

mal or abnormal outcome with a reasonable certainty, when the MRI examination either shows no focal white matter lesions and volume loss or when there is severe white mater damage, post hemorrhagic ventricular dilatation and volume loss. However, the outcome of very premature infants with mild or moderate white matter injury is still uncertain. As normal cranial ultrasound also is highly predictive for normal outcome, routine clinical MRI in every preterm infant at term equivalent age does not seem warranted. Long-term clinical follow up does remain necessary to further evaluate the predictive values of individual neuro-imaging findings and quantitative values around term equivalent age for cognitive neurodevelopmental outcome in very preterm infants.

An advanced MRI technique such as DTI is promising, and, as demonstrated in several of our studies, may help predicting clinical outcome. However, when the findings on the conventional MRI sequences are also taken into account, there is only a slight increase in sensitivity and specificity using DTI. Therefore there still is no indication for the routine use of DTI in daily clinical practice. The same is true for other advanced techniques such as volumetric and segmentation techniques, the use of which is not yet feasible in a clinical setting. Also the relation with neurodevelopmental outcome has not been studied extensively. Whole-brain statistical methods developed for DTI analysis, such as tract-based spatial statistics and atlas-based analysis might have the potential to detect mild-to-moderate white matter injury in relation to neurological outcome. Other advanced techniques such as functional resting state MRI and magnetization transfer imaging are still under active investigation. Additional research is needed to determine the clinical utility of these advanced techniques and their potential to reveal the anatomical substrate for cognitive deficits in preterm infants who do not appear to have abnormalities on other imaging techniques. In the future, serial MRI with combined grading and the application of newer techniques may provide insights into brain development and injury to the preterm infant's brain, and they may help predicting neurological outcome.

Nederlandse samenvatting

Het doel van dit proefschrift is om bij vroege (voor de 32ste week van de zwangerschap) prematuur geboren kinderen, de diagnostische en voorspellende waarde vast te stellen van MRI onderzoek van de hersenen, verricht rond de à terme datum.

Van alle kinderen die jaarlijks in Nederland worden geboren, wordt ongeveer 2% voor de 32ste week van de zwangerschap geboren. Deze kinderen hebben, ondanks de verbeterde zorg op de neonatale intensive care afdelingen, een vergrote kans op het krijgen van een motorische en cognitieve ontwikkelingsachterstand op latere leeftijd. Schade aan de hersenen, welke zich onder niet fysiologische omstandigheden na de vroeggeboorte verder ontwikkelen, lijkt hieraan ten grondslag te liggen. Hersenechografie wordt toegepast om gedurende de opname op de intensive care geïnformeerd te zijn over hersenontwikkeling en -schade. Omdat het niet duidelijk is in hoeverre hersenechografie schade aan de witte stof kan voorspellen, is in mei 2006 een prospectief onderzoek gestart waarbij, bij kinderen geboren voor de 32ste week van de zwangerschap, op de à terme leeftijd een MRI werd vervaardigd om de mate van hersenschade vast te stellen.

De medisch ethische commissie van het Leids Universitair Medisch Centrum heeft het onderzoek goed gekeurd en ouders werd om toestemming gevraagd om rond de à terme leeftijd een MRI van de hersenen van hun kind te verrichten. De kinderen werden volgens een vast gesteld protocol op een 3 Tesla MRI gescand. De bevindingen van dit MRI onderzoek worden in de diverse hoofdstukken van dit proefschrift beschreven. Bovendien worden deze ook gecorreleerd aan de ontwikkeling van de kinderen rond de leeftijd van twee jaar, het tijdstip dat zij werden terug gezien door de neonatoloog en waarbij verschillende ontwikkelingstesten werden afgenomen.

Hoofdstuk 2

In het tweede hoofdstuk wordt een overzicht gegeven van de bevindingen van witte stof schade, zowel bij hersenechografie als op MRI. Behalve conventionele MRI technieken worden er ook geavanceerde MRI technieken besproken, die mogelijk in de nabije toekomst beter in staat zullen zijn om de schade aan de witte stof vast te stellen.

Hoofdstuk 3

In dit hoofdstuk wordt beschreven of er een correlatie bestaat tussen Diffusion Tensor Imaging (DTI) en tractografie parameters van witte stof banen door het achterste been van de capsula interna en door het corpus callosum, en de mate van witte stof schade, de leeftijd van het kind en enkele bepalende klinische factoren. Wij vonden geen correlatie met witte stof schade of klinische factoren, maar wel met de leeftijd

van het kind ten tijde van de MRI, wat erop duidt dat bepaalde DTI parameters, zoals de FA en ADC, een maat zijn voor de ontwikkeling van de witte stof banen.

Hoofdstuk 4
Het doel van de studie beschreven in dit hoofdstuk, was om vast te stellen of tractografie verricht rond de à terme leeftijd, in staat is om de neurologische ontwikkeling van het kind te voorspellen. Hierbij is gebleken dat lagere FA waarden in het achterste been van de capsula interna en hogere ADC waarden in het splenium van het corpus callosum in staat zijn om psychomotore achterstand en/of cerebrale parese te voorspellen op de leeftijd van twee jaar.

Hoofdstuk 5
De onderzoeksvraag van dit hoofdstuk was of hersenechografie verricht rondom de vroeggeboorte in staat is om de mate van witte stof schade op MRI rond de à terme leeftijd te voorspellen. Het blijkt dat hersenechografie ernstige witte stof schade op MRI goed kan voorspellen, maar minder goed in staat is om milde of matig ernstige witte stof schade te voorspellen.

Hoofdstuk 6
In dit hoofdstuk is onderzocht wat de voorspellende waarde is van verschillende echografische kenmerken, voor witte stof schade op MRI rond de à terme leeftijd en voor neurologische ontwikkeling. Periventriculaire toegenomen echodensiteit van de witte stof is redelijk goed in staat om witte stof schade op MRI te voorspellen, maar de afwezigheid hiervan voorspelt niet de afwezigheid van witte stof schade op MRI. Het blijkt dat peri- en intraventriculaire bloedingen het beste in staat zijn om witte stof schade op MRI en een slechtere neurologische ontwikkeling te voorspellen. Bovendien werd vastgesteld dat de afwezigheid van echografische afwijkingen van de witte stof en een normale wijdte van de ventrikels, een hoge voorspellende waarde hebben voor een normale neurologische ontwikkeling.

Hoofdstuk 7
In dit hoofdstuk beschrijven we de klinische implicaties van diverse MRI bevindingen van witte stof schade rond de à terme leeftijd op de neurologische ontwikkeling op tweejarige leeftijd. De belangrijkste bevinding was dat periventriculaire signaal verhoging, zogenaamde DEHSI, waarvan gedacht werd dat het mogelijk witte stof schade representeert, geen correlatie had met een neurologische achterstand op twee jaar. Wij hebben daarom gepostuleerd dat DEHSI een ontwikkelingsfenomeen betreft, met een

hoge incidentie rond de à terme leeftijd en welke afwezig is na de 50ste postmenstruele week, bij prematuur geboren kinderen.

Focale punctiforme witte stof laesies en ventrikel dilatatie waren echter wel voorspellend voor ontwikkelingsachterstand en cerebrale parese.

Hoofdstuk 8

De studie beschreven in dit hoofdstuk toont aan, dat met het gebruik van echografie, de mastoid fontanel de beste benadering is om cerebellaire bloedingen te detecteren. Gradiënt echo MRI verricht rond de à terme datum, welke als gouden standaard werd gezien, detecteert echter meer, voornamelijk puntvormige hemosiderine resten van bloedingen. De conclusie van dit onderzoek was dat de betekenis van deze hemosiderine resten voor de ontwikkeling van prematuur geboren kinderen verder onderzocht moet worden.

Hoofdstuk 9

In dit hoofdstuk hebben we de klinische waarde van het detecteren van hemosiderine resten met behulp van gradiënt echo MRI onderzocht in relatie tot de neurologische ontwikkeling op twee jaar. Hemosiderine resten in de ventrikelwand waren gecorreleerd met de mate van witte stof schade op de à terme leeftijd en met een lagere psychomotore ontwikkeling op tweejarige leeftijd. Echter na correctie voor witte stof schade, leeftijd bij de geboorte en bepalende klinische factoren was de relatie niet significant meer, wat impliceert dat de kleine hemosiderine resten, die we met behulp van gradient echo MRI detecteren, geen onafhankelijke voorspellers zijn voor een achterstand in ontwikkeling.

CONCLUSIE

Het doel van dit proefschrift was om de diagnostische en voorspellende waarde van een MRI onderzoek van de hersenen rond de à terme leeftijd vast te stellen bij vroege prematuur geboren kinderen. Hoewel conventionele MRI beter in staat is om witte stof schade van de hersenen vast te stellen dan echografie, blijft het lastig om op grond van het MRI beeld, de neurologische ontwikkeling van kinderen met milde of matige witte stof schade te voorspellen. DTI en tractografie zijn beter in staat om te voorspellen of kinderen motorische achterstand of cerebrale parese zullen ontwikkelen, echter in combinatie met de bevindingen op conventionele MRI is er slechts een geringe toename in sensitiviteit en specificiteit te verwachten. Op dit moment is het klinisch verrichten

van een MRI op de à terme datum bij ieder prematuur geboren kind niet geïndiceerd, zeker niet wanneer een normale echografie van de hersenen een goede neurologische uitkomst kan voorspellen. Langdurig vervolgen van deze kinderen blijft echter noodzakelijk, om een beter inzicht te krijgen in diagnostische en voorspellende waardes van beeldvorming, met name om de cognitieve ontwikkeling op termijn te kunnen voorspellen.

Toekomst perspectief

Nieuwe technieken zoals die ontwikkeld worden voor DTI analyse van het hele brein, maar ook volume metingen en segmentatie technieken, zullen mogelijk in de toekomst beter in staat zijn om witte stof schade te kwantificeren. Verder onderzoek is noodzakelijk om met behulp van deze nieuwe technieken een anatomisch substraat te vinden, wat neurologische ontwikkelingsachterstand kan voorspellen. Evaluatie van klinische toepasbaarheid van deze nieuwe technieken, waaronder ook functionele MRI in rust (resting state fMRI) en magnetisatie transfer imaging (MTI), is noodzakelijk.

Wellicht zal in de toekomst de combinatie van opeenvolgende MRI onderzoeken en de toepassing van nieuwe technieken inzicht geven in de ontwikkeling van en schade aan de hersenen van prematuur geboren kinderen en zullen we beter in staat zijn om hun neurologische ontwikkeling in een vroeg stadium te voorspellen.

List of Abbreviations

ADC	apparent diffusion coefficient
BPD	bronchopulmonary dysplasia
BSID-III	Bayley scales of infant development - version 3
CC	corpus callosum
CBCL	child behavior check list
DEHSI	diffuse excessive high signal intensity
DWI	diffusion weighted imaging
DTI	diffusion tensor imaging
FA	fractional anisotropy
GA	gestational age
GMFCS	gross motor function classification system
GMH	germinal matrix hemorrhage
HC	head circumference
IUGR	intrauterine growth retardation
IVH	intraventricular hemorrhage
MDI	mental developmental index
PDA	persistent ductus arteriosus
PDI	psychomotor developmental index
PLIC	posterior limb of internal capsule
PMA	postmenstrual age
PWML	punctate white matter lesion
RDS	respiratory distress syndrome
TEA	term equivalent age
VPTI	very preterm infants
WM	white matter

Curriculum Vitae

The author of this thesis was born on the 2nd of January 1956 in Comodoro Rivadavia, Argentine. After completing secondary school in 1974, she started her medical studies at the University of Leiden in 1978 and obtained a medical degree in 1985.
In 1985 she commenced a radiology residency at the Academic Hospital Leiden (Head, Professor Dr. A.E. van Voorthuisen), which she completed in 1990. Since then she works as a staff member at the Radiology Department of the Leiden University Medical Center (Head, Professor Dr. J.L. Bloem). Her subspecialty is neuroradiology (Chief, Professor Dr. M.A. van Buchem), with a special interest in pediatric neuroradiology.
The research which resulted in this thesis was initiated in 2008 in close cooperation with Dr. Gerda van Wezel-Meijler, former neonatologist from the Neonatology Department of this hospital, and Dr. Jeroen van der Grond from the Radiology Department.