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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.\textsuperscript{1-5} White matter injury may eventually result in damage, underdevelopment and atrophy of the internal capsule and the corpus callosum.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{13,21}

\textbf{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\textsuperscript{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 \textit{b}-value of 1000 s/mm\textsuperscript{2}, EPI factor 56) with an image time of 5 min and 34 s.

\textbf{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\textsuperscript{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.\textsuperscript{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.\textsuperscript{5}

\textbf{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.
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.

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

*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.
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 term equivalent age (between 40 – 44 weeks). Follow up was available in 44/59 (74.6%) infants.

<table>
<thead>
<tr>
<th></th>
<th>AUC (p-value)</th>
<th>Cut-off value</th>
<th>Sensitivity % (95%CI)</th>
<th>Specificity % (95%CI)</th>
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<tr>
<td><strong>FA of the PLIC</strong></td>
<td></td>
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<tr>
<td>Motor delay</td>
<td>0.89 (&lt;0.001)</td>
<td>0.36</td>
<td>100 (48-100)</td>
<td>69 (55-82)</td>
</tr>
<tr>
<td>Cerebral palsy</td>
<td>0.79 (&lt;0.001)</td>
<td>0.36</td>
<td>80 (28-100)</td>
<td>66 (53-78)</td>
</tr>
<tr>
<td><strong>Length of the PLIC</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Motor delay</td>
<td>0.80 (0.03)</td>
<td>53.9</td>
<td>80 (28-100)</td>
<td>79 (66-90)</td>
</tr>
<tr>
<td><strong>ADC of the splenium</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Motor delay</td>
<td>0.80 (&lt;0.001)</td>
<td>1.38</td>
<td>100 (48-100)</td>
<td>65 (50-78)</td>
</tr>
</tbody>
</table>

ROC = receiver operating characteristic curve, AUC = area under the curve
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 $1.38 \times 10^{-3}$ 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. 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. 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. 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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