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ABSTRACT

BACKGROUND AND PURPOSE: In infants born very preterm, monitoring of early brain growth could contribute to prediction of later neurodevelopment. Therefore, our aim was to investigate associations between 2 early cranial ultrasound markers (corpus callosum–fastigium and corpus callosum length) and neurodevelopmental outcome and the added value of both markers in the prediction of neurodevelopmental outcome based on neonatal risk factors and head circumference in very preterm infants.

MATERIALS AND METHODS: This prospective observational study included 225 infants born at <30 weeks' gestational age, of whom 153 were without any brain injury on cranial ultrasound. Corpus callosum–fastigium and corpus callosum length and head circumference were measured at birth, 29 weeks' gestational age, transfer from the neonatal intensive care unit to a level II hospital, and 2 months' corrected age. We analyzed associations of brain markers and their growth with cognitive, motor, language, and behavioral outcome at 2 years' corrected age.

RESULTS: In infants without brain injury, greater corpus callosum–fastigium length at 2 months was associated with better cognitive outcome. Corpus callosum length at 2 months was positively associated with cognitive, motor, and language outcome. Faster growth of the corpus callosum length between birth and 2 months was associated with better cognitive and motor function. Prediction of neurodevelopmental outcome based on neonatal risk factors with or without head circumference was significantly improved by adding corpus callosum length.

CONCLUSIONS: Both corpus callosum–fastigium and corpus callosum length on cranial ultrasound are associated with neurodevelopmental outcome of very preterm infants without brain injury at 2 years, but only corpus callosum length shows the added clinical utility in predicting neurodevelopmental outcome.

ABBREVIATIONS: CA = corrected age; CBCL = Child Behavior Checklist; CC = corpus callosum; CCF = corpus callosum–fastigium; CUS = cranial ultrasound; GA = gestational age; HC = head circumference; IQR = interquartile range; NICU = neonatal intensive care unit

In infants born very preterm, adverse brain growth is an important predictor of later neurodevelopmental impairment.^{1,2} Therefore, monitoring early brain growth is important and requires reliable and clinically applicable markers. The most commonly used marker


in infancy is head circumference (HC), which is easily applicable in clinical care. In preterm infants however, head circumference often poorly reflects brain size due to head deformities and increased extracerebral fluid.^{3,4} Brain imaging techniques can add valuable information on the actual size of the brain. MR imaging is considered the most reliable method but is not bedside-available and is expensive, limiting the possibility of serial repeat imaging. Cranial ultrasound (CUS) can be performed more easily and, therefore, serially during a stay in the neonatal intensive care unit (NICU).⁵

Several previous studies in preterm infants linked corpus callosum (CC) length at term-equivalent age with neurodevelopmental outcome in childhood.^{6–8} Because CC length only reflects a small part of the brain, our study group introduced corpus callosum–fastigium (CCF) length as a new marker for brain growth.⁹ CCF length is measured on CUS in a standard midsagittal plane and covers a larger part of the brain than CC length, including several important brain structures such as the thalamus. The measurement

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can be performed both pre- and postnatally. We previously showed that CCF length has high reproducibility and applicability for monitoring brain growth during fetal life and a NICU stay.^{9,10} CCF length was found to be smaller in fetuses and neonates with fetal growth restriction compared with those with normal growth.^{10,11} However, the predictive value of CCF length for neurodevelopmental outcome needs further investigation.

In this study, we explored the associations between length and growth of the CCF and CC in early infancy and neurodevelopmental outcome at 2 years' corrected age (CA) in infants born very preterm, specifically in those without brain injury. We hypothesized that longer length and faster growth of the CCF and CC are associated with improved neurodevelopmental outcome and that both markers have added clinical value to the prediction of neurodevelopment compared with neonatal risk factors and head circumference.

MATERIALS AND METHODS

Participants

This study combined data of 2 comparable prospective observational cohort studies performed between 2010 and 2017 at the NICU of the Erasmus MC Sophia Children's Hospital, Rotterdam, the Netherlands. All preterm infants born between 24 and 30 weeks' gestational age (GA) and admitted to the NICU within 48 hours after birth were eligible for participation in Study A (Submarine study) or Study B (BOND study) (Online Supplemental Data).^{12,13} Infants with severe congenital or chromosomal abnormalities, perinatal asphyxia (cord blood/first postnatal pH, <7.0 and APGAR score at 5 minutes, <5), and congenital TORCH infection (toxoplasmosis, other agents, rubella, cytomegalovirus, and herpes simplex) were excluded. Parental informed consent was obtained for all participants. Both studies were approved by the medical ethics committee of the Erasmus MC Sophia Children's Hospital, University Medical Center, Rotterdam.

Maternal, obstetric, and neonatal characteristics were collected prospectively from the electronic medical record. Ethnicity was classified as non-Western if one or both parents were born in a non-Western country, and parental education level was based on both parents.¹⁴ Brain injury was diagnosed on CUS and included subependymal and intraventricular hemorrhage (grade 1–2+), cerebellar hemorrhage, stroke, and/or periventricular leukomalacia. Postnatal age was defined as days after birth with the day of birth as day 1.¹⁵

Markers of Brain Growth

CUS was routinely performed according to local clinical protocol by the attending neonatologist or an experienced researcher. The local protocol included CUS on postnatal age days 1, 2, 3, and 7, followed by weekly measurements until transfer from the NICU to a level II hospital. A MyLab 70 scanner (Esaote) with a convex neonatal probe (7.5 MHz) was used. Off-line measurements of CC length and CCF length on a standard midsagittal plane were performed using MyLab software (Esaote) by one of the researchers. As described previously in detail, CCF length (centimeters) was measured from the genu of the corpus callosum (outer border) to the fastigium, and CC length (centimeters), from genu to the splenium (outer-outer border) (Online Supplemental Data).⁹

Head circumference (centimeters) was measured during the NICU stay as part of standard care using a tape measure. Growth *z* scores were based on the Fenton Growth Charts from birth until discharge or 50 weeks' GA and on the World Health Organization growth charts thereafter.¹⁶

For this study, we used measurements of CCF length, CC length, and HC assessed at the following times: 1) birth (postnatal age, days 1–3); 2) around 29 weeks' GA (28–30 weeks); and 3) at NICU transfer to a level II hospital (limited to 30–36 weeks' GA). Growth rate (millimeters/week) of CCF length, CC length, and HC was calculated between birth and NICU transfer. To increase homogeneity in timing and the length of the growth periods, we only calculated growth rate when the first CUS was performed in the first week of life and the period between 2 measurements covered at least 14 days.

In study B, CUS and HC measurements were also performed at the routine outpatient clinic visit at a median of 6.9 weeks' CA (interquartile range [IQR], 6.1–8.3 weeks), further referred to as the 2 months' visit. In these infants, the growth rate of each marker was also calculated between birth and 2 months.

Neurodevelopmental Outcome

As part of the national neonatal follow-up program, all children were routinely invited to the outpatient clinic at 2 years' CA for physical and neurologic examinations by a neonatologist or pediatric neurologist. Trained physiotherapists and psychologists performed extensive testing of psychomotor and cognitive development using fine-motor and gross motor (summarized in a total motor score) and cognitive tests of the Bayley Scales of Infant and Toddler Development, 3rd edition (Bayley-III, Dutch edition), expressed as standard scores adjusted for CA at the moment of testing.¹⁷ Following Dutch guidelines, the Lexi list was used to evaluate expressive language development. This validated questionnaire is completed by parents to quantify the child's vocabulary with scores adjusted for CA at assessment and sex.¹⁸ For each child, parents were asked to complete the Child Behavior Checklist 1.5–5 years (CBCL 1.5–5), which is an internationally validated questionnaire examining behavioral and emotional problems.¹⁹ For this study, we used the CBCL Total Problems scale expressed in T-scores adjusted for CA at assessment and sex. Assessors and parents were unaware of CC or CCF length measurements.

Statistical Analysis

Because the presence and severity of brain injury in the neonatal period can influence both brain growth and neurodevelopmental outcome disproportionately,²⁰ we mainly focused on the large group of infants without any brain injury on neonatal CUS. To explore the value of the brain growth markers in the presence of brain injury, we performed additional exploratory analyses in the smaller and more heterogeneous group with any extent of neonatal brain injury on CUS. Relative risks for adverse outcomes were calculated comparing infants with and without brain injury.

First, we used nonparametric statistical tests for nonresponse analyses. Second, we used linear regression models to study the associations between length (at birth, 29 weeks' GA, NICU transfer, and 2 months' CA) and growth rate (between birth and

NICU transfer and between birth and 2 months) of the CCF, CC, and HC and the 4 neurodevelopmental outcomes (motor, cognitive, language, and behavior) at 2 years' CA in both groups. In the basic models, we adjusted for GA or CA at CUS assessment. The adjusted models were additionally corrected for sex, GA at birth, birth weight z score, and parental education on the basis of relevance reported in the literature.^{5,21} These 4 covariates were tested and confirmed to show either a statistical association with at least 1 of the 2 ultrasonic brain markers at 2 months and cognitive outcome or a change in the effect size of $>10\%$ after addition of the covariate to the basic model. Given the number of participants and variables in our models and to limit type I or II error, we only performed analyses when at least 40 measurements were available per analysis. For comparability of effect sizes, associations are reported by steps resembling the average IQR of each marker. We observed no significant interactions between any of the brain markers and sex.

Third, we evaluated the added clinical value of the brain markers in predicting neurodevelopmental outcome in infants without brain injury, compared with prediction based on neonatal risk factors and head circumference only. As baseline, a "basic neonatal" regression model was used for prediction of cognitive outcome. This model was recently created in a preterm population overlapping this cohort.²² This model included sex, GA at birth, combined parental education level, grade of bronchopulmonary dysplasia (no/mild/severe), treated patent ductus arteriosus (medically and surgically), brain injury, and the duration of the hospital admission. Because this analysis was performed in the group of infants without any brain injury, we did not include "brain injury" as a covariate in the basic neonatal model of the current study. Using linear hierarchical regression models and explained variances (R^2), we compared the basic neonatal model (both with and without HC) with models that additionally included any CUS markers associated with neurodevelopmental outcomes.

P values (2-tailed) $< .05$ were considered statistically significant. We calculated 95% confidence intervals for all effect estimates. Correction for multiple testing was not deemed necessary given the step-based and exploratory character of the analyses. Data were analyzed using SPSS Statistics, Version 25.0 (IBM) and R statistical and computing software (<http://www.r-project.org/>).

RESULTS

Study Population

Of 293 eligible children, 225 (77%) were included in this study (Online Supplemental Data), of whom 153 (68%) showed no brain injury on CUS during the neonatal period. Nonresponse analyses showed that included children more often were Western, had slightly higher birth weights, and encountered fewer complications during the NICU stay (data not shown). Parental, perinatal, and neonatal characteristics were mostly similar in infants with and without brain injury (Online Supplemental Data).

Markers of Brain Growth

Length and growth rate of the CC, CCF, and HC are presented in the Online Supplemental Data. The correlation of CC length and CCF length compared with HC during the NICU stay is plotted in the Online Supplemental Data. At all 4 time points, the

absolute length of all 3 markers appeared to be slightly larger in infants without brain injury compared with infants with brain injury. In both groups, the length of the CCF, CC, and HC increased with time. Also, the growth rate of CCF length, and even more so CC length, decreased after transfer from the NICU to a level II hospital (median 31^{+5} – 32^{+1} weeks' GA), while the growth rate of HC increased.

Neurodevelopmental and Neurologic Outcomes

Scores on the 4 neurodevelopmental tests as well as the prevalence and relative risk of neurologic complications are listed in the Online Supplemental Data. In general, outcomes were less favorable in infants with brain injury, with 11% having cerebral palsy compared with 3% (risk ratio, 3.4; 95% CI, 1.2–10.0) and 11% having visual disorders compared with 5% in those without brain injury (risk ratio, 2.1; 95% CI, 0.8–5.4). In both groups, all 4 neurodevelopmental tests showed median scores within the normal range. However, moderate or severe motor impairment was more common in those with brain injury (14% versus 5%; risk ratio, 2.7; 95% CI, 1.1–6.5).

Associations between Brain Length or Growth and Neurodevelopmental Outcome

In infants without neonatal brain injury, larger CCF length at 2 months was associated with better cognitive outcome: Every IQR (5 mm) increase in CCF length was associated with a 9.1 (95% CI, 2.4–15.8) point higher Bayley-III cognitive score (Online Supplemental Data). As for CC length, we observed a 5.9 (95% CI, 2.8–9.1) point higher Bayley-III cognitive score, a 4.6 (95% CI, 1.3–8.0) point higher total motor score, and a 6.5 (95% CI, 2.0–11.0) point higher language score for every IQR (5 mm) increase at 2 months. In addition, a 5-mm larger CC length at birth was associated with a 5.9 (95% CI, 0.4–11.4) point higher motor score. HC was also positively associated with multiple neurodevelopmental outcomes: For every IQR (20 mm) increase at 2 months, we observed a 7.2 (95% CI, 2.9–11.6) point increase in cognitive score and an 8.7 (95% CI, 2.7–14.7) point higher Lexi score.

Each IQR (0.25 mm/week) increase in the CC growth rate between birth and 2 months was associated with a 5.1 (95% CI, 0.9–9.4) point higher cognitive score and a 4.5 (95% CI, 0.1–8.9) point higher motor score. An IQR (1 mm/week) faster HC growth in this period was associated with a 5.8 (95% CI, 0.9–10.7) point higher Lexi score. We did not observe any associations between the growth rate of CCF length and neurodevelopmental outcomes. In the brain injury group, results were only available for the associations of absolute length of the CC, CCF, and HC at birth and 29 weeks' GA and neurodevelopmental outcomes, due to too-small group sizes ($n < 40$) at the other time points. None of these associations were statistically significant (Online Supplemental Data). Results of the basic models, not corrected for sex, GA at birth, birth weight z score, and parental education, are presented in the Online Supplemental Data.

Added Value of CUS Brain Markers for Outcome Prediction

In the Online Supplemental Data, we present the added values of CC and CCF length at 2 months to the prediction of the 3

associated neurodevelopmental outcomes (cognitive, motor, and language) by neonatal risk factors in infants without brain injury. Compared with the basic neonatal model with or without HC, adding CC length led to an 8.8%–9.8% increase in the explained variance (R^2) of cognitive and language outcomes ($P < .05$). There was no added value of (additionally) including CCF length in any of the models for predicting motor, cognitive and language outcomes.

DISCUSSION

In this longitudinal study of 225 infants born very preterm, larger CCF length at 2 months' CA in infants without brain injury was associated with better cognitive outcome at 2 years' CA. As for the CC, larger length at 2 months' CA and a faster growth rate from birth to 2 months were associated with higher cognitive, motor, and language scores at 2 years' CA. These associations were similar to those observed for head circumference. Prediction of neurodevelopmental outcome based on neonatal risk factors and head circumference significantly improved when CC length, but not CCF length, at 2 months was additionally taken into account.

CCF length is a new reliable marker of brain growth that captures a large part of the brain and is related to fetal growth restriction.^{10,11} We showed that in infants without brain injury, CCF length was related to cognitive outcome but had no added clinical value in the prediction of neurodevelopment. Because this is the first study to explore this association, there are no previous studies with which to compare it. The lack of predictive power may have different explanations. First, the anatomic structures that are covered by CCF length (diencephalon, thalamus, mesencephalon) are important areas of the brain, but the cerebellum and WM (as reflected by the CC) are not incorporated into CCF length. Yet these parts of the brain may be more susceptible to external factors influencing brain growth and may, therefore, be more important for outcome in this specific patient group and time period after birth.^{23,24} Second, measurement error may have played a role. However, we consider this explanation less likely because we previously showed adequate reproducibility and reliability of CCF length in a similar setting, and all measurements were performed by 2 experienced researchers.^{9,10}

The positive associations of CC length at 2 months' CA with cognitive, motor, and language outcome, and CC growth until 2 months with cognitive and motor function are in line with previous MR imaging and CUS studies and reflect the importance of the CC as the major WM pathway in the brain.^{6-8,25-27} WM is involved in different domains of neurodevelopment and is very susceptible to injury or microinjury by external factors, including neonatal complications experienced after preterm birth.²⁶ Therefore, in this specific patient group, it is likely that CC size reflects the extent of injury of the WM, which translates to later neurodevelopment. This may also explain why CC length and growth appear to be more strongly associated with neurodevelopmental outcomes than HC. Most interesting, apart from the association between CC length at birth and motor outcome, we only observed associations with outcome when CC length was measured after NICU transfer (>30–32 weeks' GA) and not during the NICU stay. These findings are comparable with those in the CUS studies of Anderson et al,^{26,27}

who reported a relation with the Bayley motor scores at 2 years for CC growth between 2 and 6 weeks after birth (30–34 weeks' GA), but not for CC growth in the first 2 weeks after birth in a similar preterm population. We hypothesize that in infants without brain damage, the period after the NICU stay may be more critical for neurodevelopment. This hypothesis is supported by the decrease in the CC growth rate after NICU transfer observed in this and other studies, likely due to the impact of more chronic complications like bronchopulmonary dysplasia.^{5,27-29}

None of the brain markers were associated with behavioral outcome. This finding may reflect the complex and multifactorial origin of behavior development, which hampers adequate prediction of later behavioral problems, especially at a young age. Furthermore, the CBCL 1.5–5 years used in this study is a screening questionnaire that roughly estimates problem behavior but is not suitable for diagnosis. In addition, underreporting of behavioral problems by parents might be an issue. Nonetheless, a very recent MR imaging study linked global brain abnormalities at term age with the CBCL total problems score at 2 years' CA.³⁰ An important difference compared with our study is that they used detailed and comprehensive Kidokoro scoring on MR images compared with a single CUS measure in our study.³¹

The observed associations of CC length at 2 months with neurodevelopmental outcomes were not stronger than those of HC. However, CC length at 2 months still showed significant added value in the prediction of neurodevelopment compared with prediction based on neonatal risk factors and head circumference only. These findings are the opposite of the conclusion that was drawn in a similar study of 87 very preterm infants by Perenyi et al,⁷ who stated that measuring CC length on CUS in early life had no additional clinical value. To further explore and improve the potential clinical value of CUS at 2 months in neonatal follow-up programs, future studies could explore combining different CUS brain markers (eg, CC length, CCF length, ventricular size, biparietal diameter, vermis length, and cerebellar width) with CUS injury scores to predict neurodevelopmental outcome.

Strengths and Limitations

This study is unique in studying CCF length in relation to neurodevelopment in preterm infants. The availability of longitudinally performed CUS enabled us to study brain markers both during and after NICU admission. Another strength of this study is the relatively large cohort of preterm infants without brain damage, representing a part of the NICU population in whom neurodevelopment has always been difficult to predict. Our data confirm that in those with neonatal brain injury, neurodevelopmental outcomes are less favorable.

Our study also has limitations. First, the group of infants with brain injury was too small and heterogeneous to perform reliable analyses at NICU transfer and 2 months or on growth of the CC and CCF length and HC. Also, the observed lack of associations at birth and 29 weeks' GA in this group should be interpreted with caution because these analyses also contained small numbers of ultrasounds/infant. Future studies should explore how the observed associations in infants without any brain injury hold in a large cohort of children with brain injury. Larger cohorts are also needed to disentangle which types of brain injury affect brain

growth and neurodevelopment most. Second, no CUS was performed around term-equivalent age because, per national policy, infants were transferred to a level II hospital when they were stable, most often around 30–32 weeks' gestation. Third, we were unable to correct for other psychological factors related to neurodevelopment, such as parenting or parental mental health. However, we believe that the most important perinatal, neonatal, and sociodemographic confounders have been covered. Last, the Bayley-III test is a commonly used—but-rough estimate of global neurodevelopment with limited predictive value for later intelligence quotient performance.³² Therefore, follow-up of this cohort into school age is needed.

CONCLUSIONS

This prospective study of infants born very preterm without asphyxia, severe congenital abnormalities, or infections showed the clinical benefit of 2 brain-growth markers, which can be easily measured on CUS. Especially, the CC (length and growth) but also CCF (length) at 2 months' CA were associated with various important neurodevelopmental outcomes at 2 years' CA. Furthermore, CC length but not CCF length showed a significant added clinical value to the prediction of neurodevelopment based on neonatal risk factors and head circumference.

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Disclosure forms provided by the authors are available with the full text and PDF of this article at www.ajnr.org.

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