Longitudinal Regional Brain Development and Clinical Risk Factors in Extremely Preterm Infants

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Objectives To investigate third-trimester extrauterine brain growth and correlate this with clinical risk factors in the neonatal period, using serially acquired brain tissue volumes in a large, unselected cohort of extremely preterm born infants.

Study design Preterm infants (gestational age <28 weeks) underwent brain magnetic resonance imaging (MRI) at around 30 weeks postmenstrual age and again around term equivalent age. MRIs were segmented in 50 different regions covering the entire brain. Multivariable regression analysis was used to determine the influence of clinical variables on volumes at both scans, as well as on volumetric growth.

Results MRIs at term equivalent age were available for 210 infants and serial data were available for 131 infants. Growth over these 10 weeks was greatest for the cerebellum, with an increase of 258%. Sex, birth weight *z*-score, and prolonged mechanical ventilation showed global effects on brain volumes on both scans. The effect of brain injury on ventricular size was already visible at 30 weeks, whereas growth data and volumes at term-equivalent age revealed the effect of brain injury on the cerebellum.

Conclusion This study provides data about third-trimester extrauterine volumetric brain growth in preterm infants. Both global and local effects of several common clinical risk factors were found to influence serial volumetric measurements, highlighting the vulnerability of the human brain, especially in the presence of brain injury, during this period. *(J Pediatr 2016;178:93-100).*

The last trimester of gestation is one of immense fetal brain growth and development. Brain volume increases approximately 5-fold, and the majority of sulcal and gyral formation takes place in the last 15 weeks of pregnancy.[1] Preterm infants spend this period outside the uterus in a neonatal intensive care environment, where they are exposed to a myriad of factors that can possibly disturb these processes.[2] Over the last decade, manual or (semi-) automatic segmentation of brain volumes using T1-weighted and T2-weighted magnetic resonance imaging (MRI) has become available for neonatal imaging. Cross-sectional studies have shown a sharp linear increase in brain volumes between 25 and 40 weeks of gestation, with a difference in regional growth rates.[3,4] Brain volumes at term-equivalent age (TEA) have been shown to be influenced by various clinical factors, among which brain injury (BI), intrauterine growth retardation, chronic lung disease, and the use of dexamethasone are the most often reported.[5,6] Several studies with limited sample sizes have studied the preterm brain longitudinally and found a difference in growth rate for gray matter versus white matter, as well as regional growth differences.[7,8]

The aim of the present study was to investigate third-trimester extrauterine volumetric brain growth, and correlate regional volumes with clinical risk factors in a longitudinally scanned cohort of extremely preterm born infants. We hypothesized that several well-known clinical risk factors, such as a lower birth weight and mechanical ventilation, would have negative effects on brain development.

Methods

Between June 2008 and March 2013, all preterm infants with gestational age <28 weeks admitted to the level-3 neonatal intensive care unit of the Wilhelmina Children's Hospital were consecutively enrolled in a prospective neuroimaging study. According to clinical protocol, infants were scanned twice, first at around 30 weeks gestation (range, 28.7-33.1 weeks), if clinically stable, and again at around TEA

BI	Brain injury
BWZ	Birth weight *z*-score
IVH	Intraventricular hemorrhage
MRI	Magnetic resonance imaging
TBV	Total brain volume
TEA	Term-equivalent age

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(range, 39.3-43.7 weeks). Infants with congenital anomalies, including chromosomal abnormalities or structural brain abnormalities, were excluded from the study. A total of 265 infants were eligible for inclusion. **Figure 1** (available at www.jpeds.com) represents the final inclusion of all infants.

Perinatal data were obtained prospectively. Birth weight *z*-scores (BWZ) were computed according to the Dutch Perinatal registry reference data.[9] Surgery (for necrotizing enterocolitis, patent ductus arteriosus, insertion of a Rickham reservoir, or retinopathy of prematurity) was scored, taking the timing of surgery in relation to when the MRI was obtained into account; for example, if the first surgery was done after the first MRI, it was scored as such only for the TEA scan. The use of morphine, including days of admnistration, was scored. Serial cranial ultrasound was performed and reported as part of standard clinical care. Intraventricular hemorrhage (IVH) grading on cranial ultrasound was scored according to Papile et al.[10] Permissions for MRI were obtained from the Medical Ethics Review Committee and parents.

MRI

MRI was performed on a 3.0-T imaging system (Achieva; Philips Medical Systems, Best, The Netherlands). At 30 weeks, infants were scanned in an MRI-compatible incubator (Dräger MR Incubator [Dräger, Lübeck, Germany] and later a Nomag IC 3.0 [Lammers Medical Technology, Lübeck, Germany], with a dedicated neonatal head coil), and at TEA, the sense head coil was used. The protocol included T2-weighted MRI in the coronal plane (30 weeks: repetition time, 10 085 ms; echo time, 120 ms; slice thickness, 2 mm; TEA: repetition time, 4847 ms; echo time, 150 ms; slice thickness, 1.2 mm).

After evaluation by a pediatric radiologist, all scans were reassessed by 2 neonatologists (L.d.V. and M.B.). The presence of IVH, periventricular hemorrhagic infarction, posthemorrhagic ventricular dilatation (defined as a ventricular index 4 mm >97th percentile, according to Levene[11]), cystic periventricular leukomalacia, punctate white matter lesions, central or cortical gray matter infarctions, or punctate or larger hemorrhagic lesions (involving >25% of a cerebellar hemisphere) in the cerebellum was scored. The presence of significant BI was defined as the presence of 1 or more of the following: IVH grade III, posthemorrhagic ventricular dilatation, periventricular hemorrhagic infarction, cystic periventricular leukomalacia, large cerebellar hemorrhages destroying more than one-half of one hemisphere, or infarctions of the central or cortical gray matter.

Volumetric Segmentation

T2-weighted MRI of both 30-week and TEA MRIs were configured using the segmentation method of Makropoulos et al.[12] This method involves an expectation-maximization scheme that combines manually labeled, age-dependent atlases with intensity information from the image to be segmented and has shown reliable results in infants scanned at postmenstrual age ranging from 28 to 44 weeks.[12,13] It segments the entire brain into 50 specific brain regions, which were subsequently used in the analysis (**Figure 2**; available at www.jpeds.com). All segmentations were manually checked, and small corrections were performed if necessary.

Statistical Analyses

Statistical procedures were performed using MATLAB and Statistics Toolbox Release 2013b (MathWorks, Natick, Massachusetts) and R version 2.15.3 (www.r-project.org). Growth was calculated as the difference between the volume of a brain region at TEA and at 30 weeks, divided by the difference in scan age in weeks and then multiplied by 10. For the analyses at 30 weeks and TEA, all data were corrected for postmenstrual age at the time of the MRI. All analyses at 30 weeks and TEA were done with both absolute volumes and with relative volumes corrected for total brain volume (TBV). First, a canonical correlation analysis was performed on the scans of all 210 infants, to determine which clinical factors accounted for the largest variance in the total set of 50 volumes, calculating the percentage of explained variance. For variables in the clinical dataset, results were compared across the time points and for the data with and without correction for TBV. This led to a set of clinical variables being most influential across all analyses, which was subsequently validated by repeating the analysis with random selections of approximately one-half of the patients.

With the restricted group of clinical variables, multiple linear regression analysis was performed to determine the effect of each clinical variable. Possible interactions between the different clinical risk factors were included in the models.

The multiple linear regression analysis was also performed using TBV. All analyses were repeated after excluding all infants with significant BI, to correct for potential confounding owing to significant BI. Because infants born small for gestational age (defined as a birth weight <10th percentile) could potentially influence the effects of BWZ, we repeated the analyses, and found that BWZ remained significant after these infants were excluded. To correct for the 50 different regions that were analyzed, a *P* value of .001 (0.05/50) was considered significant.

Results

Clinical characteristics of the included infants are summarized in **Table I** (available at www.jpeds.com). We included 131 infants with data for both the 30-week and TEA MRI (serial scans), and an additional 79 infants with data only for the TEA MRI. The infants with serial scans had lower rates of pulmonary problems and sepsis, because they needed to be clinically stable to be eligible for the earlier scan.

Growth

Growth data could be calculated for the 131 infants with serial scans. **Figure 3** shows the growth percentage change between the 2 scans for each studied brain region and also for TBV. The cerebellum showed the largest increase (258%), whereas TBV increased by 140% and ventricular volume increased by only 61%. Generally, the central regions showed less growth compared with the cortical regions. Brain regions in the temporal and occipital lobes showed more growth compared with the frontal and parietal regions.