Sonographic Maturation of Third-Trimester Cerebellar Foliation after Birth

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ABSTRACT: The development of cerebellar folia of third-trimester preterms has not been described with ultrasound before. We set out to determine normal development of the pons and cerebellar folia for future measurements of hypoplasia and atrophy. Study sonograms were made in preterms admitted to the neonatal intensive care unit with postmenstrual age (PMA) from 25 wk until term. On a weekly basis, transcranial measurements were made in the axial and coronal planes at the asterion with high-frequency transducers (8.5 and 13 MHz). The axial images showed the pons and fourth ventricle. The coronal images showed cerebellar folia and white matter. In the same coronal plane, the depth of cerebellar fissures at the rostral cerebellar convexity could be observed. A total of 172 cranial sonograms were performed on 98 neonates. The development of folia could be measured with ultrasound through the asterion. We showed that pons area, mean of three fissure depths, hemisphere area, and number of folia in the horizontal fissure correlated significantly with PMA. Hemisphere area described the same trend of growth as the transverse cerebellar diameter used in fetal sonography. (*Pediatr Res* 59: 695–699, 2006)

The development of the cerebellum has been studied extensively in different fields of research. It was studied histologically by Hochstetter in 1929 (1). Ten Donkelaar *et al.* (2) reviewed morphogenesis and histogenesis of the cerebellum and its developmental disorders. Normative data of development of folia vermis were reported by Loeser *et al.* (3). A morphologic analysis of the layers of cortex cerebelli was made by Gadson and Emery (4). Imaging techniques have also been used to study cerebellar development. Normal development of the fetal vermis cerebelli can be measured using transvaginal sonography (5). TCD can be measured with ultrasound and used to determine duration of gestation and intrauterine growth restriction (6,7). Normal values for TCD have also been established postmortem (8). Ultrasound imaging through the posterolateral fontanelle adds benefit to routine neonatal cranial sonography (9). Magnetic resonance imaging (MRI) studies of the cerebellum have been used for staging of cerebellar development and measurements of pons and cerebellum of preterms (10,11). The morphologic development of folia, however, has not been studied with MRI or ultrasound. The aim of this study was to develop a method to

measure third-trimester foliation through the asterion with high-frequency ultrasound.

SUBJECTS AND METHODS

Preterm neonates with gestational age ranging from 25 wk until term admitted to the neonatal intensive care unit (NICU) were included in this study. Preterms with an infant flow system were excluded because the required bonnet and nasal mask prevent sonographic measurements at the asterion. This is the only group of neonates that was not included; all of the other neonates were included to obtain a diverse population. Because of the high turnover of neonates, a longitudinal study was not possible, so this was essentially a cross-sectional study.

On a weekly basis, sonographic measurements were made transcranially using an Acuson Sequoia 512 Ultrasound System (Siemens Medical Solutions, Malvern, PA). These measurements were performed immediately after routine examination and, therefore, no informed consent was necessary. Sonographic images were obtained with high-frequency transducers (8.5 and 13 MHz) placed at the asterion in the coronal and axial plane. The asterion is the point of meeting of the lambdoidal, masto-occipital, and mastoparietal sutures. It lies 4 cm behind and 12 mm above the level of the auricular point in adults (12). The measurements were made on the presenting side of the head. The PA was measured first. The 8.5-MHz transducer was placed above the ear in the axial plane of the pons. To guarantee reproducibility, the plane of interest was chosen when the prepontine cistern and fourth ventricle were visible, respectively, anterior and posterior to the pons (Fig. 1A). The ellipse tool was used to measure PA. The second measurement was made with a 13-MHz transducer in the coronal plane of the cerebellum. The image obtained in this way showed cortex and white matter of one cerebellar hemisphere. The direction of the plane was chosen where the fourth ventricle has the shape of an isosceles triangle. The craniocaudal distance (CCD4) and the width (W4) of the fourth ventricle were measured (Fig. 1C). Fissures with their folia could be distinguished in the cortex. The depths of the three largest fissures were measured at the cranial convexity (Fig. 1C). MFID was used for statistical analysis. Next, a "free trace tool" was used to measure the HA (Fig. 1D). In the same coronal plane, the NFOF was counted (Fig. 1, E and G). The depth of the image was set at 20 mm and the contrast level was set to maximum for better discrimination of the folia. The folia can be recognized as white spots, resembling apples on a tree (Fig. 1, F and H). Only large dots were counted. These dots probably represent the interface between adjacent folia. The transducer was then rotated to obtain an axial image of the surface of the inferior part of the cerebellum. This way the number of folia at the surface (NFOS) of the cerebellum can be counted over a fixed distance (Fig. 1B).

The data were analyzed with SPSS software (SPSS, Inc., Chicago, IL). Linear regression analysis was performed and Pearson's correlation coefficient was calculated.

RESULTS

A total of 172 cranial sonograms were performed on 98 neonates. The number of follow-up scans ranged from 1 to 3,

Abbreviations: HA, hemisphere area; MFID, mean of three fissure depths; NFOF, number of folia in the horizontal fissure; PA, pons area; PMA, postmenstrual age; PVL, periventricular leukomalacia; TCD, transverse cerebellar diameter

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Figure 1. (A) Echo of PA; (B) echo in oblique plane of section through inferior cerebellar surface at 40 wk PMA; (C) echo of fissure depth; (D) echo of HA; (E) echo of a preterm's hemisphere at 26 wk PMA; (F) NFOF at 26 wk PMA; (G) echo at 42 wk PMA; (H) NFOF at 42 wk PMA. The cranial side of the head is to the left and lateral side is on the top of each echo image, except in A, where anterior is to the left.

depending on the duration of admission at the NICU. Gestational age at birth in this patient population ranged from 25 2/7 to 42 wk. PMA at scanning ranged from 25 4/7 to 44 1/7 wk. Most measurements were performed on neonates between 27 and 34 wk PMA, as shown in Figure 2A. Birth weight ranged from 635 to 4525 g. A summary and analysis of measurements are given in Table 1. CCD4 and W4 did not correlate with PMA. A halfway evaluation of the measurements showed that NFOS was not a promising method to measure third-trimester development of folia. Hence, we developed the concept of NFOF. This measurement was then added to the sonogram and NFOS was removed. The missing measurements of PA (9), HA (11), and MFID (4) were due to incomplete digital data storage. The linear regression equations in Table 1 were calculated and described the relationships between the measured data and PMA. Figure 2, B–F, shows the relationships of PA, MFID, HA, NFOF, and NFOS with PMA in a graph. PA, MFID, HA, and NFOF correlated positively with PMA. NFOS correlated negatively with PMA. Data analysis showed that MFID and HA were significant for a quadratic fit, contrary to the linear fit for PA, NFOF, and NFOS. Table 1 shows the correlations between HA, MFID, PA, NFOF, and NFOS. HA correlated significantly with MFID ($r = 0.792$, $p < 0.01$), PA ($r = 0.732$, $p < 0.01$), and NFOF ($r = 0.712$, $p < 0.01$).

DISCUSSION

This study demonstrates that sonographic examination of the posterior fossa through the asterion can be used to measure

PA, HA, MFID, NFOF, and NFOS. Some of these measurements are directly related to maturation of cerebellar folia. All of the measurements with the exception of NFOS relate significantly to PMA. Most measurements were performed on neonates between 27 and 34 wk PMA. We therefore assume our measurements are most reliable between 27 and 34 wk. Further studies should focus on neonates older than 34 wk or younger than 27 wk.

PA in a sagittal section through the fourth ventricle has not been measured with ultrasound before. PA measurements with MRI were performed by Argyropoulou *et al.* (11). That report describes the relationship between PVL and size of pons and cerebellum. In patients with PVL, the corpus callosum (used as a marker for assessment of the extent of white matter injury), pons, and cerebellum were smaller than in controls. A positive correlation was found between corpus callosum, pons, and vermis in preterms and a control group. The cerebellum only correlated with the corpus callosum in the control group. PVL is a common finding during neurosonography of preterm infants (13,14). Measurements of PA may add to the sonographic study of PVL.

TCD is one way of measuring development of the cerebellum (8,15–17). TCD consists of the width of two hemispheres and the vermis. We have measured the area of one hemisphere (HA), because this can be readily done without repositioning a sick preterm infant. HA turned out to be a reproducible method to follow hemisphere growth ($r = 0.890$, $p < 0.05$). Comparing our measurements of HA with the measurements

Figure 2. (A) Distribution of number of sonograms with PMA. (B) Linear fit of regression for PA with PMA. (C) Quadratic fit of regression for MFID with PMA. (D) Quadratic fit of regression for HA with PMA. (E) Linear fit of regression for NFOF with PMA. (F) Linear fit of regression for NFOS with PMA. (G) Correlation of TCD and HA with PMA, TCD (cm) with PMA (8), predicted TCD (cm) with PMA (18), and quadratic fit for HA (cm²) with PMA. The 95% confidence intervals are shown for individual (outer lines) and for group mean measurements.

of TCD by Hill *et al.* (18) and Pinar *et al.* (8), we can observe an identical trend of growth (Fig. 2G).

MFID was chosen as a reproducible method to appreciate the depth of the secondary fissures at the rostral cerebellar hemisphere convexity. The depth of major fissures of vermis cerebelli has been related by Makori *et al.* (19) to exposure to 13-*cis*-retinoic acid (cRA). Table 2 gives an overview of toxins that have the potential to disturb cerebellar foliation. MFID turned out to be a reproducible method ($r = 0.717$, $p <$

0.01). In future studies, MFID may be used to measure the affect of toxins on cerebellar foliation.

Hochstetter (1) studied the growth of folia vermis in sagittal histologic sections. The sections show the increase in number of folia from 12 to 28 wk PMA. Loeser *et al.* (3) reported the growth of folia for each lobule from 24 to 37 wk PMA. Our measurements using ultrasonographic inspection of the maturation of the folial pattern (NFOF) confirm this increase in number of folia with increase in PMA. NFOS shows a nega-

Table 1. Summary and analysis of measurements

Measurement	No. of measurements	Mean	Range	SD	<i>r</i>	SE of the estimate	
PA	163	1.32 cm ²	0.60–2.49 cm ²	0.35	0.748**	0.235	
MFID	168	0.37 cm	0.14–0.80 cm	0.13	0.717**	0.104	
HA	161	2.23 cm ²	1.10–5.28 cm ²	0.84	0.890*	0.384	
NFOF	95	4.46	0–16	2.73	0.735**	1.86	
NFOS	79	16.7	10–26	3.17	0.285*	3.06	

Linear regression equations for PMA			Correlation	PA	MFID	HA	NFOF	NFOS
PA (cm ²) = 0.06662 × PMA – 0.821			PA	1	0.593**	0.732**	0.481**	–0.227
MFID (cm) = –0.000546 × PMA ² + 0.060015 × PMA – 0.991365			MFID		1	0.792**	0.626**	–0.031
HA (cm ²) = 0.003188 × PMA ² – 0.033557 × PMA – 0.053502			HA			1	0.712**	–0.298**
NFOF = 0.486 × PMA – 11.085			NFOF				1	–0.232
NFOS = –0.219 × PMA + 23.870			NFOS					1

* $p < 0.05$, ** $p < 0.01$.

Table 2. Overview of toxins that affect cerebellar folia

Toxin	Place of effect	Cytotoxic effect	Reference
Cis-dichlorodi-ammineplatinum	Purkinje neurons	Affected morphology of folia on lobules V–VII of cerebellar vermis	(24)
Ethanol	Defects in the glia limitans, Purkinje cell dendrites external granular layer, inhibition of insulin-, IRS1-, and PI3 kinase	Fusion of folia V and VI of the cerebellar vermis, cortical laminar structure was disrupted around the fusion, cerebellar hypoplasia	(25–27)
<i>N</i> -ethyl- <i>N</i> -nitrosourea (ENU)	Mitotically active cells of the external granular layer and on non-mitotic differentiating neurons, including Purkinje cells	Absence of secondary folia, and the cerebellum as a whole was small in size	(28)
X-irradiation	Purkinje cells, Bergman glial fibers	Impaired foliation of the cerebellum	(29)
Chloro-organic pesticides		Retarded differentiation of the nervous structure of cerebellum	(30)

tive relation with PMA, but this is expected as with an increase in size of the folia, the number of folia over a fixed distance will decrease. NFOF and NFOS are probably not suited for comparison between individual patients because we expect limited interobserver reproducibility. Improved ultrasound imaging techniques may, in the future, produce images with greater detail and could result in a more reproducible method for counting folia. NFOF and NFOS as used in this report may be used to study cohorts of infants with bronchopulmonary dysplasia or fetal growth retardation. A comparison of NFOF and NFOS can be made between a group of preterms with, for instance, intrauterine growth retardation or chronic lung disease—disorders with chronic hypoxia that might influence development of the external and internal granular layers—and a control group (20–22).

An MRI study of preterm infants by Lin *et al.* (23) showed the ratio of thalami area to the cerebellum area was significantly smaller in infants with moderate and severe PVL than in the control group. Also, the ratio of cerebral hemisphere area to the cerebellum area was smaller in infants with severe PVL than in the control group. A ratio of NFOF and NFOS with cerebral hemisphere area and thalami area may be determined with ultrasound imaging in a group of preterms with PVL and a control group.

In conclusion, maturation of folia can be measured with ultrasound through the asterion in preterm infants. We showed that PA, MFID, HA, and NFOF correlate significantly with PMA. HA described the same trend of growth as TCD. Further cohort studies should provide evidence for the use of this method for recognizing impaired cerebellar foliation. Insonation *via* the asterion may prove useful for the description of malformations of the cerebellum presenting in the neonatal period.

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