Radiology

Normal Cerebellar Growth by Using Three-dimensional US in the Preterm Infant from Birth to Term-corrected Age[1]

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Conflicts of interest are listed at the end of this article.

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Purpose: To establish cross-sectional and longitudinal reference values for cerebellar size in preterm infants with normal neuroimaging findings and normal 2-year neurodevelopmental outcome by using cranial ultrasonography (US).

Materials and Methods: This prospective study consecutively enrolled preterm infants admitted to a neonatal intensive care unit from June 2011 to June 2014 with a birth weight of less than or equal to 1500 g and/or gestational age (GA) of less than or equal to 32 weeks. They underwent weekly cranial US from birth to term-equivalent age and magnetic resonance (MR) imaging at term-equivalent age. The infants underwent neurodevelopmental assessments at age 2 years with Bayley Scales of Infant and Toddler Development, 3rd edition (BSID-III). Patients with adverse outcomes (death or abnormal neuroimaging findings and/or BSID-III score of <85) were excluded. The following measurements were performed: vermis height, craniocaudal diameter, superior width, inferior width, vermis area, and transcerebellar diameter. Statistical analyses were conducted by using multilevel analyses.

Results: A total of 137 infants with a mean GA at birth of 29.4 weeks (range, 25–32 weeks) were included. Transcerebellar diameter increased by 1.04 mm per week on average; vermis height and craniocaudal diameter increased by 0.55 mm and 0.59 mm, respectively. Superior vermian width increased by an average of 0.45 mm, whereas inferior vermian width increased by an average of 0.51 mm per week. Vermis area was found to increase by 0.22 cm^2 per week on average. The sex effect was significant (female lower than male) for vermis height ($P < .05$), craniocaudal diameter ($P < .05$), inferior vermian width ($P <. 05$), and vermis area ($P <. 05$).

Conclusion: Cross-sectional and longitudinal reference values were established for cerebellar growth in preterm infants, which may be included in routine cranial US.

Online supplemental material is available for this article.

Although survival of preterm infants has increased over the past decades, survivors are still at considerable risk for cognitive, behavioral, neurosensory, and motor disabilities (1). Currently, predicting neurodevelopmental outcome is an important challenge for pediatricians.

The third trimester of gestation is a critical period of brain growth and development (2), and exposure to extrauterine environment in preterm infants may disrupt brain growth (3). The slower growth of certain brain regions has been related to poorer cognitive and motor outcome in infancy and early childhood (4,5).

The importance of cerebellar injury and growth in preterm infants has become increasingly recognized (6,7). The cerebellar growth rate is rapid when compared with the cerebrum during the last half of gestation and the first postnatal year (8), and its volume may be reduced because of multiple risk factors (infection, medication administration, and respiratory complications) (9–11). Cerebellar volume has been used as a predictor of short- and long-term developmental outcomes in preterm-born children (4,12).

To date, most inferences about third-trimester cerebellar growth in the absence of brain injury after early exposure to extrauterine life in preterm infants have been based on cross-sectional quantitative magnetic resonance (MR) imaging measures (13). However, MR imaging may not be readily available and is difficult to perform repeatedly in infants; furthermore, most neonatal intensive care units do not have MR imaging facilities. Ultrasonography (US) remains the most accessible neuroimaging tool in neonatal intensive care units. Cranial US is safe, noninvasive, repeatable, and reproducible (14), and it can be used for assessing cerebellar growth as accurately as MR imaging (15).

Only a few neonatal US studies have performed serial measurements of cerebellar structures and estimated cerebellar growth rate in the preterm infant, but they were

Abbreviations

BSID-III = Bayley Scales of Infant and Toddler Development, 3rd edition, CI = confidence interval, GA = gestational age

Summary

This study establishes cross-sectional and longitudinal reference values for the preterm cerebellar growth from birth to term-corrected age though US.

Implications for Patient Care

- Cerebellar size is easy to assess by using cranial US, and monitoring cerebellar growth could become part of standard US examinations.
- Normative data of extrauterine cerebellar growth have been established for US monitoring from birth to term-corrected age in the preterm infant.

cross-sectional (16) and/or studies with small sample sizes of infants at lower gestational ages (GAs), without adequate verification of the reliability of the measures and without long-term follow-up (16–19). Currently, there is a lack of normative data of cerebellar size in the preterm infant and it remains largely unknown whether extrauterine life affects cerebellar development.

The aim of our study was to assess cerebellar growth in preterm infants by using serial cranial US in the absence of brain injury and with normal 2-year neurodevelopmental outcome.

Materials and Methods

Institutional review board approval and written parental informed consent were obtained for our prospective observational cohort study. This research project was partially granted by the Spanish Neonatal Society (FSEN-ALTER 2013).

Patients

From June 2011 to June 2014, we consecutively included infants with very low birth weight admitted to our neonatal intensive care unit at University Hospital Puerta del Mar, Cadiz, Spain. Inclusion criteria were birth weight of less than or equal to 1500 g and/or GA less than or equal to 32 weeks. Exclusion criteria included congenital and chromosomal anomalies, metabolic disorders, central nervous system infections, and lack of informed consent. Perinatal data and clinical courses were prospectively collected. All patients were followed prospectively and underwent weekly cranial US until either discharge or term-equivalent age. All infants were included in a neurologic follow-up program, and a neurodevelopmental assessment was performed at age 2 years with the Bayley Scales of Infant and Toddler Development, 3rd Edition (BSID-III) (20) by a developmental psychologist (R.C.F.C, with 8 years of experience) who was blinded to the patients' neuroimaging findings and neonatal course.

For the purpose of our prospective study, we assessed cerebellar growth only in those patients with a normal outcome. We defined normal outcome as the absence of brain injury (normal serial cranial US and normal MR imaging at term-equivalent age), normal neurologic examination at term-equivalent age, and BSID-III score greater than 85 at 24 months of corrected age.

US Examination

The first US examination was performed within the first 48 hours of life and on a weekly basis thereafter (I.B.F. and S.P.L.L., both with 10 years of experience). All studies were performed with the infant lying supine with his or her head turned to the right.

Three-dimensional US

Three-dimensional US was performed by using the four-dimensional option in the US system (Voluson i 3D/4D Portable Ultrasound System; GE Healthcare, Milwaukee, Wis). First, the transducer (S-VNA 5-8B, 5 MHz to 8 MHz) was situated in the third coronal plane by using a center frequency of 6.5 MHz, and the scan angle was set at 90°. With the transducer fixed in that position, the beam moved from anterior to posterior planes and from side to side through lateral planes. Next, the transducer was situated on a midsagittal view, and another scan was performed. Scans were saved and analysis was performed off-line by using 4D View software (version 10.0; GE Healthcare).

The acquired scans were displayed in three perpendicular planes on the screen simultaneously. This multiplanar mode allows for exact orientation and each of the three planes can be rotated around the x-, y-, and z-axes, thus allowing the examiner to choose the planes of interest. In the coronal plane, maximal transverse cerebellar diameter was measured between the cerebellar hemispheres in a posterior coronal scan via the anterior fontanelle at the level of the quadrigeminal cistern (see Appendix E1 [online] for details of the measurements). In the midsagittal plane, the cerebellar vermis height was measured from the anterosuperior portion to the inferoposterior portion. Craniocaudal diameter was defined as the maximum distance between the most cranial portion of the culmen and the most caudal portion of the uvula, lying perpendicular to the fastigium-declive line (14,21). For superior vermian width, the longest diameter of the anterosuperior vermis was taken from the fastigial point to the periphery. For inferior vermian width, the longest diameter of the inferoposterior vermis was taken from the fastigial point to the periphery. For vermis area, a manually contoured area was drawn on cerebellar margins with indentations considered at fastigial point and primary fissure.

Intra- and Interobserver Reliability

The intra- and interobserver reliabilities were assessed in 15 US examinations of different patients (randomly selected). The two observers (I.B.F.; E.R.Z., with 2 years of experience), blinded to clinical information of the patients separately repeated all linear measurements three times to evaluate the intraobserver variability. To control for memory effects potentially biasing subsequent measurements, each observer performed all measurements for the 15 patients before starting again with the first one, allowing a 24-hour interval, and recorded them while blinded to previous measurements. The interobserver reliability was evaluated by comparing the mean of the three measures performed by each observer while blinded to the

other's measurements. The intraclass correlation coefficients were calculated by using the two-way random model for absolute agreement and interpreted according to the strength of agreement scale by Brennan and Silman (22).

Statistical Analysis

Our study group characteristics were evaluated by using *t* tests for continuous variables and χ^2 tests for categorical variables. Exploratory analysis indicated that brain tissue metrics were approximately normally distributed and were expressed as mean and standard deviation. Missing data were found to be less than 5% for each measurement; therefore, no further analyses or adjustments were required.

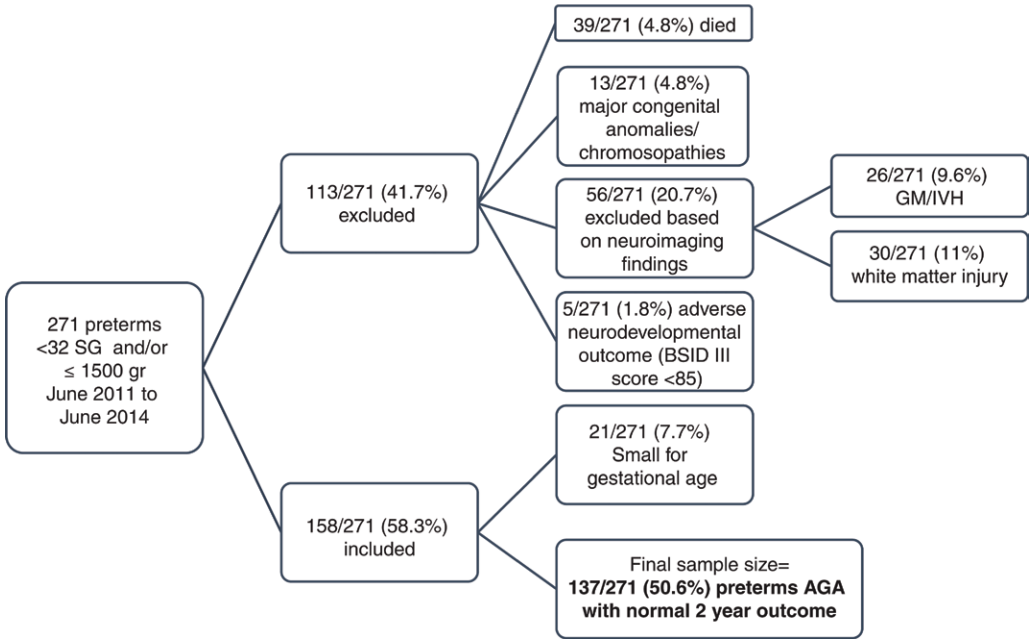

Figure 1: Flowchart shows prospective inclusion and final sample size of the study. GM/IVH = germinal matrix-intraventricular hemorrhage; BSID-III = Bayley Scales of Infant and Toddler Development, 3rd edition; AGA = adequate birth weight for gestational age; SG = small for gestational age.

To assess whether extrauterine life affects brain growth, we compared measurements in the first US examination to those made at different postnatal ages for the same corrected GA.

For every measure, we developed multilevel models for continuous responses fitting a random-effects model to achieve growth curve models. Random effects were normally distributed. This growth model allowed for variation between individuals in the rate of change in y (the slope of t_{ij}) as well as in their level of y at any occasion (the intercept) (23).

We tested models that allow for differences between groups in an analysis of sex differences. First, allowing for a sex difference at any age, and in the event that sex effect was strongly significant, we then allowed the effect of age on growth (the rate of increase) to differ for male and female infants by including interactions between sex and the age variables. The null hypothesis for the test was that the coefficient on the interaction term is zero. If the null was not rejected, then we concluded that the rate of increase in the measurement with age does not differ for male and female infants.

Results

Study Population

Our cohort included 271 patients. One hundred thirteen of 271 (41.7%) infants were excluded for adverse neurologic outcome as follows: 39 of 271 (14.4%) died, 13 of 271 (4.8%) had major congenital anomalies or genetic disorders, 56 of 271 (20.7%) had abnormal neuroimaging findings (26 of 271 [9.6%] sustained germinal matrix-intraventricular hemorrhage of any degree and 30 of 271 [11%] sustained white matter injury), and five of 271 (1.8%) had a BSID-III score of less than 85 at 2 years of corrected age with previously normal neuroimaging findings. Among these 158 patients, we further excluded 21 of 158 (13.3%) patients for being small for GA. Thus, our final sample size included 137 preterm infants with adequate birth weight for GA and normal outcome at 2 years of corrected age (Fig 1). Perinatal characteristics are described in Table 1.

US Examinations

Serial US examinations performed in 137 patients accounted for a total of 437 US examinations. The distribution of patients and the number of US examinations based on GA is described in Table 2.

Intra- and Interobserver Reliability

The mean intraclass correlation coefficient for interrater agreement was 95.2% and 98.6% for intrarater agreement, which are considered excellent. A full description of intraclass correlation coefficients with 95% confidence intervals (CIs) for each measurement is detailed in Table 3.

Linear Measurements

We found measurements from the first US examination to be greater than those at the same but corrected GA. The average differences were 3.36 mm for transcerebellar diameter (P = .02), 1.55 mm for vermis height (P = .01), 1.55 mm for craniocaudal diameter (P = .006), 1.5 mm for superior vermian width (P = .002), 1.78 mm for inferior vermian width (P = .0042), and 0.68 cm^2 for vermis area (P = .0018) (Table 4).

Given these differences, we then considered measurements from the first US examination when defining cross-sectional data. For longitudinal normative data, we used the postnatal corrected age. Cross-sectional mean and standard deviation values for each measurement, as well as percentile charts and graphs based on longitudinal data, can be found in Appendix E1 (online).

Table 1: Perinatal Characteristics of the Study Population

Parameter	No. of Patients (*n* = 137)
Male sex	67/137 (48.9)
Female sex	70/137 (51.1)
Gestational age at birth (wk)*	29.4 ± 2.2
Birth weight (g)*	1333.5 ± 364.7
Birth cephalic perimeter (cm)*	27.4 ± 2.8
Cesarean section	109/137 (79.6)
Apgar score at 1 min†	7 (0–9)
Apgar score at 5 min†	8 (4–10)
Clinical Risk Index for Babies score†	1 (0–15)

Note.—Unless otherwise specified, data are numerators and denominators, with percentages in parentheses.
* Data are means ± standard deviation.
† Data are medians, with ranges in parentheses.

Table 2: Gestational Age at the Time of Entry into the Study, Number of Preterm Infants Entered, Sex, and Total Number of Studies Performed within This Gestational Age Group

Gestational Age (wk)	No. of Patients	Female Sex*	No. of US Studies
25	5	5/5 (100)	32
26	16	9/16 (56)	80
27	10	7/10 (70)	54
28	14	4/14 (29)	34
29	17	7/17 (41)	53
30	22	11/22 (50)	62
31	30	17/30 (57)	76
32	23	10/23 (43)	46
Total	137	70/137 (51)	437

* Data are numerators and denominators, with percentages in parentheses.

Transverse Cerebellar Diameter

Optimal planes for transcerebellar diameter measurements were obtained in 422 (96.6%) of the total 437 US examinations. Inter- and intrarrater intraclass correlation coefficients were 0.978 (95% CI: 0.938, 0.995) and 0.993 (95% CI: 0.975, 0.999), respectively. We found an average increase of 1.04 mm (95% CI: 0.93, 1.16) per week (Fig 2). The sex effect was not found to be significant (P = .898) for the rate of growth of transcerebellar diameter. The final equation to estimate transcerebellar diameter according to GA is as follows: 40.81 + 1.04 × (GA − 30). A preterm infant at 30 weeks GA would therefore be expected to have a transcerebellar diameter of 40.81 mm. For more detailed data on multilevel analysis performed for each measurement, see Appendix E2 (online).

Vermis Height

Optimal planes for vermis height measurements were obtained in 435 (99.54%) of the total 437 US examinations. Inter- and intrarrater intraclass correlation coefficients were 0.974 (95% CI: 0.93, 0.993) and 0.98 (95% CI: 0.929, 0.996), respectively. We found an average increase of 0.55 mm (95% CI: 0.5, 0.61) per week (Fig 3). The sex effect was significant ($P < .05$), with an absolute mean difference of 0.68 mm (female lower than male). This difference remained constant from birth to term-corrected age. The final equation for predicting estimated vermis height in accordance with sex and GA was as follows: vermis height (male) = 17.69 + 0.34 (male) + 0.55 × (GA − 30) and vermis height (female) = 17.69 − 0.34 (female) + 0.55 × (GA − 30), where GA is a patient's GA in weeks. Therefore, a preterm infant at 30 weeks GA would be expected to have a vermis height of 18.03 mm (male infant) or 17.35 mm (female infant).

Craniocaudal Diameter

Optimal planes for craniocaudal diameter measurements were obtained in 434 (99.31%) of the total 437 US examinations. Inter- and intrarrater intraclass correlation coefficients were 0.976 (95% CI: 0.935, 0.994) and 0.99 (95% CI: 0.990, 0.998), respectively. We found an average increase of 0.59 mm (95% CI: 0.53, 0.65) per week (Fig 4). The sex effect is significant ($P <$ .05), with an absolute mean difference of 0.64 mm (female lower than male). This difference remained constant from birth to term-corrected age. The final equation to estimate craniocaudal diameter in accordance with sex and GA was as follows: craniocaudal diameter (male) = 17.30 + 0.32 (male) + 0.59 × (GA − 30) and craniocaudal diameter (female) = 17.30 − 0.32 (female) + 0.59 × (GA − 30). By using this equation, a preterm infant at 30 weeks GA is expected to have a craniocaudal diameter of 17.62 mm (male infant) or 16.98 mm (female infant).

Table 3: Inter- and Intraobserver Reliability of Performed Measurements

Linear Measurements	Interrater ICC	Intrarater ICC
Transverse cerebellar diameter	0.978 (0.938, 0.995)	0.993 (0.975, 0.999)
Vermis height	0.974 (0.930, 0-993)	0.980 (0.929, 0-996)
Craniocaudal diameter	0.976 (0.935, 0.994)	0.990 (0.964, 0.998)
Superior vermian width	0.942 (0.849, 0.985)	0.974 (0.908, 0.995)
Inferior vermian width	0.890 (0.733, 0.970)	0.994 (0.976, 0.999)
Vermis area	0.982 (0.950, 0.995)	0.983 (0.938, 0.997)

Note.—Data in parentheses are 95% confidence intervals. ICC = intraclass correlation coefficient.

Superior Vermian Width

Optimal planes for superior vermian width measurements were obtained in 433 (99.08%) of the total 437 US examinations.

Inter- and intrarrater intraclass correlation coefficients were 0.942 (95% CI: 0.849, 0.985) and 0.974 (95% CI: 0.908, 0.995), respectively. We found an average increase of 0.45 mm (95% CI: 0.39, 0.5) per week (Fig 5). The sex effect was not found to be significant (P = .9) in the rate of growth of superior vermian width. The final equation to estimate the expected superior vermian width in accordance with GA would be as follows: 10.12 + 0.45 × (GA − 30). A preterm infant at 30 weeks GA would therefore be expected to have a superior vermian width of 10.12 mm.

Table 4: Mean Differences of the Initial US Measurements Performed within 48 Hours Compared with Grouped Mean Measurement for Corrected Gestational Age

Parameter	Mean	*P* Value
Transverse cerebellar diameter (mm)	3.36 (0.56, 6.18)	.02
Vermis height (mm)	1.54 (0.3, 2.78)	.01
Craneocaudal diameter (mm)	1.55 (0.39, 2.71)	.006
Superior vermis width (mm)	1.50 (0.54, 2.46)	.002
Inferior vermis width (mm)	1.78 (0.54. 3.03)	.0042
Vermis area (cm^2)	0.68 (0.27, 1.09)	.0018

Note.—Data in parentheses are 95% confidence intervals.

Inferior Vermian Width

Optimal planes for inferior vermian width measurements were obtained in 434 (99.31%) of the total 437 US examinations. Inter- and intrarrater intraclass correlation coefficients were 0.89 (95% CI: 0.733, 0.97) and 0.994 (95% CI: 0.976, 0.999), respectively. We found an average increase of 0.51 mm (95% CI: 0.45, 0.56) per week (Fig 6). The sex effect was significant ($P < .05$), with an absolute mean difference of 1.02 mm (female lower than male). This difference remained constant from birth to term-corrected age. The final equation to estimate inferior vermian width in accordance with sex and GA was as follows: inferior vermian width (male) = 11.92 + 0.51 (male) + 0.51 × (GA − 30) and inferior vermian width (female) = 11.92 − 0.51 (female) + 0.51 × (GA − 30). A preterm infant at 30 weeks GA is therefore expected to have an inferior vermian width of 12.43 mm (male infant) or 11.41 mm (female infant).

Vermis Area

Optimal planes for vermis area measurements were obtained in 434 (99.31%) of the total 437 US examinations. Inter- and intrarrater intraclass correlation coefficients were 0.982 (95% CI: 0.95, 0.995) and 0.983 (95% CI: 0.938, 0.997), respectively. We found an average increase of 0.22 cm^2 (95%

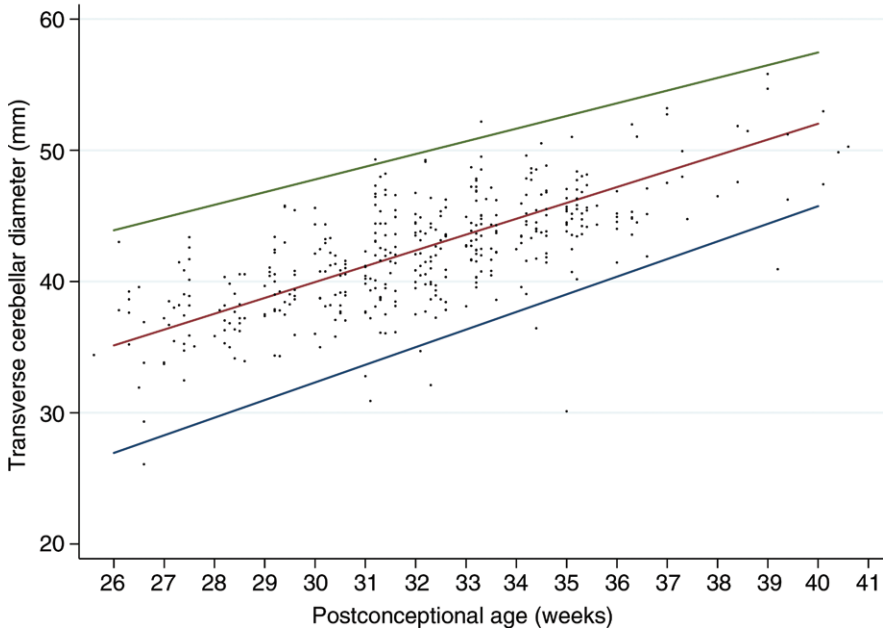

Figure 2: Scatterplot of transverse cerebellar diameter by postnatal age shows observed measurements and mean ± 2 (standard deviation).

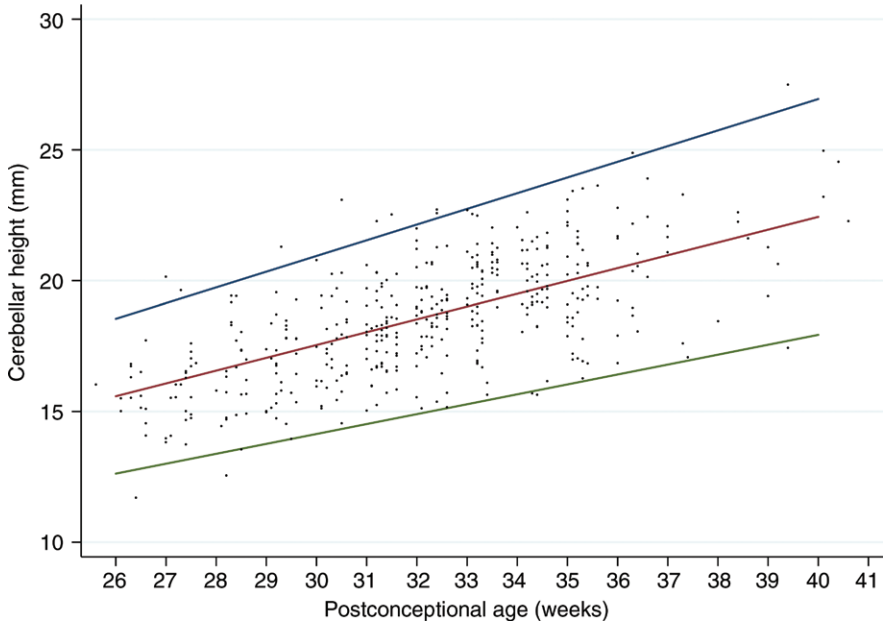

Figure 3: Scatterplot of vermis height by postnatal age shows observed measurements and mean ± 2 (standard deviation).

CI: 0.21, 0.24) per week (Fig 7). The sex effect is significant ($P < .05$), with an absolute mean difference of 0.18 cm^2 (female lower than male). This difference remained constant from birth to term-corrected age. The final equation to estimate vermis area in accordance with sex and GA was as follows: area (male) = 2.58 + 0.09 (male) + 0.22 × (GA − 30) and area (female) = 2.58 − 0.09 (female) + 0.22 × (GA − 30). A preterm infant at 30 weeks GA would therefore be expected to have a vermis area of 2.67 cm^2 (male infant) or 2.49 cm^2 (female infant).

Discussion

We established the cerebellar growth rate in preterm infants exposed to extrauterine life with normal neuroimaging findings and normal 2-year neurodevelopment. Cross–sectional and longitudinal normative data of cerebellar growth in the preterm infant based on serial cranial US have not been previously well established in the literature.

Interestingly, the first US biometric data set obtained within 48 hours of delivery was consistently greater than the mean averages of the serial studies. This raises the notion that intrauterine cerebellar growth is slightly greater than growth outside the uterus in premature infants during their stay in the neonatal intensive care unit.

Previous studies in preterm cerebellar growth have focused mainly on identifying factors that contribute to impaired cerebellar development (9,11). Only a few MR imaging–based studies have investigated third-trimester brain maturation in the absence of brain injury after early exposure to extrauterine life (24,25). Although advanced quantitative MR imaging techniques have become key to enhancing our knowledge of central nervous system maturation and growth from fetal life to adulthood, it cannot replace US as an immediate bedside tool.

Moreover, performing linear measurements in routine two-dimensional cranial US is a simple way to recognize normal versus impaired cerebellar growth.

Three-dimensional US can be a valuable tool in the neonatal intensive care unit because it allows navigation in all planes and enables volume calculations. It has mainly been used in fetal US (26,27), but there has been increased interest in postnatal brain studies in the last decade (26,28,29). It can complement two-dimensional US. Both three-dimensional and two-dimensional US face the challenge of potential motion artifacts. In our study, three-dimensional US was used to obtain optimal two-dimensional images of the studied planes because it allows for correction of symmetry and orientation offline. This allowed us to include optimal imaging planes in most of the US examinations in our study, in contrast to previous two-dimensional US studies where only 67% of optimal midsagittal planes were achieved (14). Reliability and reproducibility were found to be excellent in our study, whereas former studies have reported lower rates of intraclass correlation coefficient

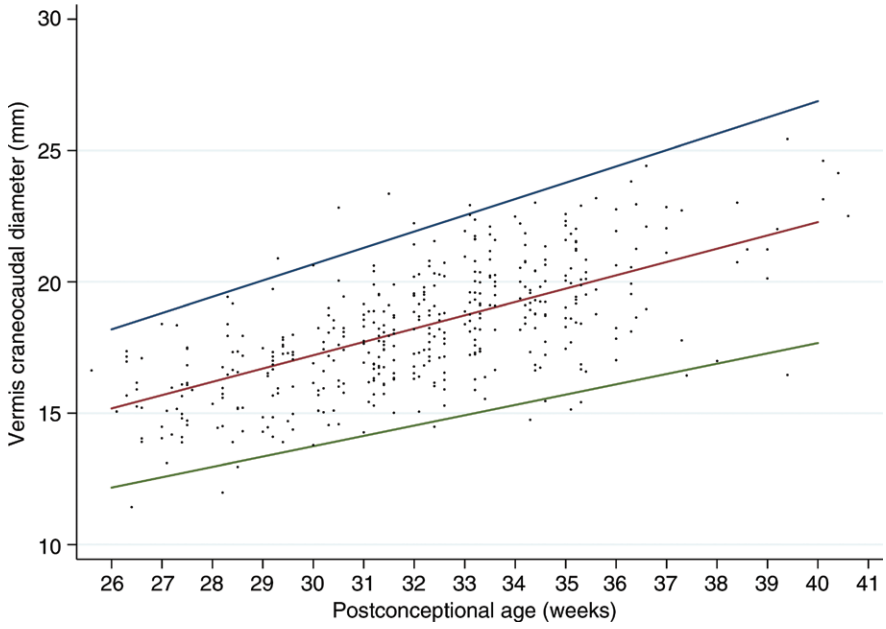

Figure 4: Scattterplot of craniocaudal vermian diameter by postnatal age shows observed measurements and mean ± 2 (standard deviation).

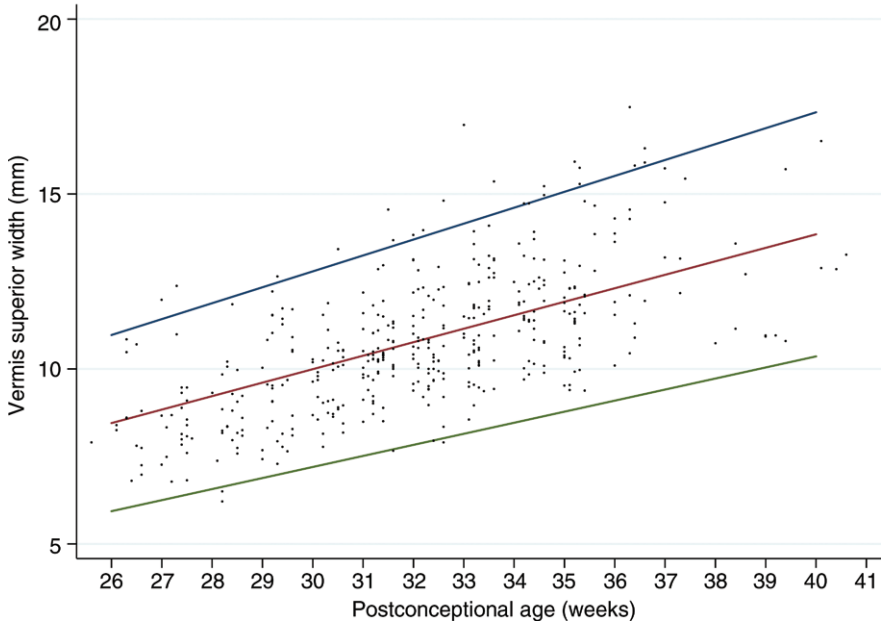

Figure 5: Scatterplot of superior vermian width by postnatal age shows observed measurements and mean ± 2 (standard deviation).

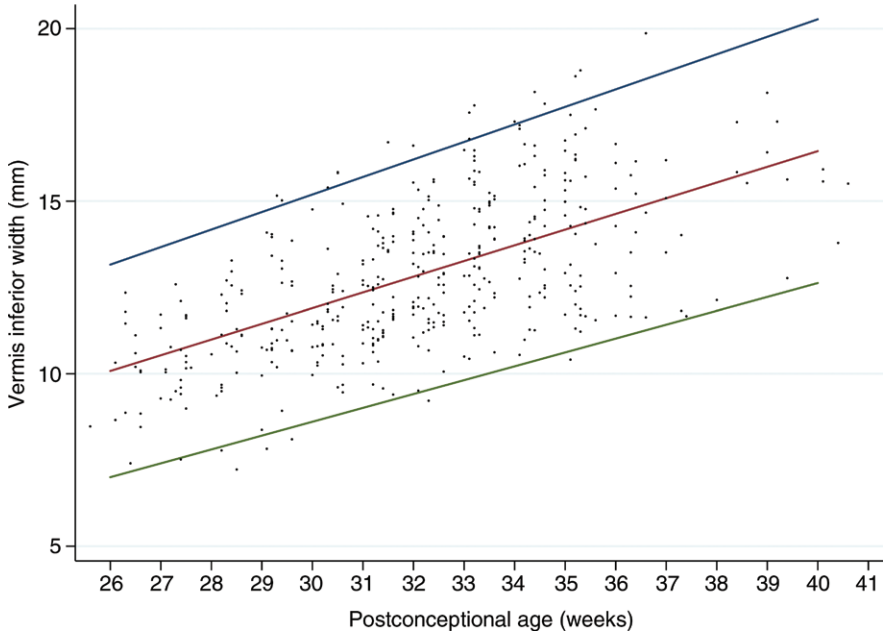

Figure 6: Scatterplot of inferior vermian width by postnatal age shows observed measurements and mean ± 2 (standard deviation).

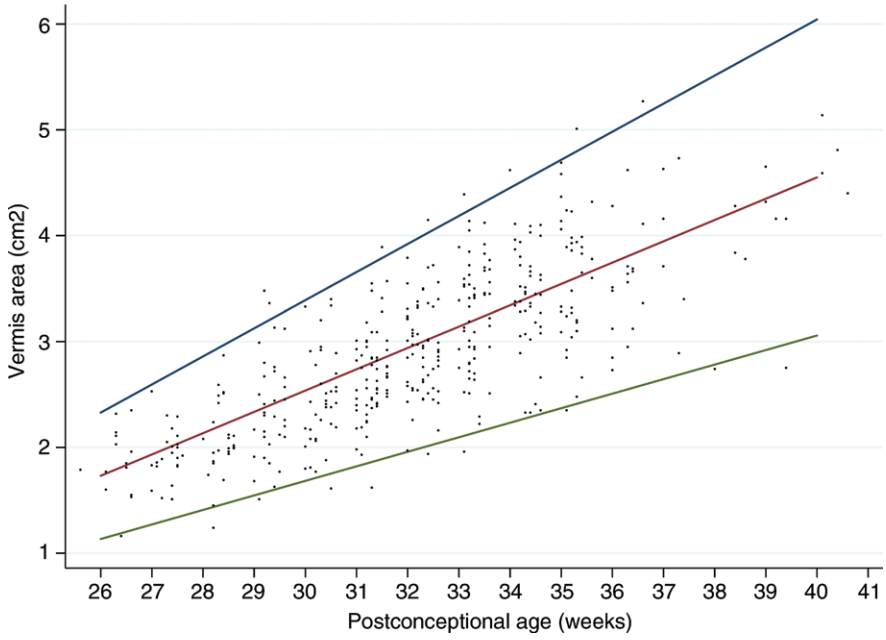

Figure 7: Scatterplot of vermis area by postnatal age shows observed measurements and mean ± 2 (standard deviation).

(14). This may be related to a greater proportion of optimal planes in our study.

We found a significant difference in cerebellar size when adjusting for sex, with smaller vermis height, craniocaudal diameter, and inferior vermian width in female infants. We found this difference to be present at birth and to remain constant until term-equivalent age. Sexual dimorphism has been studied from fetal life to adulthood mainly by using MR imaging (30–32); whether these differences depend on chromosomal sex, the influence of sex hormones, or other factors remains to be elucidated. Our findings are consistent with previously reported larger cerebellar volumes in male infants than in female infants, even after correction for larger brain volumes (31). Size-by-age trajectories of brain development rather than group averages across broad age ranges seems to be the best way to assess sexual dimorphism (30), but previously published data suggest that adult patterns of sexual dimorphism arise before birth and persist throughout postnatal brain development (33).

Although the role of sex differences remains uncertain and warrants future research, we reported absolute quantitative measurements of the cerebellum to generate normative values for general brain US practice.

Our study was subjected to several limitations that need to be addressed. The sample size in the infants with extremely low GA was small—which is expected for these extremely preterm patients—given the exclusion criteria of our study. We excluded preterm infants who were small for GA; however, no other perinatal and postnatal events were explored that could possibly affect extrauterine brain growth. Moreover, longer-term neurodevelopment would be preferable to outcome at 2 years of corrected age, because some cognitive and behavioral deficiencies may only develop later. Comparison with normal fetal growth and measurements at term, as well as measurements performed in supratentorial and/or cerebellar injury patterns, will need to be addressed in future research. Increasing evidence suggests that cerebellar injury can be studied in better detail through the mastoid fontanelle (34); however, this technique requires more training and is more operator dependent. Routinely performed US examinations are more commonly performed through the anterior fontanelle and we intended our results to be generalizable to the general practice.

In conclusion, cross-sectional and longitudinal reference values were established for cerebellar growth in preterm infants, which may be included in standard US examinations. Our data suggest that extrauterine life affects cerebellar growth, which may indicate impairment of cerebellar development after preterm birth. Further research is warranted in both normal outcome preterm infants, as well as in the presence of brain injury.

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