Original Article

⚕ Thieme

Quantitative Evaluation of Brain Echogenicity in Hypoxic-Ischemic Encephalopathy in Term Neonates Compared with Controls

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ABSTRACT

Purpose   Neurosonography evaluation of neonatal hypoxic-ischemic encephalopathy (HIE) is mainly qualitative. We aimed to quantitatively compare the echogenicity of several brain regions in patients with HIE to healthy controls.

Materials and Methods   20 term neonates with clinical/MRI evidence of HIE and 20 term healthy neonates were evaluated. Seven brain regions were assessed [frontal, parietal, occipital, and perirolandic white matter (WM), caudate nucleus head, lentiform nucleus, and thalamus]. The echogenicity of the calvarial bones (bone) and the choroid plexus (CP) was used for ratio calculation. Differences in the ratios were determined between neonates with HIE and controls.

Results   Ratios were significantly higher for HIE neonates in each region ($p < 0.05$). The differences were greatest for the perirolandic WM, with CP and bone ratios being 0.23 and 0.22 greater, respectively, for the HIE compared to the healthy neonates ($p < 0.001$). The perirolandic WM had a high AUC, at 0.980 for both the CP and bone ratios. The intra-observer reliability for all ratios was high, with the caudate to bone ratio being the lowest at 0.832 and the anterior WM to CP ratio being the highest at 0.992.

Conclusion   When coupled with internal controls, quantitative neurosonography represents a potential tool to identify early neonatal HIE changes. Larger cohort studies could reveal whether a quantitative approach can discern between degrees of severity of HIE. Future neurosonography protocols should be tailored to evaluate the perirolandic region, which requires posterior coronal scanning.

Introduction

Neurosonography is the initial imaging modality of choice in the evaluation of neonatal hypoxic-ischemic encephalopathy (HIE). This evaluation, which has been mainly performed qualitatively by comparing intracranial structures with different echogenicities, requires visual recognition of patterns by trained radiologists [1].

Qualitative neurosonography has proven reliable in diagnosing focal intracerebral lesions such as intraventricular hemorrhage, periventricular hemorrhagic infarction, and cystic periventricular leukomalacia [2].

However, diffuse processes resulting from neonatal HIE, such as white matter (WM) edema, are more challenging to recognize and

seem to be more dependent on the expertise of the radiologist. It is well known that WM edema is depicted in neurosonography images as areas of increased echogenicity as well as enhanced or diminished gray-WM differentiation [3–5]. The echogenicity of WM is typically higher than that of the less echogenic and more compact cortical gray matter [6], but the accentuation or reversal of this difference in echogenicity can be seen in evolving HIE. Nevertheless, since cerebral edema takes time to evolve, qualitative neurosonography can be inconspicuous and negative during the first 24 to 48 hours following HIE. Increased echogenicity commonly found in the deep brain structures such as the basal ganglia and thalami may not be evident until approximately 2 to 3 days after HIE. Moreover, increased cortical and subcortical echogenicity changes may even take 5 to 7 days to become conspicuous [2].

Quantitative neurosonography is not performed routinely in clinical practice, particularly in cases of neonatal HIE. Quantitative neurosonography has been previously attempted in infants by Barr et al. [7], Simaeys et al. [8], Vansteenkiste et al. [9], and Padilla et al. [10], although these infants were rarely term.

We hypothesized that quantitative neurosonography could demonstrate differences in echogenicity in term HIE patients compared to controls during the first days following injury. If this is correct, quantitative neurosonography may increase the sensitivity of neurosonography in the early detection of HIE. Therefore, our study aimed to compare the echogenicity measurements of several brain regions in patients with HIE with those of the same regions in healthy controls.

Methods

Study design

Retrospective study performed in an institutional review board-approved and HIPAA-compliant manner.

Participants

Two groups of term neonates were retrospectively identified from the period Jan 2018 to Dec 2019 in a major tertiary pediatric hospital. The first group (HIE group) was composed of 20 neonates (15 males and 5 females). The number of individuals in both the HIE and control groups was based on similar prior studies. The diagnosis of HIE was made by combining clinical history, blood gas measurement, and magnetic resonance imaging (MRI) findings consistent with HIE. Numbers are expressed as mean ± standard deviation (SD). Only patients with MRI reports consistent with HIE were included. Neurosonography was performed at 0.40 ± 0.60 days of life. Images correlating neurosonography and MRI findings from two patients (▶ Figs. 1,2) and from the group HIE (▶ Figs. 3–5) are shown. All patients in the HIE group underwent hypothermia treatment at 0.4 ± 0.49 days of life. MRI examinations were performed at 5.1 ± 1.48 days of life. APGAR (1, 5, and 10 minutes), pH and basis excess (arterial blood gas), age at neurosonography, age at hypothermia treatment, age at MRI, and short-term survival outcomes (by the end of the study period) of all patients in the HIE group can be found in ▶ Table 1.

The second (control group) was composed of 20 healthy term neonates (11 males and 9 females). None of the neonates had any

clinical evidence of HIE. Blood gas measurement during labor and delivery showed no signs of hypoxia. Neurosonography examinations were performed at 3.50 ± 2.72 days of life. Indications for ultrasound in the 20 healthy newborns were increased head circumference (50%), retrocerebellar cyst on previous gestational ultrasound (15%), large anterior fontanelle (10%), hypoplastic vermis on gestational MRI (10%), hyperreflexia (5%), ventricular asymmetry (5%), and ventricular cyst (5%).

The exclusion criteria included congenital heart diseases, chromosomal abnormalities, absence of MRI, (or, in the HIE group, no evidence of injury on their MRI), congenital infections, multiple pregnancies, lack of an anteroposterior scan from the neurosonography study, anteroposterior scan not involving all the brain from the frontal lobes to the occipital lobes, hydrocephalus, intracranial hemorrhage, and cephalohematoma. Control infants meeting these criteria with similar ages to the HIE neonates were challenging to enroll, and therefore scans up to 10 days of life were included for the control neonates.

Neurosonography Protocol

Neurosonography examinations were performed using one of two scanners, one Philips and one GE, each with a 5–8 MHz micro convex transducer using the anterior fontanelle as the acoustic window. Examinations were performed by four ultrasonographers with at least ten years of experience each. The examinations included sagittal and coronal standard still images and continuous anteroposterior scan in the coronal plane with the same settings of the ultrasound machine during the scan. This continuous coronal neurosonographic acquisition included all brain structures from the frontal to the occipital poles in the coronal plane.

Post-Processing and Data Collection

Neurosonography images were post-processed by Parametric MRI (pMRI) software available for common use and downloaded from *https://www.parametricmri.com*. Images were imported from an online PACS server or offline DICOM files and arranged by study and series in searchable tables. Selected series were loaded for analysis. Regions of interest were placed over grayscale images. In the case of ultrasound images, mean grayscale values were computed and stored automatically. Results were exported in DICOM or tabulated text format per case or in batch. Video tutorials on the use of the software, including loading data and image segmentation, are available on the website listed above.

Brain regions of interest were segmented (frontal, parietal, parieto-occipital, and perirolandic WM, caudate nucleus head, lentiform nucleus, and thalamus), and their internal mean grayscale values were determined with segmented regions of bone and the choroid plexus (CP) of the lateral ventricle atria as internal controls (▶ Fig. 6a–g).

The mean grayscale value, which will be referred to as echogenicity, of the different brain regions was measured as follows:
1) Frontal WM, two slices before the rostral portion of the frontal horn to avoid volume averaging (▶ Fig. 6a)
2) Caudate nucleus head, immediately before the level of the caudothalamic groove (▶ Fig. 6b)
3) Lentiform nucleus at the level of the middle cerebral artery (▶ Fig. 6c)
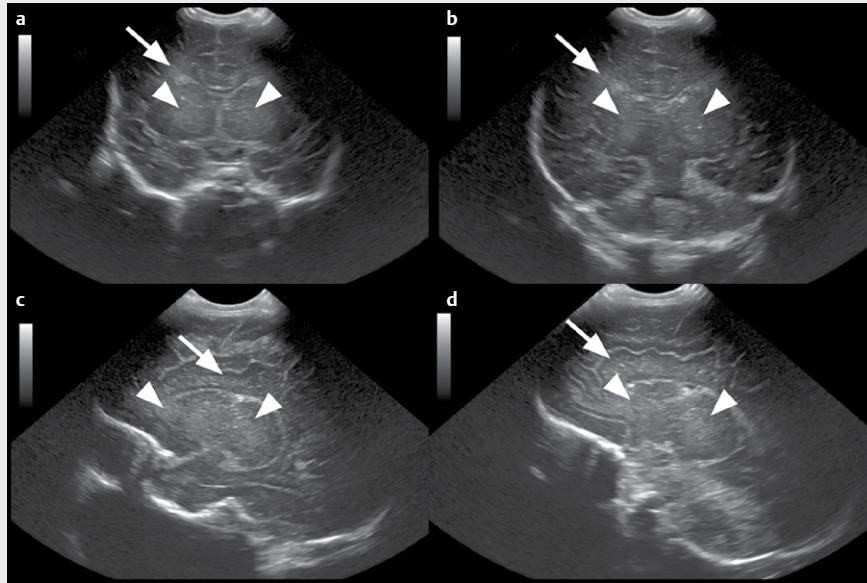
▶ **Fig. 1** Diffusely increased echogenicity involving the white matter (white arrows) and bilateral basal ganglia/thalami (white arrowheads) in a patient with HIE.
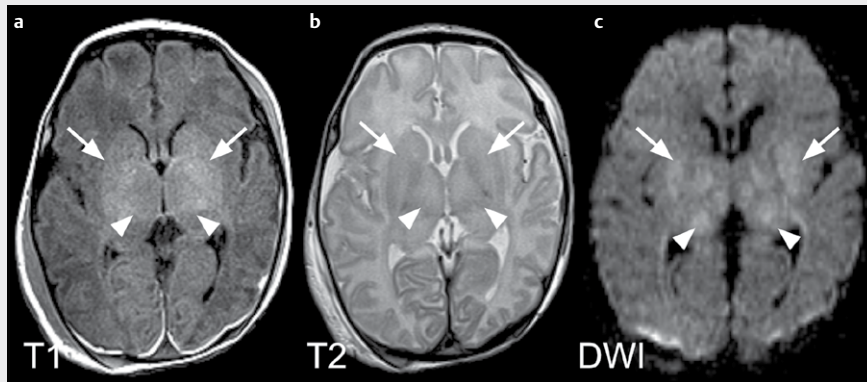
▶ **Fig. 2** Signal changes involving the bilateral basal ganglia (white arrows) and thalami (white arrowheads) in **a** neonate with hypoxic-ischemic encephalopathy, with increased T1-weighted signal in a, increase T2-weighted signal in **b**, and increased DWI signal in **c**.
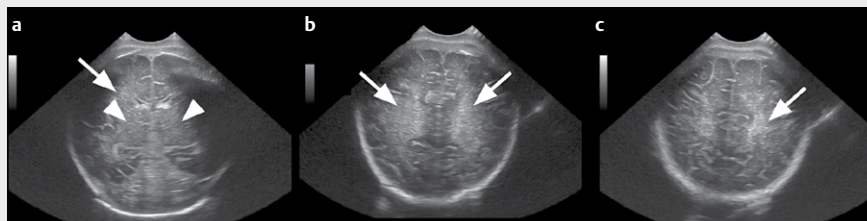
▶ **Fig. 3** Diffusely increased echogenicity involving the white matter (white arrows) in **a**, **b**, **c** and also in the bilateral basal ganglia and thalami (white arrowheads) in a.
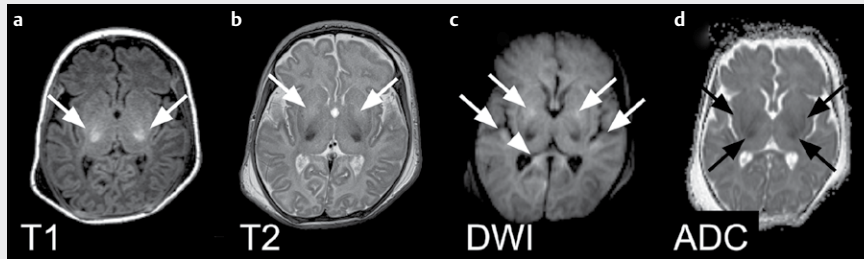
▶ **Fig. 4** Diffuse and bilateral signal changes in the basal ganglia, corpus callosum, cortex, and posterior limbs of the internal capsules (PLIC). In **a**, increased T1-weighted signal in the bilateral PLIC. In **b**, increased T2-weighted signal in the bilateral basal ganglia. In **c** and **d**, restricted diffusion in the perisylvian cortex and basal ganglia (white arrows) and splenium of the corpus callosum (white arrowhead). Corresponding ADC map demonstrates dark signal in these regions (black arrows).
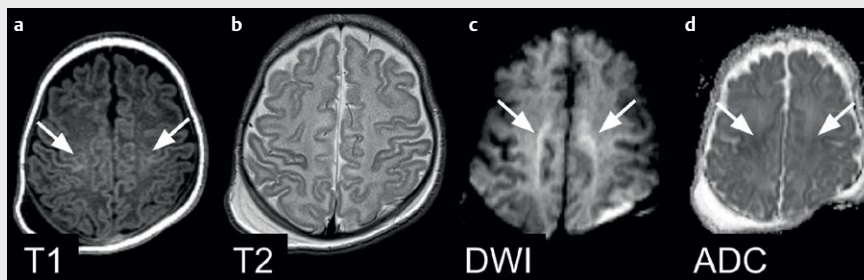
▶ **Fig. 5** Bilateral signal changes in the perirolandic cortex. In **a**, increased T1-weighted signal, no evident signal changes in the T2-weighted images in **b**, with evident DWI signal changes in **c**, and restricted diffusion in **d**.

4) Thalamus, two slices before the quadrigeminal plate (▶ Fig. 6d)
5) Parietal WM, at the level of the thalamus (▶ Fig. 6e)
6) Parieto-occipital WM, two slices after the caudal portion of the occipital horn, to avoid volume averaging (▶ Fig. 6f)
7) Perirolandic WM, at the level of the *pars marginalis* (▶ Fig. 6g)
8) Bone, at the level of the thalamus (▶ Fig. 6h)
9) CP, the level of the glomus (▶ Fig. 6i).

Since the frontal and the perirolandic WM are the farest anterior and posterior planes of a neurosonography examination, they may be challenging to measure, especially in infants with a limited acoustic window due to a narrow or partially closed fontanelle.

The echogenicity ratios of the brain regions of interest to the bone and CP were calculated. The rationale for using two different ratios was exploratory and based on the understanding that these two structures represent the most echogenic regions in the field of view. HIE and intraventricular hemorrhage can coexist in up to 10% of cases, being more prevalent in those treated with hypothermia [11]. In the event of intraventricular hemorrhage, the CP ratio calculation may be artefactual. For intraobserver reliability evaluation, each segmentation was performed twice, approximately a month apart, by the same user (FGG), a fellowship-trained neuroradiologist with ten years of experience.

Analysis

Statistical testing was performed using R: A language and environment for statistical computing version 3.6.1. The alpha error was set to 0.05. Demographics were compared using proportion tests, including the proportion of the control and HIE groups with Cesarean and vaginal deliveries and the proportion of males and females.

A permutational multivariate analysis of variance using distance matrices (permANOVA) was used to analyze the effect of several factors on the echogenicity ratios for each brain region of interest to CP and bone. These factors were: patient group (control versus HIE), age at neurosonography, sex, length of pregnancy, and delivery type (vaginal versus Cesarean). Multiple post-hoc two-sample permutation tests were performed with the Hommel adjustment for multiple comparisons to control for type I error. Pearson correlation coefficient was calculated for the two replicates of each ratio to evaluate intra-observer reliability.

The empirical receiver operating characteristic curves were calculated for each ratio. The area under the curve was calculated for each receiver operating characteristic curve.

Results

Participants

The neonates in the HIE group were born between 37 weeks, 2 days and 44 weeks of gestation (39.7 weeks ± 11.5 days) with a birth

Table 1 APGAR at 1, 5, and 10 minutes, pH, basis excess, age at neurosonography, age at hypothermia treatment, age at magnetic resonance imaging, and short-term survival outcomes of all patients in the hypoxic ischemic encephalopathy group. Ages are shown in days.

CASE	APGAR 1, 5, 10	pH	BASIS EXCESS	AGE AT US	AGE AT HYPOTHERMIA	AGE AT MRI	ALIVE
1	2/4/7	6.95	−17	1	1	5	YES
2	2/3/2	7	−7	0	1	3	NO
3	1/7/8	7	−21	1	1	7	YES
4	0/0/0	7	−23	0	0	5	NO
5	1/3/7	6.9	−18	1	0	7	YES
6	1/1/7	7	−15	0	0	6	YES
7	4/5/6	6.4	−15	0	1	5	YES
8	1/5/6	7	−16	1	1	7	YES
9	1/2/6	7	−20	0	0	4	YES
10	2/3/4	6.97	−19	0	1	6	NO
11	6/7/9	7	−14	1	0	7	YES
12	1/5/8	6.89	−23	0	0	5	YES
13	1/2/6	6.9	−23	0	1	3	YES
14	2/3/5	6.97	−17	0	0	6	YES
15	2/3/2	6.97	−18	0	0	7	YES
16	2/5/6	6.95	−18	0	0	5	YES
17	0/1/1	6.97	−20	0	0	4	YES
18	NA/4/9	6.9	−15	0	0	2	YES
19	2/6/7	7	−16	2	1	4	YES
20	0/2/4	7	−13	1	0	4	YES

NA - not available.
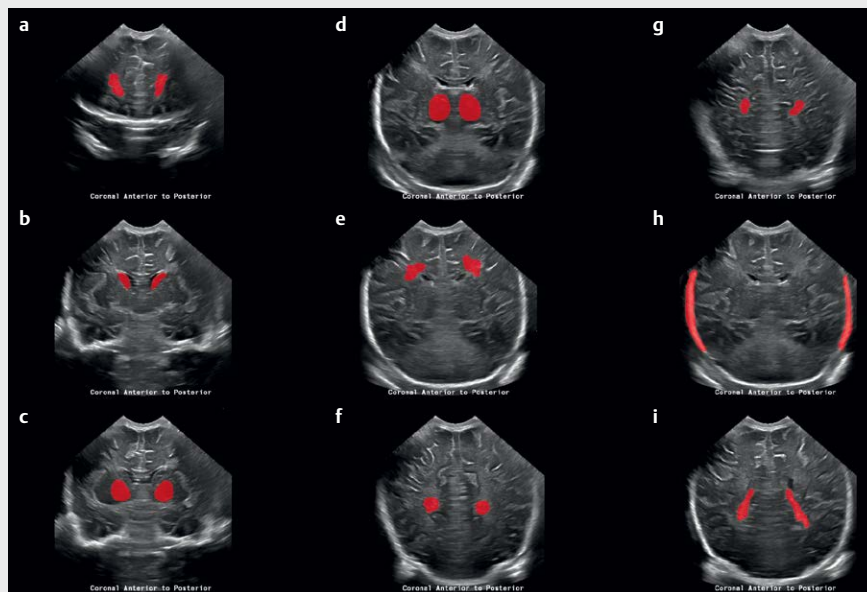
Fig. 6 Segmentation of brain regions of interest that were post-processed by the parametric software: **a**) frontal white matter, **b**) caudate nucleus head, **c**) lentiform nucleus, **d**) thalamus, **e**) parietal white matter, **f**) parieto-occipital white matter, and **g**) perirolandic white matter. For the internal controls, the echogenicity of the calvarial bones was measured at the level of the thalamus **h**), and the echogenicity of the choroid plexus was measured at the level of the glomus **i**).

▶ **Table 2** Results of permutational multifactorial analysis of variance evaluating the effects of multiple factors group (control versus hypoxic-ischemic injury), subject age, length of pregnancy, and delivery type (vaginal versus Cesarean) on echogenicity ratios of brain regions of interest to the choroid plexus and calvarial bone.

	F	p-value
Group	40.594	0.001***
Age	1.177	0.290
Gender	2.537	0.104
Length	0.737	0.460
Delivery	3.453	0.046*
Group:Age	0.852	0.421
Group:Sex	0.900	0.404
Age:Sex	1.402	0.270
Group:Length	1.910	0.153
Age:Length	2.786	0.087
Sex:Length	1.696	0.192
Group:Delivery	1.018	0.323
Age:Delivery	3.351	0.046*
Sex:Delivery	1.949	0.150
Length:Delivery	0.807	0.444
Group:Age:Sex	1.169	0.283
Group:Age:Length	0.425	0.703
Group:Sex:Length	0.962	0.375
Age:Sex:Length	1.410	0.227
Group:Age:Delivery	2.353	0.091
Group:Sex:Delivery	3.064	0.078
Age:Sex:Delivery	0.652	0.527
Group:Length:Delivery	1.289	0.263
Age:Birth:Delivery	0.954	0.413
Sex:Length:Delivery	2.612	0.085
Group:Sex:Length:Delivery	2.075	0.114

▶ **Table 3** Results of multiple post-hoc two-sample permutation tests comparing echogenicity ratios of the control and hypoxic-ischemic injury groups. CP – choroid plexus ratio; B – calvarial bone ratio.

Echogenicity ratio	p-value
Caudate nucleus head_CP	0.017
Caudate_B	0.008
Lentiform nucleus_CP	0.017
Lentiform nucleus_B	0.011
Thalamus_CP	0.013
Thalamus_B	0.011
Occipital white matter_CP	<0.001
Occipital white matter_B	<0.001
Parietal white matter_CP	<0.001
Parietal white matter_B	<0.001
Frontal white matter_CP	<0.001
Frontal white matter_B	0.012
Perirolandic white matter_CP	<0.001
Perirolandic white matter_B	<0.001

weight of 3495 ± 555 g. Cesarean section was performed in 14 and vaginal delivery in 6 pregnancies. The neonates in the control group were born between 37 weeks, 5 days and 41 weeks of gestation (39.3 weeks ± 6.5 days) with a birth weight of 3336.7 g ± 403. In the control group, cesarean section was performed in 8 and vaginal delivery in 12 pregnancies. The echogenicity in the frontal and perirolandic WM was not measured in 4 patients due to technical challenges, such as motion artifacts and lack of acoustic window.

Test results

There was no significant difference between the proportions of vaginal and cesarean deliveries in the control and HIE groups ($p = 0.057$) or between the proportions of males and females in the control and HIE groups ($p = 0.185$).

Patient group (control versus HIE) and delivery type (vaginal versus cesarean) significantly affected the brain region echogenicity ratios ($p < 0.001$ and $p = 0.046$, respectively; ▶ **Table 2**). Echogenicity

ratios were significantly lower for control neonates than for HIE neonates in each brain region (▶ **Table 3**; ▶ **Fig. 2**). The differences in ratios between the control and HIE subject ratios were smallest for the caudate and putamen ratios. Caudate and putamen to CP ratios were both 0.08 greater in HIE neonates, and caudate and putamen to bone ratios were both 0.10 greater in the HIE subjects. The differences were greatest for the perirolandic WM region, with the ratios to CP and bone being 0.23 and 0.22 greater, respectively, for the HIE patients compared to the controls (▶ **Fig. 7**). Although delivery type had a significant p-value in our overall multivariate analysis, when pairwise permutation tests were performed comparing vaginal and cesarean deliveries within the HIE and control groups, there were no significant differences.

The generated receiver operating characteristic curves and area under the curve calculations revealed a similar trend (▶ **Fig. 8**; ▶ **Table 4**). The lowest areas under the curve, which were still in the acceptable range, belonged to the caudate and putamen to CP ratios at 0.733 and 0.745, respectively. The perirolandic WM had a high area under the curve, at 0.980 for both the CP and bone ratios.

The intra-observer reliability for all ratios was high, with the caudate to bone ratio being the lowest at 0.832 and the anterior WM to CP ratio being the highest at 0.992 (▶ **Table 5**).

Discussion

HIE is a major cause of morbidity and mortality in neonates and can result in long-term, persistent motor, sensory, and cognitive impairment [12]. Early detection of neonates with HIE is of paramount importance for prompt treatment in order to reduce neurodevelopmental damage and improve outcomes [2]. In the early phases of HIE, dysfunction of membrane ion transport from hypoxia leads
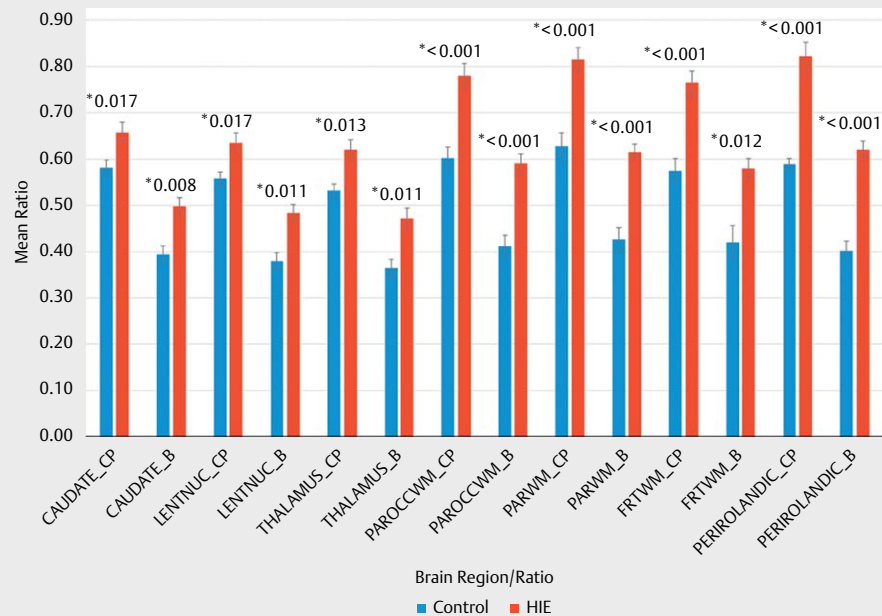
► **Fig. 7** Mean echogenicity ratios for each brain region to the echogenicity of two internal controls, the choroid plexus (CP), and calvarial bone (B), for both healthy control infants and infants with a diagnosis of hypoxic-ischemic encephalopathy. HIE – Hypoxic ischemic encephalopathy LENTNUC – Lentiform nucleus OCCWM – Occipital white matter PARWM – Parietal white matter FRTWM – Frontal white matter

to cellular edema, which later activates cellular pathways that ultimately lead to cell death and tissue necrosis [13].The most sensitive imaging modality to detect cytotoxic edema is diffusion-weighted imaging [14]. Diffusion-weighted imaging is highly recommended in the evaluation of cases of HIE, as it can be employed to assess the extent of the injury and for outcome prediction [15].

Despite advances in other imaging techniques, neurosonography, which is radiation-free, cost-effective, and portable, remains a fundamental screening method in the evaluation of focal or diffuse changes in the brain, particularly in cases of HIE. These changes alter the echogenicity of brain structures, which translates into either increasing or decreasing echogenicity relative to the unaffected areas of the brain (qualitative neurosonography) [2].

Though neurosonographic evaluation of HIE is the most convenient imaging method for this type of injury, it has historically been qualitative and subjective, which may give rise to ambiguity and inconsistency [8, 9]. Accurate and reliable interpretation of HIE by neurosonography is heavily dependent on the experience of trained radiologists and visual recognition of patterns [1]. Moreover, depending upon the acquisition settings, transducer, size of the fontanelle, and the selected window/level settings of the neurosonography images, the degree of brain injury may be under- or overestimated if the ultrasound images are reviewed only qualitatively. Even experienced pediatric radiologists may misinterpret the degree of changes of WM echogenicity [6]. In addition to these technical variations, the severity of HIE can lower radiologists' sensitivity for subtle findings of HIE, and mild HIE may appear normal or near-normal on neurosonography, especially if the neurosonog-

raphy examination is performed during the first two or three days of life [2]. The presence of coexistent complications, such as leukomalacia or intracranial hemorrhage, could also present challenges for radiologists.

These limitations of the typical application of neurosonography may be overcome by quantitative neurosonography. Quantitative data may also serve as a biomarker for outcome prediction in neonates suffering from HIE. These biomarkers may play a major role, especially in settings in which MRI is less available or not feasible due to critical conditions. Previous studies [8, 16] have proposed different quantitative techniques to quantify neonatal WM lesions. Simaeys et al. [8] used a software-based method to compensate for the variable ultrasound acquisition factors by comparing the echogenicity of intracerebral lesions with that of the CP, whose echogenicity is considered to be relatively constant. Padilla et al. [10] showed that calculation of the relative echogenicity (ratio of the mean pixel brightness measured in brain regions and the bone at the same depth of sampling) might offer a semiquantitative method to evaluate various anatomical regions of the neurosonography examination. However, there is a lack of data showing the diagnostic accuracy of the quantitative evaluation of brain echogenicity in neonatal HIE.

Technical factors may also limit the validity of quantitative measures used to identify HIE. Studies that have attempted to quantify ultrasound signals from the neonatal brain have often used raw gray levels instead of ratios. Barr et al. [7] found higher gray levels in the brain parenchyma of infants with brain injury. These authors used an 8-bit image with optimized scanning parameters for each patient to obtain the best images. However, no
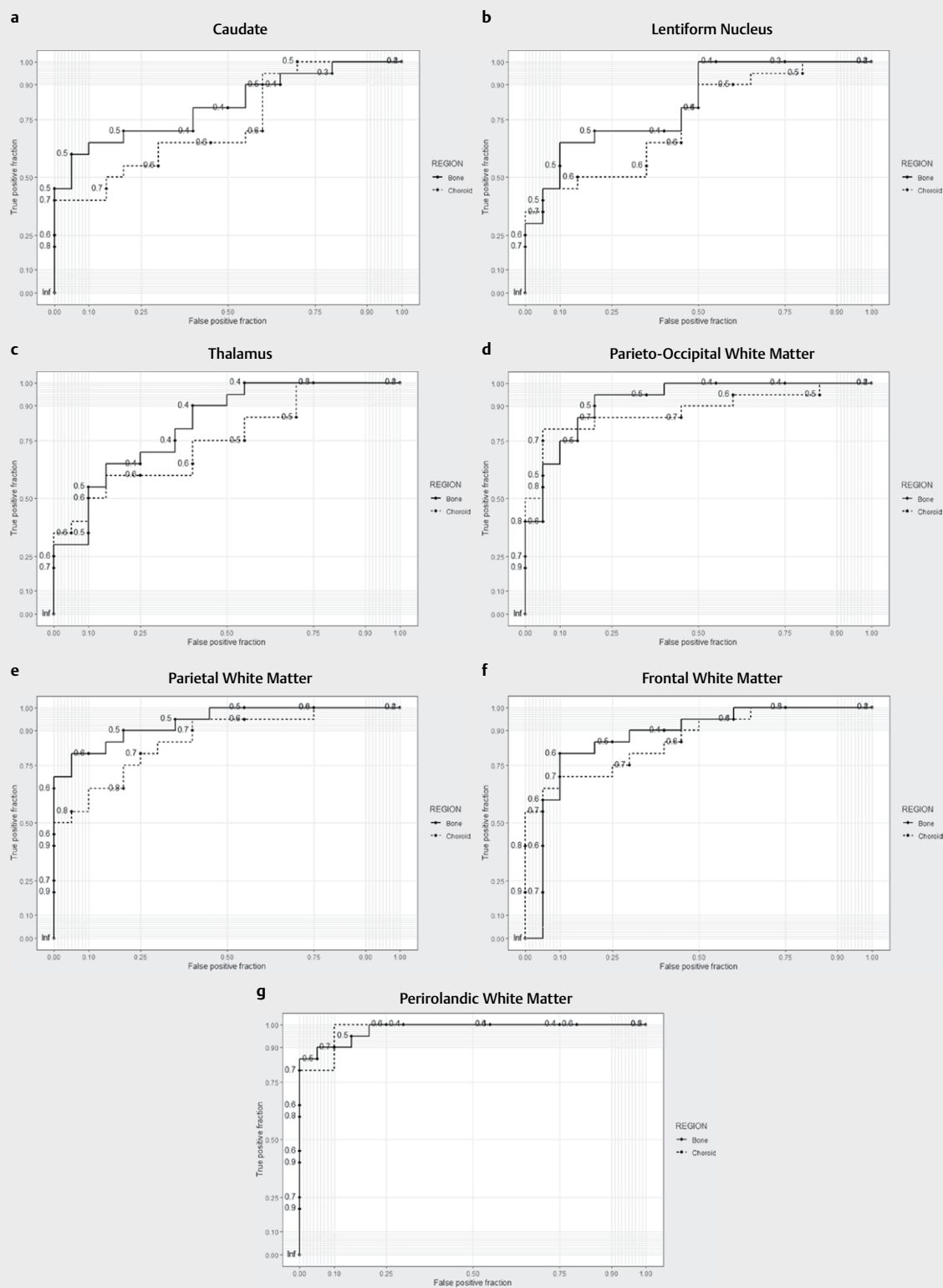
▶ **Fig. 8** Empirical receiver operating characteristic curves for the echogenicity ratios of the brain region of interest, with either the choroid plexus or calvarial bone as the internal control.

normalization was made, and only the raw pixel intensity was evaluated. This limits the generalizability of the protocol of these authors secondary to bias introduced by technical differences. In our study, we employed internal controls to help determine the effects of technical factors. Similarly, Simaeys et al. [8] compared the echo-

▶ Table 4 Areas under the curve for each brain region echogenicity ratio (CP – choroid plexus; B – calvarial bone) calculated from the empirical receiver operating characteristic curves.

Echogenicity Ratio	Area Under the Curve
Caudate nucleus head_CP	0.733
Caudate_B	0.810
Lentiform nucleus_CP	0.745
Lentiform nucleus_B	0.818
Thalamus_CP	0.753
Thalamus_B	0.820
Occipital white matter_CP	0.880
Occipital white matter_B	0.923
Parietal white matter_CP	0.863
Parietal white matter_B	0.938
Frontal white matter_CP	0.863
Frontal white matter_B	0.873
Perirolandic white matter_CP	0.980
Perirolandic white matter_B	0.980

▶ Table 5 Pearson correlation coefficients demonstrating intra-observer reliability between the two measurements of each echogenicity ratio.

Echogenicity Ratio	Pearson Correlation Coefficient
Caudate nucleus head_CP	0.963
Caudate_B	0.832
Lentiform nucleus_CP	0.954
Lentiform nucleus_B	0.834
Thalamus_CP	0.973
Thalamus_B	0.878
Occipital white matter_CP	0.981
Occipital white matter_B	0.937
Parietal white matter_CP	0.988
Parietal white matter_B	0.915
Frontal white matter_CP	0.992
Frontal white matter_B	0.924
Perirolandic white matter_CP	0.988
Perirolandic white matter_B	0.914

genicity of the periventricular WM with that of the CP and found elevated ratios in neonates with HIE.

Here, we describe multiple brain region echogenicity (mean grayscale value) ratios that could help identify early sonographic evidence of HIE. These echogenicity ratios of multiple brain regions that are known to be involved in HIE compared to internal controls (CP and calvarial bone) were able to discriminate between healthy neonates and neonates with HIE. We focused our measurements

on those areas of the brain most vulnerable to damage from HIE, specifically the deep gray nuclei [2]. The frontal, parietal, parieto-occipital, and perirolandic WM, while more challenging to identify with consistency, also represent vulnerable areas in neonates with hypoxia-ischemia [17, 18]. Our results show that while the deep gray matter demonstrated an acceptable to a good degree of discriminatory capacity, WM, mainly in the perirolandic region, had a greater ability to identify individuals with HIE.

The echogenicity of the neonatal CP may be potentially affected in cases of infections (ventriculitis, ependymitis, and choroid plexitis), hemorrhage, or congenital CP tumors. CP hemorrhage is more commonly found in premature infants but may occur in term neonates with HIE [11] and is rarely described in healthy neonates. Heibel et al. found eleven cases of CP hemorrhage (two isolated and nine associated with intraventricular extravasation) among 1000 consecutive neurosonography examinations of clinically normal, full-term neonates [19]. CP infarcts (commonly associated with thalamic infarct) are very uncommon in the pediatric population and are more commonly reported in adults [20, 21]. In animal studies, the CP has been shown to be highly vulnerable to HIE [22]. However, there is no clear evidence that this occurs in humans. There were no signs of CP hemorrhage in neurosonography examinations in the HIE and control groups and no mention of CP hemorrhage in the magnetic resonance reports of the HIE group. Both CP and bone were chosen as internal controls. Calvarial bones may be a potentially better internal control since they are seen in all images and are unaffected by HIE [10].

Sagittal scans were not used for analysis since these were only available in a few neonates. Sagittal scans would be beneficial to better delineate deep and periventricular WM, the majority of the length of the caudate nucleus, and larger sections of the thalamus. In sagittal scans, the immediate comparison between the hemispheres would be lost in paramedian and periventricular sections. However, since they are standard sections, reliability should be high. Alternative acoustic windows such as the posterior fontanelle or mastoid would help measure the echogenicity of the posterior fossa structures.

Beyond sensitivity and specificity, it is essential to have a test that is reliable and reproducible. Limited intra-observer reliability testing with our ratios demonstrated an excellent correlation between two independent segmentation events spaced approximately one month apart. The individual performing the measurements in our study was a fellowship-trained neuroradiologist, and as such, likely has greater knowledge of anatomy on neonatal neurosonography than a radiologist with less specialized training. However, it is possible to teach radiologists how to identify these regions, and our research could also form the basis for automated, computer-aided segmentation in the future.

Our study has limitations. The study was retrospective, and only a limited number of healthy control neonates were available. Also related to the retrospective nature of our study, the ultrasound studies were performed on multiple different machines with different technologists. Future studies addressing interobserver variability should be performed to validate widespread clinical use of the method. Our protocol, which utilized internal controls, was designed to help mitigate this limitation. Also, it was not possible to completely blind the individual (FGG) analyzing the studies because

the reader is a trained neuroradiologist who can recognize the sonographic signs of HIE. Lastly, since neurosonography in controls occurred three days (mean) later than in HIE patients, perhaps a transient increase in echogenicity ratios could be found after stressful but not asphyxiating spontaneous vaginal delivery.

In conclusion, quantification of brain parenchyma echogenicity, when coupled with internal controls, demonstrated differences in echogenicity in term HIE patients compared to controls during the first days following the injury. Quantitative neurosonography represents a potential method for early identification of HIE and increases diagnostic accuracy, which warrants further validation. Our ratios were elevated in cases of HIE and produced promising receiver operating characteristics. Further work with a larger cohort could reveal whether a quantitative approach can discern between degrees of severity of HIE. In the future, neurosonography protocols should be tailored to evaluate this region, which requires dedicated posterior coronal scanning. Furthermore, our method could, in principle, especially prospectively, offer an opportunity to employ machine learning/artificial intelligence under standardized conditions to improve the assessment of HIE.

Acknowledgements

Conflict of Interest

Colbey Freeman has received a GPU grant from NVIDIA to study the use of machine learning to track ultrasound contrast microbubbles. Misun Hwang has received an investigator-initiated pilot grant from Bracco for the study of contrast-enhanced ultrasound in neonatal brain injury.

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