Feasibility and Safety of Contrast-Enhanced Ultrasound of the Neonatal Brain: A Prospective Study Using MRI as the Reference Standard

Judy H. Squires, MD[1,2], Nancy H. Beluk, RT[1], Vince K. Lee, BS[1], Toby D. Yanowitz, MD, MS[3], Serter Gumus, MD[1,2], Subramanian Subramanian, MD[1,2], Ashok Panigrahy, MD[1,2]

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BACKGROUND. MRI is the reference standard for neonatal brain imaging, but it is expensive, time-consuming, potentially limited by availability and accessibility, and contraindicated in some patients. Transfontanelle neonatal head ultrasound is an excellent alternative but may be less sensitive and specific than MRI. Contrast-enhanced ultrasound (CEUS) has the potential to improve the capabilities of ultrasound.

OBJECTIVE. The purpose of this study is to prospectively evaluate the feasibility, safety, and diagnostic performance of transfontanelle neonatal brain CEUS, with MRI used as the reference standard.

METHODS. Neonates in the institutional neonatal ICU who were undergoing MRI as part of their clinical care were prospectively recruited to undergo portable brain ultrasound and CEUS for research purposes. Brain ultrasound and CEUS were performed portably without moving the patient from the isolette or crib in the neonatal ICU. Adverse events were recorded. Two radiologists independently evaluated ultrasound and CEUS images for abnormalities and then reached consensus regarding discrepancies. A separate radiologist reviewed MRI examinations. Sensitivity, specificity, and interreader agreement were evaluated, with MRI used as the reference. Qualitative post hoc image review was performed.

RESULTS. Twenty-six neonates (nine boys and 17 girls; mean [± SD] age, 15.2 ± 14.0 days) were included. No significant alteration in patient vital signs or adverse reaction to the ultrasound contrast agent (UCA) occurred. The mean duration of the examination was significantly shorter for combined ultrasound and CEUS than for MRI (21.1 ± 4.7 vs 74.2 ± 34.8 minutes; $p < .001$). Interrater agreement for any abnormality was almost perfect for both ultrasound and CEUS ($\kappa = 0.92$ and 0.85, respectively). Sensitivity for any abnormality was 86.7% for ultrasound and 93.3% for CEUS; specificity was 100.0% for both. CEUS had sensitivity of 87.5% for acute or subacute ischemia and 100.0% for chronic ischemia; its specificity was 100.0% for acute or subacute ischemia and chronic ischemia. For both ultrasound and CEUS, sensitivity for subdural and intraparenchymal hemorrhage was poor (22.2–50.0%). On CEUS but not on MRI, post hoc review showed a case of postischemic hyperperfusion, which was confirmed by subsequently performed contrast-enhanced CT.

CONCLUSION. The use of portable brain CEUS in neonates is feasible, safe, and more rapid than MRI.

CLINICAL IMPACT. The potential diagnostic utility of brain neonatal CEUS relative to conventional ultrasound, particularly for ischemia, warrants further investigation.

Brain injury in neonates is a significant source of morbidity and mortality among preterm and term infants, often resulting in adverse neurodevelopmental outcomes [1–3]. Early and accurate characterization of brain injury is important for prognostication and early intervention. The reference standard for neonatal brain imaging is MRI

[1]Department of Radiology, University of Pittsburgh School of Medicine, Pittsburgh, PA.

[2]Department of Pediatric Radiology, UPMC Children's Hospital of Pittsburgh, 4401 Penn Ave, 2nd Fl Radiology, Pittsburgh, PA 15224. Address correspondence to J. H. Squires (judysquires@chp.edu).

[3]Department of Neonatology, Magee-Womens Hospital, University of Pittsburgh School of Medicine, Pittsburgh, PA.

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[4]. However, use of MRI in the neonatal period is limited given the logistic challenges and medical risks of scanning preterm and very-low-birth-weight patients. These include patient motion, the need for temperature control and vital sign monitoring, and the possible need for sedation. Systems barriers include expense, limited availability and accessibility, and the need for personnel who have the technical and clinical expertise to perform imaging appropriately.

For neonates, transfontanelle brain ultrasound is a well-established alternative neuroimaging modality that avoids many of the pitfalls of MRI. The technique is safe, is comparatively inexpensive, offers superb spatial resolution, and most importantly can be performed quickly and portably at the patient's bedside, resulting in limited disturbance of the neonate. Brain ultrasound is therefore incorporated into nationally published and hospital-based guidelines [5–7]. The major disadvantage of brain ultrasound is its lower sensitivity and specificity compared with those of MRI, with MRI able to better show disease extent [2, 8]. Anticipated improvements in the capabilities of screening brain ultrasound will enhance the quality of care provided to critically ill neonates.

Microbubble contrast-enhanced ultrasound (CEUS) is a well-established safe, low-cost, and efficient imaging modality [9–14]. Microbubbles of ultrasound contrast agents (UCAs) remain intravascular without diffusing into the interstitial space and are easily visible compared with the adjacent tissue, thus improving the visibility of very small vascular structures. Therefore, real-time perfusion can be readily visualized. The application of this technology has expanded the diagnostic potential of ultrasound across organ systems. One UCA (sulfur hexafluoride lipid–type A microspheres [Lumason, also known as SonoVue, Bracco Diagnostics]) is approved by the FDA for IV use to characterize focal liver lesions in children. Although this represents off-label use based on current approval status, several reports have described the potential capability of transfontanelle brain CEUS, including in the setting of hypoxic ischemic injury and in the intraoperative setting, to monitor brain perfusion in neonates during congenital heart disease surgery [15–20]. However, to our knowledge, the safety and diagnostic performance of CEUS for neonatal brain imaging have not been specifically evaluated.

The purpose of the present study was to prospectively evaluate the feasibility, safety, and diagnostic performance of transfontanelle neonatal brain CEUS, with MRI used as the reference standard.

HIGHLIGHTS

Key Finding

- *This prospective study of 26 neonates showed that brain CEUS performed portably in the NICU is feasible, safe, and more rapid than MRI, with almost perfect interreader agreement.*

Importance

- *Although further study is warranted, the findings suggest the potential utility of bedside CEUS in detecting neurovascular abnormalities in critically ill infants.*

Methods

Patients

This prospective study was approved by the institutional review board at the University of Pittsburgh. All activities were performed in compliance with HIPAA. Informed consent was obtained from both legal guardians.

The clinical MRI schedule was reviewed from August 2017 to May 2019 to identify consecutive potentially eligible patients in the neonatal ICU (NICU) at the UPMC Children's Hospital of Pittsburgh. Neonates were eligible for the study if they either were scheduled to undergo brain MRI for any indication or had already undergone brain MRI within the previous 48 hours and if they already had functioning IV access for clinical care purposes. Neonates who were actively treated with extracorporeal life support were ineligible, to minimize any potential confounding effects of microbubble destruction in the circuit. Study participants underwent brain ultrasound and CEUS, performed portably in the NICU, for research purposes.

Image Acquisition

Patients first underwent clinical MRI performed using a standard neonatal brain protocol at our institution, with the exception of one patient who underwent MRI after brain CEUS due to ease of scheduling. The MRI sequences obtained varied depending on the clinical indication and the protocol used, but at a minimum they included T1- and T2-weighted images, susceptibility-weighted images, and DWI with ADC mapping. Use of IV gadolinium-based contrast material depended on the indication and selected protocol.

Brain ultrasound and CEUS were performed portably, without moving the patient from the isolette or crib in the NICU, as soon as possible after MRI was performed and after informed consent was obtained. Per institutional protocol, a radiologist was present for all CEUS examinations. Specifically, all CEUS examinations were performed by a single board-certified pediatric radiologist (J.H.S., who had 3 years of CEUS experience) who was blinded to the clinical indication for MRI and the results of MRI. However, when available, prior head ultrasound images were reviewed, thereby mimicking clinical workflow. A routine ultrasound examination was always performed before CEUS, consistent with standard implementation of CEUS for all organ systems. A Logiq E9 unit (GE Healthcare) was used for patients who underwent imaging through August 20, 2018, at which time a Logiq E10 unit (GE Healthcare) was acquired and used for the remaining patients. Brain ultrasound was performed using the routine clinical protocol at our institution, including acquisition of both coronal and sagittal still images and cinematic sweeps through the anterior fontanelle with the use of both a curved-array transducer with a small footprint (3-10 MHz) and a linear high-frequency (9 MHz) transducer, as well as acquisition of transmastoid images with attention given to the posterior fossa. Additional spectral Doppler imaging of the cerebral arteries was not performed, and compression imaging also was not performed. Brain CEUS was conducted immediately after ultrasound. When the Logiq E9 unit was used, only the linear 9-MHz transducer had contrast preset capability, which was utilized with the contrast ultrasound software and baseline settings of the machine. However, for this machine, the 3-10–MHz transducer did not have contrast preset capability. Therefore, contrast settings were emulated as closely as pos-

sible, with use of coded harmonic imaging, decreased acoustic output (power) of 8%, and decreased frequency of 6 MHz based on manufacturer recommendations. After our upgrade to use of the Logiq E10 unit, the ultrasound contrast software and contrast presets of the machine were used for both the curved-array 3-10–MHz transducer and the linear 9-MHz transducer. The mechanical index (MI) ranged from 0.09 to 0.14, whereas the thermal index was 0.0 for all studies. After the UCA was administered, imaging was performed only in contrast mode to minimize any potential cavitary, thermal, or mechanical bioeffects. There was no flash pulse or intentional destruction of microbubbles.

Any type of functioning IV access was used. Although UCA was used off label, the FDA-approved dose, route, and number of allowable UCA injections were followed. One dose of sulfur hexafluoride lipid–type A microspheres (0.03 mL/kg; rounded up to the nearest 0.1 mL) was administered IV by either the bedside nurse or the pediatric sonographer with use of a 1-mL syringe via the parallel hub of a three-way stopcock; a 3-mL 0.9% normal saline flush was then administered via the perpendicular hub of the stopcock at a rate of 1 mL/s. All contrast-enhanced imaging was performed using cinematic clips that were saved. First, a wash-in cinematic clip obtained at the level of the third ventricle in the coronal plane for 30 seconds after UCA administration was saved to allow visualization of both gray and white matter. This was followed by coronal anterior-to-posterior and sagittal right-to-left cinematic sweeps performed using the curved-array 3-10–MHz transducer and, finally, axial transmastoid superior-to-inferior cinematic sweeps. Imaging time was kept as short as possible while visualizing all possible structures, to minimize any potential bioeffects related to CEUS. After adequate imaging was completed, only intermittent scanning in contrast mode was performed to evaluate for spontaneous UCA clearance. After complete microbubble clearance, a second dose of UCA was administered, and the same cinematic clips were saved using the linear 9-mHz transducer. Two injections were used to mimic the clinical protocol for evaluation of liver lesions with CEUS, which is the current FDA-approved use of IV sulfur hexafluoride lipid–type A microspheres in children, and to provide a backup acquisition given the use of two types of transducers in this feasibility study.

Patients were monitored by the bedside nurse for potential adverse reactions to the UCA before, during, and for 30 minutes after the CEUS examination. Patient monitoring included the oxygen saturation percentage, respiratory rate, and continuous cardiac rate and rhythm monitoring using the standard NICU extremity pulse oximeter and cutaneous leads. A 15% change in the heart rate from baseline, a 15% change in the respiratory rate from baseline, and a 5% change in oxygen saturation were considered clinically significant. In addition, patients were visually monitored for any evidence of hypersensitivity by the radiologist performing the examination and the bedside NICU nurse.

Image Analysis

The radiologist who performed the brain ultrasound and CEUS examinations (J.H.S.) also reviewed the ultrasound images and all CEUS images and clips obtained using both transducers in the same review setting. This radiologist subjectively assessed whether each CEUS image showed diagnostic quality of enhancement. This radiologist also scored examinations using a binomial approach, evaluating for the presence or absence of germinal matrix hemorrhage, ventriculomegaly, subdural or intraparenchymal hemorrhage, ischemia, and brain malformation. The location of all identified abnormalities was recorded. The Papile grade of intraventricular hemorrhage was recorded [21]. Ischemia, if present, was classified as acute or subacute if the area appeared echogenic or chronic (i.e., having a duration of more than 1 week) if hypoechoic or anechoic changes suggesting encephalomalacia were seen in the region. Quantitative analysis was not performed.

The brain ultrasound images and all CEUS images and clips were then separately and independently reviewed and graded by a second pediatric radiologist (S.G., who had 2 years of CEUS experience). No training specific to this investigation occurred before image interpretation was conducted by either radiologist, primarily because brain CEUS findings are not yet well described. Discrepancies in brain ultrasound and CEUS interpretation were then reviewed in a consensus session for ultrasound and CEUS, and the consensus interpretation was used for analysis. Independent analysis of the two contrast injections and transducer type results was not performed.

A third pediatric radiologist (S.S., who had 4 years of experience in pediatric neuroradiology), who was blinded to the clinical report and ultrasound and CEUS results, independently reviewed all MRI examinations. This radiologist evaluated MRI for the presence or absence of the abnormalities that were also assessed on ultrasound and CEUS. After the initial separate evaluations of each modality, the first radiologist (J.H.S.) performed a qualitative post hoc assessment of cases showing discrepancy between modalities.

Statistical Analysis

Continuous data are presented as mean ± SD. The examination duration was compared between CEUS and MRI by use of a *t* test. The examination time for CEUS included both the initial ultrasound examination and the subsequent CEUS examination, as well as time for spontaneous microbubble clearance between imaging. The examination time for MRI did not include the patient transportation time, which is not tracked and readily available at our institution. Interrater agreement for individual abnormalities and for any abnormality was determined using Cohen kappa coefficients with 95% CIs; kappa coefficients were classified using the benchmarks described by Landis and Koch [22]. Sensitivity and specificity for each abnormality type and for any abnormality overall were computed separately for ultrasound and CEUS, with MRI used as the reference standard; 95% CIs were provided. A $p < .05$ was considered statistically significant. Statistical analyses were performed using SAS/STAT software (version 9.4, SAS Institute).

Results

Patients and Examinations

A total of 97 neonates with a scheduled or recently completed brain MRI examination were screened for study participation. Of these neonates, 35 were ineligible because of lack of IV access. No screened neonate was ineligible on the basis of active treatment with extracorporeal life support. In addition, legal guardians were unavailable for 15 neonates. Thus, the guardians of 47 neonates were approached for consent. The guardians of 20 neonates declined participation. Twenty-seven neonates were there-

fore enrolled. One neonate was later excluded because of subsequent loss of IV access. This exclusion resulted in a final study sample of 26 neonates (nine boys and 17 girls; mean [± SD] age, 15.2 ± 14.0 days; range, 1–59 days) (Fig. 1).

Six patients had congenital heart disease and/or a right-to-left vascular shunt. CEUS used a mean dose of sulfur hexafluoride lipid–type A microspheres of 0.2 mL ± 0.1 mL per injection, as based on patient weight. The mean time between MRI and CEUS was 22.1 ± 13.9 hours. The mean duration of brain MRI was 74.2 ± 34.8 minutes. The mean duration of combined ultrasound and CEUS was significantly shorter (21.1 ± 4.7 minutes; $p < .001$). Two MRI examinations were performed using IV gadolinium-based contrast material because of clinical concern about infection; the remaining 24 MRI examinations were performed without contrast material. For the first 14 patients, CEUS was performed using the Logiq E9 unit, which did not have preset capability for the curved-array transducer, whereas CEUS was performed using the Logiq E10 unit for the remaining 12 patients.

Safety

No clinically significant alteration in patient vital signs occurred before, during, or within 30 minutes after UCA administration, including in patients with cardiac disease and shunts. No adverse reaction to the UCA occurred.

Image Assessment

All CEUS examinations (regardless of size, type, or location of the IV access used for contrast agent administration and use of the linear high-frequency transducer or the curved-array transducer with a small footprint) subjectively showed diagnostic quality of enhancement.

Agreement for any abnormality was almost perfect between the two readers for the independent ultrasound and CEUS interpretations (κ = 0.92 and 0.85, respectively). For individual findings, agreement, which was expressed using the kappa coefficient, ranged from 0.46 to 1.00 for ultrasound and from 0.43 to 1.00 for CEUS. Cases of discrepancy between the two readers that were resolved by consensus included one case each of small grade 1, 2, and 3 germinal matrix hemorrhages; acute thalamus ischemia; acute temporal lobe ischemia; and posterior fossa subdural hemorrhage. Additional results regarding interreader agreement are presented in Table 1.

Results of the ultrasound, CEUS, and MRI examinations are provided in Table 2. Table 3 shows the diagnostic performance of ultrasound and CEUS, with MRI used as the reference standard. Sensitivity of ultrasound was 86.7%, and that of CEUS was 93.3%. Specificity was 100.0% for both ultrasound and CEUS. Nine patients had both normal ultrasound and CEUS findings that were confirmed by MRI (Fig. 2 and Video S1, the latter of which can be viewed in the *AJR* electronic supplement to this article, available at doi.org/10.2214/AJR.21.26274). Ultrasound and CEUS both had 100.0% sensitivity and 100.0% specificity for ventriculomegaly. No patient had a brain malformation observed on any imaging modality. CEUS had sensitivity of 75.0% and specificity of 86.4% for germinal matrix hemorrhage, sensitivity of 33.3% and specificity of 100.0% for subdural hemorrhage, sensitivity of 50.0% and specificity of 100.0% for intraparenchymal hemorrhage, sensitivity of 87.5% and specificity of 100.0% for acute or subacute ischemia, and sensitivity of 100.0% and specificity of 95.8% for chronic ischemia. Differences between ultrasound and CEUS were not significant given wide 95% CIs.

Post Hoc Imaging Review

All cases of subdural hemorrhage that were missed at ultrasound or CEUS were posterior fossa hemorrhages. In one patient with posterior fossa subdural hemorrhage, the finding was prospectively seen on CEUS but not on ultrasound (Fig. 3). Cases of intraparenchymal hemorrhage that were not seen on ultrasound or CEUS were hemorrhagic areas within areas of ischemia, most likely representing hemorrhagic transformation. Areas of ischemia in these patients were detected by ultrasound and CEUS. In one neonate with subacute ischemia in the middle cerebral artery territory, hyperenhancement of the area of infarct was seen on CEUS but not on ultrasound or MRI, although MRI showed ischemia (Fig. 4 and Video S2, the latter of which can be viewed in the *AJR* electronic supplement to this article, available at doi.org/10.2214/AJR.21.26274). Hyperperfusion was confirmed in this patient on contrast-enhanced head CT performed for separate clinical reasons 6 hours later.

In one patient with intraventricular pus, which was caused by *Escherichia coli* on the basis of CSF culture, ultrasound and CEUS findings were interpreted at both independent and consensus review as grade 3 germinal matrix hemorrhage. However, in retrospect, the case showed avid enhancement of the ependymal

TABLE 1: Agreement Between Two Readers for Ultrasound and Contrast-Enhanced Ultrasound (CEUS)

Finding	Ultrasound		CEUS	
	κ (95% CI)	Agreement	κ (95% CI)	Agreement
Germinal matrix hemorrhage	0.61 (0.2–1.00)	Substantial	0.43 (0.00–0.92)	Moderate
Ventriculomegaly	0.84 (0.52–1.00)	Almost perfect	1.00 (1.00–1.00)	Almost perfect
Subdural hemorrhage	0.65 (0.01–1.00)	Substantial	0.65 (0.01–1.00)	Substantial
Intraparenchymal hemorrhage	1.00 (1.00–1.00)	Almost perfect	0.65 (0.01–1.00)	Substantial
Acute or subacute ischemia	0.69 (0.37–1.00)	Substantial	0.79 (0.50–1.00)	Substantial
Chronic (> 1 wk) ischemia	0.46 (0.00–1.00)	Moderate	0.65 (0.01–1.00)	Substantial
Brain malformation	1.00 (1.00–1.00)	Almost perfect	1.00 (1.00–1.00)	Almost perfect
Any abnormality	0.92 (0.78–1.00)	Almost perfect	0.85 (0.64–1.00)	Almost perfect

TABLE 2: Patient Characteristics, Including Indication for MRI and Consensus on Ultrasound Results, Contrast-Enhanced Ultrasound (CEUS) Results, and MRI Results

Patient Age (d)	Gestational Age at Birth	Vascular Catheter Type	Indication for MRI and Other Diagnoses	Ultrasound Result (Consensus Reading)	CEUS Result (Consensus Reading)	MRI Result (Single Reader Interpretation)
1	38 wk 1 d	24-gauge PIV catheter	Intracranial glioma	Ventriculomegaly, intraparenchymal hemorrhage	Ventriculomegaly, intraparenchymal hemorrhage, intraventricular hemorrhage	Ventriculomegaly, intraparenchymal hemorrhage, intraventricular hemorrhage
1	28 wk 1 d	24-gauge PIV catheter	Intracranial hemorrhage on prior HUS, prematurity	GMH grade 4, ventriculomegaly; subdural, intraparenchymal, and intraventricular hemorrhage; acute and chronic ischemia	GMH grade 4, ventriculomegaly; subdural, intraparenchymal, and intraventricular hemorrhage; acute and chronic ischemia	Ventriculomegaly; subdural, intraparenchymal, and intraventricular hemorrhage; acute ischemia; **no GMH grade 4 or chronic ischemia**
2	36 wk 1 d	24-gauge PIV catheter	Follow-up ventriculomegaly	GMH grade 3	GMH grade 3, subdural hemorrhage (posterior fossa)	GMH grade 3, subdural hemorrhage (posterior fossa)
3	41 wk 2 d	UVC	Meconium aspiration syndrome and completion of ECMO therapy, severe pulmonary hypertension, seizures, sepsis	Acute ischemia	Extensive acute ischemia	Extensive acute ischemia, **intraparenchymal hemorrhage (hemorrhagic transformation of ischemia)**
3	38 wk 5 d	1.9-French PICC in right basilic vein	Outside hospital HUS with reported grade 4 GMH, abnormal neurologic examination	Acute ischemia (MCA territory)	Acute ischemia (MCA territory) with hyperperfusion	Acute ischemia (MCA territory)
3	38 wk 3 d	24-gauge PIV catheter	Apnea, abnormal findings on prior HUS	GMH grade 2, acute ischemia	GMH grade 2, acute ischemia	GMH grade 2, acute ischemia and **intraparenchymal hemorrhage (hemorrhagic conversion of ischemia), posterior fossa subdural hemorrhage**
4	40 wk 2 d	24-gauge PIV catheter	Seizures	Normal	Normal	Normal
4	39 wk 6 d	24-gauge PIV catheter	Abnormal findings on neurologic examination	Normal	Normal	Normal
6	39 wk 6 d	UVC	Seizure, sepsis, ASD	Normal	Normal	Normal
6	41 wk 0 d	24-gauge PIV catheter	HII, seizures	Acute ischemia	Extensive acute ischemia	Extensive acute ischemia, **posterior fossa subdural hemorrhage**
6	39 wk 2 d	24-gauge PIV catheter	Hypotonia	Normal	Normal	Normal
8	39 wk 0 d	24-gauge PIV catheter	Persistent hypoglycemia	Normal	Normal	Normal
10	37 wk 0 d	3-French PICC in right femoral vein	HII	Extraaxial and intraventricular hemorrhage	Extraaxial and intraventricular hemorrhage	Extraaxial and intraventricular hemorrhage
12	39 wk 0 d	24-gauge PIV catheter	Seizure	Normal	Normal	Normal
16	39 wk 0 d	24-gauge PIV catheter	Infection	Normal	Normal	Normal
16	37 wk 0 d	24-gauge PIV catheter	Abnormal head ultrasound, dilated ventricles	Enlarged subarachnoid spaces	Enlarged subarachnoid spaces	Enlarged subarachnoid spaces

(Table 2 continues on next page)

TABLE 2: Patient Characteristics, Including Indication for MRI and Consensus on Ultrasound Results, Contrast-Enhanced Ultrasound (CEUS) Results, and MRI Results (continued)

Patient Age (d)	Gestational Age at Birth	Vascular Catheter Type	Indication for MRI and Other Diagnoses	Ultrasound Result (Consensus Reading)	CEUS Result (Consensus Reading)	MRI Result (Single Reader Interpretation)
18	38 wk 0 d	7-French Hohn catheter in right internal jugular vein	Meconium aspiration syndrome and completion of ECMO therapy	Chronic ischemia	Chronic ischemia	Chronic ischemia
20	27 wk 5 d	24-gauge PIV catheter	Sepsis, evaluate for ventriculitis, PFO versus ASD	Ventriculomegaly, GMH grade 3	Ventriculomegaly, GMH grade 3, ventriculitis	Ventriculomegaly, **no GMH,** ventriculitis with **intraventricular pus**
21	40 wk 0 d	24-gauge PIV catheter	HII	Acute ischemia	Extensive acute ischemia	Extensive acute ischemia, **grade 2 GMH, subdural and intraparenchymal hemorrhage (hemorrhagic transformation of ischemia)**
26	34 wk 0 d	24-gauge PIV catheter	Concern for possible nonaccidental trauma	Normal	Normal	Normal
28	37 wk 4 d	24-gauge PIV catheter	Cyanotic episodes, PFO with left to right shunting, abnormal findings on HUS	Normal	Normal	Normal
28	37 wk 1 d	2.7-French tunneled Broviac catheter in right facial vein	Gastroschisis, ASD and VSD, intracranial calcifications	Normal	Normal	**Posterior fossa subdural hemorrhage**
29	37 wk 3 d	1.9-French single-lumen PICC in right leg	Seizure, infection	Normal	Normal	Normal
31	38 wk 0 d	24-gauge PIV catheter	IUGR, failure to thrive, concern for infection, PFO	Intraventricular hemorrhage	Intraventricular hemorrhage	**Acute ischemia (tiny right frontal lobe infarct)**, intraventricular hemorrhage
35	37 wk 2 d	3-French PICC in left arm	Infection	Acute ischemia and hemorrhage	Extensive acute ischemia and hemorrhage	Extensive acute and **chronic** ischemia and hemorrhage
59	25 wk 3 d	24-gauge PIV catheter	Prematurity, bronchopulmonary dysplasia with pulmonary hypertension, ASD, grade 1 IVH on HUS, ventilated	Grade 1 GMH	Grade 1 GMH	**Grade 2** GMH, **subdural (tentorial) hemorrhage**

Note—Discordant CEUS and MRI results are indicated in boldface. PIV = peripheral IV, HUS = head ultrasound, GMH = germinal matrix hemorrhage, UVC = umbilical venous catheter, ECMO = extracorporeal membrane oxygenation, PICC = peripherally inserted central catheter, MCA = middle cerebral artery, ASD = atrial septal defect, HII = hypoxic ischemic injury, PFO = patent foramen ovale, VSD = ventricular septal defect, IUGR = intrauterine growth restriction, IVH = intraventricular hemorrhage.

TABLE 3: Diagnostic Performance of Ultrasound and Contrast-Enhanced Ultrasound (CEUS) for Individual Abnormalities and for Any Abnormality, With MRI Used as the Reference Standard

Abnormality	Sensitivity			Specificity		
	Percentage (95% CI)	Numerator	Denominator	Percentage (95% CI)	Numerator	Denominator
Ultrasound						
GMH	75.0 (32.6–100.0)	3	4	90.9 (78.9–100.0)	20	22
Ventriculomegaly	100.0 (100.0–100.0)	4	4	100.0 (100.0–100.0)	22	22
Subdural hemorrhage	22.2 (0.0–49.4)	2	9	100.0 (100.0–100.0	17	17
Intraparenchymal hemorrhage	50.0 (10.0–90.0)	3	6	100.0 (100.0–100.0)	20	20
Acute or subacute ischemia	87.5 (64.6–100.0)	7	8	100.0 (100.0–100.0)	18	18
Chronic (> 1 wk) ischemia	100.0 (100.0–100.0)	2	2	95.8 (87.8–100.0)	23	24
Brain malformation	NA	NA	NA	100.0 (100.0–100.0)	26	26
Any abnormality	86.7 (69.5–100.0)	13	15	100.0 (100.0–100.0)	11	11
CEUS						
GMH	75.0 (32.6–100.0)	3	4	86.4 (72.0–100.0)	19	22
Ventriculomegaly	100.0 (100.0–100.0)	4	4	100.0 (100.0–100.0)	22	22
Subdural hemorrhage	33.3 (2.5– 64.1)	3	9	100.0 (100.0–100.0)	17	17
Intraparenchymal hemorrhage	50.0 (10.0–90.0)	3	6	100.0 (100.0–100.0)	20	20
Acute or subacute ischemia	87.5 (64.6–100.0)	7	8	100.0 (100.0–100.0)	18	18
Chronic (> 1 wk) ischemia	100.0 (100.0–100.0)	2	2	95.8 (87.8–100.0)	23	24
Brain malformation	NA	NA	NA	100.0 (100.0–100.0)	26	26
Any abnormality	93.3 (80.7–100.0)	14	15	100.0 (100.0–100.0)	11	11

Note—GMH = germinal matrix hemorrhage, NA = not applicable.

ventricular lining (Fig. 5), which was not seen in other patients with intraventricular hemorrhage.

Discussion

To our knowledge, this is the first prospective evaluation of brain CEUS in neonates that has used MRI as the reference standard. The addition of a UCA-enhanced examination to traditional ultrasound was both feasible and safe for this off-label use in this small cohort. The small catheters placed for clinical purposes were used to administer the UCA, with diagnostic quality of enhancement observed across all catheter types and patient gestational ages. Of importance, in this potentially vulnerable population, no adverse events occurred during or after UCA administration, consistent with the reported low rate of adverse events for UCAs [9–12]. Patient transport and sedation were not needed, and imaging was performed faster by use of the combination of ultrasound and CEUS than by MRI. Inclusion of transportation in the MRI examination time would have further increased the difference in imaging time between the two modalities. These neonates also usually already have IV access in place for clinical care purposes. Thus, brain CEUS is a particularly attractive option for imaging critically ill neonates.

The potential bioeffects of CEUS were minimized by use of meticulous technique. High MI imaging or prolonged imaging have a theoretic risk of microbubble rupture causing damage to adjacent structures, called sonoporation. Potential sonoporation of developing intracranial structures was minimized by imaging exclusively with low MI after UCA administration. Total CEUS imaging time was kept as short as possible while still visualizing all intracranial structures. Finally, between injections, while waiting for spontaneous UCA clearance, we performed no high MI imaging or flash technique, and we conducted only intermittent scanning with low MI to assess spontaneous UCA clearance.

Early and accurate detection of acute brain injury in the neonatal period assists with prognostication as well as decision making and early treatment [23, 24]. Although abnormalities in individual patients were detected on CEUS but not on ultrasound, larger studies are needed to provide a more comprehensive comparison of the accuracy of the two techniques. Nonetheless, interreader agreement for any abnormality observed on CEUS was almost perfect, despite the lack of a training session for the two readers.

CEUS identified hyperenhancement of an area of subacute stroke in one patient. Postischemic hypervascularity using arterial spin labeling perfusion MRI has been described elsewhere [25]. To our knowledge, this is the first such case reported on CEUS. Hyperperfusion is thought to be related to revascularization after vessel recanalization or, possibly, to increased neuronal activity. However, the cause and clinical significance are unknown. Imaging patients with stroke over time can be easily performed using CEUS, which could provide a more accessible differentiation of postischemic hyperperfusion versus hypoperfusion than that offered by MRI. This in turn could facilitate improved insight of the correlation of the finding with neurodevelopmental outcomes.

Identification of hemorrhage remains a limitation of both ultrasound and CEUS. The sensitivity of these two modalities for

subdural or intraparenchymal hemorrhage ranged from 22.2% to 50.0%. In our cohort, areas of ischemia were identified on CEUS, but superimposed hemorrhagic transformation was not. Because microbubbles travel within vascular structures, enhancement is impeded by lack of blood flow due to either ischemia or hemorrhage. Thus, ischemia and hemorrhage appear similar on CEUS, with both manifesting as nonenhancing areas. In addition, subdural hemorrhages were all located in the posterior fossa, which may be birth related and may not be clinically significant.

An important finding on CEUS in one patient, although identified only at the post hoc review, was ventriculitis related to infection. To our knowledge, this is the first reported case of infectious ventriculitis at CEUS. We suggest that this diagnosis be considered when the appearance that we have described is encountered, particularly in the presence of clinical suspicion for intracranial infection.

Although this is a prospective study, we acknowledge potential limitations. First, the sample size was small. In addition, ultrasound contrast presets were not available for both transducers until the machine upgrade occurred. The performance of CEUS for patients who underwent imaging before the upgrade may have been underestimated, particularly for abnormalities in the peripheral brain. Nonetheless, all examinations were deemed to subjectively exhibit diagnostic quality of enhancement. The authors recommend use of transducers with software presets and use of a curved-array transducer with a small footprint when possible. Also, the radiologist performing CEUS was blinded to the MRI indication and results. However, it was not possible to blind the radiologist who was performing bedside ultrasound and CEUS to the clinical status of the patients based on visual inspection, which may have introduced bias. However, the second CEUS reader was not present at the time of imaging, and interrater agreement of the independent interpretations was almost perfect. Further, consistent with clinical workflows, ultrasound and CEUS were interpreted during the same setting, which may have also biased the CEUS results. Finally, quantitative CEUS analysis was not performed; all analyses were based on readers' qualitative visual assessment. This limitation reflects current CEUS implementation given the current lack of widely available quantification software for CEUS in the United States. Proprietary quantification software has been developed for research purposes at some U.S. institutions but in general is not externally available. We recognize the availability of CEUS quantification software for research purposes in humans that is currently available outside of the United States (e.g., VueBox, Bracco Imaging).

In conclusion, use of brain CEUS is feasible to image the neonatal brain portably and safely and offers almost perfect interreader agreement. The potential diagnostic utility of brain CEUS relative to conventional ultrasound warrants further investigation.

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(Figures start on next page)

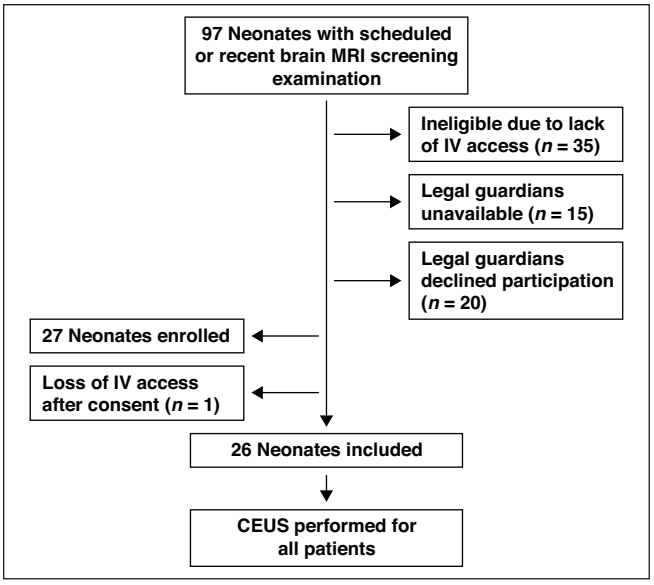

Fig. 1—Flow diagram of patients screened, including those excluded and included. CEUS = contrast-enhanced ultrasound.

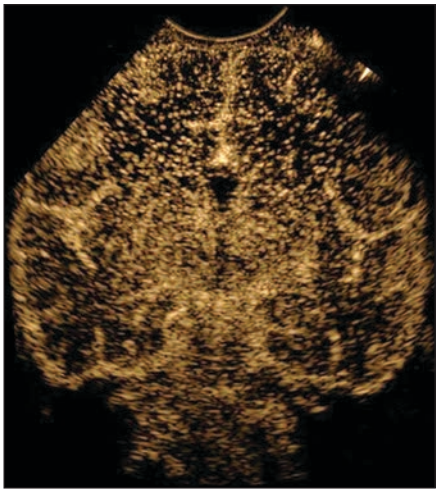

Fig. 2—12-day-old boy, born at gestational age of 39 weeks 0 days, who had normal results of contrast-enhanced ultrasound (CEUS) performed using Logiq E10 ultrasound machine (GE Healthcare) and curved-array 3-10–MHz transducer at 17 seconds after administration of ultrasound contrast agent. Coronal CEUS image shows homogeneous supratentorial and infratentorial enhancement. Findings of previously performed MRI examination (not shown) were also normal.

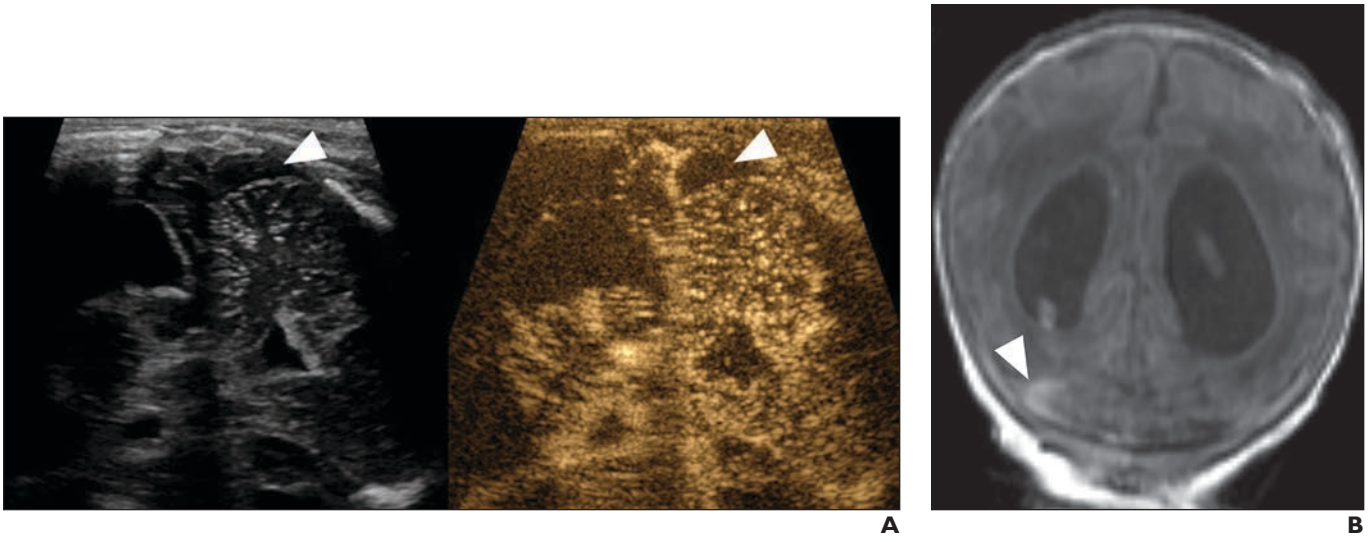

Fig. 3—2-day-old girl, born at gestational age of 36 weeks 3 days (late preterm), who had bilateral in utero grade 3 germinal matrix hemorrhages and posterior fossa subdural hemorrhage.
A, Split-screen axial transmastoid image shows gray-scale image (*left*) and contrast-enhanced ultrasound (CEUS) image (*right*) obtained 1 minute and 29 seconds after administration of ultrasound contrast agent. CEUS was performed using Logiq E10 ultrasound machine (GE Healthcare) and linear 9-MHz transducer. Images show heterogeneously hypoechoic right posterior fossa subdural hemorrhage with no enhancement (*arrowheads*) adjacent to sigmoid dural venous sinus overlying cerebellar hemisphere.
B, Coronal 3D gradient-echo MR image confirms right posterior fossa subdural hemorrhage (*arrowhead*).

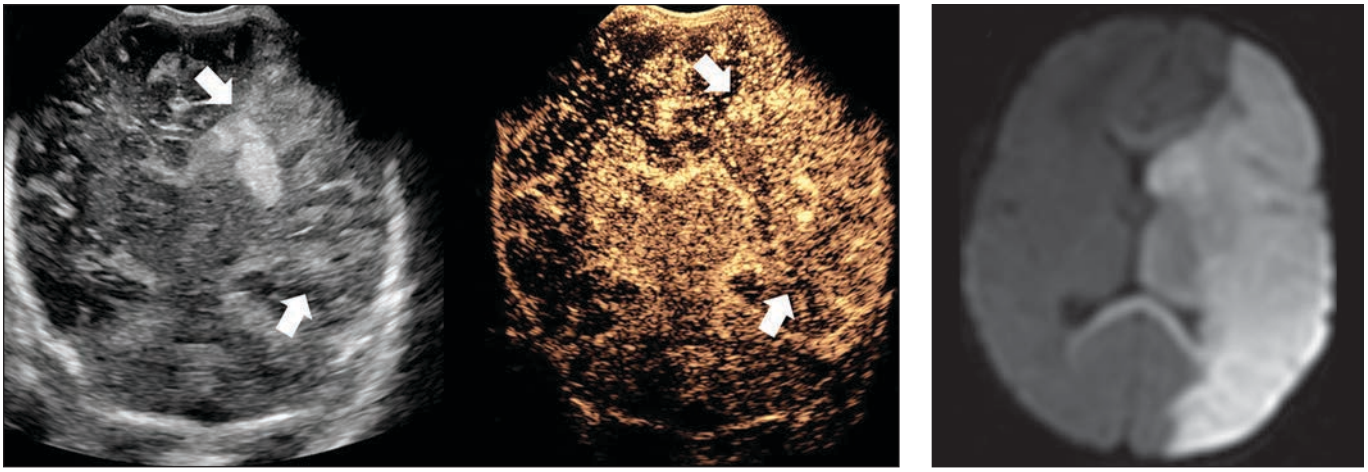

Fig. 4—3-day-old girl, born at gestational age of 38 weeks 5 days, who had hyperenhancing subacute large left middle cerebral artery (MCA) territory infarct.
A, Coronal contrast-enhanced ultrasound image image was performed using Logiq E10 ultrasound machine (GE Healthcare) and curved-array 3-10–MHz transducer. Split-screen display shows gray-scale image (*left*) and contrast-only image (*right*) obtained 20 seconds after injection of contrast agent. On contrast-only image, intrinsic hyperechogenicity of background soft tissue is subtracted, such that only microbubbles are displayed brightly. Gray-scale image shows heterogeneous hyperechogenicity of left middle cerebral artery territory (*arrows*), compatible with cytotoxic edema from subacute infarct. Contrast-only image shows hyperenhancement of infarction (*arrows*), compatible with hyperperfusion. Hyperperfusion was also present on subsequent contrast-enhanced head CT (not shown).
B, Axial diffused-weighted MR image shows diffusion restriction, compatible with infarct.

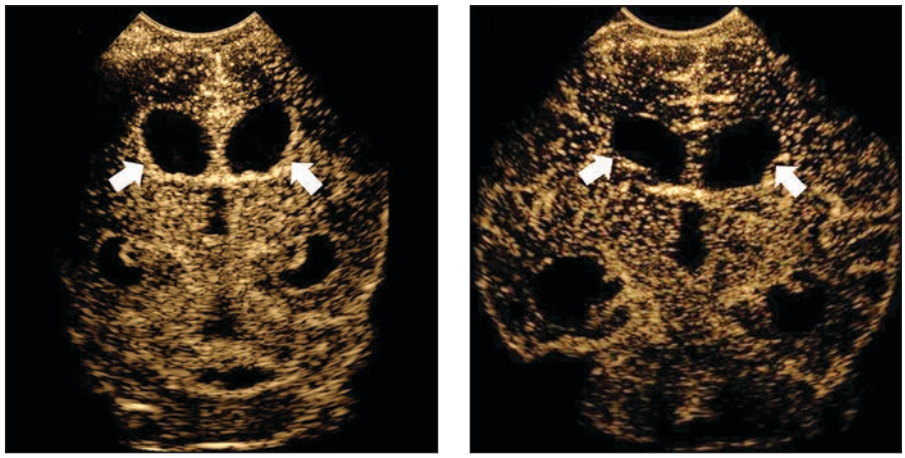

Fig. 5—Contrast-enhanced ultrasound (CEUS) images of brain in two infants with ventriculomegaly of different causes. Both images were obtained using Logiq E10 ultrasound machine (GE Healthcare) and curved-array 3-10–MHz transducer.
A, 20-day-old girl, born at gestational age of 27 weeks 5 days, who had *Escherichia coli* ventriculitis. CEUS image obtained 17 seconds after administration of ultrasound contrast agent shows hyperenhancement of ependymal lining of lateral ventricles (*arrows*), compatible with ventriculitis. CSF within dilated ventricles is nonenhancing.
B, 2-day-old girl (same patient as Fig. 3), born at gestational age of 36 weeks 3 days, who had bilateral in utero grade 3 germinal matrix hemorrhages and posterior fossa subdural hemorrhage. CEUS image obtained 20 seconds after administration of ultrasound contrast agent shows lateral ventricular ependymal enhancement (*arrows*) similar to adjacent supratentorial white matter, in contradistinction to hyperenhancing ependyma seen in Figure 5A.

(Editorial Comment starts on next page)

Editorial Comment: Contrast-Enhanced Ultrasound of the Brain—Potential Applications in the Neonatal ICU

Assessment of the brain in critically ill neonates is crucial for the detection of abnormalities that may influence prognostication and patient management. Current technologies include both gray-scale and color Doppler ultrasound, which are relatively insensitive to ischemic changes, and MRI, which poses challenges related to patient transportation and the potential need for sedation. Contrast-enhanced ultrasound (CEUS) is an alternative modality for addressing these limitations; early evaluation of CEUS for assessment of the pediatric brain has been promising [1, 2].

This study expands on earlier preliminary work by prospectively evaluating neonatal brain CEUS performed portably in the neonatal ICU, in comparison with MRI, which was also performed for all patients. The authors show excellent performance of CEUS for diagnosing ischemia and also provide further evidence of the safety of administration of ultrasound contrast agents to neonates. Their findings support the use of CEUS for evaluation of the neonatal brain not only as an adjunct to MRI but also as a potential replacement used for patients who are too ill to be transported or who have a contraindication to MRI. In conjunction with the existing literature, the results should encourage practicing radiologists to add CEUS to their diagnostic imaging armamentarium.

Given the reliable detection of intracranial abnormalities, CEUS provides radiologists and referring physicians with an attractive option for patients in the neonatal ICU who would benefit from a safe bedside alternative or supplement to MRI. Furthermore, as the authors discuss, CEUS can be used for real-time visualization of intracranial perfusion and for longitudinal follow-up of findings identified on initial ultrasound or MRI examinations (e.g., to assess for revascularization). Ultimately, this study provides a foundation for wider implementation of CEUS in the ongoing efforts to help these vulnerable patients.

Ryne A. Didier, MD
Children's Hospital of Philadelphia
Philadelphia, PA
didierr@chop.edu

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