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Elucidation of Renal Scars in Children With Vesicoureteral Reflux Using Contrast-Enhanced Ultrasound: A Pilot Study

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Introduction: Vesicoureteral reflux is a common disorder in children but can result in kidney scarring following acute pyelonephritis. The gold standard diagnostic to detect renal scars in children is ^{99m}Tc-dimercaptosuccinic acid (DMSA) scintigraphy. DMSA has a number of limitations including radiation exposure, need for sedation, and radiotracer supply shortages. Contrast-enhanced ultrasound (CEUS) is a technique whereby biocompatible microspheres of inert gas are administered i.v. that reflect ultrasonography sound waves and do not involve radiation. Because the contrast agent is rapidly cleared, contrast images must be obtained within minutes of administration. CEUS has been used in a variety of organ systems, but its use in pediatric kidney diseases is limited.

Methods: In this study, we performed CEUS in 7 children with documented renal scars by radiographic imaging consistent with reflux nephropathy.

Results: In all subjects, CEUS detected all previously known radiologic abnormalities as well as detecting new areas of hypoenhancing renal parenchyma. None of the patients experienced any serious adverse events.

Discussion: This study represents the first report of using CEUS to characterize renal scars in children with reflux nephropathy. We conclude that CEUS is a highly sensitive, rapid, and cost-effective diagnostic imaging modality for detecting and monitoring renal scars in children with vesicoureteral reflux.

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Vesicoureteral reflux (VUR) affects 1% to 2% of all children, and up to one-third of these patients will experience urinary tract infection (UTI). Acute pyelonephritis associated with VUR can lead to renal scarring and ultimately chronic/end-stage kidney disease known as reflux nephropathy.[1] The Randomized Intervention for Children with Vesicoureteral Reflux (RIVUR) trial, a randomized, placebo-controlled trial in children with VUR and a history of UTI, demonstrated a rate of new scarring of almost 12% during the 2-year study period.[2] Classically, acquired reflux nephropathy scars arise following an episode of acute pyelonephritis. The pathognomonic scar in acquired reflux nephropathy fans out from the entry point in the medulla to the cortical segment in a wedge-shaped fashion. A number of imaging modalities have been used to identify and to characterize these cortical scars in children with VUR.

^{99m}Tc-dimercaptosuccinic acid (DMSA) scintigraphy is a radionuclide scan performed to detect pyelonephritis and renal scars and is considered the gold standard. The study involves a peripheral IV line and injection of radiotracer. To allow for cortical uptake, most centers wait 1 to 4 hours before imaging.[3] In the multicenter RIVUR study, 90% of centers used

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sedation to obtain images.[4] Although less than in an abdominal computed tomography (CT) scan, a substantial amount of radiation is involved in DMSA scanning and amounts to roughly 10 times the radiation exposure of pulsed fluoroscopy voiding cystourethrograms and 50 chest X-rays.[5,6] Finally, in the United States, we have experienced 2 major shortages of DMSA radiotracer in the past 10 years. Due to DMSA's unavailability since 2014, clinicians must consider other options to detect and to monitor renal scars. Magnetic resonance (MR) urography has also shown promise, but this modality frequently requires sedation and is expensive compared to other imaging modalities.[7] Conventional ultrasound's sensitivity is low as 37% to detect renal scars.[8] A rapidly obtained, cost-effective modality that is highly sensitive, does not require sedation, and does not expose the patient to radiation does not exist currently for detection of kidney scars.

Contrast-enhanced ultrasound (CEUS) is a technique that has the potential to replace imaging studies that involve radiation.[9] Ultrasound contrast agents comprised phospholipid or protein microspheres that encase an inert gas. The microspheres approximate the size of a red blood cell and remain within the vascular space. These agents are not filtered or secreted by the kidneys, and therefore allow visualization of the renal parenchyma without interfering with enhancement of the collecting system. Unlike other imaging contrast agents that cannot be administered to patients with renal insufficiency, ultrasound contrast agents are safe for use in this patient population. Because gas is highly reflective on ultrasound imaging, these agents can be administered i.v. in very small doses (0.3–2.5 ml) and are detectable down to the capillary level. In pediatrics, CEUS has been used to improve visualization of the heart, liver, and bladder.[10] Lumason (Bracco, Milan, Italy) is a contrast agent that consists of sulfur hexafluoride gas surrounded by a thin phospholipid shell roughly 2.4 μm in size. The sulfur hexafluoride gas has an extremely short half-life, and 82% is expired by the lungs unchanged within 20 minutes of administration.[11] Given that reflux nephropathy scars are areas of kidney parenchyma with abnormal blood flow, we hypothesized that CEUS would elucidate areas of abnormal parenchyma (scars) in children with known reflux nephropathy.

METHODS

Patient Population

Eight children, adolescents, and young adults aged 8 to 21 years of age were enrolled in the pilot study between May and August 2016 at Le Bonheur Children's Hospital in Memphis, TN. Two healthy adults were imaged at the beginning of the study to optimize image acquisition and contrast administration. The study was approved by the Institutional Review Board of the University of Tennessee Health Science Center and was performed under U.S. Food and Drug Administration investigational new drug application number 129000.

Inclusion and Exclusion Criteria

Patients who were 8 years or older and were eligible for renal scar detection via DMSA were approached. All patients were required to have a history of previous abnormal renal parenchyma via an imaging modality suggestive of renal scarring and/or evidence of reflux nephropathy by an abnormal serum creatinine value. All patients and legal representatives gave informed consent and assent prior to the study. Exclusion criteria included allergy to sulfur hexafluoride or other related products, known cardiac congenital abnormalities, abnormal baseline electrocardiogram (ECG), or a history of open-heart surgery, retinopathy, emphysema, or pregnancy.

Image Acquisition and Contrast-Enhanced Ultrasound Technique

After consent and pretesting screening, a 20-gauge peripheral IV line was placed. All patients underwent conventional nonenhanced renal ultrasound initially using a LOGIQ General Electric E9 version 5 (Milwaukee, WI) ultrasound machine and curved 1-6 transducer. The study radiologists and nephrologist reviewed previous images and study day images to plan study image acquisition. For the CEUS examinations, the LogicE9 contrast-specific software version R4, 3.0 was used, and dynamic imaging during renal perfusion was obtained, followed by static imaging. The ultrasound transducer was held in a single longitudinal plane that best depicted the suspected renal scar on grayscale imaging and was held in that position throughout the dynamic phase of imaging. Dynamic imaging was recorded beginning with the contrast injection and continued for 30 to 60 seconds afterward. Following the dynamic phase, additional transverse and longitudinal static images of the entire kidney were obtained for an additional 3 to 5 minutes until enhancement waned. All subjects received Lumason sulfur hexafluoride contrast agent through the 20-gauge peripheral IV line. Subjects received 0.03 ml/kg per manufacturer's recommendations up to a maximum of 1.5 ml per injection. Each injection was immediately followed by a 5-ml sterile normal saline solution flush. Subjects received a separate injection

and imaging study for each kidney, spaced at least 10 minutes apart.

Patient Monitoring and Assessments

The subject's heart rate, blood pressure, and oxygen saturation were measured at baseline before the ultrasound was performed. Heart rate and oxygen saturation were monitored continuously during the study. Vital signs were then measured again after the last injection, and then every 15 minutes during a 30-minute postinjection observation period. The subjects were called 24 hours posttest and 1 week posttest and were interviewed about any related symptoms that they experienced.

Image Review

All CEUS images and previous images were reviewed after the completion of the study. The official radiologist ultrasound report and images were reviewed with each family/study patient, with a discussion of clinical relevance and future recommendations.

RESULTS

We imaged 2 healthy adults and 1 child with previous imaging consistent with pyelonephritis but no sustained parenchymal defect on subsequent imaging. These study subjects were used to assess "normal" renal enhancement as well as dynamic scan length. Seven study subjects with evidence of cortical scars underwent CEUS. All 7 subjects had previous imaging demonstrating abnormal renal parenchyma. Patient characteristics, previous imaging summaries, and CEUS results are presented in Table 1. Six of the 7 subjects had a history of vesicoureteral reflux, and 1 subject had scarring of unknown etiology. Of these 6 subjects, all had undergone ureteral reimplantation at least 5 years before the CEUS study. In addition, all 7 subjects had a history of febrile UTI. No subject had a serious adverse event related to the contrast. One patient did complain of a mild headache 1 hour postinjection that resolved with a single dose of acetaminophen.

Overall, multiple renal parenchymal defects previously identified were detected with CEUS. All 7 subjects had hypoenhancing areas in the kidney parenchyma in corresponding abnormal regions on previous imaging. CEUS detected all abnormalities that were present on previous imaging in all subjects. Furthermore, we detected a number of previously unidentified unenhanced regions in each patient. The perspicuity of renal contours was excellent, with well-defined margins, and renal contours were better visualized in patients that may have had limited resolution with conventional grayscale ultrasound secondary to large body habitus. Findings included wedge-shaped contrast filling defects that appeared as hypoenhancing areas (Figure 1). We also discovered a number of areas of flattening and irregularity of the outer renal contour (Figure 2). One patient had moderate, left-sided, nonobstructive hydronephrosis and cortical thinning (Figure 3). In this example, CEUS accentuated the parenchymal thinning and elucidated areas devoid of contrast enhancement. No parenchymal or contour defects were noted in any healthy controls or unaffected subject kidneys.

DISCUSSION

In recent years, CEUS has become a cost-effective option to image various organs that does not expose the patient to radiation and can be used even with altered

Table 1. Patient characteristics and imaging findings

Patient	Gender	Age (yr)	Pertinent previous imaging (years previous to CEUS)	CEUS findings
CEUS-003[a]	Male	15	DMSA (2.5): Focal wedge-shaped defect in upper pole of right kidney	Normal left kidney; right kidney with upper pole hypoenhanced area; lower pole with severe blunting of renal contour
CEUS-004	Female	15	RUS (0.75): Atrophic left kidney with multifocal scarring	Atrophic left kidney with irregular renal contour and severe lower pole cortical thinning
CEUS-005	Female	15	RUS (0.75): Right parenchymal thinning. Left kidney with upper and lower pole scarring	Multiple areas of hypoenhancement in left kidney cortex in upper and lower poles, including a cystic structure in upper pole not previously seen
CEUS-006[b]	Female	9	RUS (2): Moderate hydronephrosis of left kidney with lower pole scarring	Moderate left-sided hydronephrosis; mid and lower pole scarring of left kidney
CEUS-007	Female	13	RUS (2): Left upper pole scarring, DMSA (4): no focal scars	Large upper pole and small mid pole hypoenhanced area in left kidney
CEUS-008[c]	Male	19	RUS (1): Asymmetric kidney sizes with left echogenic areas of unknown significance	Irregular mid pole renal contour of right kidney; moderate mid pole and large lower pole wedge-shaped hypoenhanced areas of left kidney
CEUS-010	Female	16	RUS (1): Right kidney with multifocal scarring	Large area of hypoenhancement in lower pole of right kidney with irregular renal contour of lower pole

CEUS, contrast-enhanced ultrasound; DMSA, ^{99m}Tc-dimercaptosuccinic acid [scintigraphy]; RUS, renal ultrasound.
[a]Figure 2.
[b]Figure 3.
[c]Figure 1.

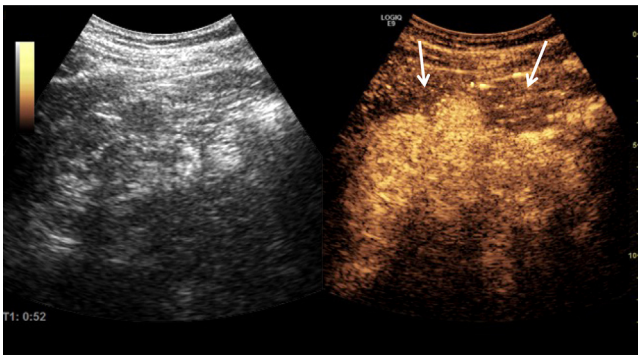

Figure 1. (Left) in patient CEUS-008, left kidney has poor corticomedullary differentiation on conventional grayscale ultrasound. (Right) contrast-enhanced ultrasound image taken at same time and in same plane as grayscale image. Arrows highlight large, wedge-shaped, hypoenhancing areas not visualized on grayscale image on left.

renal function. Avoiding radiation and sedation are extremely important in the pediatric population. CEUS has been used in adults, but its use in renal imaging in children is limited. In this study, we present pilot study data demonstrating the feasibility of CEUS to characterize renal parenchymal defects in those with reflux nephropathy. This modality may offer a fast, comparatively inexpensive, and safe option to detect and monitor renal scars in children with vesicoureteral reflux.

A clinical debate has been ongoing regarding the imaging and clinical management of children with vesicoureteral reflux. Because more than 90% of children with vesicoureteral reflux will not develop scars, the core of the debate revolves around limiting children to unneeded exposures such as bladder catheterization or radiation.[12] Conventional grayscale ultrasound has a low sensitivity in detecting renal scars; thus, its universal use is limited. Further complicating matters, the radiotracer used in DMSA, the gold standard, has been unavailable in the United States for the past 2 years.

In this pilot study, we have demonstrated that CEUS can detect, with high-resolution, parenchymal defects in children with a history of vesicoureteral reflux nephropathy. We have shown that CEUS shows defects that are apparent on other forms of imaging including DMSA. Interestingly, we have also detected areas of suspected scar that were not visible on previous imaging. We cannot determine whether these are new areas of hypoenhancement on CEUS represent false-positive or false-negative DMSA results. If the latter is true, CEUS may provide more detailed information about renal scars than previously offered by other modalities such as conventional ultrasound and DMSA. Ultimately, histological examination of kidney scarring with correlation to CEUS and DMSA findings using an animal model is needed.

If a clinician has suspicion of renal scarring or would like to monitor the evolution of previous abnormal renal parenchyma, the clinician must weigh the potential risks of the imaging modality with the clinical information gained. DMSA scans and CT with contrast both involve a significant radiation load to the patient; thus, longitudinal, repeated imaging must be tempered to avoid significant radiation exposure. Magnetic resonance urography provides high-resolution images, but patients often need sedation to obtain optimal images, and MRI is the most expensive imaging modality. CEUS provides high-resolution images at a fraction of the cost without any need for sedation. Because the microbubbles are cleared in 3 to 5 minutes, the images must be obtained rapidly. In our study, the imaging

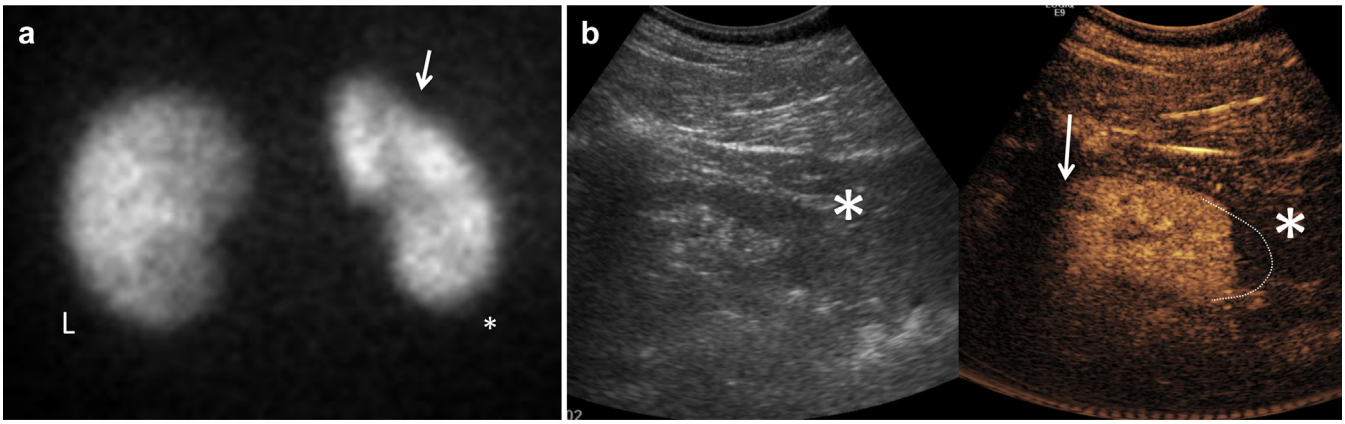

Figure 2. (a) ^{99m}Tc-dimercaptosuccinic acid (DMSA) scan of subject (patient CEUS-003) with normal size and shape of left kidney (L). The right kidney has a wedge-shaped filling defect in the upper pole (arrow). Lower pole of right kidney has subtle blunting of renal contour (*). (b) (Left) grayscale conventional ultrasound image with normal corticomedullary differentiation and lower pole contour of right kidney (*). (Right) large area of hypoenhancement of lower pole (*) highlighted by dashed line. Arrow corresponds to wedge-shaped defect demonstrated in a (arrow) DMSA scan.

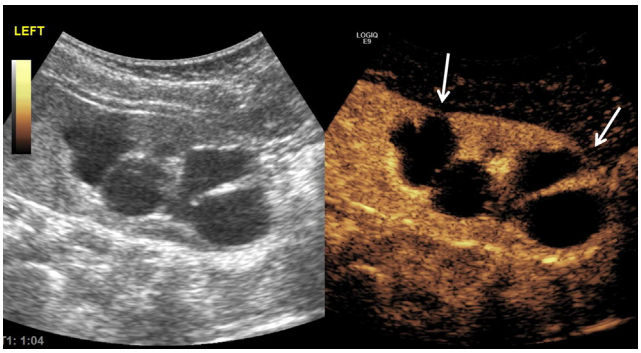

Figure 3. (Left) in patient CEUS-006, grayscale conventional ultrasound image demonstrating moderate hydronephrosis of the left kidney. (Right) contrast-enhanced ultrasound image taken at the same time and in the same plane as the grayscale image. Arrows highlight areas of parenchymal thinning and demonstrate the collecting system to apparently extend to the renal capsule with no intervening parenchyma between the surrounding soft tissue and collecting system.

time was extremely short. Furthermore, a vial of ultrasound contrast agent costs roughly 100 US dollars; thus, the overall expense is not significantly increased compared to conventional ultrasound.

The safety of CEUS in children for noncardiac imaging is well established.[13] In more than 1000 children imaged, no serious/severe adverse events were reported. Minor events that possibly could be attributed to the contrast agent were reported in 0.5% to 1% of the children, depending on the study. These included urticaria, taste alteration, and hyperventilation. None of the children in our pilot study had any serious adverse events.

The study presented here offers the first known report of characterizing renal scars using CEUS in children with a history of reflux nephropathy. Further studies are needed to compare the gold standard DMSA or MR urography with CEUS. A major limitation of our study is that DMSA radiotracer is not available currently, so we needed to use a historical study for comparison. Over time, scars should not spontaneously resolve; thus, our CEUS findings most likely represent scars. A clinical trial to compare the efficacy of CEUS to DMSA or MRI is needed, as are studies using animal models of reflux nephropathy to compare histologic evidence of scar with CEUS findings. If validated, CEUS represents a cost-effective, relatively quick and safe diagnostic imaging modality to monitor renal scars longitudinally.

DISCLOSURE

MBC received a royalty for a single speaking engagement from GE Healthcare. All the other authors declared no competing interests.

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