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USE OF CONTRAST-ENHANCED ULTRASOUND IN THE ASSESSMENT OF UTERINE FIBROIDS: A FEASIBILITY STUDY

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Abstract—Contrast-enhanced ultrasound (CEUS) is an innovative ultrasound technique capable of visualizing both the macro- and microvasculature of tissues. In this prospective pilot study, we evaluated the feasibility of using CEUS to visualize the microvasculature of uterine fibroids and compared CEUS with conventional ultrasound. Four women with fibroids underwent gray-scale ultrasound, sonoelastography and power/color Doppler scans followed by CEUS examination. Analysis of CEUS images revealed initial perfusion of the peripheral rim, that is, a pseudo-capsule, followed by enhancement of the entire lesion through vessels traveling from the exterior to the interior of the fibroid. The pseudo-capsules exhibited slight hyper-enhancement, making a clear delineation of the fibroids possible. The centers of three fibroids exhibited areas lacking vascularization, information not obtainable with the other imaging techniques. CEUS is a feasible technique for imaging and quantifying the microvasculature of fibroids. In comparison with conventional ultrasound imaging modalities, CEUS can provide additional diagnostic information based on the microvasculature. (E-mail: ljm.juffermans@vumc.nl) © 2018 World Federation for Ultrasound in Medicine & Biology. All rights reserved.

Key Words: Uterine fibroids, Microvasculature, Contrast-enhanced ultrasound, Ultrasound imaging, Ultrasound contrast agents, Microbubbles.

INTRODUCTION

Uterine fibroids are benign monoclonal tumors arising from a single smooth muscle cell of the myometrium. The reported prevalence of symptomatic fibroids ranges from 25% to 46% (Stewart 2001; Wegienka et al. 2013). Pathologic examination suggests that the prevalence is even higher, up to 70% to 80% by the age of 50 (Baird et al. 2003). The presence of fibroids often goes unnoticed; however, possible symptoms vary from excessive bleeding and anemia, to pelvic pain, bowel and bladder dysfunction, miscarriages and subfertility dependent on their location and deformation of the uterine cavity (Brölmann and Huirne 2007; Pritts et al. 2009). Furthermore, fibroids are the most common indication for hysterectomies worldwide (Farquhar and Steiner 2002) and accounted for 45% of all hysterectomies in the United States in 2010 (Wright et al. 2013).

It is thought that fibroids affect angiogenesis and the vascular structure in the adjacent myometrium, leading to increased vessel number and size (Stewart 2001; Stewart and Nowak 1996). Fibroids typically have a peripheral rim of vascularization, the pseudo-capsule, from which vessels penetrate the center of the fibroid (Fleischer 2003). Malignant lesions, such as sarcomas, may have a distinct vascular pattern and increased vessel diameter compared with normal tissue and benign lesions (Abramowicz 2005; Exacoustos et al. 2007; Van den Bosch et al. 2015). A clear depiction of the vasculature is therefore of importance for accurate discrimination between fibroids and sarcomas and is in fact crucial for choosing the appropriate treatment of fibroids.

To get an impression of the microvasculature, Doppler imaging can be used. Contrast-enhanced ultrasound (CEUS) is an innovative imaging technique capable of visualizing both the macro- and microvasculature (Testa et al. 2005) and offers the unique opportunity to visualize the microvasculature of uterine fibroids.
Currently used contrast agents are gas-filled microbubbles stabilized by a shell. Microbubbles (2–5 μm) are injected intravenously and are capable of passing through the smallest capillaries (Smeenge et al. 2011). Microbubbles oscillate particularly at frequencies used for diagnostic imaging (1–10 MHz), reflecting a unique nonlinear echo (Blomley et al. 2001). Although the use of CEUS is already quite established in the assessment of liver lesions (Brannigan et al. 2004), renal carcinoma (Dong et al. 2009) and cardiac imaging (Porter et al. 2014), the use of this novel technique is still limited in the field of gynecology (Testa et al. 2005; Zhang et al. 2010).

The objective of the current feasibility study was to visualize fibroid microvasculature with CEUS and to compare fibroid characteristics with gray-scale ultrasound, sono-elastography, power/color Doppler results, to explore the added clinical value of CEUS in imaging fibroids.

METHODS

Patients

Patients enrolled in this prospective observational feasibility study were women with uterine fibroids who visited the outpatient clinic of the Vrije Universiteit Medical Center (VUMc) between June and August 2014. This study was performed at both VUMc and the Academic Medical Center (AMC), both in Amsterdam, The Netherlands. The study was approved by the institutional review board. All patients with fibroids on conventional ultrasound were asked to participate in the study.

Patients gave written informed consent before inclusion. Exclusion criteria were post-menopausal, pregnant or lactating status; known allergy to Sonovue; history of any clinically unstable cardiac condition; severe cardiac rhythm disorders 7 d before CEUS; severe pulmonary or systemic hypertension; and respiratory distress syndrome.

Equipment and conventional ultrasound

All sonographic examinations were performed using a Philips iU22 scanner equipped with a C10-3 v transvaginal probe (Philips, Bothell, WA, USA) at the AMC. Conventional ultrasound examinations were performed in a standardized manner before CEUS and consisted of 2-D gray-scale ultrasound, sonoelastography, color Doppler and 3-D power Doppler. Two-dimensional gray-scale ultrasound was performed using the following settings: resolution R1 (optimized settings for maximum image quality), general mid-range frequencies, dynamic range 56, gain at 71% and 15-Hz frame rate.

Sonoelastography is an ultrasound technique used to estimate strain and discriminate soft from stiff tissue. Sonoelastography images were obtained according to a standardized method described by Stoelinga et al. (2014), with the following settings: resolution R1, high persistence level and a 15-Hz frame rate.

The Doppler ultrasound had a frequency of 5–8 MHz. The settings used for color Doppler were as follows: resolution RP (optimized settings for color sensitivity), wall filter 47 Hz at low color persistence, pulse repetition frequency (PRF) of 500 Hz, gain fixed at 60% and a frame rate of 15 Hz. After completion of 2-D gray-scale and color Doppler analysis, 3-D power Doppler was activated at the 700-Hz PRF, resolution RP, a 49-Hz wall filter, fixed gain of 62% and 15-Hz frame rate. All Doppler scans were performed with the same settings.

Subjective assessments of blood flow (low, average, high) in the pseudo-capsule and the center of the fibroid were made on color and power Doppler. The cardiac phase was not included in the analysis of color and power Doppler images. All images were transferred to an external hard disk in the digital imaging and communications in medicine (DICOM) format.

Contrast-enhanced ultrasound procedure

During gray-scale ultrasound, the fibroid of interest was identified in a sagittal plane, and machine settings were converted to contrast mode at 3.5-MHz power modulation, resolution RS (optimized settings to improve speed), fixed gain at 68%, low mechanical index of 0.06 and 10-Hz frame rate. These settings were fixed for all four patients. A bolus of 1.2 mL contrast agent, that is, Sonovue (Bracco, Geneva, Switzerland), was administered through a periphery-placed intravenous cannula and followed by a flush of saline (5.0 mL) to push the agent into the central venous stream. The target lesion was continuously monitored for 2 min from the start of contrast injection (t = 0 s). This procedure was repeated by injection of a second 1.2-mL bolus to obtain contrast-enhanced images of the myometrium without fibroid tissue. The entire procedure was recorded and transferred to an external hard disk connected to the ultrasound machine. The CEUS examinations were performed by a single gynecologist (J.A.H.) with more than 15 y of experience in ultrasonography.

Contrast-enhanced ultrasound images were analyzed offline using VueBox (Bracco Suisse, SA, Trial Version 5.0.1.50339). The complete clip was reviewed to describe the contrast-enhancement characteristics of fibroid and normal uterine tissue. Next, time–intensity curves were obtained from manually selected regions of interest: entire fibroid, pseudo-capsule, center of fibroid, myometrium adjacent to fibroid, myometrium distant from fibroid and endometrium. Three parameters were calculated from these time–intensity curves: peak enhancement (maximal level of enhancement, associated with relative blood volume); rise time (time from baseline to peak enhancement, related to blood flow velocity) and wash-in rate (peak enhancement/rise time).
Statistical analysis
Baseline characteristics are expressed as the mean ± standard deviation or, for categorical data, as the number (percentage). Results of the time–intensity curve parameters are expressed as the median [range], with individual values displayed in scatterplots.

RESULTS
Patient baseline characteristics
Four women between 29 and 47 y old (mean age: 39 ± 9.1 y) were included in this feasibility study. Two patients had a solitary fibroid and two had multiple fibroids. Three patients presented with abnormal uterine bleeding; in one patient the fibroid was diagnosed during transvaginal ultrasound after placement of an intrauterine contraceptive device. All patients were pre-menopausal. Baseline characteristics are listed in Table 1.

Contrast-enhanced ultrasound characteristics
The obtained CEUS images of normal myometrium and endometrium, as well as of the uterine fibroids, were of good quality, allowing assessment of the enhancement characteristics in all four women.

Normal myometrium and endometrium on CEUS. Contrast-enhanced ultrasound examinations of normal myometrium and endometrium revealed comparable results for all four patients. During the wash-in phase, enhancement was first observed in the outer layer of the myometrium, the arterial arcade, followed by the inner myometrial layer and finally the endometrium. In one patient a clear boundary between endometrium and myometrium was visible because of hypo-enhancement of the endometrium compared with adjacent myometrium (Fig. 1). During wash-out of contrast, a clear boundary was visible in all patients, caused by an earlier wash-out of contrast from the endometrium rather than from the myometrium.

Fibroid characteristics on CEUS. In general, all four fibroids could be easily depicted and delineated using CEUS. The duration from start of contrast injection to the beginning of enhancement of the fibroid ranged from 11 to 17 s. Wash-in of contrast was first observed in the pseudo-capsule of all fibroids. This was followed by heterogeneous enhancement of the center of the fibroid through branched vessels extending from the exterior to the interior of the fibroid. The enhancement of the pseudo-capsule was of greater intensity than that of the surrounding myometrium, making a clear delineation of the fibroid possible. The center of the fibroids exhibited hypo-enhancement compared with surrounding myometrium. In the center of fibroids 2, 3 and 4, hypo-echogenic areas were observed, indicating lack of vascularization (Figs. 2e–g and 3e–g). During wash-out, signal intensity decreased first from the center of the fibroid, followed by gradual wash-out from the entire fibroid and surrounding myometrium.

Table 1. Baseline patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>39 ± 9.1</td>
</tr>
<tr>
<td>Nulliparous</td>
<td>2 (50)</td>
</tr>
<tr>
<td>Mean body mass index (kg/m²)</td>
<td>22.3 ± 1.2</td>
</tr>
<tr>
<td>Solitaire fibroid</td>
<td>2 (50)</td>
</tr>
<tr>
<td>Presenting symptoms</td>
<td></td>
</tr>
<tr>
<td>Abnormal uterine bleeding</td>
<td>3 (75)</td>
</tr>
<tr>
<td>Dysmenorrhea</td>
<td>1 (25)</td>
</tr>
<tr>
<td>Pelvic pain/pressure</td>
<td>1 (25)</td>
</tr>
<tr>
<td>None</td>
<td>1 (25)</td>
</tr>
</tbody>
</table>

* Data are expressed as the mean ± standard deviation or number (%).

Fig. 1. Gray-scale ultrasound and contrast-enhanced ultrasound scans of a normal uterus: (a) Gray-scale ultrasound image. (b) Contrast-enhanced ultrasound image obtained 14 s after contrast injection (t = 14 s), revealing initial enhancement of the myometrium. (c) Contrast-enhanced ultrasound image obtained 95 s after contrast injection (t = 95 s), revealing hypo-enhancement of the endometrium compared with surrounding myometrium. E = endometrium, M = myometrium.
CEUS versus gray-scale ultrasound. All fibroids were initially diagnosed using gray-scale ultrasound, by which they could be well delineated based on differences in tissue echogenicity. Heterogeneity in gray-scale pattern within the fibroid was seen; however, the cause of this heterogeneous gray-scale pattern remained unclear.

CEUS versus sonoelastography. Sonoelastography is a technique used to discriminate soft from stiff tissue. Although blood vessels, and thus the pseudo-capsule, are graded as soft tissue, sonoelastography does not provide detailed information on tissue vascularization. Small green regions within the fibroid (Figs. 2b and 3b) are characterized...
as soft tissue, however; sonoelastography also is not conclusive as these regions could either be vessels or degenerating areas.

**CEUS versus power Doppler.** Doppler imaging does provide information on the vasculature, however, only on the macrovasculature. The highly vascularized pseudo-capsule was clearly depicted (Figs. 2c, d and 3c, d), especially in the area located close to the probe. At larger distance penetration was less optimal, and the pseudo-capsule distal of the fibroid was not depicted, in contrast to CEUS images that displayed signals from the entire fibroid. Virtually no Doppler signal was observed in the center of the fibroid. Subjective analysis revealed, in general, average-high blood flow in the pseudo-capsule and low-average blood flow in the center of the fibroid (Table 2).

**Time–intensity curve parameters**

In addition to the enhancement characteristics, original time–intensity curves for different regions of interest were obtained for both normal endometrium/myometrium

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**Fig. 3.** Subserosal fibroid (3.2 cm). All images were obtained from the third patient. (a) Gray-scale ultrasound revealing a well-delineated fibroid. (b) Sonoelastography image revealing the fibroid’s center in blue (stiff tissue) with a pseudo-capsule in red (soft tissue). Small green areas indicate softer tissue present in the fibroid’s center. (c) Power Doppler image revealing the pseudo-capsule. (d) Color Doppler image partially revealing the proximal site of the pseudo-capsule. (e) Contrast-enhanced ultrasound image at $t = 19$ s revealing initial enhancement of the pseudo-capsule. (f) Contrast-enhanced ultrasound image at $t = 40$ s revealing peripheral enhancement without enhancement in the center of the fibroid, that is, a large hypo-echogenic region. (g) Contrast-enhanced ultrasound image at $t = 108$ s revealing gradual wash-out of contrast from the fibroid.
Table 2. Subjective assessment of blood flow

<table>
<thead>
<tr>
<th>Fibroid region</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Power Doppler</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudo-capsule</td>
<td>+/-</td>
<td>+</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td>Center</td>
<td>+/-</td>
<td>+/-</td>
<td>-</td>
<td>+/-</td>
</tr>
<tr>
<td>Color Doppler</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudo-capsule</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td>Center</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+/-</td>
</tr>
</tbody>
</table>

+ = high flow; +/- = average flow; - = low flow.

(Fig. 4) and the fibroids (Fig. 5). The perfusion parameters for each patient are depicted in scatterplots in Figure 6. Peak enhancement was highest in the pseudo-capsule (46.4 dB [44.4–51.3]), followed by the myometrium at a distance from the fibroid, endometrium, myometrium adjacent to fibroid, entire fibroid and finally the center of the fibroid (39.5 dB [30.4–46.2]). The rise time of the pseudo-capsule (20.9 s [10.7–29.1]) was higher, compared with that of the center of the fibroid (8.9 s [6.7–24.8]). Also,
the wash-in rate was higher in the pseudo-capsule (36.1 dB/s [34.6–45.0]) than in the center of the fibroid (32.2 dB/s [18.9–39.4]). The data from these parameters corresponded to the order of fibroid vascularization, which started in the pseudo-capsule continued as vessels traveling to the center of the fibroid.

**Adverse events**

The injection of ultrasound contrast agent was well tolerated by all patients; no adverse events occurred.

**DISCUSSION**

These preliminary results indicated that CEUS is a promising, tolerable, innovative and easy-to-perform technique capable of visualizing the microcirculation of normal myometrium, endometrium and uterine fibroids. Fibroids were easy to recognize and delineate on CEUS because of the slight hyper-enhancement of the pseudo-capsule compared with adjacent myometrium. After initial perfusion of the pseudo-capsule, vessels traveling from the exterior to the interior of the fibroid were enhanced. In this stage hypo-echogenic areas appeared as either small areas (Fig. 2e–g) or a large area (Fig. 3e–g). Hypo-echogenic areas on CEUS images indicate lack of vascularization. Here, the additional information obtained by CEUS is illustrated, as no indication for such lack of vascularization could be obtained from gray-scale images. Neither could this be detected by Doppler as the size of the microvasculature within the fibroid is beyond the resolution of Doppler imaging. The fact that no signals appeared in the fibroid’s center on Doppler could mean either that no larger vessels reached the center or that the penetration of the ultrasound wave was not sufficient for the depiction of blood flow, leading to an underestimation of the degree of vascularization.

Knowing whether a fibroid is highly vascularized or nearly avascular is important when selecting the most appropriate treatment. There is a strong correlation between the degree of vascularity and the success of uterine artery embolization (Isonishi et al. 2008). Whereas for ablation therapy the opposite applies; higher vascularity translates into poor ablation efficacy (Liu et al. 2014). In addition, several studies have already reported that CEUS may also be used to assess the non-perfused volume pre- and post-uterine artery embolization or ablation therapy.

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**Fig. 6. Scatterplots of time–intensity curve parameters.** For all the different regions of interest, individual data points are shown. (a) Peak enhancement (dB). (b) Rise time (s). (c) Wash-in rate (dB/s). Myometrum 1 = myometrium adjacent to fibroid; myometrum 2 = myometrium distant from the fibroid. Horizontal lines represent the median.
to assess the effectiveness of the therapy. Although the diagnostic accuracy of CEUS still has to be validated, it may become an easy-to-apply, cost-effective alternative to the current reference test magnetic resonance imaging (Dorenberg et al. 2007; Lei et al. 2014; Sconfienza et al. 2008; Wang et al. 2014; Zhou et al. 2007). To date, studies illustrating the feasibility of CEUS in the diagnosis of fibroids or other benign uterine disorders, for example, adenomyosis, are limited (Testa et al. 2005; Zhang et al. 2010). The enhancement characteristics of fibroids on CEUS in our study were in line with the characteristics described in the two articles cited.

Next to enhancement characteristics, blood supply to and vascularization of the fibroid can be quantified by determining perfusion parameters, which can easily be calculated from the time–intensity curves. Quantification of such perfusion parameters allows for monitoring of embolization or ablation therapies, and comparison with other techniques assessing perfusion of tissue such as dynamic MRI. Our data from the perfusion parameters agreed with a previous study in which, in particularly larger fibroids, the center of the fibroid was less vascularized than the pseudo-capsule using 3-D power Doppler (Nieuwenhuis et al. 2015).

The ability of CEUS to provide a clear depiction of the microvasculature may become of great value in differentiating fibroids from malignant lesions such as sarcomas. To date there is no reliable method to predict whether a patient with fibroids may actually have a uterine sarcoma (Aviram et al. 2005). Histologic examination of tissue obtained after surgery is required to diagnose uterine sarcomas. Malignant lesions, in general, have a distinct vascular pattern characterized by numerous and complex vessels, increased blood flow, incomplete vessel wall muscularization and larger vessel diameter resulting in low resistance to flow (Exacoustos et al. 2007; Liu et al. 2012; Song et al. 2009; Zhang et al. 2010). In contrast to gray-scale ultrasound, which does not depict vascularization, CEUS could in theory be capable of depicting these malignant vascular features (Zhang et al. 2010). The importance in discriminating between fibroids and sarcomas is highlighted by the recent recommendation of the U.S. Food and Drug Administration (U.S. Food and Drug Administration (FDA 2014; Wallis 2014) not to use laparoscopic power morcellation during hysterectomy or myomectomy for the treatment of fibroids, as there is a risk of spreading cancerous tissue in patients with unsuspected uterine sarcoma (Wallis 2014). Although European guidelines are less strict and recommend a laparotomic approach or contained morcellation when there is a matter of raised suspicion for sarcoma (Bröllmann et al. 2015), the demand for an imaging technique that can accurately distinguish the malignant sarcoma vasculature from benign fibroid vasculature is clear.

Though, we were not able to compare our results with a reference test to determine the diagnostic accuracy of CEUS, much can be learned from each case because we compared the CEUS images with gray-scale ultrasound, sonoelastography and power/color Doppler. CEUS has several advantages over the other techniques: (i) CEUS is capable of visualizing macro- and microvasculature. (ii) Compared with Doppler, CEUS has the ability to obtain more information on non-perfused tissue or necrosis. (iii) CEUS renders a uniform image because of better tissue/fibroid penetration compared with Doppler. (iv) CEUS identifies enhancement characteristics of uterus and fibroids, as well as the direction of blood flow, that is, from the periphery to the fibroid’s center. (v) CEUS quantifies tissue-specific perfusion parameters. (vi) CEUS is an easy-to-perform technique providing high-quality images, which are not dependent on the pressure applied with the probe, as is the case in sonoelastography.

Although CEUS is a promising technique, it also has its limitations. Only one fibroid can be continuously monitored with a single injection of contrast. The scanning field is relatively small; thus, larger fibroids (>8 cm) cannot be displayed at once. Therefore, this technique is not suitable for characterizing a large uterus with multiple fibroids, because this will probably not alter the therapeutic options. However, it may become more relevant to examine one or two fibroids in more detail and evaluate fibroid features to predict the effect of various fibroid therapies. In the future, this detailed information obtained using CEUS may potentially be used for differentiation between fibroids and adenomyosis, and maybe even sarcomas, which is challenging with current imaging modalities (Agostinho et al. 2017; Gaetke-Udager et al. 2016). Finally, CEUS is more invasive than conventional ultrasound because venous access is required to administer the contrast agent. Limitations of the present study were the small sample of only four patients and the continuous monitoring of the target lesion for only 2 min after injection of contrast, allowing evaluation of the complete wash-in phase, but only partial evaluation of the wash-out phase. The latter requires a longer observation period. In addition, the patients included did not undergo a myomectomy or hysterectomy; hence no histopathology analysis could be performed. Therefore, this study should be seen as a first attempt to illustrate feasibility and describe enhancement patterns of fibroids using CEUS in comparison with conventional ultrasound imaging modalities. More data are needed to confirm the added clinical value and utility of CEUS in gynecology, including reproducibility and accuracy in the diagnosis of uterine pathologies.

In conclusion, we found that CEUS is a tolerable and feasible technique for detailed depiction and quantification of fibroid macro- and microvasculization. In comparison with conventional ultrasound imaging modalities, CEUS
is able to provide additional diagnostic information based on the microvasculature.

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