Ultrasound in Med. & Biol., Vol. ■■, No. ■■, pp. ■■–■■, 2017

0301-5629/$ - see front matter

https://doi.org/10.1016/j.ultrasmedbio.2017.08.1881

● *Clinical Note*

FIRST-IN-HUMAN STUDY OF ACOUSTIC ANGIOGRAPHY IN THE BREAST AND PERIPHERAL VASCULATURE

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(*Received* 16 *March* 2017; *revised* 20 *August* 2017; *in final form* 21 *August* 2017)

Abstract—Screening with mammography has been found to increase breast cancer survival rates by about 20%. However, the current system in which mammography is used to direct patients toward biopsy or surgical excision also results in relatively high rates of unnecessary biopsy, as 66.8% of biopsies are benign. A non-ionizing radiation imaging approach with increased specificity might reduce the rate of unnecessary biopsies. Quantifying the vascular characteristics within and surrounding lesions represents one potential target for assessing likelihood of malignancy *via* imaging. In this clinical note, we describe the translation of a contrast-enhanced ultrasound technique, acoustic angiography, to human imaging. We illustrate the feasibility of this technique with initial studies in imaging the hand, wrist and breast using Definity microbubble contrast agent and a mechanically steered prototype dual-frequency transducer in healthy volunteers. Finally, this approach was used to image pre-biopsy Breast Imaging Reporting and Data System (BI-RADS) 4 and 5 lesions <2 cm in depth in 11 patients. Results indicate that sensitivity and spatial resolution are sufficient to image vessels as small as 0.2 mm in diameter at depths of ~15 mm in the human breast. Challenges observed include motion artifacts, as well as limited depth of field and sensitivity, which could be improved by correction algorithms and improved transducer technologies. padayton@email.unc.edu

Key Words: **Contrast-enhanced ultrasound, Breast imaging, Angiography, Superharmonic, Microbubble, Microvasculature, Dual frequency.**

INTRODUCTION

Nearly 250,000 women were diagnosed with breast cancer in the United States in 2016, with more than 40,000 deaths in the same year (Howlader et al. 2016). Currently, mammography is used to screen women over the age of 40 and has been reported to increase survival by about 20% (Alexander et al. 1999; Bjurstam et al. 2003). Based on mammographic results, the lesion is assigned a score from 1 to 6 according to the Breast Imaging Reporting and Data System (BI-RADS) (American College of Radiology 2013). For individuals with a suspicious mammogram (BI-RADS categories 3–5), follow-up imaging with other approaches, including diagnostic mammography, breast tomosynthesis (Rafferty et al. 2013; Shan et al. 2015; Tucker et al. 2014), magnetic resonance imaging (Elsamaloty et al. 2009; Mountford et al. 2009) and ultrasound (Moon et al. 2002), may be used to clarify the nature of the lesion. Lesions ultimately categorized as BI-RADS 4 and 5 have a high likelihood of malignancy and are directed toward biopsy or surgical excision.

Pathology results from more than 26,000 patients receiving breast biopsy indicate that 66.8% of the biopsies were benign, suggesting that many of these biopsies are unnecessary (Weaver et al. 2006). In addition, breast biopsies are known to yield false negatives at a rate of about 2% (Boba et al. 2011), and many patients are subjected to repeat biopsies depending primarily on the physician's judgment (Shachar et al. 2016). About 10% of lesions require repeat biopsy, and only approximately 17% of these are malignant (Youk et al. 2007), suggesting many patients may be subjected to multiple unnecessary biopsies. If there existed a non-invasive, non-ionizing radiation imaging approach with sufficient specificity, it might be possible to reduce the rate of unnecessary biopsies, sparing patients pain and anxiety (Hayes Balmadrid et al. 2017).

Conflicts of Interest: P.A.D. declares that he is an inventor on a patent describing the dual-frequency imaging technology, and is a co-founder of SonoVol, Inc., a company that has licensed this technology.

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For solid tumors to grow beyond a certain size (typically ~2 mm), new vessels must form (angiogenesis) (Bergers and Benjamin 2003; Folkman and Shing 1992). In breast lesions, in particular, elevated microvessel density is correlated with the occurrence of metastases and, thus, has been identified as a potential prognostic indicator, as microvessel density in the region of highest neovascularization has been reported to predict overall and relapse-free survival (Weidner et al. 1991, 1992). Several non-invasive imaging techniques have sought to use this knowledge for diagnosis, including magnetic resonance imaging (MRI) (Daldrup-Link et al. 2003), computed tomography (CT) (Boone et al. 2006), color Doppler ultrasound (Adler et al. 1990) and conventional contrast-enhanced ultrasound imaging (Barnard et al. 2008; Forsberg et al. 2008; Hoyt et al. 2015; Sridharan et al. 2015; Wan et al. 2012). However, none of these approaches has yet been found able to improve diagnostic accuracy or reduce the need for biopsy in clinical studies.

From an imaging perspective, a key challenge is the ability to resolve the microvessels formed early in tumor angiogenesis, as the vessels observed in histologic evaluation of invasive carcinomas typically have diameters <100 μm (Ottinetti and Sapino 1988). To form vascular or "angiographic" images, many imaging techniques use exogenous contrast agents to image vascular structures in the breast, for example, iodine in computed tomography (CT) (Boone et al. 2006), gadolinium in MRI (Rahbar et al. 2015) and perfluorocarbon-filled microbubbles in contrast-enhanced ultrasound (Hoyt et al. 2015). Even with the use of contrast agents, the ability to both detect and resolve the microvessels of clinical interest remains challenging, as typical spatial resolutions for clinical imaging systems are ~700 μm for MRI (Pinker et al. 2014), ~600 μm for CT (Reiner et al. 2013) and 300–500 μm for conventional ultrasound (Rissanen et al. 2008). Special small animal imaging systems have exhibited higher resolutions in all modalities, including as high as 100–200 μm in MRI (Herrmann et al. 2012; Jansen et al. 2009), as high as 40 μm in CT (for scan times >50 min) (Starosolski et al. 2015) and 30–200 μm for high-frequency ultrasound (Foster et al. 2002, 2009).

Contrast-enhanced ultrasound (CEUS) imaging has been performed in the breast for tumor characterization and diagnosis. Ricci et al. (2007) characterized CEUS enhancement as being equal in accuracy to MRI for breast cancer diagnosis in humans. Subsequent studies have described contrast enhancement and wash-out patterns, but contrast-enhanced ultrasound imaging is still not routine in the breast (Liu et al. 2008; Zhao et al. 2010). Ultrasound molecular imaging also exhibits promise as a clinical indicator of malignancy. Microbubbles targeted to vascular endothelial growth factor receptor (VEGFR2) have been validated in model systems (Bzyl et al. 2013; Pochon et al. 2010), and it was recently found in humans that higher targeting was observed in malignant than in benign lesions and that targeting intensity was related to the level of VEGFR2 expression as measured with immunohistochemistry (Willmann et al. 2017).

We have recently developed a new contrast-enhanced ultrasound microvascular imaging approach based on the superharmonic signal produced by microbubbles, detected with a multi-frequency transducer, and reconstructed in three dimensions. In this approach, microbubbles are excited using a low-frequency (<6 MHz) pulse, and images are formed from high-frequency (>20 MHz) signals produced by microbubbles. Resulting images have higher contrast-to-tissue ratio (CTR, ~ 25 dB) and spatial resolution (100–200 μm) than conventional contrast-enhanced ultrasound (Lindsey et al. 2014). Because these images reveal vascular structures alone, we call this technique acoustic angiography as it is similar to other forms of angiographic imaging, for example, computed tomography angiography, magnetic resonance angiography (Rubin and Rofsky 2009). In addition, these images can be segmented and vessel tortuosity computed using previously established quantitative metrics (Bullitt et al. 2003, 2006). This is potentially useful because previous studies using intra-vital microscopy in animal models have reported that tumor vascular remodeling occurs when tumors consist of <100 cells (Li et al. 2000), providing another potential quantitative metric beyond microvessel density. In imaging a genetically engineered mouse model of ductal carcinoma, quantification of vessel tortuosity has enabled 2- to 3-mm tumors to be distinguished from healthy tissue (Shelton et al. 2015).

In this work, we present the first translation of acoustic angiography imaging to humans. Imaging volumes have been acquired of the vasculature in the wrist of healthy volunteers, as well as of the breast of both healthy volunteers and patients. Because of the high spatial resolution of this technique—comparable to that of small animal CT and MRI—it represents a potential tool for quantifying the high microvascular density associated with invasive tumors in the breast. Contrast-enhanced ultrasound imaging using the superharmonic response of microbubbles has been described in *in vitro* and *in vivo* studies, but clinical studies in humans are limited, and are restricted to examinations of the heart at low frequencies (0.8 MHz/2.8 MHz) (Bouakaz et al. 2003). Although CEUS allows imaging of tissue perfusion and power Doppler allows imaging of individual vessels at these spatial scales, acoustic angiography reveals vascular morphology and enables quantification of vessel tortuosity, a marker of malignancy.

METHODS

This study was approved by the institutional review board of the University of North Carolina at Chapel