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Research article

Vascular branching point counts using photoacoustic imaging in the superficial layer of the breast: A potential biomarker for breast cancer

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ABSTRACT

This study aimed to identify the characteristics of the vascular network in the superficial subcutaneous layer of the breast and to analyze differences between breasts with cancer and contralateral unaffected breasts using vessel branching points (VBPs) detected by three-dimensional photoacoustic imaging with a hemispherical detector array. In 22 patients with unilateral breast cancer, the average VBP counts to a depth of 7 mm below the skin surface were significantly greater in breasts with cancer than in the contralateral unaffected breasts ($p < 0.01$). The ratio of the VBP count in the breasts with cancer to that in the contralateral breasts was significantly increased in patients with a high histologic grade ($p = 0.03$), those with estrogen receptor-negative disease ($p < 0.01$), and those with highly proliferative disease ($p < 0.01$). These preliminary findings indicate that a higher number of VBPs in the superficial subcutaneous layer of the breast might be a biomarker for primary breast cancer.

1. Introduction

Ultrasonography (US), mammography, magnetic resonance imaging (MRI), computed tomography and positron emission tomography are used to diagnose breast cancer based on the morphology and properties of the tumor [1]. Features of tumor-associated blood vessels have been reported not only to be intratumoral such as tumor angiogenesis [2], but also to include the vascular architecture around the tumor [3,4]. The modalities presently available for imaging of the vasculature include Doppler US, contrast-enhanced US, and contrast-enhanced MRI [3,5–9]. However, the contrast agents used can cause allergic reactions and agents such as gadolinium cannot be used in patients with severe renal dysfunction. Diffuse optical tomography using near-infrared light can measure the amount of water, fat, and total hemoglobin as well as its oxygen saturation in tissues [10]. These parameters do not require a contrast agent and can be measured noninvasively. However, the resolution is in the order of centimeters, so it is difficult to discriminate tumor tissue from normal breast tissue when the tumor is less than a few centimeters in size. Tumor-related vascular changes in the superficial layer of breasts had not been reported in detail. Photoacoustic imaging provides good visualization of these superficial areas, and is able to highlight changes in the superficial vasculature due to tumor.

We have developed a photoacoustic imaging (PAI) system for assessment of blood vessels [4,11–13]. The third iteration of our prototype system (PAI-03) has high performance to a depth of more than 20 mm from the skin surface with submillimeter resolution, allowing

Abbreviations: BP, blood pressure; DCIS, ductal carcinoma in situ; HDA, hemispherical detector array; MRI, magnetic resonance imaging; PAI, photoacoustic imaging; US, ultrasonography; VBP, vessel branching point

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the structure of fine blood vessels to be visualized [4]. Use of a hemispherical detector array (HDA) and three-dimensional constructed images provides detailed images of the vascular structures. We have successfully used the PAI-03 system to detect and analyze blood vessels associated with cancer.

The PAI system can visualize the vascular network of the breast as well as the tumor-related vasculature with high resolution. In a previous study using the PAI-03 system, we identified certain features of cancer-associated blood vessels [4]. Adjacent blood vessels oriented toward the tumor were visualized in patients with invasive breast cancer, and photoacoustic signals were found to be increased in the tumor in some cases that had received preoperative chemotherapy. Tumor angiogenesis results in the formation of immature blood vessels, which increases the vascular permeability [2]. Increased vascular permeability elevates the interstitial pressure and stagnates blood flow, despite the increase in metabolism and the blood flow requirements [2,14]. Therefore, we hypothesized that blood volume around the tumor including the subcutaneous layer might be increased due to the influence of increase blood flow requirements on the tumor.

We have been using this system in patients with breast cancer to evaluate the characteristics of the vascular network in the breast in detail, including vessel branching points (VBPs) in both the superficial area just below the skin surface and in the whole breast within the visual range. In this study, VBPs were defined as the points where blood vessels divide into two or more branches. We have been attempting to detect differences in blood flow between breasts with cancer and contralateral breasts with no detectable cancer using the number of visible VBPs.

In this report, we present our preliminary data on the relationship between VBP counts in breasts with cancer and those in contralateral breasts with no detectable cancer to determine the potential utility of VBP as a biomarker of primary breast cancer.

2. Material and methods

2.1. Patients

Data of 22 patients with unilateral breast cancer who had participated in another clinical trial (UMIN00012251) were analyzed in this study. Patients were enrolled in the clinical trial between December 2014 and December 2015 at Kyoto University Hospital, Japan. The inclusion criteria for the clinical trial were a primary breast lesion, age 20 years or older at the time of diagnosis, and an Eastern Cooperative Oncology Group performance status of 0 or 1. The exclusion criteria included pregnancy, cardiac pacemaker implantation, and any other condition judged by the clinical investigators to make the patient unsuitable for inclusion. Patients with benign lesions were also excluded. The contralateral breasts were diagnosed as cancer-free by mammography, US, and MRI. The study was approved by the institutional review board at Kyoto University (UMIN00012251) and was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2008. All participants provided written informed consent.

2.2. Configuration of the PAI system

The VBPs were counted using a PAI-03 system with an HDA (Canon Inc., Tokyo, Japan; in collaboration with Optosonics Inc., Oriental, NC) [4,15]. The system was modified by reducing the depth of the breast-holding cup from 50 mm to 38 mm. A laser capable of irradiating at wavelengths of 755 nm and 795 nm was also used. The detector array was scanned continuously in a spiral pattern in one plane. The spiral scanning pattern was modified to gradually increase the density of data acquisition points as the scan approached the center of the spiral scan. AQ-switched alexandrite laser with selectable wavelengths of 755 nm and 795 nm was used. The laser energy used in the PAI-03 system is approximately 200 mJ/pulse and is constant for both wavelengths. The light illumination was 60 mm in diameter at the surface of the breast cup. The maximum light energy was set to less than 10 mJ/cm^2, which is half the maximum permissible exposure recommended by the American National Standards Institute.

A piezoelectric zirconate titanate transducer was used to detect the PA signal. The HDA used contains 512 elements on its internal surface, each within a circle measuring 3 mm in diameter. The central frequency was 2 MHz. The imaging area for each laser shot was just within the range of the 30-mm radius of the cylinder. To enhance the imaging area, the HDA was scanned spirally in the horizontal plane; this allowed the imaging area to be selected at radii of 50 mm, 70 mm, and 100 mm. For each scan data acquisition was set to at either 1024 or 2048 corresponding to an acquisition time of 51.2 s and 102.4 s, respectively. Using a spherical phantom with a diameter of 0.3 mm, the full width at half maximum was 0.57 mm in the x and y directions and 0.37 mm in the z direction.

2.3. Acquisition of PAI data

For each patient, both breasts were scanned in water at body temperature, first at a wavelength of 795 nm and then at a wavelength of 755 nm. In each case, a PA image acquired at 795 nm that had equal oxyhemoglobin and deoxyhemoglobin absorption coefficients and the image corresponding to the total hemoglobin distribution were used to analyze the structure of the blood vessels. Hemoglobin oxygen saturation was not analyzed, so the images taken at a wavelength of 755 nm were not used. PA images were reconstructed using a universal back-projection algorithm [16], taking into account the average speed of sound in breast tissue and that in water for impedance matching. The variations in light fluence in tissue from patient to patient were compensated for by calculating the result of the diffusion equation. The PAI images in this study were constructed using the absorption coefficient at 795 nm and the color signal intensity.

2.4. Extraction of the breast surface data

A PAI image of the breast in the coronal plane was used to extract the breast surface. The areas where the skin pigment signal started were contoured, and the plotted points were obtained manually at intervals of about 1–2 cm per slice of a 2D image. The same contour extraction procedure was performed for slices at least every 10 slices (1.25 mm) to obtain a three-dimensional breast contour point group. When the deviation between the spline curve based on the original plot points and the breast shape by skin pigment signal was more than 3 mm, the contour was extracted by adding more plot points. Typically, there were 200–300 plot points per breast. The surface shape of the breast was reconstructed by approximating these contours as a curved surface. The moving least squares method [17] was used for surface approximation.

2.5. MRI data acquisition

Breast MRI was performed using a 3.0-T scanner (MAGNETOM Trio, A Tim System, Siemens AG, Germany) with a dedicated 16-channel breast array coil. Fat-suppressed T1-weighted dynamic contrast-enhanced images were obtained pre-contrast and then at 1–2 min (early) and 5–6 min (delayed) after gadolinium injection. Gadoteridol (ProHance, Eisai Inc., Tokyo, Japan) was power-injected at a dose of 0.2 mL/kg and a speed of 2.0 mL/s, then flushed with 20 mL of saline at the same rate. Whole-breast axial scanning at a high temporal resolution was performed for 1 min (3D-VIBE: TR/TE 3.70/1.36 ms, FA 15 and FOV 330 mm × 330 mm, matrix 384 × 346, thickness 1.0 mm). Subtraction images were computed pixelwise by subtracting the signal intensity of the pre-contrast images from that of the early post-contrast images.

Deformed MRI images were constructed based on pre-contrast and early post-contrast MRI subtraction images in which blood vessels could