Detection and Quantification of Microbleeds on Fixed Brain Specimens

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Background and purpose:

MRI has defined "cerebral microbleeds" as a common phenomena of the aging brain, becoming increasingly frequent beginning at age 60. By age 80, nearly 40% of the population is likely to show evidence of microbleeds using MRI. However, in pathological examination very small microbleeds are often detected, and it is believed that the prevalence of cerebral microbleeds could easily be higher when more sensitive imaging techniques are used. Brain hemorrhagic phenomena have traditionally received attention in the context of classical hemorrhagic stroke syndromes, specifically intracerebral hemorrhage and subarachnoid hemorrhage. The principal risk factors for cerebral microbleeds, in addition to age, are known to be hypertension and cerebral amyloid angiopathy [1], with cerebral amyloid angiopathy-dependent cerebral microbleeds understood to be primarily cortical in location while hypertension-dependent microbleeds are thought to be primarily located in the deep hemispheres [1,2]. We have found the presence of iron deposits in pericytes immediately adjacent to brain capillary endothelial tight junctions[3]. This finding, along with presence of typical perivascular hemosiderin deposits, lead us to propose that the formation of microbleeds may be in part associated with the transient opening of tight blood-brain-barrier junctions; therefore, it is not only a vascular risk factor for developing stroke, but also a risk factor for worsening disease progression in patients diagnosed with Alzheimer's or other neurological diseases. In order to better understand the role of microbleeds, we designed a study to image brain specimens with high spatial resolution MRI.

Methods:

The imaging sequence for detection of microbleeds is heavily T2*-weighted, thus very sensitive to the presence of bubbles on the surface of the brain specimen. One very challenging task was to remove bubbles on the specimen. For each brain, half-hemisphere were cut into 1.5 cm slices and fixed. We have built a special specimen-processing chamber for de-bubbling, as shown in **Figure 1**. It is made of a vacuum chamber (diameter 25 cm) connected to a powerful vacuum pump. On the cover of the chamber, a hole was drilled for passing through the ultrasound sonicator probe. All connections were carefully sealed to achieve the highest vacuum power. The brain specimen was submerged into PBS in a plastic container, and placed into the chamber. Then the ultrasound sonicator probe was inserted into the container. The sonication and vacuum were turned on together to de-bubble the specimen for approximately one hour. Then the container was carefully removed to ensure that the specimen was fully submerged in PBS and covered for MR imaging. MR imaging was performed on a Philips 3T scanner using a gradient echo sequence with TR=6.1 ms, TE= 47 ms; flip angle=20°; matrix size 800x800; in plane resolution, 0.25x 0.25 mm; slice thickness= 0.7 mm.

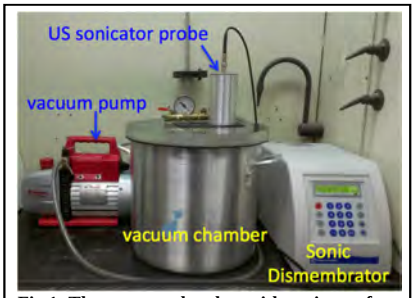

Fig.1: The vacuum chamber with sonicator for de-bubbling of the brain specimen.

Although a great effort has been put into de-bubbling, some micro bubbles were still present on the surface or in the sulci. In order to detect the true microbleeds, the specimen was flipped, gone through the de-bubble procedures and imaged again. If the dark spots were only seen on one set of image, they were likely to be bubbles; those seen on both sets of images were identified as microbleeds. One example is shown in **Figure 2**. In order to standardize the evaluation procedure, we have developed a post-processing image analysis software for objective detection and quantification of the size of microbleeds. The first step was to segment the contour of specimen, and remove dark spots on the surface. The second step was to apply the registration algorithm to confirm that a detected microbleed was seen on both sets of images from "side-A" and the flipped "side-B" of the same specimen. An example is shown in **Figure 3**.

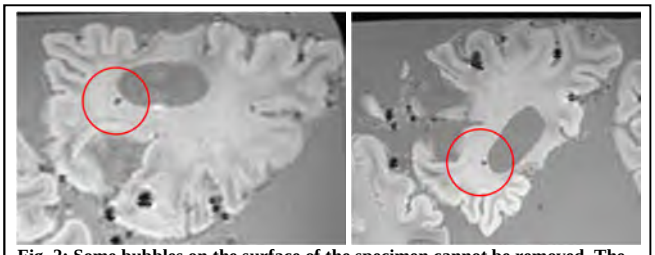

Fig. 2: Some bubbles on the surface of the specimen cannot be removed. The specimen is flipped, de-bubbled and imaged again. If a microbleed is seen at the same location on both sides, then it is determined as a true microbleed.

Results:

A total of 27 cases were imaged, and microbleeds were found in 16 cases (16/27= 59%). The age of subjects ranged from 46 to 102, with a mean of 83 and a median of 90 years old. Of these, 16 patients had confirmed pathological diagnosis, including 5 Alzheimer's disease, 9 normal aging with mild Braak changes, and 2 other diseases. Microbleeds were found in 3 of 9 normal aging cases (3/9=33%), and in 3 of 5 Alzheimer's disease cases (3/5=60%). We have tried to quantify the size of microbleeds. In the case shown in **Fig. 3**, it contained 5x7 pixels on the image plane and was seen on 3 consecutive slices, so this microbleed was estimated to be 1.25 x 1.75 x 2.1 mm. In order to improve the detection sensitivity, the phase images were acquired for susceptibly-weighted processing, as shown in **Figure 4**. After a suspicious microbleed is identified, registration can be applied to confirm that it is seen on both side-A and flipped side-B. With the SWI enhancement effect, we should be able to detect smaller microbleeds.

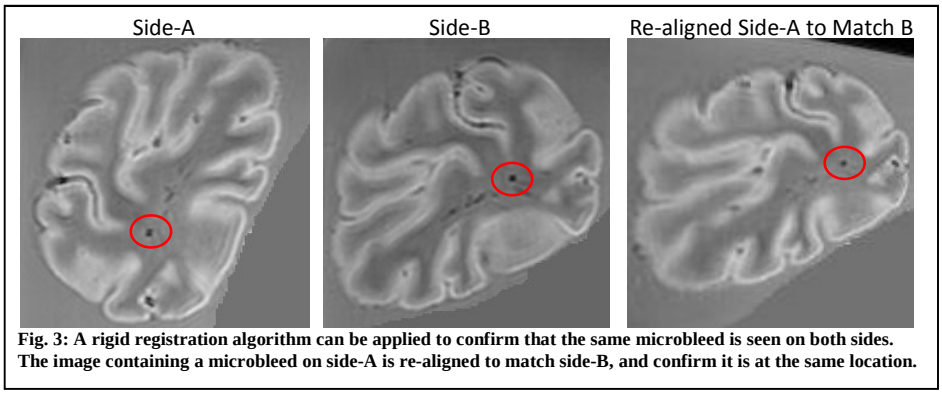

Fig. 3: A rigid registration algorithm can be applied to confirm that the same microbleed is seen on both sides. The image containing a microbleed on side-A is re-aligned to match side-B, and confirm it is at the same location.

Discussion:

For microbleeds detected in in-vivo MRI, it is extremely difficult to locate the tissues seen on MRI. Specimen imaging can be used to link and confirm in-vivo imaging findings with histopathological examination results. After the quantification is completed, the number and the size of detected microbleeds will be used to correlate with patients' cognitive function while they were alive and the final pathological diagnosis to understand its role on disease progression.

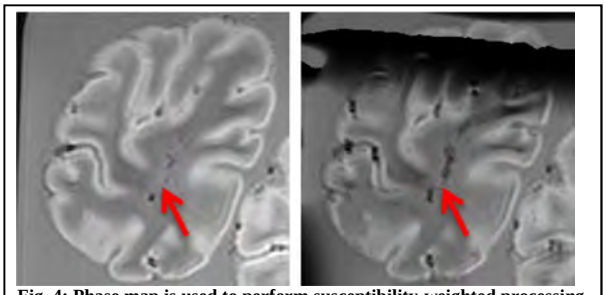

Fig. 4: Phase map is used to perform susceptibility-weighted processing to enhance the detection of small microbleeds (right image).

References: [1] Lee et al. J Neurol Sci. 2007;258(1-2):111-4. [2] Wiegman et al. J Neurol Sci. 2014;345(1-2):125-30. [3] Fisher et al. Stroke. 2010;41(12):2782-5.