Can High Frequency Ultrasound and MRI Diagnose Malignant Atheromatous Plaque In Vitro?

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SUMMARY

It remains a vital clinical issue how to diagnose malignant atheromatous plaques consisting of ulcerative plaque and hemorrhagic plaque, which are potential risks for thrombosis and the arterial spasm. This study proposes further investigations to develop methods in order to detect this type of lesions by echocardiography. In this study, we tested high frequency (7.5 MHz) US and 1.0 T MRI (T1 weighted SE, STIR; short time inversion recovery sequence, and T1 weighted fat suppression technique) for their precision in the diagnosis of atheromatous plaque as malignant or benign in post mortem human aorta.

Ten hemorrhagic plaques were imaged as heterogeneous echo-pattern in the shoulder of plaques covered with high-echo capsule with US; however, these findings were also obtained from 2 of 16 non-hemorrhagic plaques. With T1SE, hemorrhagic plaques were revealed as mixed areas of reduced intensity and high intensity which were differentiated from fatty deposition with T1 weighted fat suppression technique. Ulcerative plaques were revealed as concave shaped plaques and diagnosed correctly with both methods. US was superior to MRI from the viewpoints of examination time and measuring wall thickness. US indicated intimal plus medial thickness of hemorrhagic plaque and non-hemorrhagic plaque at 4.3 ± 1.1 mm and 3.0 ± 1.0 mm (p < 0.05) respectively. MRI was vulnerable to artifacts and its image was poorer in quality due to its lower resolution: however, probably because of its superior ability to distinguish fatty deposition from hemorrhage, MRI ultimately enabled more accurate diagnosis than US, as long as its image was fairly clear. The overall accuracies were 80% with US and 85.7% with MRI as confirmed by histological tests. From these results, the careful analysis of the two images obtained from US and MRI enables clinical diagnosis of malignant atheromatous plaques. (Jpn. Heart J 36: 235–245, 1995)

Key words: Ultrasound (US) MRI Image diagnosis Atherosclerosis

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ATHEROSCLEROTIC disease brings about a variety of lesions on the walls of arteries, from fatty streaks to complex lesions characterized by the accumulation of lipid, hyperplasia of smooth muscle cells and fibrous tissue, and calcification.

In postmortem arteriograms and histological studies, arterial thrombosis or spasm have been linked to myocardial infarction or unstable angina. Recent pathological studies have shown that arterial thrombi and spasm were related to rupture or fissuring and hemorrhagic episodes of atheromatous plaques.

Therefore, there are two clinical types of atheromatous plaques, benign and malignant, which have the potential to cause arterial thrombosis or spasms. However, there is no data on how to evaluate atheroma plaques as malignant. This study assumed that ulcerative plaque and hemorrhagic plaque are malignant, and we tested high frequency US and MRI for their ability to differentiate these malignant plaques from benign plaques compared to histology.

**Methods**

Twenty-nine atheromatous plaques from six fresh descending aortas were taken from humans at autopsy. The excised aorta were cut, opened flat with wooden pins on the wooden plates and preserved in 10% formalin solution for two or three days. After each aortic specimen was examined with the naked eye for confirmation of atheromatous plaques, each plaque region was pinned to the backing with wood picks and sketched for later blind examination (US, MRI and histology were done independently). The high resolution (7.5 MHz, Auxson 128XP) ultrasound transducer was placed over the aortic tissue manually. The ultrasound beam was aimed to penetrate the aorta in the perpendicular direction. The transducer-to-aortic wall distance was between 1.5 and 2.0 cm. In this setting, the axial and lateral resolutions were 0.25 mm and 0.6 mm, respectively.

The images were recorded in four different B color modes (sepia, rainbow, magenta, temperature) and ordinary gray scale images by the SONY recorder system and still photographs were taken on Polaroid film after retrieval. From the images the intima plus medial thickness of the atheromatous plaque was measured. The tissue characteristics were assessed by the following standards: surface irregularity, echogenisity and heterogeneity of the plaque.

After the US examination, MRI (Magnetom SP 42, 1.0 T unit) studies were performed at room temperature, 20–21°C: T1 weighted spin echo sequence (T1 image), short time inversion recovery sequence (STIR) and T1 weighted fat suppression technique (FATSUP). FATSUP reduces the intensity of signals from fat tissue by RF presaturation. Field of view was 180–200 mm and imaging matrix was between 160 × 256 and 256 × 256. Most images were taken with a
slice thickness of 2 mm. Effective pixel size was thus at maximum $1.5 \times 1.3 \times 3.0$ mm.

In the T1 weighted images repetition time (TR) was $200-500$ msec and echo time (TE) was $15-20$ msec. In the STIR images the parameters were TR $1000-3200$ msec, TE was $20$ msec and inversion time (IRM) was $150$ msec. In FATSUP, the saturation frequency used was varied between $133-200$ Hz depending on the degree of fat suppression gain. Number of acquisitions was 4–6. We used a surface receiving coil (elliptical spine, Siemens) in the MRI experiments.

Histological studies were performed with hematoxylin-eosin and von Gieson stain after US and MRI studies. Eight normal areas were also analyzed with these three methods.

**Statistical analysis:** All data are expressed as mean ± standard deviations. Statistical analyses of these data were compared using the student t test. Differences were considered significant at $p < 0.05$.

**Results**

We evaluated 16 plaques and 5 normal areas with US, MRI and histology, while the other 13 plaques and 3 normal areas were only evaluated with US and histology because of technical difficulties and space limitations with MRI. US and MRI could evaluate plaque characteristics and wall thickness, however, two MRI images were not of usable quality due to artifacts. US was superior to MRI for measuring intimal plus medial thickness, which is thought to be a good indicator of arterial wall thickness.

**US image of atheromatous plaques:** Normal aorta was revealed as thin three layers with US (Figure 1). Non-hemorrhagic plaque was revealed as a homogeneous echo hump (Figure 2), and hemorrhagic plaque was revealed as a heterogeneous hump with a low echo area in the plaque (Figure 3). Ulcerative lesions took on a fissured appearance at the surface (Figure 4). Calcified lesions revealed intense echos with shadows within the plaque. Figure 5 is a diagram of US images of atheromatous plaques. In sum, malignant atheromatous plaques, i.e. hemorrhagic plaques and ulcerative lesions, were distinguishable with high frequency US. Table I shows the relationship between US results and histological results.

The overall accuracy of US was 75.7%, and US was able to diagnose 20 atheromas (80%) correctly as non-hemorrhagic and hemorrhagic atheromatous plaques. Some misdiagnoses of nonhemorrhagic plaques for normal areas were made because the thickening was too slight to notice the lesions. A plaque with a minor hemorrhagic lesion was also misdiagnosed as a non-hemorrhagic plaque.
US (upper) and MRI (lower) images of normal aorta. Normal areas were revealed as three thin layers with US, and a 2–3 mm thick wall was seen as high intensity area in T1 weighted images.

US images of fatty depositions in the atheroma plaque were quite similar to those from hemorrhagic plaques (Figure 6), and these plaques were overestimated as hemorrhagic plaques.

US measured intimal plus medial thickness of hemorrhagic plaque and non-hemorrhagic plaque at 4.3 ± 1.1 and 3.0 ± 1.0 mm ($p < 0.05$) respectively; both were thicker than those of normal areas (1.8 ± 0.2 mm, $p < 0.01$, Table II).

**MRI image of atheromatous plaques:** In normal areas, a 2–3 mm thick wall was seen as a high intensity area in T1 weighted images (Figure 1). A black line between intima and water was sometimes imaged in T1 weighted images, though this may have been an artifact as this never appeared on the spin echo sequences. FATSUP did not affect the signal intensity of normal vessel walls. Calcification did not give any signal or shadow, and was represented as a black area on all sequences.

Non-hemorrhagic plaques were observed to be thicker humps of almost homogenous intensity (Figure 2). In the hemorrhagic plaques, MRI showed a mixed area of reduced intensity and high intensity at the shoulder portion (Figure 3). Ulcerative lesions had a fissured appearance on the surface and intimal defect...
Figure 2. US (upper) and MRI (lower) of non-hemorrhagic plaque. A non-hemorrhagic plaque was revealed as a homogenous hump with US and MRI. The intense echo with a shadow (US) and a black area (MRI) represents calcification.

Figure 3. US (upper), MRI (lower) and histological photography (right) of hemorrhagic plaque. Hemorrhagic plaque was observed to be a heterogenous hump with a low echo area in the plaque (US); MRI showed a mixed area of reduced intensity and high intensity at the shoulder of the plaque. Black arrows show hemorrhages in the US and MRI images and histological photographs.
Figure 4. US (upper), MRI (lower) and histological photography (left) of ulcerative lesions. Ulcerative lesions had a fissured appearance on the surface and intimal defects (white arrows) using US and MRI. Black arrows show hemorrhages in the US and MRI images and histological photographs.

Non-hemorrhagic plaque

- homogenous echo pattern

Hemorrhagic plaque

- ulcerative lesion (sometimes)
- thicker wall
- heterogenous echo pattern

Figure 5. Diagram of US and MRI images of each atheroma plaque.
Table I. Relationship between US Results and Histological Results

<table>
<thead>
<tr>
<th>US results</th>
<th>Pathological results</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>Normal</td>
<td>8</td>
</tr>
<tr>
<td>Non-hemo plaque</td>
<td>10</td>
</tr>
<tr>
<td>Hemo plaque</td>
<td>2</td>
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</tbody>
</table>

Figure 6. US (upper) and MRI (lower) of local fat deposition in the atheroma plaque. Some non-hemorrhagic atheroma with fatty deposition revealed heterogenous humps with relatively low echo areas (US). FATSUP image shows low intensity area.

Table II. Intimal Plus Medial Thickness of Each Plaque Measured with US

<table>
<thead>
<tr>
<th>Wall thickness (US: mm)</th>
<th>Normal (n = 8)</th>
<th>Non-Hemo (n = 10)</th>
<th>Hemo (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.8 + 0.2</td>
<td>3.0 + 1.0</td>
<td>4.3 + 1.1</td>
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<tr>
<td>ρ &lt; 0.01</td>
<td>ρ &lt; 0.05</td>
<td>ρ &lt; 0.01</td>
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</table>
Table III. Relationship between US Results and MRI Results

<table>
<thead>
<tr>
<th>US</th>
<th>MRI</th>
<th>Calc (+)</th>
<th>Calc (-)</th>
<th>Ulcer (+)</th>
<th>Ulcer (-)</th>
<th>Hemo (+)</th>
<th>Hemo (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>US</td>
<td>Calc (+)</td>
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<td>2</td>
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</tr>
<tr>
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<td>Calc (-)</td>
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<td>16</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>US</td>
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<td>0</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>US</td>
<td>Ulcer (-)</td>
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<td></td>
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<tr>
<td>US</td>
<td>Hemo (+)</td>
<td>6</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>US</td>
<td>Hemo (-)</td>
<td>0</td>
<td>13</td>
<td></td>
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</tbody>
</table>

(Figure 4).

With the exception of inadequate images (artifact, inadequate examination), the overall accuracy of MRI compared to histology was 85.7% (18/21).

**Diagnosis of malignant atheromatous plaque with US and MRI:** In the normal area, the aorta had a thin wall and a uniform thickness. The endothelial surface was revealed to be a smooth layer by both US and MRI. Non-hemorrhagic plaque displayed an irregular endothelial surface overlying homogenous isoechoic tissue with US, and a rather high intensity signal with MRI. Hemorrhagic plaque displayed an irregular surface with a heterogenous low echo area which was usually detected at the shoulder in the plaque. These low echo areas had high intensity signals under both MRI and FATSUP-MRI.

Presumably the high signal intensity in these low echo regions reflects high hemosiderin and fluid depositions due to bleeding and inflammation. On the other hand, lipid-laden areas showed high intensity for the T1 image but low intensity for FATSUP. Calcification produces high echos with shadows with US and low or zero NMR signal intensity with MRI.

**DISCUSSION**

The major objective of this study was to evaluate the diagnostic capabilities of high frequency US and MR imaging for the detection of malignant atheromatous plaque lesions, defined as ulcerative and hemorrhagic lesions,\(^{12,13}\) as they are known to be able to induce arterial thrombo-embolism and arterial spasms.

The most important problem in diagnostic imaging of malignant atherosclerosis is tissue characterization, especially detecting hemorrhage. Hemorrhagic plaque yielded mixed low and normal intensity echos with US (Figure 3), and these low echos were usually located at the shoulder portion of the atheroma.
However, some atheromatous plaque with fat deposition was indistinguishable from hemorrhagic plaque with US. That method alone is thus of dubious value for distinguishing between the two lesions, though it is worth pointing out that hemorrhagic atheroma plaque was thicker and usually located at the shoulder portion of the atheroma, while fatty deposition occurred anywhere throughout the atheromatous plaque.

High resolution trans-esophageal US providing information about atherosclerosis of the thoracic aorta is considered valuable for the evaluation of systemic atherosclerotic gradings, which are judged according to pathological grading.\(^\text{14,15}\) However, clinically, arterial thrombi and spasm were related to rupture and hemorrhagic episodes of the atheroma plaques.

Another difficulty was the MRI images obtained with the fat suppression technique; Fatty deposition was imaged as a low area, while hemorrhagic plaque appeared as a hump with heterogenous high and low NMR signal intensities. However, MRI had difficulty distinguishing hemorrhagic and non-hemorrhagic plaques, especially small hemorrhagic or small calcified atheromatous plaques.

In intracerebral hemorrhage, heterogenous NMR signals are a sign of the deposition of mixed hemosiderine and hemoglobin forms.\(^\text{16}\) In this study, we used three MRI techniques: T1 image, short time inversion recovery sequence and a fat suppression technique, to differentiate between hemorrhagic change and fat deposition. These techniques were useful for evaluating hemorrhagic plaque with MRI.

MRI has been shown to assess arterial disease non-invasively. From our results, MRI can image atheroma accurately and is especially reliable for assessing lipid content. This is very useful for differentiating a fatty deposition from a hemorrhagic lesion in the atheromatous plaque, especially since some fat depositions are indistinguishable from hemorrhagic plaque when using US. Conventional techniques have not exploited the chemical shift in the resonant frequency between the hydrogens in water and fat.\(^\text{17}\)

MRI is potentially capable of identifying the anatomic consequences of atherosclerotic vascular disease. Kamman et al\(^\text{18}\) reported that formalin fixation causes changes in MR relaxation times. There were visible intensity differences in the MR images, both increased and decreased in comparison to images of fresh tissue. However, there was no fabrication in MR images by the formalin fixation.

Lamont et al\(^\text{19}\) and Tovi and Ericsson\(^\text{20}\) have evaluated the signal intensity changes in formalin fixed tissue as compared to fresh tissue samples, but these differences do not impede identification of structures.

This is the first finding, to our knowledge, of repeatable measurements by MRI and US which exhibit results inconsistent with each other (Table III). Potential reasons for this are differences in resolution and that the observers might
inadvertently have taken measurements of different areas or the same areas at different orientations in the series of measurements with the two methods. We used 7.5 MHz US and 1.0 T MRI, which have spatial resolutions of 0.25 mm and 1.7 mm, respectively. They were of course marked with wooden pins; however, the pins were slightly thicker for US and thinner for MRI. Calcified lesions produced low or zero NMR signal intensity on MRI. This technique seems to have a certain risk of over-diagnosis of ulcerative lesions, as the low signal of the attendant calcified lesion is virtually identical to the signal from the vessel lumen. We believe the spatial resolution of 1.7 mm to be another liability of MRI for analysis of atherosclerotic lesions.

From our data, careful analysis of the two images obtained from US and MRI may enable the correct diagnosis of hemorrhagic and ulcerative atheromatous plaques not only in vitro but also in situ. However, more examinations with US and MRI in situ are required before clinically applying the data obtained in this study.

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