Identification of Atherosclerotic Plaque Components With Intravascular Ultrasound Elastography In Vivo
A Yucatan Pig Study

Chris L. de Korte, PhD; Marion J. Sierevogel, MD; Frits Mastik; Chaylendra Strijder, BSc; Johannes A. Schaar, MD; Evelyn Velema; Gerard Pasterkamp, MD, PhD; P.W. Serruys, MD, PhD; Anton F.W. van der Steen, PhD

Background—Intravascular ultrasound elastography assesses the local strain of the atherosclerotic vessel wall. In the present study, the potential to identify different plaque components in vivo was investigated.

Methods and Results—Atherosclerotic external iliac and femoral arteries (n=24) of 6 Yucatan pigs were investigated. Before termination, elastographic data were acquired with a 20-MHz Visions catheter. Two frames acquired at end-diastole with a pressure differential of ≈4 mm Hg were acquired to obtain the elastograms. After dissection, x-ray was used to identify the arterial segments that had been investigated by ultrasound. Specimens were stained for collagen, fat, and macrophages. Plaques were classified as absent, early fibrous lesion, early fatty lesion, or advanced fibrous plaque. The average strains in the plaque-free arterial wall (0.21%) and the early (0.24%) and advanced fibrous plaques (0.22%) were similar. Higher average strain values were observed in fatty lesions (0.46%) compared with fibrous plaques (P=0.007). After correction for confounding by lipid content, no additional differences in average strain were found between plaques with and without macrophages (P=0.966). Receiver operating characteristic analysis revealed a sensitivity and a specificity of 100% and 80%, respectively, to identify fatty plaques. The presence of a high-strain spot (strain >1%) has 92% sensitivity and 92% specificity to identify macrophages.

Conclusions—To the best of our knowledge, this is the first time that intravascular ultrasound elastography has been validated in vivo. Fatty plaques have an increased mean strain value. High-strain spots are associated with the presence of macrophages. (Circulation. 2002;105:1627-1630.)

Key Words: arteriosclerosis imaging inflammation lipids

The composition of an atherosclerotic plaque is an important determinant for clinical syndromes. Determinants of ruptured plaques are a large lipid core covered by a thin fibrous cap with a dense infiltration of macrophages. Although some promising invasive and noninvasive techniques are being developed, no technique is currently clinically available to identify these vulnerable plaques.

Intravascular ultrasound (IVUS) has proven to be a powerful technique to assess the geometry of the vessel wall and plaque. However, the sensitivity and specificity to detect lipid cores remains low. IVUS elastography assesses the local radial strain in the tissue caused by an intraluminal pressure differential. In vitro experiments revealed different strain values in fibrous and fatty plaques in human coronary and femoral arteries. Feasibility experiments in patients showed that reproducible elastograms could be obtained. Additionally, we studied whether atheroma and macrophages were related with strain values.

Methods

Animals Six atherosclerotic Yucatan pigs, average weight 40 kg, were studied. To induce atherosclerosis, pigs were put on an atherogenic diet. Two weeks thereafter, the external iliac and femoral arteries were denuded with a 4-F endothelial Fogarty catheter. The atherogenic diet was continued for 9 to 10 months after which femoral and internal iliac arteries were stented for the purpose of another study. Subsequently, the atherogenic diet was replaced by a regular diet. No intervention was performed in the denuded external iliac arteries that were visualized for the present study. The following 42 days, 3 pigs were treated with a drug and 3 pigs served as controls for the purpose of another study. ANOVA revealed that the intake of the drug had no influence on the measurements performed in this study. After the elastographic acquisition, animals were euthanized and vessels were harvested. The Ethical Committee on Animal Experimentation of the Faculty of Medicine, Utrecht University, approved the investigation.
Atherogenic Diet
In addition to essential nutrients, vitamins, salts, 2% cholesterol, 18% casein, and 6% peanut oil formed the basic atherogenic components of the diet.

Anesthesia
During denudation, intervention, and termination, the animals were anesthetized with intravenous midazolam 0.3 mg/kg per h and sufentanil 2.5 μg/kg per h and ventilated with a mixture of O2:air 1:1 and 1% halothane after a premedication with 4 mg/kg azaperone, 10 mg/kg ketamine, and 4 mg/kg thiopental.

Elastographic Acquisition
At termination, the arterial tree was accessed through a left carotid approach. An arterial 8-F sheath was introduced into the descending aorta, and an 8-F guiding catheter was advanced to the aortic bifurcation. Through the guiding catheter, contrast angiography was performed and a 20-MHz Visions catheter (JoMed) was advanced. IVUS data were acquired in the external iliac and the proximal, unstenoted part of the femoral artery. The position of the IVUS catheter was registered with angiography and radiopaque rulers. Frames that contained 512 angles with high-frequency raw ultrasound signals (7.5 mm) were acquired at 30 frames/second. The data were captured together with the pressure and ECG signals with a workstation that contained a framegrabber (Coreco Inc) connected to the digital interface of an InVision Echo machine (JoMed). Blood pressure was measured with the introduction sheath that was located distally from the aortic arch. Each acquisition of 4 seconds that contained 120 frames was stored on a CD-ROM for off-line processing. The animals were euthanized by an overdose of pentobarbital.

After euthanization, the surrounding tissue was removed from the arteries without changing the position of the legs. The investigated cross-sections were identified by angiography by comparison with the stored angiogram and the anatomic landmarks and radio-opaque rulers. The investigated locations were marked with suture in the adventitia. Next, the artery was excised and frozen in liquid nitrogen.

Histology
Cross-sections (7 μm) were stained for general morphology (elastin-van Gieson), collagen (picro-Sirius red and imaged with polarized light), and macrophages (acid phosphatase). The lipid content was assessed by the empty spaces in the picro-Sirius red stain imaged with polarized light microscopy. A lesion was classified as fatty when >40% of the plaque area consisted of fat. A lesion had positive macrophage staining when the acid phosphatase stain revealed clusters of cells with >10 cells. A lesion was classified as advanced when occupying an area within the internal elastic lamina of >40%; otherwise, it was classified as early lesions. Plaques were classified as absent, early fatty lesions, early fibrous lesions, and advanced fibrous plaques by observers blinded to the elastographic results. In this animal model, no advanced fatty plaque and no calcified components were found.

Data Analysis
Elastograms were determined with 2 frames acquired near end-diastole because motion of the catheter is minimal in this phase. Strain values up to 2% were obtained for a pressure differential of 4 mm Hg (±0.5) (100 milliseconds interframe time). These strain values are detectable with the chosen window length. For each angle, the radial strain is determined with cross-correlation analysis of the high-frequency ultrasound data. All signal processing was performed in Matlab (MathWorks). The strain values were color coded from red for low strain via yellow to green for 2% strain and were plotted as a complementary image to the IVUS echogram. The resolution of an elastogram in the radial direction is 200 μm.

The strain value of a plaque was determined by averaging all strain values found in the plaque. Additionally, the presence of a high-
strain spot (strain >1%) in the elastogram was related to the presence of fat and macrophages. The alignment of the ultrasound data and the histologic cross-sections was performed by the use of the IVUS echogram and the histology as described earlier.5

Statistics
All statistical analysis was performed with SPSS statistical software. Values are expressed as mean and the range. Bivariable linear regression analysis was performed to study the relation between the presence of fat and macrophages and the mean radial strain. Receiver operating characteristic analysis was performed to assess the optimal strain value and to evaluate the predictive power to identify fatty plaques. Furthermore, the sensitivity and specificity of a high-strain spot to identify fat and macrophages was determined.

Results
In 50% of the arterial segments, early plaques were found (n=12). Nine of these early lesions were classified as fatty. Advanced fibrous lesions were found in 25% of the segments (n=6), and the remaining 25% contained no plaque (n=6). Macrophages were found in cross-sections with early plaque (n=11) and in 1 fibrous plaque.

Typical examples of an advanced fibrous plaque and an early fatty plaque are shown in Figures 1 and 2. The elastogram (Figure 1) shows low-strain values, which indicate relatively hard material. The histology reveals the presence of collagen. Macrophages and fat are not found. High-strain values were found in a cross-section with early fatty plaque (Figure 2).

Higher average strain values were found in the fatty plaques with fatty material than in the other pathologies that showed similar mean strain values (Table 1). Regression analysis revealed a highly significant difference in average strain values between plaques with and without fat (P=0.007). After correction for the confounding effect of fat, the presence of macrophages had no additional effect (P=0.966). Receiver operating characteristic analysis revealed a maximum sensitivity of 100% with a corresponding specificity of 80% to identify fatty plaques for a strain value of 0.35%. The area under the curve was 0.952.

A high-strain spot has 75% sensitivity and 100% specificity to identify fat (Table 2). A 92% sensitivity and 92% specificity was found to identify the presence of macrophages.

Discussion
In this study, IVUS elastography is validated in vivo with an atherosclerotic Yucatan minipig model. An earlier in vitro study revealed different mean strain values in fibrous, fibrofatty, and fatty plaques in human coronary and femoral arteries.5 In this in vivo study, significant higher strain values were found in fatty plaques than in fibrous plaques.

| TABLE 1. Mean Strain Value in Plaque Types and Normal Artery |

<table>
<thead>
<tr>
<th>Plaque</th>
<th>Strain, %</th>
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<tbody>
<tr>
<td>Absent (n=6)</td>
<td>0.21</td>
</tr>
<tr>
<td>Early fatty lesions (n=9)</td>
<td>0.46</td>
</tr>
<tr>
<td>Early fibrous lesions (n=3)</td>
<td>0.24</td>
</tr>
<tr>
<td>Advanced fibrous plaque (n=6)</td>
<td>0.22</td>
</tr>
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In this animal model, only homogeneous plaque types and no calcified material were found. Because human plaques are mainly heterogeneous, these results cannot be directly transferred to the human situation. However, the main components of rupture prone plaques (ie, fibrous and fatty tissue and macrophages) were all present in this model.

Elastography has a high sensitivity to identify fatty material: A maximum sensitivity of 100% with corresponding specificity of 80% was achieved when the threshold was set at a mean strain in the plaque of 0.35%. This sensitivity and specificity is higher than values obtained with conventional IVUS in vitro. Prati et al4 found a sensitivity of 65% and a specificity of 95%. In another study by Komiyama et al,5 a sensitivity and a specificity of 55% and 72%, respectively, were found. This corroborates our findings in vitro in which we found a high correlation between strain and plaque composition and no relation between echogenicity and plaque components.5

No independent relation between mean strain of the plaque and macrophages was found. These results indicate that the mean strain value of the plaque is dominated by the tissue type (fibrous or fatty). However, a high-strain spot has a sensitivity and specificity of 92% to identify macrophages. It should be realized that the presence of macrophages should not necessarily be interpreted as plaque inflammation. However, increased strain values probably refer to the presence of active macrophages, because these cause plaque weakening.

**Conclusions**

With the use of in vivo intravascular elastography, higher mean strain values were found in fatty plaques than in fibrous plaques. Elastography has a high sensitivity and specificity to identify fatty plaques. The presence of macrophages results in localized high-strain spots.

**Acknowledgments**

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**References**


**TABLE 2. Relation Between a High Strain Spot and Fat or Macrophages**

<table>
<thead>
<tr>
<th>High Strain Spot</th>
<th>Fat</th>
<th>Macrophages</th>
</tr>
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<tbody>
<tr>
<td>Present</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>Absent</td>
<td>0</td>
<td>1</td>
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