Characterizing Vulnerable Plaque Features With Intravascular Elastography

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Background—In vivo detection of vulnerable plaques is presently limited by a lack of diagnostic tools. Intravascular ultrasound elastography is a new technique based on intravascular ultrasound and has the potential to differentiate between different plaques phenotypes. However, the predictive value of intravascular elastography to detect vulnerable plaques had not been studied.

Methods and Results—Postmortem coronary arteries were investigated with intravascular elastography and subsequently processed for histology. In histology, a vulnerable plaque was defined as a plaque consisting of a thin cap (<250 µm) with moderate to heavy macrophage infiltration and at least 40% of atheroma. In elastography, a vulnerable plaque was defined as a plaque with a high strain region at the surface with adjacent low strain regions. In 24 diseased coronary arteries, we studied 54 cross sections. In histology, 26 vulnerable plaques and 28 nonvulnerable plaques were found. Receiver operator characteristic analysis revealed a maximum predictive power for a strain value threshold of 1.26%. The area under the receiver operator characteristic curve was 0.85. The sensitivity was 88%, and the specificity was 89% to detect vulnerable plaques. Linear regression showed high correlation between the strain in caps and the amount of macrophages (P<0.006) and an inverse relation between the amount of smooth muscle cells and strain (P<0.0001). Plaques, which are declared vulnerable in elastography, have a thinner cap than nonvulnerable plaques (P<0.0001).

Conclusions—Intravascular elastography has a high sensitivity and specificity to detect vulnerable plaques in vitro. (Circulation. 2003;108:2636-2641.)

Key Words: atherosclerosis ■ elasticity ■ plaque ■ ultrasonics ■ catheters
In this study, we evaluated the ability of intravascular elastography to identify vulnerable plaque features. We tested the hypothesis that a pattern consisting of a high-strain region at the surface adjacent to low-strain regions corresponds to vulnerable plaque in histology. Furthermore, we looked at the relation between vulnerable plaque features and local high-strain regions.

Methods

Atherosclerotic human coronary (n=24) artery segments of 23 patients were excised and measured within 24 hours postmortem. All patients (mean age, 65±6 years) died of noncoronary causes, such as pneumonia (n=9), cancer (n=11), aortic dissection (n=1), and liver failure (n=2). Fifteen left coronary arteries and 9 right coronary arteries were used. The arteries were mounted between 2 sheaths. Before a defined constant pressure in the artery was achieved, the vessel was pressurized several times to detect leakage caused by even the smallest side branches still left open. After closing the remaining side branches, the vessel was pressurized several times to ensure the absence of leakage. Immediately after the ultrasound, experiments were performed in physiological saline solution in a water tank at room temperature.

A water column system, also containing physiological saline solution, was connected to the proximal sheath. This sheath was used to insert a 20-MHz IVUS catheter (JoMed Inc) connected to an InVision echo apparatus (JoMed Inc).

First, an IVUS frame was acquired at 80 mm Hg intravascular pressure. After 10 seconds, another IVUS frame was acquired at 100 mm Hg to obtain the incremental strain of the material. The data were captured using a PC-based acquisition system, connected to the digital interface of the echo machine, and stored on a CD-ROM for offline processing.

Fifty-eight cross sections were scanned with an interval of more than 10 mm to ensure uncorrelated observations. After the elastographic acquisition, the position of the ultrasonic cross sections was marked in the echogram with a clearly visible surgical needle inserted in the adventitia. On the surgical needle, a suture was mounted. Before cutting, the needle was removed but the suture remained in place. This suture was used as a marker for sectioning location.

Subsequently, the coronary arteries were pressure-fixed (80 mm Hg) and imaged segments were stained for the presence of collagen and fat (picro Sirius red and polarized microscopy), smooth muscle cells (α-actin), and macrophages (CD 68).

Elastograms were calculated as described by de Korte et al.6 The local strain was calculated from the gated radiofrequency traces and displayed color-coded from blue for 0% strain via red to yellow for 2% strain and plotted as a complementary image to the IVUS echogram. The resolution of the strain measurement in the radial direction was 200 μm.

Observers blinded for the outcome of each respective technique analyzed elastograms and histology. The thickness of the plaque cap and the atheroma size were measured using quantitative analysis of light microscopic images (Clemex Vision PE 3.5). To compare histology with elastography, matching between IVUS pictures and histology was performed using anatomical or artificial landmarks like calcium spots or the above-described suture. The matching was performed without knowledge of the elastographic results. Cross sections (n=4), for which a match between histology and IVUS echogram was not obvious, were excluded from the study.

In histology, a vulnerable plaque was defined as a plaque whose content consists of more than 40% atheroma covered by a thin cap with moderate to heavy macrophage infiltration, as described by Davies.12 A thin cap was defined as smaller than 250 μm.13 Smooth muscle cells (SMCs), collagen, and macrophages were graded as none, minor, moderate, and heavy according to their staining.14

In elastography, the potentially vulnerable location was defined as the region with the highest strain at the surface with adjacent low-strain regions (Figure 1). These spots were correlated with tissue components, which indicate vulnerability.

For statistical analysis, SPSS 11.0.1 (SPSS Inc) was used. Continuous variables are presented as median values and corresponding 25th and 75th percentiles. A receiver operator characteristic (ROC) curve analysis was performed to evaluate the value of local strain for the prediction of plaque vulnerability. According to this method, sensitivity (number of identified vulnerable plaques per total number of vulnerable plaques) and specificity (number of identified nonvulnerable plaques per total number of nonvulnerable plaques) were determined over the entire range of strain measurements using each observed value as a diagnostic threshold and plotted against each other. The area under the obtained curve, which may range from 0.5 to 1.0, represents the diagnostic accuracy of local strain. The strain...
value with the highest sum of sensitivity and specificity was considered to best discriminate between vulnerable and nonvulnerable plaques. Subsequently, multiple linear regression analyses were performed to evaluate the relationship between local strain and several plaque components, such as macrophages, smooth muscle cells, collagen content, and cap thickness. Regression coefficients with corresponding standard errors are reported, as well as significance levels. Nonlinear regression was used to test the relation between cap thickness and strain according to the model of Loree et al.3 The Kolmogorov-Smirnov test was used to assess normal distribution.

Results

Predictive Value of Intravascular Elastography to Detect Vulnerable Plaque

Average strain was obtained in the high-strain region of the plaques (n=54) of 24 diseased coronary arteries and compared with the histological grading of vulnerability. Twenty-six vulnerable plaques and 28 nonvulnerable plaques were found. The optimal sensitivity and specificity were obtained for a threshold value of 1.26% strain (Figure 2). The area under the curve of the ROC analysis was 0.85 (95% CI, 0.74 to 0.97).

Regarding vulnerable plaques, elastography was positive in 23 cases but negative in 3 cases. This results in a sensitivity of 88%. Nonvulnerable plaque were seen by histology in 28 cases and detected by elastography in 25 cases but were falsely diagnosed as positive in 3 cases. This results in a specificity of 89%. Intravascular elastography has a positive predictive value of 88% and negative predictive value of 89% based on comparison with histology. Comparing vulnerability in elastograms with cap thickness measured in histology, vulnerable plaques have a statistically thinner cap (258.7 ± 18.1 μm) than nonvulnerable plaques (362.7 ± 29.7 μm), with P<0.005.

Relation Between Features Associated With Plaque Vulnerability and Strain

The box-and-whisker plots show the relation between the 4 classes of macrophage content divided versus strain (Figure 3), SMC versus strain (Figure 4), and collagen versus strain (Figure 5). The relation between strain and cap thickness is reported in Figure 6. There is an inverse relationship between strain and cap thickness. A curve estimation, based on finite element modeling,3 showed $R=0.68$ with $P<0.0001$. It should be noticed that a cap >400 μm results in a strain <1%, whereas the data are more scattered for lower cap thickness. The $R$ of the linear regression model with all tissue components mentioned above as independent variables and
strain as dependent variable is 0.48, with a value of \( P < 0.0001 \). Detailed analysis is given in the Table.

**Discussion**

There is a need for a technique to diagnose the vulnerability of a plaque. With the availability of such a technique, a vulnerable plaque can be identified and followed and treatment can be monitored. Studies have shown that different strain values were obtained with intravascular elastography for different plaque components in vitro and in vivo.\(^{15}\)

One aim of the study was to assess the predictive value of IVUS elastography to identify the vulnerable plaque. This study demonstrates that intravascular elastography can accurately diagnose vulnerable plaques, as defined by Davies\(^{12}\) in vitro with a high sensitivity and specificity. Because detection of vulnerability is presently not feasible in vivo with other available techniques, intravascular elastography may offer a unique opportunity to learn more about the etiology of plaque rupture and to assess the effect of pharmacological agents on plaque stabilization.

Rupturing of a lipid-rich plaque may initiate the development of acute cardiac ischemic events. We measured the mechanical properties of plaques, as depicted by the strain in an elastogram. The vulnerable plaque is characterized by a high strain value on the surface of the plaque (Figure 7); conversely, a stable plaque has a low strain caused by a stable cap.

Another aim of the study was to find out which anatomical features in particular generate the high strain spots in an elastogram. To answer this question, elastograms and histology were compared in 54 cross sections of human coronary arteries. The principal findings of this part of the study were as follows. First, there is a high correlation between strain and macrophages. Second, there is an inverse correlation between the amount of SMCs and strain. Third, plaques, which are declared vulnerable in elastography, have a thinner cap than nonvulnerable plaques and a higher macrophage concentration.

The high correlation between strain and macrophages can be explained by weakening of the cap. Macrophages play a central role in the degradation process of the extracellular matrix by secreting proteolytic enzymes.\(^{16}\) In our study, this is reflected by the relationship between the amount of macrophages and the level of strain in the high strain spot in the elastogram.

Compared with macrophages, less is known about the influence of SMCs on the vulnerable plaque. Nevertheless, it has been suggested that plaques are more likely to break if fewer SMCs are present.\(^{17}\) The reason is not quite clear, but it seems that SMCs play an important role in preserving the integrity of the extracellular matrix in the cap.\(^{18,19}\) Furthermore, mechanical overstretching has proven to induce apoptosis in isolated vascular smooth muscle cells.\(^{20}\) In our study, this phenomenon may be exemplified by the fact that high strain is correlated with a low amount of SMCs.

There is also a significant relation between strain and collagen. However, after correcting for the confounding effect of macrophages and smooth muscle cells, no statistically significant effect of collagen on strain could be demonstrated. Thus, these results demonstrate that high strain is mainly caused by the presence of macrophages and the paucity of smooth muscle cells.

Rupture of the plaque occurs mostly at the thinnest part of the cap. In our study, we found an inverse relation between cap thickness and strain. Loree et al\(^{13}\) have already shown using a finite element model that a reduction of the fibrous cap thickness under 250 \( \mu m \) dramatically increases peak circumferential stress in the plaque. However, this relation was never demonstrated by measurements in coronary plaques. In our study, the cap thickness of plaques that are considered vulnerable was 250 \( \mu m \). Furthermore, no regions with a strain higher than 1% were found in plaques with a cap thickness of greater than 260 \( \mu m \).

In this study, all patients died from noncoronary causes. This population was chosen to detect rupture-prone plaques and not already-ruptured plaques. However, taking into ac-

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**Description of the Linear Regression Model Regarding the Influence of Tissue Components (Cap Thickness, Macrophages, SMCs, Collagen) on Strain**

<table>
<thead>
<tr>
<th>Model</th>
<th>Coefficient</th>
<th>SD</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cap thickness, ( \mu m )</td>
<td>-0.0004</td>
<td>0.001</td>
<td>0.14</td>
</tr>
<tr>
<td>Macrophages</td>
<td>0.552</td>
<td>0.192</td>
<td>0.006</td>
</tr>
<tr>
<td>SMCs</td>
<td>-0.738</td>
<td>0.166</td>
<td>0.0001</td>
</tr>
<tr>
<td>Collagen</td>
<td>-0.217</td>
<td>0.177</td>
<td>0.227</td>
</tr>
</tbody>
</table>

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**Figure 6.** Relation between strain (percent) and cap thickness (micrograms).

**Figure 7.** A typical observational example of an eccentric plaque with high strain spots on the shoulders is given. The image was recorded in a patient with unstable angina pectoris (Braunwald IIIb).
count that plaque rupture is a process triggered by inflammation, using cap thickness as an exclusive marker may be too simple. The often-used upper bound of 65 μm, as defined by Burke et al., described that it was not likely to have ruptured caps thicker than 65 μm. However, their conclusion was not that caps that are larger than 65 μm could not be considered vulnerable. Davies et al. focused more on the presence of inflammation rather than the cap thickness, which degrades the integrity and leads to a rupture. Nevertheless, in vulnerable patients who died from coronary artery disease, Mann and Davies found that the mean minimal cap thickness, i.e., the thinnest part of a cap, was 250 μm. In view of this, we considered caps thinner than 250 μm with moderate or heavy infiltration of macrophages as rupture prone. Studies have shown that inflammation contributes extensively to mechanical behavior of fibrous caps. Therefore, an exact cap thickness and the relation to triggering processes-like inflammation have to be exactly investigated in the future.

The impact of a diagnostic technique is highly dependent on its feasibility in vivo. Intravascular elastography has been applied in patients. Because IVUS is a commercially available technique that is routinely used by cardiologists, no additional catheters are needed to perform elastography. Elastography is able to detect vulnerable plaque features. This may enable us to follow the natural history of plaques suspected to be vulnerable (Figure 7). Furthermore, the influence of treatment of vulnerable plaques can be studied. Because palpography is based on computer processing, it can be easily implemented in the daily diagnostic procedure of patients with acute coronary syndrome.

Motion, either from the heart or the catheter, can lead to unreliable results in the clinical setting. These motion artifacts can be avoided by acquisition of the signals in end diastole, where a pressure change still exists but the catheter motion reaches its minimum. Furthermore, contrary to light-based techniques, ultrasound at the frequency used in this study does not suffer from the presence of blood in the artery.

However, the use of intravascular elastography is limited by its invasive nature. In the future, noninvasive tests like computed tomography or MRI may serve as screening tests to identify high-risk lesions and triage the patients to a more invasive testing once they have reached sufficient spatial and parametric resolution.

Other intravascular techniques are presently under development to detect vulnerable plaque features. Optical coherence tomography has a much higher resolution (approximately 10 μm) than IVUS and may provide a more detailed description of the morphology of a plaque. However, it has limited penetration depth and requires flushing the lumen with saline or inflating a balloon before images can be acquired. The same problems occur with angioscopy, where flushing is needed to identify yellow plaques, which are associated with acute coronary syndromes. Thermography can detect plaques with high temperature, which is related to inflammation. Results of preliminary investigation in humans have been reported. However, the method needs additional validation, especially regarding the influence of external parameters like intracoronary blood flow. Near-infrared spectroscopy obtains pictures based on absorbance of light by organic molecules. This technique has proven to detect plaque components with a high accuracy in vitro, although blood particles may hinder light waves to reach the plaque, a fact that limits all light-emitting diagnostic tools in their ability to detect plaque components.

Several groups have investigated the potential of radiofrequency signal analysis for tissue characterization. Many of these studies revealed the potential to identify calcified plaques, but although promising, none of them has converged yet to a technique with sufficient spatial and parametric resolution to identify a lipid pool covered by a thin fibrous cap. The power of elastography is that it is assessing the weak spot of a plaque and thus not needing high resolution.

Limitations

Strain estimation in this study is based on tissue mechanics ex vivo. The level of deformation of tissue in vivo may be different to the in vitro measurements. This may be attributable to lack of external parameters, such as innervations, tonus, and pulsatility. Future studies will address these questions. To extrapolate the results to a clinical situation, a prospective study is needed as in vivo validation.

All measurements of this study were performed at room temperature (23°C). In a previous study, we found that this temperature difference in vitro does not affect strain measurements.

The phrase and definition of vulnerable plaque are critically chosen. From a prospective view, it is unknown which plaques are rupture prone. The result of the ROC analysis may have been different if another definition were chosen.

The arteries are preconditioned by repetitively pressurizing them from baseline. The arteries were initially pressurized at 80 mm Hg and then pressurized 10 seconds later to 100 mm Hg. The effects of preconditioning and hysteresis were not assessed in these experiments, limiting our ability to directly extrapolate to steady-state pulsatile environment. Although there is a difference between the static and dynamic mechanical properties of vascular tissue, the ratio between the different plaque components remains similar. Nevertheless, in a pulsatile environment, the locations with increased strain may change between static or pulsatile experiments. However, the optimal threshold value (1.26% strain) for detecting a vulnerable plaque cannot be directly transferred from static in vitro experiments to pulsatile in patient recordings. This is corroborated by the initial elastographic in vivo acquisitions, where similar strain values were measured as in the in vitro experiments but with a significantly lower pressure differential. This study shows that you can get sensitivity and specificity of approximately 90%. For in vivo measurements in patients, the strain threshold still has to be determined.

Plaques, in which elastography was assessed, were selected from the IVUS pictures. However, because the coronary artery specimens were short and recording close to the sheets was avoided, only a limited number of plaques were suited for recording and a preselection of certain plaque features was hindered.
Conclusions

Intravascular elastography identifies successfully vulnerable plaque features in postmortem coronary arteries. Thus, intravascular elastography may play an important role in diagnosing the vulnerable plaque and help to gain deeper insight in the pathophysiology of acute coronary syndrome and identify the high-risk patient.

If unstable angina, plaque progression, and myocardial infarction are to be understood and prevented, vulnerable plaques must be identified and stabilized. Endeavors to prevent acute events are limited by identifying the high-risk plaque. The identification of vulnerable plaques may allow establishing therapies to reduce the risk of cardiac death in the future and opens the door to a preventive strategy for our patients.

Acknowledgments

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