Validation of Noninvasive In Vivo Compound Ultrasound Strain Imaging Using Histologic Plaque Vulnerability Features

Hendrik H.G. Hansen, PhD; Gert Jan de Borst, MD, PhD; Michiel L. Bots, MD, PhD; Frans L. Moll, MD, PhD; Gerard Pasterkamp, MD, PhD; Chris L. de Korte, PhD

Background and Purpose—Carotid plaque rupture is a major cause of stroke. Key issue for risk stratification is early identification of rupture-prone plaques. A noninvasive technique, compound ultrasound strain imaging, was developed providing high-resolution radial deformation/strain images of atherosclerotic plaques. This study aims at in vivo validation of compound ultrasound strain imaging in patients by relating the measured strains to typical features of vulnerable plaques derived from histology after carotid endarterectomy.

Materials and Methods—Strains were measured in 34 severely stenotic (>70%) carotid arteries at the culprit lesion site within 48 hours before carotid endarterectomy. In all cases, the lumen-wall boundary was identifiable on B-mode ultrasound, and the imaged cross-section did not move out of the imaging plane from systole to diastole. After endarterectomy, the plaques were processed using a validated histology analysis technique.

Results—Locally elevated strain values were observed in regions containing predominantly components related to plaque vulnerability, whereas lower values were observed in fibrous, collagen-rich plaques. The median strain of the inner plaque layer (1 mm thickness) was significantly higher (P<0.01) for (fibro)atheromatous (n=20, strain=0.27%) than that for fibrous plaques (n=14, strain=-0.75%). Also, a significantly larger area percentage of the inner layer revealed strains above 0.5% for (fibro)atheromatous (45.30%) compared with fibrous plaques (31.59%). (Fibro)atheromatous plaques were detected with a sensitivity, specificity, positive predictive value, and negative predictive value of 75%, 86%, 88%, and 71%, respectively. Strain did not significantly correlate with fibrous cap thickness, smooth muscle cell, or macrophage concentration.

Conclusions—Compound ultrasound strain imaging allows differentiating (fibro)atheromatous from fibrous carotid artery plaques. (Stroke. 2016;47:2770-2775. DOI: 10.1161/STROKEAHA.116.014139.)

Key Words: atherosclerotic plaque ▪ carotid artery diseases ▪ elasticity imaging techniques ▪ lipids ▪ stroke ▪ ultrasonography

Atherosclerotic carotid plaque rupture is a major cause of cerebrovascular mortality and morbidity. The propensity of a plaque to rupture is mainly related to its composition and geometry.1,2 Rupture-prone plaques typically contain a medium to large necrotic/lipid-rich core, which is separated from the lumen by a thin fibrous cap, whereas stable plaques have a thicker cap and often no necrotic/lipid-rich core.1,2 Furthermore, vulnerable plaques frequently present with intra-plaque hemorrhage, a high concentration of macrophages, and a decreased number of smooth muscle cells (SMCs).2,3

Local features of plaque vulnerability are correlated with an increased risk of future cerebrovascular events (CVE).4,5 This explains why local detection of these features is important. Detection of local features might also serve as a surrogate marker for plaque progression and subsequent CVE in other vascular beds. For instance, catheter-based techniques such as virtual histology or near-infrared spectroscopy have demonstrated that detection of lipid in the vascular tree has potential for accurate risk stratification of patients with acute coronary syndromes.6 However, because plaques are often asymptomatic before the first CVE, a patient-friendly noninvasive technique is required. Ultrasound is nonionizing, fast, and relatively inexpensive, and, therefore, has many advantages for assessment of plaque characteristics. Unfortunately, conventional ultrasound does not allow an accurate determination of the ratio of lipids and fibrous tissue throughout the plaque.

A relatively new technique is ultrasound strain imaging, also referred to as elastography or palpography,3,7-13
Vascular strain imaging estimates deformations/strains in the plaque and arterial wall induced by the pulsating blood. As intravascular studies have shown, strain values differ significantly for fibrous, fibroatheromatous, and atheromatous plaques. Furthermore, an increased number of high strain spots was found in coronary arteries of patients with unstable angina pectoris compared with patients with stable angina. Although one study reported that strain values did not differ between plaques in patients with and without major adverse cardiac events, in that study, strain parameters of all plaques of a patient were averaged that probably masked the increased strain regions present in the plaque responsible for the event.

A noninvasive technique would have allowed checking which characteristics the plaque had that actually ruptured. With a noninvasive technique, also long-term monitoring to study the effectiveness of treatment on local plaques will become available. Lately, noninvasive variants were introduced for carotid arteries, mostly for longitudinal imaging planes. However, imaging in longitudinal planes only allows visualization of the anterior and posterior wall. Because plaque can be present all over the circumference, we developed a technique for accurate estimation of radial strains in transverse imaging planes by using radiofrequency ultrasound data acquired at 3 insonification angles. This technique, called compound ultrasound strain imaging (CUSI), was validated in phantom experiments and simulations.

This study aims at validating noninvasive CUSI in vivo in patients by relating the measured strains to the typical features of vulnerable plaques derived from histology after carotid endarterectomy.

**Methods**

**Study Population**

Initially, in-patient strain data of 44 severely stenotic carotid arteries were noninvasively obtained using CUSI before carotid endarterectomy. Arteries of symptomatic patients (amaurosis fugax, transient ischemic attack, or stroke [Rankin ≤3]), with a stenosis of >70%, and asymptomatic patients (no ipsilateral symptoms during the past 6 months), with a stenosis of >80%, were included. Duplex ultrasound combined with either magnetic resonance angiography or computed tomography angiography was used to determine the level of stenosis. Selection for carotid endarterectomy was always discussed in a multidisciplinary team using international guidelines for symptomatic and asymptomatic carotid stenosis. The protocol was approved by the local ethics committee and in accordance with the World Medical Association Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects. All patients agreed to participate, and written informed consent was obtained. For reasons described further on, plaques of 10 patients were excluded from analysis. The median age of the included patients was 69 years with interquartile values ranging from 61 to 75 years. Seventy-six percent of the patients were men. Fifteen plaques were detected in the left internal carotid artery, 15 in the right internal carotid artery, 3 in the left common carotid artery, and 1 in the right common carotid artery. Three plaques of asymptomatic patients were included and 31 of symptomatic patients who experienced amaurosis fugax (n=10), transient ischemic attack amaurosis fugax (n=11), or stroke (n=10), respectively.

**Imaging Protocol**

The common carotid artery, bifurcation, and internal carotid artery of each patient were examined in a longitudinal imaging plane by an experienced sonographer using a Samsung Medison Accuvix V10 equipped with an L5-13IS linear transducer (f_m=5.8.5 MHz). In this longitudinal view, the cross-section with the highest amount of luminal narrowing was visually determined: the culprit lesion. The distance of the culprit lesion to either the flow divider or the origin of the carotid bulb was measured using a caliper and used to relocate the culprit lesion in the endarterectomy specimen. The sonographer rotated the transducer to image the culprit lesion in a transverse view. While having the vessel centered in this view, a dedicated imaging mode, called radial zone mode, was used to record ultrasound radiofrequency data (f_m=6.16 MHz) for 3 seconds for insonification angles of 0°, +40°, and −40°±20°±20° at a frame rate of at least 129 Hz (43 Hz/angle). Sequentially switching between the insonification angles was done automatically and did not require repositioning of the probe. Because larger insonification angles enable an improved estimation of the arterial deformations in the horizontal direction, sizes of the positive and negative angles were maximized, while keeping the entire vessel cross-section in the image view for all angles. To improve delineation between lumen and plaque, which is essential for radial strain estimation, the sonographer additionally recorded data in duplex mode. Before and after the described measurement series, systolic and diastolic blood pressures were determined using a sphygmomanometer. The entire protocol took ≈15 minutes.

**Cardiot Endarterectomy and Histological Analysis**

During carotid endarterectomy, the vascular surgeon made a longitudinal incision on the ventral side of the plaque, after which the intima and media layer containing the plaque were excised. The culprit lesion segment was identified using the aforementioned caliper distance and cut and stained with 5 different stainings according to the procedures of a Biobank study called Athero-express: CD68, α-actin, picrosirius red, hematoxylin and eosin, and Elastin van Gieson. On the basis of the stained slices, an expert (G.P.) blinded to the strain results scored 4 distinct plaque characteristics: plaque phenotype (fibrous or (fibro)atheromatous), SMC concentration, macrophage concentration, and cap thickness (thin or intermediate to thick). Percentage concentration of macrophages and SMCs were determined on a continuous scale using the previously published quantitative analysis. The other characteristics were analyzed semiquantitatively. A plaque’s phenotype was classified as fibrous when a plaque had no lipid core or a small (10% of plaque area) lipid core, low macrophage infiltration, and high SMC and collagen content; otherwise, it was classified as (fibro)atheromatous. Although fragments of thrombus, intraplaque bleeding, and calcifications were occasionally observed in the stained slices, these components were not used for analysis, because it was questionable if these components remained present throughout the endarterectomy and histological preparation.

The orientation and rotation of the slices with respect to the ultrasound recordings was determined before strain estimation using photographs taken during the plaque preparation process (before and after slicing) together with the echograms (calculated from the radiofrequency data). If present, the location of calcifications was also used to deduce the orientation of the slice with respect to the echograms. Ten arteries were excluded from analysis. Two because the plaque became fragmented during preparation, which impeded proper histological analysis. Two because the imaged cross-section obviously did not match with the histology slice. Five because the lumen–vesel boundaries could not be identified on the ultrasound images and 1 because the longitudinal motion of the artery caused the imaged cross-section to move out of plane from systole to diastole.

**Radial Strain Estimation**

Radial strain estimation was performed off-line using custom-made software (MATLAB 2010b, Mathworks, Natick, MA). First, ultrasonic frames were identified that corresponded to the systolic and diastolic phases (maximum and minimum lumen diameters derived from a diameter curve obtained by postprocessing, respectively). Next, from the 3 seconds acquisition, the pressure cycle (systole to systole), which visually showed the least out-of-plane motion, was selected.
for strain analysis for each patient. Within this cycle, the cumulative
eradial wall strain was determined from systole to diastole using
a coarse-to-fine 2-dimensional cross-correlation–based displacement
estimation algorithm followed by displacement compounding, tracking,
rotation, and 2-dimensional least-squares strain estimation.\textsuperscript{12,23–24}

For detailed settings of the algorithm, please see Table I in the online-
only Data Supplement. All strain estimation was performed on raw
tissue ultrasound radiofrequency data, because the phase information of the
radiofrequency data allows more accurate strain estimation than can
be obtained when using envelope or B-mode data.\textsuperscript{23} A strain value
was obtained for every 62.5 and 200 \( \text{\mu m} \) of tissue in the vertical and
horizontal directions, respectively. Because the amount of deformation
is not only related to tissue stiffness but also to the force applied
to the tissue, all strains were normalized with respect to a reference
pulse pressure \( P_{\text{norm}} \) of 5333 Pa (40 mm Hg):

\[
\varepsilon_{\text{norm}} = \frac{P_{\text{norm}}}{P_{\text{sys}} - P_{\text{diast}}} \varepsilon_{\text{meas}}
\]

where \( \varepsilon_{\text{norm}} \) and \( \varepsilon_{\text{meas}} \) are the estimated and normalized strain, and \( P_{\text{sys}} \)
and \( P_{\text{diast}} \) are the brachial systolic and diastolic pressures in Pascals
(Pa) for a certain patient. As a final result, the algorithm provided a
color-coded image (similar to Color Doppler) representing the radial
strain from systole to diastole (Figures 1A, 2C, and 3C). Yellow and
black indicate wall thickening and thinning (in radial direction),
respectively, in these images.

Analysis

First, a qualitative comparison (visual match) between the strain
images and the histological composition of plaques was performed
to assess whether increased radial strains matched with histologically
vulnerable plaque regions. Because the ultimate goal is to have one
value based on the strain image that identifies whether a plaque is vul-
nerable or not, 2 types of parameters were defined: a percentile strain
parameter: the \( x \)-th-percentile of the strain values (25th, 50th, 75th,
90th, 95th, and 99th percentile), and a high strain area parameter: the
percentage of strain values (representing an area) exceeding a certain
strain level. On the basis of the obtained strain images, strain levels
of 0.0%, 0.5%, 1.0%, and 1.5% were tested. Because strain for tissue
distal from the lumen is not only related to the local plaque com-
position but also to the tissue composition in between this location
and the lumen, only strain values within a 1-mm-thick ring adjacent
to the lumen–plaque border were considered when calculating the
parameter values. Plaque rupture that leads to thrombus formation
was observed in fibrous, collagen-rich regions.

Typical examples of strain images and corresponding histol-
ogy of different plaques are presented in Figures 1 through
3. In general, locally elevated strain values were observed in
regions that predominantly contained vulnerability-related
components according to histology, whereas lower values
were observed in fibrous, collagen-rich regions.

The AUC and cross-correlation results for all settings of
both strain parameters for all histological features are pre-

tented in Tables II and III in the online-only Data Supplement.

Fourteen plaques were classified as fibrous and 20 as
fibroatheromatous. Thirteen had a thin cap and 18 a thick
cap. In 3 cases, the cap thickness could not be estimated from
the staining. SMC concentration could also not be determined
for 1 plaque.

Results

Histological Results

Fourteen plaques were classified as fibrous and 20 as
fibroatheromatous. Thirteen had a thin cap and 18 a thick
cap. In 3 cases, the cap thickness could not be estimated from

Figure 1. A, Histology. B, Duplex image. 
C, Strain image. D, Photograph of the
culprit lesion cross-section of a fibrous
plaque. Strain values are close to zero
all over the plaque, indicating a low level
of deformation as expected for a fibrous
plaque fully composed of stiff collagen-rich material (recognizable as purple on the
Elastin van Gieson image and red on the
picrosirius red image).
and negative predictive value were 75%, 86%, 88% and 71%, respectively, at a threshold area of 37.3%. The Mann–Whitney tests also reveal a significant difference between the values of both strain parameters when differentiating between (fibro) atheromatous and fibrous plaques. For cap thickness, no significant difference in strain parameter values was found. Also, no significant correlation with SMC or macrophage concentration was observed.

**Discussion**

In this study, noninvasive CUSI was validated in patients using histological data of plaque specimen obtained with endarterectomy. The principal findings of this study are that: (1) strain parameters obtained with CUSI allow differentiation between fibrous and (fibro) atheromatous plaques, (2) noninvasive CUSI of carotid arteries is feasible in patients, and (3) no significant correlation was found between strain parameters and cap thickness, SMC concentration, or macrophage concentration.

**Relation Between Strain and Histology**

Both strain parameters were significantly higher for (fibro) atheromatous plaques than that for fibrous plaques, which was also observed with intravascular elastography. Opposed to that study, no negative correlation with cap thickness was observed. Probably, this is because the plaques were obtained by endarterectomy in this study. Especially for thin caps, it is difficult to perform the excision and longitudinal cut without loss of cap material. Therefore, classification of the absolute cap thickness might be inaccurate, which might also explain the large variance in the AUC for this parameter. Also, no significant difference was found in strain for various concentrations of SMCs and macrophages. This might be because of the fact that strain is not a direct result of the presence of one histological component, but a representation of the interaction between all components. This is perfectly illustrated by the plaque shown in Figure 1, which contains a high concentration of macrophages and is simultaneously predominantly composed of collagen and SMCs. Because of the collagen and SMCs, this plaque is stiff resulting in low strain values despite the macrophages. This might also explain why plaque phenotype correlates best with the strain parameters because it takes into account multiple features of plaque vulnerability. The fact that strain not directly reflects the presence of a single plaque component was also the reason why we did not choose to quantify the degree of local matching between strain and histology. Nevertheless, regions that mainly consist of soft tissue and a high concentration of macrophages that destabilize the plaque integrity can be expected to deform more than collagen-rich regions, as confirmed by the images shown in Figures 1 through 3.

**Comparison With Other Imaging Techniques**

(Fibro)atheromatous plaques were detected with a sensitivity and specificity of 75% and 86%, respectively. Imaging modalities, such as magnetic resonance imaging and positron emission tomography–computed tomography have shown similar and occasionally higher values, although a one-to-one comparison with CUSI still has to be performed. For a discussion on the performance of these imaging modalities for vulnerable plaque imaging, we would like to refer to a recent review article. The main advantages of an ultrasound-based technique,
such as CUSI, are the short measurement times, the fact that is nonionizing, easily applicable, relatively inexpensive, and noninvasive. Therefore, CUSI might make screening for vulnerable plaques in patients in an earlier (subclinical) stage of atherosclerosis possible. CUSI might also be the first patient-friendly technique to study the effect of lipid-lowering medication at plaque level. Prospective studies such as the ECST-2 trial (http://www.ecst2.com), in which large populations with less advanced plaques are imaged and strain parameters are related to traditional cardiovascular risk factors and clinical parameters like an acute event or the need for an interventional procedure, are crucial to show the usefulness for cardiovascular risk stratification of the measured strain parameters. It would also be interesting to investigate whether the proposed strain parameters can be used as a surrogate marker for CVE when studying the effect of medication and interventions, as has also been done for intima–media thickness measurements.26

Limitations
Although extreme care was taken not to damage the tissue, 2 plaques were no longer intact after endarterectomy and histological preparation. In the surgical procedure, the arterial wall is longitudinally dissected, and during histological preparation, calcified plaques first undergo a decalcification step to enable microdissection. Both procedures affect plaque integrity and probably also affect the most vulnerable regions of the plaque. Consequently, histology-based vulnerability might have been underestimated for some vulnerable plaques and overestimated for calcified plaques, leading to mismatches with the strain-based classification and possibly to underestimation of the performance of the technique.

During the endarterectomy, the specimens become deflated and cut, which influences geometry. This probably led to small mismatches in the alignment of the imaged and histology-stained cross-sections and, thus, also introduced noise in the performance analysis. En-bloc excision instead of the current excision procedure would have allowed a better preservation of plaque morphology, although it can only be performed safely in certain patients.

The optimal settings of the 2 strain parameters were chosen as those providing the highest area under the receiver–operator characteristics curves or Spearman correlation values. This procedure depends to a certain extent on the characteristics of the studied samples. Especially because the sample size was relatively small, this might have introduced some error in the performance assessment.

The final limitation that might have affected the performance assessment of the strain parameters is the fact that some histology parameters (phenotype and cap thickness) were determined semiquantitatively. Unfortunately, for these parameters, no quantitative methods were available yet. This might have made the analysis more objective and would have enabled calculation of correlation on a continuous scale. Nevertheless, as previous Athero-express studies have shown, the intra- and interobserver reproducibility of the semiquantitative analysis is moderate to substantial.20,21

Of the 10 excluded plaques, 6 were excluded because of technique-related issues. We only included cases (34 out of 40) for which the imaged cross-section remained in the imaging view from systole to diastole and for which the lumen–plaque boundary was identifiable on the B-mode data, because otherwise strain results would be based on noise. If we consider the studied cases to be representative of a standard population, this implies that the current implementation of the technique should not be used in 15% of cases. Future versions of CUSI might be developed to allow successful plaque differentiation in more cases. For instance, to deal with out-of-plane motion in future, the technique is being extended into the third dimension. For those cases in which plaques are located too deep to have a proper delineation between lumen and plaque border (n=2 in this study), improvements in sensitivity of ultrasound equipment might be a solution. Acoustic shadowing caused by calcifications, which also lead to an unidentifiable lumen–plaque boundary in 3 cases, cannot be overcome by technical innovation. However, it should be noted that dense calcifications are mainly present in advanced plaques. Imaging at an earlier stage of atherosclerosis might not have this problem. As long as plaques are not smaller than 2 by 2 mm², which is the largest window size used by the algorithm, we expect the technique to have at least equal performance. Current clinical guidelines do not indicate a need for intervention when stenosis is <50%. At ≥50% stenosis, this size requirement is usually fulfilled.

As mentioned earlier, the entire protocol took 15 minutes followed by an off-line strain calculation. The off-line analysis requires user interaction and takes ≈2 hours per patient, which is too slow for routine clinical use. However, most commercial vendors have already incorporated real-time radiofrequency-based strain estimation using cross-correlations in their systems. Therefore, real-time CUSI should be feasible.

Summary
Noninvasive compound ultrasound strain imaging allows in vivo estimation of cumulative radial strains in transverse cross-sections of severely stenotic human carotid artery plaques. Local matches were observed between the strain patterns and the histology-based plaque composition. Furthermore, 2 strain parameters were defined that positively correlated with the histology-based plaque composition. Furthermore, 2 strain parameters were defined that positively correlated with the presence of an atheromatous core. No significant correlation was found with fibrous cap thickness, SMC concentration, or macrophage concentration. Nevertheless, because the technique is noninvasive, easily applicable, relatively inexpensive, fast, and nonionizing, it seems promising for early assessment of carotid artery plaque vulnerability, although more studies are needed to fully explore its potential.

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Disclosures

None.

References

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**Supplemental Tables**

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<tr>
<th>Setting</th>
<th>Iteration 1</th>
<th>Iteration 2</th>
<th>Iteration 3</th>
<th>Iteration 4</th>
<th>Strain Estimation</th>
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<td>RF data</td>
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<td>Lateral kernel overlap (%)</td>
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<td>Median filter size (mm x mm)</td>
<td>2.2 x 1.8*</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>9° x 0.5 mm³</td>
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*Axial x lateral direction; †with sub-sample aligning; ‡filter settings after compounding for the cumulated horizontal and vertical displacements, respectively; §filter sizes defined in circumferential x radial direction, because of conversion to polar grid with a spacing of 1° circumferentially and 100 µm radially.
Table II. Performance of the strain parameters at different settings for detecting differences in histology-based plaque phenotype and cap thickness

<table>
<thead>
<tr>
<th>Strain parameter</th>
<th>Plaque phenotype (fibrous / (fibro)atheromatous)</th>
<th>Cap thickness (thick / thin)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AUC (CI)</td>
<td>Median (IQ values)</td>
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<tr>
<td>25&lt;sup&gt;th&lt;/sup&gt; percentile</td>
<td>0.71 (0.53, 0.89)</td>
<td>-2.07 (-3.10, -1.38) / -1.27 (-2.30, -0.40)&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>50&lt;sup&gt;th&lt;/sup&gt; percentile</td>
<td>0.78 (0.61, 0.94)</td>
<td>-0.75 (-1.04, -0.08) / 0.27 (-0.40, 1.36)&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>75&lt;sup&gt;th&lt;/sup&gt; percentile</td>
<td>0.74 (0.55, 0.93)</td>
<td>0.96 (0.09, 2.02) / 2.10 (1.28, 2.94)&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>90&lt;sup&gt;th&lt;/sup&gt; percentile</td>
<td>0.66 (0.47, 0.86)</td>
<td>2.48 (1.19, 4.49) / 4.07 (2.93, 5.45)&lt;sup&gt;*&lt;/sup&gt;</td>
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<tr>
<td>95&lt;sup&gt;th&lt;/sup&gt; percentile</td>
<td>0.63 (0.43, 0.83)</td>
<td>3.73 (2.16, 5.89) / 5.42 (4.04, 7.39)</td>
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<td>99&lt;sup&gt;th&lt;/sup&gt; percentile</td>
<td>0.58 (0.38, 0.79)</td>
<td>5.30 (3.35, 9.31) / 6.96 (5.54, 10.24)</td>
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<tr>
<td>Area strain &gt; 0.0%</td>
<td>0.78 (0.61, 0.94)</td>
<td>38.81 (27.67, 48.32) / 55.02 (43.72, 70.83)&lt;sup&gt;*&lt;/sup&gt;</td>
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<td>Area strain &gt; 0.5%</td>
<td>0.80 (0.63, 0.96)</td>
<td>31.59 (16.07, 37.09) / 45.30 (36.03, 63.64)&lt;sup&gt;*&lt;/sup&gt;</td>
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<td>Area strain &gt; 1.0%</td>
<td>0.76 (0.59, 0.94)</td>
<td>24.49 (11.57, 32.08) / 39.44 (29.02, 55.77)&lt;sup&gt;*&lt;/sup&gt;</td>
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<tr>
<td>Area strain &gt; 1.5%</td>
<td>0.73 (0.55, 0.90)</td>
<td>17.83 (8.43, 27.50) / 32.59 (21.18, 46.73)&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
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</table>

*P < 0.01 (Mann-Whitney test). AUC = area under the receiver-operating-characteristics curve, CI = binominal exact confidence interval, and IQ = interquartile.
Table III. Performance of the strain parameters at different settings for detecting differences in the histology-based concentration of macrophages and smooth muscle cells

<table>
<thead>
<tr>
<th>Strain parameter</th>
<th>Macrophage concentration (low / high)</th>
<th>Smooth muscle cell concentration (high / low)</th>
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</thead>
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<tr>
<td></td>
<td>Spearman correlation coefficient</td>
<td>Spearman correlation coefficient</td>
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<td>P-value</td>
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<td>25th percentile</td>
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<td>50th percentile</td>
<td>0.00</td>
<td>-0.08</td>
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<td>75th percentile</td>
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<td>90th percentile</td>
<td>0.04</td>
<td>-0.02</td>
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<td>95th percentile</td>
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<tr>
<td>99th percentile</td>
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<td>Area strain&gt; 0.0%</td>
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<td>-0.11</td>
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<tr>
<td>Area strain&gt; 0.5%</td>
<td>-0.23</td>
<td>-0.07</td>
</tr>
<tr>
<td>Area strain&gt; 1.0%</td>
<td>-0.20</td>
<td>-0.04</td>
</tr>
<tr>
<td>Area strain&gt; 1.5%</td>
<td>-0.12</td>
<td>-0.01</td>
</tr>
</tbody>
</table>

Supplemental References

