To target patients at high risk of a cerebrovascular event, adequate discrimination between vulnerable and stable carotid plaques is important. The current approach of surgical intervention guided by the severity of luminal stenosis is imperfect and unsustainable. Two thirds of all carotid plaque thromboembolisms result from plaques with <60% stenosis, whereas numerous surgeries are needed to prevent just 1 ischemic stroke caused by carotid plaque thromboembolism. This carries unnecessary operative risk and costs. Because the degree of stenosis inadequately reflects plaque vulnerability, current research focuses on improving vulnerable plaque identification.

One approach to distinguish vulnerable from stable plaques is a morphological classification. Virmani et al introduced a histopathologic classification scheme for plaques. Carotid plaques with a thin fibrous cap (<0.2 mm) and a distinct lipid-rich necrotic core are classified as thin fibrous cap atheroma and presumed to be at an increased rupture-risk. Plaques classified as pathological intimal thickening (an early stage extracellular lipid pool while mostly fibrous) and fibrous cap atheroma (similar as a thin fibrous cap atheroma but with a cap >0.2 mm) are considered more stable. Although this classification scheme is oft-used, it does not always adequately distinguish vulnerable from stable plaques. Not all, and only all, thin fibrous cap atheromas are vulnerable: numerous thin fibrous cap atheromas do not rupture, whereas numerous differently classified plaques do.

Another approach to distinguish vulnerable from stable plaques is based on a biomechanical stress analysis. Plaque rupture is, in essence, the mechanical failure of the fibrous cap. A biomechanical analysis incorporates the morphology, tissue properties, and hemodynamics to model the mechanical response of carotid plaques.

Background and Purpose—Two approaches to target plaque vulnerability—a histopathologic classification scheme and a biomechanical analysis—were compared and the implications for noninvasive risk stratification of carotid plaques using magnetic resonance imaging were assessed.

Methods—Seventy-five histological plaque cross sections were obtained from carotid endarterectomy specimens from 34 patients (>70% stenosis) and subjected to both a Virmani histopathologic classification (thin fibrous cap atheroma with <0.2-mm cap thickness, presumed vulnerable) and a peak cap stress computation (<140 kPa: presumed stable; >300 kPa: presumed vulnerable). To demonstrate the implications for noninvasive plaque assessment, numeric simulations of a typical carotid magnetic resonance imaging protocol were performed (0.62×0.62 mm² in-plane acquired voxel size) and used to obtain the magnetic resonance imaging–based peak cap stress.

Results—Peak cap stress was generally associated with histological classification. However, only 16 of 25 plaque cross sections could be labeled as high-risk (peak cap stress>300 kPa and classified as a thin fibrous cap atheroma). Twenty-eight of 50 plaque cross sections could be labeled as low-risk (a peak cap stress<140 kPa and not a thin fibrous cap atheroma), leading to a κ=0.39. 31 plaques (41%) had a disagreement between both classifications. Because of the limited magnetic resonance imaging voxel size with regard to cap thickness, a noninvasive identification of only a group of low-risk, thick-cap plaques was reliable.

Conclusions—Instead of trying to target only vulnerable plaques, a more reliable noninvasive identification of a select group of stable plaques with a thick cap and low stress might be a more fruitful approach to start reducing surgical interventions on carotid plaques. (Stroke. 2015;46:2124-2128. DOI: 10.1161/STROKEAHA.115.009707.)

Key Words: carotid artery plaque ■ histology ■ magnetic resonance imaging ■ mechanics ■ risk assessment ■ stroke
environment. The peak stress in the fibrous cap computed with finite element analysis is used as a rupture-risk marker. It depends not only on fibrous cap thickness\textsuperscript{10,11} but also on, for example, local lumen curvatures, lipid core size, lumen area, and plaque composition.\textsuperscript{2} Plaques with a peak cap stress exceeding 300 kPa are presumed to be at an increased rupture-risk.\textsuperscript{12} Plaques with a stress lower than 140 kPa are considered more stable.\textsuperscript{10,12,13} The efficacy of current plaque biomechanical models to distinguish vulnerable from stable plaques is unknown.

The merits of these 2 classifications lies in their use of quantitative thresholds which can be assessed through noninvasive imaging. Carotid magnetic resonance imaging is currently the only noninvasive imaging modality to obtain the entire plaque geometry/morphology with high soft-tissue contrast in a clinical setting.\textsuperscript{14} MRI segmentation data of the vascular wall including plaque components can be used as input for finite element analysis.\textsuperscript{8} However, recent MRI simulation studies addressed limitations of carotid MRI about the spatial resolution for thickness measurements of thin fibrous caps.\textsuperscript{15} This also has consequences for the reliability of stress computations of thin-cap, high-stress plaques.\textsuperscript{16} Numeric computer simulations of MRI can be used as an effective means to investigate in vivo MRI segmentation accuracy.\textsuperscript{17} Their principal advantage is the availability of a perfect ground truth (i.e., the object being imaged) on a submillimeter scale. This study consists of 2 parts. In the first part, we acquired carotid plaque cross sections from endarterectomy specimens from patients with >70\% stenosis, which underwent surgery. We subjected the cross sections to both a histological classification and a peak cap stress computation and investigated the cases of agreement and disagreement. In the second part, we demonstrated the implications for a noninvasive imaging-based risk assessment with MRI. We performed that demonstration with a subset of the plaques using computer simulations of a clinical carotid MRI pulse sequence.

### Methods

#### Histology

Histological cross sections of excised carotid plaques causing <70\% stenosis (North American Symptomatic Carotid Endarterectomy Trial [NASCET]) from 34 patients who had been scheduled for carotid endarterectomy were used. Written informed consent was obtained from all patients. The plaques were decalcified, embedded in paraffin, sliced at intervals of 1 mm with a thickness of 5 \( \mu \)m, and stained with either an Elastica van Gieson or Resorcine Fuchsine staining to distinguish fibrous tissue and lipid-rich necrotic core. These tissues were manually delineated on \( \mu \)m-resolution microscopy images to create the ground truth plaque models. We randomly selected 25 cross-sections morphologically classified as pathological intimal thickening, 25 as fibrous cap atheroma, and 25 classified as thin fibrous cap atheroma. From each patient at least 1 plaque cross section was included. For the histological classification, the definitions introduced by Virmani et al\textsuperscript{1} were followed. Pathological intimal thickening was defined as a lesion mainly consisting of extracellular matrix and fibrous tissue, with only dispersed extracellular lipid. Fibroatheroma show a necrotic core with free cholesterol and cell debris. A thick cap fibroatheroma is separated from a thin cap fibroatheroma by a cap thickness of 200 \( \mu \)m.

#### Finite Element Analysis

The spatial stress distributions were computed with Abaqus Standard 6.11 (Dassault Systèmes Simulia Corp, Providence, RI). Fibrous and lipid-rich necrotic core tissues were modeled as incompressible with a nonlinear neo-Hookean constitutive model (material constant \( C_1=167 \) kPa for fibrous tissue; \( C_1=1 \) kPa for lipid-rich necrotic core).\textsuperscript{14} Each two-dimensional (2D) model was meshed with \( \approx 50000 \) four-node linear hybrid quadrilateral elements (mesh-independent solutions). The loading condition consisted of a static intraluminal pressure of 16.7 kPa (125 mmHg). The maximum principal stress was used as the scalar stress measure. Before MRI simulations, the ground truth models were deformed with finite element analysis by applying 80 mmHg to recover an in vivo shape. The initial stresses in the resulting MRI-based plaque models were computed with the backward incremental method.\textsuperscript{19}

#### Noninvasive Imaging Demonstration: MRI Simulations

To demonstrate the influence of MRI-based in vivo peak cap stress computations on the agreement with the histological classification of the underlying ground truth plaque, we performed numeric MRI simulations on a subset of the plaques (32 plaque cross sections). These 32 cross sections were used in our previous studies.\textsuperscript{15,16} A numeric MRI simulation consists of modeling MRI physics by solving the Bloch equations for a provided pulse sequence and ground truth computer sample model. The Jülich Extensible MRI Simulator was used for simulations.\textsuperscript{17} A clinically applied, 2D, T1-weighted, turbo spin-echo, gadolinium contrast enhanced, black-blood sequence on a 3.0T full-body system was simulated.\textsuperscript{18} The repetition/echo times were 800 ms/10 ms respectively. A reduced field-of-view of 37×37 mm\(^2\) was simulated with a matrix size of 60×60. This resulted in an in-plane acquisition voxel size of 0.62×0.62 mm\(^2\), identical to the clinical protocol. Fibrous, lipid-rich necrotic core, and background tissues were assigned apparent T\(_1\) relaxation times of 2500, 1220, and 1412 ms, respectively (all T\(_2\)=50 ms).

#### Analysis

When comparing the means of groups of data, a nonparametrical Mann–Whitney \textit{U} test was used (significant if \( P<0.05 \)). For test to correlations, the Pearson correlation coefficient (\( R \)) was used (significant if \( P<0.05 \)). Cohen \( \kappa \) analysis was performed to investigate the agreement between the classification schemes. For the demonstration with MRI simulations, the simulated carotid MRI were independently segmented by 3 MRI readers (M.B., J.S., and G.H.). The MRI readers were blind to the ground truth histology segmentations and the stress computations. MRI-based plaque models were created from their segmentations and subjected to finite element analysis. We compared the MRI-based peak cap stress with the histological classification of the ground truth plaque cross section using quartiles for stress. We defined 3 groups: low stress<third quartile; first quartile-medium stress<third quartile; and high stress>third quartile. That analysis was done for each MR reader separately.

### Results

#### Examples

Three examples of histological plaque cross sections and their stress distributions are shown in Figure 1. The first example (left) is a cross section classified as pathological intimal thickening containing some deep extracellular lipid while being mostly fibrous. The minimum fibrous cap thickness is 1.16 mm and the peak cap stress is 66 kPa. The second example (middle) is a cross section classified as a fibrous cap atheroma containing a large distinct lipid-rich necrotic core with a cap thickness of 0.24 mm and a peak cap stress of 264 kPa. The third example (right) is a cross section classified as a thin fibrous cap atheroma with a cap thickness of 0.12 mm and a peak cap stress of 404 kPa. The plaque cross sections had low stresses in the soft lipid regions and high stresses at the plaque shoulders (high lumen curvature) and at the location of the minimum fibrous cap thickness.
The results for all plaque cross sections are shown in Figure 2. On average cross sections classified as pathological intimal thickening had the lowest stress (median, 105 kPa; interquartile range, 78 kPa). Cross sections classified as fibrous cap atheroma had a slightly higher stress (median, 150 kPa; interquartile range, 142 kPa), \( P=0.03 \). Cross sections classified as thin fibrous cap atheroma had the highest peak cap stress (median, 404 kPa; interquartile range, 268 kPa), \( P<10^{-3} \) with respect to both other groups. The peak cap stress as a function of the minimum fibrous cap thickness is plotted in Figure 3. There was a significant inverse correlation between peak cap stress and minimum fibrous cap thickness: \( R=-0.68; P<10^{-3} \). However, the large spread in the data (especially for caps <0.2 mm) indicates that minimum fibrous cap thickness was not the only parameter influencing peak cap stress.

### Peak Cap Stress is Associated With Histological Classification

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### High-Risk Plaques, Low-Risk Plaques, and the Cases of Disagreement

Despite the aforementioned general association, only a limited number of plaque cross sections had an agreement between both classifications. We found that 64% (16/25) of the thin fibrous cap atheromas had a stress >300 kPa threshold; we labeled these 16 plaques as high risk. We also found that 56% (28/50) of the cross sections, which were not classified as a thin fibrous cap atheroma had a stress <140 kPa threshold; we labeled these 28 plaques as low risk. This resulted in a \( \kappa=0.39 \), indicating a slight agreement between the classification schemes. The remaining 31 of 75 plaque cross sections (41%) did not belong to either group as they had a disagreement between both classifications. That group consisted of 9 thin fibrous cap atheromas, 15 fibrous cap atheromas, and 7 pathological intimal thickenings. These plaque cross sections could not be labeled as either high risk or low risk.

### Noninvasive Imaging: MRI Simulations

Of the 32 plaques used for the MRI simulations, 8 (25%) were classified as pathological intimal thickening, 14 (44%) as fibrous cap atheroma, and 10 (31%) as thin fibrous cap atheroma. An MRI-based stress computation resulted in a severely underestimated and imprecise peak cap stress because of overestimation of cap thickness, in particular for plaques with caps <0.62 mm.
Discussion

To adequately treat patients at risk of an acute cerebrovascular event, plaque vulnerability needs to be incorporated. In this study, we compared 2 approaches: a morphological classification and a biomechanical analysis. Although such a comparison has been reported in the literature, our study focused specifically on the cases of agreement and disagreement and their implications instead of merely general associations. In addition, we also demonstrated the implications for a noninvasive assessment of carotid plaque vulnerability.

Although this study focuses on analyses on histological cross sections, our main findings can be translated to the clinical arena. If a carotid plaque of a symptomatic patient is scanned with MRI, individual cross-sections can be segmented and computational analyses can be performed. The most dangerous cross-section—representing the location where plaque rupture is most likely to occur—can then be selected, and subjected to the criteria discussed in this study. In theory, it is possible to identify a group of patients with high-risk plaques (ie, both high peak cap stress and classified as a thin fibrous cap atheroma). There are, however, 2 practical problems associated with this approach. First: the unreliability of a noninvasive assessment of thin-cap (<0.2 mm) plaques because of the limited voxel size (=0.6 mm) of current carotid MRI inhibits a reliable identification of a group of high-risk plaques in clinical practice. Second: even if sufficiently high-resolution imaging is available, one would then still miss many plaques of which it is currently uncertain if they are vulnerable or stable. We identified numerous thin fibrous cap atheromas with a low peak cap stress and numerous differently classified plaques with a high peak cap stress. These plaques had a disagreement between the 2 classification schemes we investigated. This reflects our current inability to identify all, and only all, vulnerable plaques. Leaving vulnerable carotid plaques untreated could result in undertreatment of patients with carotid artery disease.

It is in theory also possible to identify a group of patients with low-risk plaques (ie, both low peak cap stress and not classified as a thin fibrous cap atheroma). The 2 practical problems associated with the identification of high-risk plaques do not apply here anymore. First: low-risk plaques typically have thick fibrous caps, which can be reliably measured with MRI. This also yields a more reliable peak cap stress computation associated with minimum fibrous cap thickness. Indeed, our demonstration with MRI simulations suggests that only a noninvasive identification of a select group of low-risk plaques is feasible in clinical practice. Although it was not the focus of their study, Esposito-Bauer et al recently reported a perfect identification of a group of stenosis-inducing stable plaques (58 months event-free) with MRI but an imperfect identification of all, and only all, unstable plaques. Second: instead of focusing on the few patients who would benefit from surgical intervention, it might also be useful and practicable to focus on a group of patients who would not benefit. This means a shift of focus from vulnerable plaques to stable plaques. By reliably identifying low-risk plaques and exempting them from surgery, one could also effectively achieve a reduction in the large number of surgical interventions. This hypothesis, the stable plaque paradigm, needs to be tested in a randomized control trial. We note that in order improve stable plaque detection, additional

Figure 3. Peak cap stress as a function of minimum fibrous cap thickness. Dotted lines indicate the 0.2 mm and the 140/300 kPa thresholds. The gradient qualitatively illustrates the reliability of a noninvasive assessment of the variable with magnetic resonance imaging (MRI).

Figure 4. Magnetic resonance imaging (MRI)–peak cap stress versus histological classification of the underlying (ground truth) plaque cross section for the 3 MRI readers.
parameters such as intraplaque hemorrhage, calcifications, and cap inflammation/strength should be incorporated.

Limitations

The number of patients used was limited and in some cases multiple cross sections from the same plaques were used. We furthermore selected equal group sizes of differently histologically classified plaques. This may not reflect the actual prevalence of entire-plaque classifications in carotid artery disease patients. Such a population-based investigation was beyond this study’s scope. Our biomechanical analyses were residual stress-free, static, 2D and used literature-based plaque tissue elasticity values. Microcalcifications (which cannot be noninvasively imaged) and localized tissue strength/elasticity were not incorporated. Note that the aforementioned issues become relatively less influential when targeting thick-cap, low-stress plaques. For example, knowledge of cap strength becomes important only for thinner caps with high peak cap stresses within the range of experimental cap-failure observations (>140 kPa). In the MRI simulations, motion, axial partial volume effects, high image noise, and imperfect blood signal-suppression were neglected which yielded an ideal-case imaging scenario. This means that thickness measurements of thin caps would be even more unreliable than we determined here. The 70 kPa threshold we introduced in this study is MRI-protocol dependent and does not directly apply to stress modeling based on images from other protocols. In all, we do note that although the limitations and assumptions of this study influence our reported quantitative data, they do not devalue our main arguments and conclusions.

Conclusions

The limited spatial resolution of current clinical MRI renders the targeting of thin-cap, high-stress plaques unreliable. Instead of focusing on vulnerable plaques, a reliable identification of stable plaques might be a more fruitful approach to start reducing carotid surgical interventions.

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Disclosures

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Carotid Plaque Morphological Classification Compared With Biomechanical Cap Stress: Implications for a Magnetic Resonance Imaging–Based Assessment
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背景および目的：プラックの不安定性を特定する2つ手法として病理組織学的分類法と生体力学的解析を比較し、磁気共鳴画像法（MRI）を用いて頜動脈プラックのリスクを非侵襲的に判別する意義を評価した。

方法：頜動脈内膜剝離術を行った患者34例（＞70%狭帯）の検体から組織学的プラック75病変の横断切片を得、Virmaniらによる病理組織学的分類（被膜厚0.2mm未満で線維性被膜が薄いアテローム：不安定プラックと推定）と被膜への最高圧力の算出（＞140kPa：安定プラックと推定、＞300kPa：不安定プラックと推定）を行った。プラックの非侵襲的評価の意義を示すため、典型的な頜動脈MRIプロトコルの数値シミュレーションを実施（面内で得られたポルタサイズ=0.62×0.62mm²）MRIを用いた被膜への最高圧力の算出に使用した。

結果：被膜への最高圧力は一般に組織学的分類に関連してい。しかし、25のプラック横断切片のうち16のみが高リスク（被膜への最高圧力＞300kPaで、線維性被膜が薄いアテローム）と分類された。プラック横断切片50のうち28は低リスク（被膜への最高圧力＜140kPaで、線維性被膜が厚いアテローム）と分類され、κ=0.39であった。2つの分類法間でプラック31病変（41%）が不一致となった。被膜厚に関しては、MRIのポールサイズの測定限界のため、非侵襲的分類はプラックの線維性被膜が厚い低リスク群においてのみ信頼できるものであった。

結論：不安定プラックのみを対象にしようとせず、被膜が厚く低圧力の安定プラック群を対象とした信頼性の高い非侵襲的分類を行えばより有益な手法となり、頜動脈プラックに対する外科的介入を減少させる可能性がある。

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