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Title: Plaque structural stress estimations improve prediction of future major adverse cardiovascular events following intracoronary imaging

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Plaque structural stress estimations improve prediction of future major adverse cardiovascular events following intracoronary imaging

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**Short Title:** Plaque stress and MACE

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ABSTRACT

**Background:** Although plaque rupture is responsible for most myocardial infarctions, few high-risk plaques identified by intracoronary imaging actually result in future major adverse cardiovascular events (MACE). Non-imaging markers of individual plaque behavior are therefore required. Rupture occurs when plaque structural stress (PSS) exceeds material strength. We therefore assessed whether PSS could predict future MACE in high-risk non-culprit lesions (NCLs) identified on virtual-histology intravascular ultrasound (VH-IVUS).

**Methods and Results:** Baseline NCL features associated with MACE during long-term follow-up (median 1,115 days) were determined in 170 patients undergoing three-vessel VH-IVUS. MACE was associated with plaque burden (PB) ≥70% (hazard ratio (HR) 8.6, 95%CI 2.5-30.6, p<0.001) and minimal luminal area (MLA) ≤4mm² (HR 6.6, 95%CI 2.1-20.1, p=0.036), although absolute event rates for high-risk lesions remained <10%. PSS derived from VH-IVUS was subsequently estimated in NCLs responsible for MACE (n=22) versus matched control lesions (n=22). PSS showed marked heterogeneity across and between similar lesions, but was significantly increased in MACE lesions at high-risk regions, including PB≥70% (13.9±11.5 vs. 10.2±4.7, p<0.001) and thin-cap fibroatheroma (14.0±8.9 vs. 11.6±4.5, p=0.02). Furthermore, PSS improved the ability of VH-IVUS to predict MACE in plaques with PB≥70% (adjusted log-rank, p=0.003) and MLA≤4mm² (p=0.002). Plaques responsible for MACE had larger superficial calcium inclusions, which acted to increase PSS (p<0.05).

**Conclusion:** Baseline PSS is increased in plaques responsible for MACE, and improves the ability of intra-coronary imaging to predict events. Biomechanical modeling may complement plaque imaging for risk stratification of coronary NCLs.

**Key Words:** Arteriosclerosis; Coronary disease; Intravascular ultrasound; Imaging
INTRODUCTION

Atherosclerosis is a multifocal disease, with myocardial infarction (MI) remaining a leading cause of morbidity and mortality. Around two thirds of all spontaneous thrombotic coronary events resulting in MI or sudden cardiac death are due to rupture of an atheromatous plaque\(^1,2\). Repeated cycles of subclinical rupture and repair also underlie rapid plaque growth\(^3\), leading to luminal encroachment and symptoms of progressive angina. Morphologically, ruptured plaques exhibit large necrotic lipid cores, thin overlying fibrous caps and evidence of microcalcification\(^4\). The precursor lesion for rupture, termed a 'thin-cap fibroatheroma' (TCFA), displays several of these compositional features\(^4\). However, prospective studies have shown that future clinical event rates attributable to 'high-risk' plaques were <10% over three years\(^5\), highlighting that novel, non-imaging based markers are required to improve plaque-based risk stratification.

Plaque rupture occurs when the plaque structural stress (PSS) exceeds the material strength of the tissue\(^6\). Autopsy studies have shown that PSS is increased following plaque rupture, and that the location of peak PSS can accurately predict rupture site\(^7,8\). Plaque composition can also affect PSS, with large lipid cores, thin fibrous caps, and superficial microcalcification all acting to increase PSS\(^9\text{--}11\). Although direct in vivo measurement of PSS is currently impossible, it can be estimated using finite element analysis (FEA) based upon coronary plaque imaging. The imaging modality must be able to display whole plaque architecture, while also discriminating between plaque tissue components. At present, virtual-histology intravascular ultrasound (VH-IVUS) remains one of the few imaging modalities that possesses these qualities, permitting reliable characterization of coronary plaques through automated analysis of ultrasound backscatter signal\(^12\).
We recently developed a novel tool to calculate PSS derived from VH-IVUS data and showed that PSS levels were increased in coronary plaques in patients presenting with MI compared with stable angina\textsuperscript{11}. Based on these results, we hypothesized that PSS may be increased in plaques responsible for MACE and that integration of PSS with high-risk VH-IVUS characteristics could improve our ability to predict future plaque rupture. Here we prospectively studied the ability of combined PSS/VH-IVUS to predict major adverse cardiovascular events (MACE) in the Virtual Histology in Vulnerable Atherosclerosis (VIVA) study\textsuperscript{13}. We show that: (a) Short-term VH-IVUS imaging predictors of MACE are also associated with longer-term MACE, (b) PSS estimations from VH-IVUS and histology are significantly correlated, (c) baseline PSS varies markedly both between and within higher-risk plaques, (d) baseline PSS is significantly elevated in plaques responsible for MACE compared with matched control lesions, (e) PSS significantly improves the ability of VH-IVUS to predict MACE, (f) subtle differences in plaque architecture increase PSS. Combining PSS calculations with plaque imaging data may significantly improve risk stratification of coronary plaques.
METHODS

Patient recruitment

The VIVA (Virtual Histology in Vulnerable Atherosclerosis) study design, including exclusion/inclusion criteria, has been described previously\(^\text{13}\) (Supplemental Methods). Patients (n=170) undergoing percutaneous coronary intervention (PCI) with either stable angina or acute coronary syndrome were prospectively enrolled. VH-IVUS imaging was performed in the culprit vessel before PCI and in all three major coronary arteries after PCI. Exclusion criteria for VH-IVUS was any vessel with visual reference diameter <2.5mm or with excessive tortuosity preventing catheter delivery. All data were obtained using 20MHz Eagle-Eye Gold catheters (Volcano Corporation, Rancho Cordova, US) after administration of glyceryl trinitrate, using a motorized pullback at 0.5mm/sec from the most distal safe position in the vessel. In total, 30,372.2mm of IVUS were included in the analysis (median 177.2 [145.3-216.4] mm per patient). Non-culprit lesions (NCLs) were defined as any lesions imaged at baseline that did not undergo PCI. The study protocol was approved by the regional ethics committee (Ref: 07/Q0106/47) and all participants provided written informed consent.

Ex vivo validation

Permission to use autopsied human hearts for research was sought from relatives, with the study protocol approved by the regional ethics committee (07/H0306/123). At autopsy, the left anterior descending artery was identified and excised along with approximately 40mm of surrounding tissue to maintain structural integrity\(^\text{14}\). A guiding catheter was then sutured into the ostium of the artery and side branches ligated. The specimen was fixed to an imaging rig, pre-warmed (to 37°C) and subsequently pressure-perfused at a constant 100mmHg. Electrocardiographic signals were obtained through use of an automated generator,
permitting capture of VH-IVUS data using 20MHz Eagle-Eye Gold catheters (Volcano Corporation, Rancho Cordova, US), again at a constant motorized pullback of 0.5mm/sec. Specimens were then fixed in formalin, after de-calcification if necessary, with each plaque undergoing histological processing. Slides were digitally imported (NanoZoomer, Hamamatsu, Japan) and stored for subsequent analysis. Careful co-registration between VH-IVUS data and plaques was performed using measurements documented at time of imaging, with the assistance of fiduciary landmarks. Matched histological and VH-IVUS plaques (n=48) were then included in the validation analysis.

Clinical endpoints

Major adverse cardiovascular events (MACE) data were collected by telephone interview and clinic follow-up (median 1,115 days). MACE comprised events driven by presumed plaque rupture, defined as a composite of cardiac death, MI, unplanned revascularization or hospitalization from progressive angina, according to the Braunwald Unstable Angina Classification. An independent review panel composed of four independent cardiologists adjudicated and categorized each clinical event. MACE occurring without angiographic follow-up (n=3) were classified as 'indeterminate' and excluded from analysis. Following adjudication and repeat angiography, MACE were linked to a plaque imaged at the index procedure either at a previously untreated site (NCLs) or an initially treated site (culprit lesion), consistent with previous reports. Overall, 17 events (40.5%) were deemed to be attributable to a culprit lesion at baseline (restenotic) and were excluded from all NCL analysis. The study flow diagram is presented online (Supplemental Figure 1).

IVUS analysis
Independent IVUS analysis was performed by Krakow Cardiovascular Research Institute core laboratory using Volcano Image Analysis Software 3.0.394 (Volcano Corporation). Luminal and external elastic membrane borders were manually corrected, allowing calculation of plaque burden (PB) and luminal area. Positive remodeling on IVUS was defined as a remodeling index of >1.05, being calculated as the external elastic membrane area at the MLA, divided by the mean external elastic membrane of the proximal and distal reference segments. ECG-gated radiofrequency data were recorded at the R-wave peak, permitting identification and quantification of individual plaque components, each displayed as separate colors; fibrous (dark green), fibrofatty (light green), necrotic core (red) and dense calcium (white). VH-defined plaque classification was performed with a plaque (PB>40%) classified as VH-T DFA if >10% confluent necrotic core was in contact with the lumen for 3 consecutive frames13. Full details of the VH-IVUS plaque classification scheme employed are online (Supplemental Methods and Supplemental Figure 2).

Biomechanical analysis
NCLs responsible for MACE (n=22) were matched to control lesions based on plaque characteristics that are known to affect PSS, including luminal area, plaque burden, necrotic core/dense calcium volume and plaque classification. Predicted probabilities for every NCL were determined by logistic regression analysis, allowing propensity matching to be performed between plaques responsible for MACE and a control arm (n=22). Following matching, these two plaque groups then underwent dynamic 2D finite element analysis (FEA) simulations, as described previously11. Vessel geometry and plaque architecture/composition were obtained at diastole (Figure 1A) and imported into dedicated analysis software (proprietary code, MATLAB R2012b, The MathWorks Inc, US), allowing construction of 5,120 segmented plaque models (Figure 1B). Non-uniform circumferential vessel shrinkage
was applied to VH-IVUS images to create a zero-pressure configuration, as required for FEA\textsuperscript{11, 15}. Individual plaque components (n=100,843) were modeled as incompressible, piecewise homogeneous, non-linear isotropic and hyper-elastic as described by the modified Mooney-Rivlin strain energy density function:

\[ W = c_1 (I_1 - 3) + D_1 \left[ \exp \left( D_2 (I_1 - 3) \right) - 1 \right] + \kappa (J - 1) \]

where \( I_1 \) is the first invariant of the modified right Cauchy-Green tensor \( \bar{C} = J^{-2/3} C \); \( J \) is the Jacobian of the deformation gradient tensor, \( C \); \( \kappa \) is the Lagrange multiplier; and \( c_1, D_1 \) and \( D_2 \) are material constants derived from previous experimental work\textsuperscript{16} and include: arterial vessel wall, \( c_1=0.138 \text{ kPa}, D_1=3.833 \text{ kPa}, D_2=18.803 \); fibrous tissue, \( c_1=0.186 \text{ kPa}, D_1=5.769 \text{ kPa}, D_2=18.219 \text{ kPa} \); and necrotic core, \( c_1=0.046 \text{ kPa}, D_1=4.885 \text{ kPa}, D_2=5.426 \). The material properties of dense calcification were derived by fitting a Young’s modulus of 184 MPa based on experimental data\textsuperscript{17}: \( c_1=1.147 \times 10^5 \text{ kPa}, D_1=7.673 \times 10^4 \text{ kPa} \) and \( D_2=2.838 \times 10^{-8} \). The motion of each plaque component was governed by kinetic equations as:

\[ \rho v_{i,t,t} = \sigma_{i,j} \quad (i,j = 1, 2) \]

where \([v_i]\) and \([\sigma_{ij}]\) are the displacement vector and stress tensor, respectively, \( \rho \) = density of each component, and \( t \) = time. The entire plaque model was then meshed using 9-node quadrilaterals (Figure 1C), generating ~40,000 nodes and ~10,000 elements per model. Both displacement and strain were assumed to be large. There was no relative movement at the interface of individual atherosclerotic components and the relative energy tolerance was set to 0.005. Two adjacent points were fixed at the outer wall of the model to prevent rigid body movement. The loading conditions for each simulation were taken from coronary pressure recordings taken during VH-IVUS acquisition, with outer boundary pressure set at zero. FEA simulations were performed using ADINA 8.6.1 (ADINA R&D Inc., Watertown, US). As plaque rupture is thought to result from superficial plaque destabilisation, PSS was calculated
≤200μm depth from the luminal contour, representing the axial resolution of VH-IVUS\textsuperscript{18}. Variation in PSS during one cardiac cycle was also calculated, being defined as,

\[
\text{Variation of PSS}_i = \max(\text{PSS}_i^t) - \min(\text{PSS}_i^t)
\]

in which the subscript \(i\) is the \(i\)\textsuperscript{th} integration node and the superscript \(t\) = time were computed.

All PSS values presented refer to maximum principal stress normalised by coronary pressure (Figure 1D), creating a dimensionless index allowing comparisons between patients\textsuperscript{11}. Researchers performing simulations were blinded both to plaque classification and patient outcome. Intra-class correlation coefficients for PSS absolute agreement on identical VH-IVUS images were excellent for both intra and inter-observer variability (all >0.97, p<0.001).

**Statistical analysis**

Data were assessed for normality using the Shapiro-Wilk test. Normally distributed data are presented as mean (SD or SEM) and compared using an unpaired \(t\) test, with non-normal data as medians [Q1-Q3] and compared using a Mann-Whitney \(U\) test. Categorical variables were analysed using \(\chi^2\) or Fisher's exact tests, where appropriate. Paired analyses were performed using either a paired \(t\) test or McNemar’s test, as appropriate. Cox proportional hazard regression models using a robust sandwich estimator to account for patient clustering were fitted to assess the effect of NCL on MACE. To assess predictive performance, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), with 95% confidence intervals, were calculated for plaque characteristics associated with MACE. As plaques comprise several VH-IVUS frames, linear mixed-effect models were used to compare PSS values between patients and clinical outcomes. Receiver operating characteristic (ROC) curves were calculated by plotting sensitivity versus (1-specificity), allowing calculation of area under the curve (AUC). Integrated PSS and plaque imaging time-to-event data are presented as Kaplan-Meier estimates of cumulative hazard and compared
using a clustering adjusted log-rank method. Power calculations from pilot data suggested that 20 plaques per group were required to detect a 1.3 difference for PSS between MACE and non-MACE plaques (SD 1.7, α=0.05, β=0.1). All calculations were two-tailed with p<0.05 considered statistically significant. Statistical analyses were performed in SPSS 21.0.0 (SPSS Inc., IBM Computing, US), R 2.10.1 (The R Foundation for Statistical Computing) and Stata MP 13.1 (StataCorp LP, US).
RESULTS

Baseline patient characteristics and MACE

The VIVA study enrolled 170 patients with stable angina or ACS who were undergoing PCI for three-vessel VH-IVUS imaging. Overall, 42 MACE occurred in 28 patients over a median of 1,115 days [968-1,537], which included 3 deaths, 6 MIs, 18 unplanned revascularization procedures and 15 hospitalizations due to progressive angina. Patient demographics were similar between patients that did or did not sustain MACE, as presented in Table 1.

Baseline non-culprit lesion characteristics

Baseline IVUS plaque characteristics for the whole NCL lesion cohort (n=931 plaques) and those responsible for MACE are presented in Table 2. On gray-scale IVUS, MACE NCLs had smaller lumens (lower MLA and luminal volume; both p<0.001), and were larger (increased PB and plaque volume; both p<0.001). Plaque composition, defined either by percentage VH-IVUS tissue type or classification, was not significantly different between groups. Plaque-level characteristics associated with long-term MACE on univariate analysis included PB≥70% (HR 8.6, 95%CI 2.5-30.6; p<0.001) and MLA≤4mm² (HR 6.6, 95%CI 2.1-20.1; p=0.001), while VH-TCFA was not statistically significant (HR 3.6, 95%CI 0.8-16.5; p=0.10). However, these higher risk regions had clearly different temporal relationships with MACE. MLA≤4mm² and PB≥70% were associated with MACE throughout the study period, whereas MACE attributable to VH-TCFA appeared only after ~600 days follow-up (Figure 2). Absolute MACE rates for plaques exhibiting PB≥70%, MLA≤4mm² or VH-TCFA were 9.5%, 6.3% and 3.3%, respectively, with PPV of 9.6%, 6.8% and 2.9%, again respectively. Full details of predictive performance for each plaque characteristic are presented in Supplemental Table 1. Overall, 122 plaques (13.1%) exhibited all three high-risk VH
features (PB≥70%+MLA≤4mm²+VH-TCFA), with this combination increasing MACE to 10.7%.

**PSS calculated by VH-IVUS and histology is comparable**

Late follow-up of the VIVA cohort confirms results of previously published prospective studies\(^5\). However, event rates of only ~10% for the highest risk plaques indicate that additional markers are required to improve MACE prediction. We have previously found that PSS estimated from VH-IVUS is increased in patients presenting with ACS vs. stable angina\(^11\), suggesting that PSS may be increased in unstable lesions and might help predict future MACE.

To assess whether VH-IVUS can identify plaques with high PSS, we first estimated PSS in 48 plaques imaged at autopsy by VH-IVUS and compared these with co-registered histology specimens (**Supplemental Figure 3**), including 16 (33.3%) classified as fibroatheromas\(^4\). PSS values derived from histology were located superficially in 81.3% plaques, consistent with a role for PSS in superficial plaque rupture. Although histology-derived PSS was higher than VH-IVUS-derived PSS (mean: 1.67 [1.24-2.21] vs. 1.34 [1.01-1.69], p<0.001 and 95\(^{th}\) centile: 4.84 [3.42-6.26] vs. 3.53 [3.10-4.02], p<0.001), PSS derived from VH-IVUS showed a moderate and positive correlation with PSS derived from histology (mean: r\(_S\)=0.57, p<0.001 and 95\(^{th}\) centile: PSS r\(_S\)=0.41, p=0.004)(**Supplemental Figure 4**). In addition, there was no significant difference in either mean (1.16 [0.77-1.48] vs. 1.00 [0.84-1.31], p=0.92) or 95\(^{th}\) centile PSS (3.62 [2.48-4.50] vs. 3.58 [3.01-3.91], p=0.93) between PSS derived from VH-IVUS or histology in fibroatheroma, maintaining the positive correlation (r\(_S\)=0.56, p=0.02).

Thus, PSS derived from VH-IVUS provides reliable estimates of stress experienced by coronary plaques *in vivo.*
Plaque structural stress is increased in MACE lesions

We next examined PSS in NCL plaques responsible for MACE (n=22) compared with control (non-MACE) plaques (n=22). Plaques were matched based on their VH-IVUS composition and classification as shown in Table 2. PSS was determined throughout whole plaques and within discrete, pre-defined, higher-risk regions (e.g. PB≥70%). There were marked differences in PSS in higher-risk regions between plaques, and PSS varied markedly even within a higher-risk region of the same plaque (Figure 3). These findings indicate that PSS is determined by differences in plaque composition and geometry even within the same lesion, and does not just reflect plaque size or lumen area. Despite this heterogeneity, PSS was significantly increased in MACE plaques compared with non-MACE controls at specific high-risk regions, including PB≥70% (mean±SEM 13.9±0.66 vs. 10.2±0.24, p<0.001) and in VH-TCFA (14.0±0.28 vs. 11.6±0.12, p=0.02)(Table 3). In contrast, PSS was similar at MLA≤4mm² sites or within other plaque regions (both p>0.05).

Plaque structural stress significantly improves prediction of MACE

These findings suggest that PSS is not just a surrogate for geometric features of a plaque, and might discriminate between NCLs that do or do not cause MACE, for example, those higher risk regions that also have high PSS. To assess whether PSS improves the ability to predict MACE when combined with VH-IVUS, we performed a lesion-based analysis in 10% of the NCL population (96 plaques). Using a separate developmental cohort (n=20), PSS was calculated for each high-risk plaque region defined by VH-IVUS (PB≥70%, MLA≤4mm² and VH-TCFA) and ROC analysis performed to determine cut-off points for PSS to predict MACE (Supplemental Figure 5). Time-to-event curves, adjusted for the presence of multiple plaques within a patient, confirmed that PSS markedly increased the ability of VH-
IVUS to predict MACE in plaques with PB≥70% (p=0.003) and MLA≤4mm² (p=0.002) (Figure 4). A similar pattern, albeit not statistically significant (p=0.22), was observed for plaques classified as VH-TCFA.

Influence of plaque composition on plaque structural stress

Our biomechanical analysis shows a difference in PSS values between lesions responsible for MACE vs. control plaques. As plaques were matched for higher risk features (PB, MLA, VH-TCFA), other features that increase PSS must be responsible. We therefore studied the sub-plaque architecture within these regions to determine the microstructural differences that may be responsible, focusing on features that increase risk of rupture, including necrotic core (NC) and microcalcification¹⁹ (Figure 5). Mean or maximal component NC areas were similar within PB≥70% or VH-TCFA regions in MACE vs. non-MACE plaques (Supplemental Table 2). However, MACE plaques exhibited larger superficial dense calcium inclusions in both PB≥70% (0.16±0.47 vs. 0.13±0.31mm², p=0.047) and VH-TCFA (0.28±0.57 vs. 0.20±0.41mm², p=0.049).
DISCUSSION

Atherosclerosis plaques are typically demonstrable at different stages of development throughout the coronary tree. Consequently, NCLs greatly outnumber culprit lesions in patients undergoing PCI, and underlie the majority of subsequent MACE. Although VH-IVUS can identify NCLs at higher-risk of causing future events, the low event rates observed in prospective studies suggest that factors additional to plaque composition determine rupture. PSS is a proposed driver of rupture that integrates plaque anatomy and composition with physical forces that plaques experience, but has been largely neglected in clinical studies. We therefore examined whether PSS could discriminate those NCL that generate future MACE from those that do not, and could therefore guide therapies to prevent plaque rupture.

There are several important and novel features in this study that complement and enhance our understanding of coronary plaque behavior. First, we confirm previous observations that lesions with MLA≤4mm² and PB≥70% on IVUS are associated with long-term MACE. Second, PSS values derived from VH-IVUS and histology correlate well, suggesting that VH-IVUS-derived PSS is biologically meaningful and of potential clinical use. Third, plaques responsible for MACE have increased PSS values in higher-risk regions compared with matched controls, most marked at PB≥70%. Fourth, PSS is not homogenous across higher risk regions, and does not track with features such as PB or MLA. Fifth, calculation of PSS has potential to improve the ability of VH-IVUS to predict MACE. Finally, increased PSS is associated with plaque microarchitecture, particularly larger superficial calcium inclusions.
Although we observed an association between baseline VH-IVUS plaque features and long-term clinical events, absolute rates were low, suggesting that while intra-coronary imaging may identify the substrate for rupture, morphology alone is insufficient to identify plaques at highest risk of MACE. In contrast, mechanical forces have been hypothesised as determinants of plaque rupture\(^5\), and factors that increase PSS including hypertension, strenuous exercise or emotional stimuli, also increase risk of cardiovascular events\(^{20,21}\). However, PSS has been largely overlooked or excluded from clinical studies, most likely due to limitations in imaging resolution and concerns that values obtained may be incorrect. Our study is the first to show that PSS estimates from VH-IVUS are highly reproducible and correlate positively with values derived from histology. More importantly, our study suggests that plaques exposed to high structural stresses \textit{in vivo} can be identified correctly.

Our most important finding is that lesions resulting in MACE had significantly higher baseline PSS at specific plaque regions (e.g. PB\(\geq\)70\%) compared with matched controls. Although previous studies showed increased PSS in plaques responsible for MI or sudden cardiac death\(^{7,11}\), this is the first report showing that increased coronary PSS may occur prior to clinical events in higher-risk lesions. Plaque rupture results from failure of the fibrous cap, indicating that factors acting directly on the cap or within the superficial plaque are more likely to promote rupture\(^{22}\). Indeed, we find that superficial (but not deep) PSS is associated with MACE. Recent trials have shown that NCL intervention can improve outcomes\(^{23}\), although treatment was based on angiographic stenosis, which may miss high-risk lesions, or over-treat low-risk lesions. In contrast, integration of PSS with VH-IVUS improved our ability to predict future MACE by 2-3 fold, suggesting that PSS may represent a novel and complementary parameter to plaque imaging in predicting future NCL events. This incremental gain in predictive accuracy could identify patients with a high burden of high-
stress plaques, and direct more intense pharmacotherapy or possibly even protective intervention.

PSS estimation assimilates several variables critical to plaque stability, including architecture, tissue material properties, luminal geometry, and patient hemodynamics. Although changes to these factors can alter PSS, the microstructural differences responsible for MACE were unknown. Indeed, PSS appears independent of other higher risk features, as increasing PB or reducing luminal area does not increase PSS\textsuperscript{11}. In contrast, we find that PSS varies markedly both between and within higher-risk plaques. Furthermore, MACE lesions had increased areas of superficial calcification that can act as 'stress amplifiers' depending on size, orientation and relative location to one another\textsuperscript{22}. Thus, plaque microstructure influences PSS, and the overall risk of rupture for each lesion may be influenced by small changes in plaque composition. Established plaque classification algorithms rely on summary statistics to describe anticipated behavior of individual lesions, but this dilutes the overall effect of discrete, subtle changes in plaque structure that increase risk of rupture. In contrast, estimation of PSS at specific higher-risk plaque regions may represent an objective method to quantify these microstructural differences and determine risk of rupture.

Our study has some limitations. First, although consistent with other studies, MACE numbers were relatively low; however, low MACE rates identified by imaging alone are the major driver for additional modalities to predict risk. Second, proportional hazards models were all performed at a ‘lesion-level’ and the regression estimates presented apply to individual plaques and not patients. Third, other pathological processes may result in rapid plaque growth and MI, including plaque erosions and calcified nodules and the role of PSS in these processes is unknown. Fourth, the overall calculation of PSS relies on accurate identification
of plaque morphology, and debate continues regarding the accuracy of VH-IVUS. However, independent \textit{ex vivo} validation has confirmed that VH-IVUS-defined plaque classification is reliable\textsuperscript{14}, the biological importance of VH-IVUS-defined plaque subtypes has been validated in prospective studies\textsuperscript{5, 13, 24}, and VH-IVUS-derived PSS correlated well with histology-derived PSS. Fifth, the incremental benefit of combined FEA/VH-IVUS was conducted in 10\% of NCLs imaged at baseline and validation of these findings in a larger cohort should be performed. Finally, biomechanical simulations were performed in 2D without modeling residual stresses or blood flow. However, residual stresses are not currently quantifiable \textit{in vivo}, and although endothelial shear stress may affect plaque development, it is $10^{-5}$ times smaller than PSS and unlikely induce plaque rupture\textsuperscript{25}.

**CONCLUSIONS**

We show that atherosclerotic plaques resulting in MACE show increased PSS compared with matched controls, and PSS estimates markedly improve the ability of VH-IVUS to predict MACE. Our results suggest that biomechanical modeling may complement plaque imaging risk stratification for coronary NCLs.
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**CONFLICT OF INTEREST**
None declared.
CLINICAL PERSPECTIVE

Coronary plaque rupture underlies the majority of myocardial infarctions (MI), with repeated cycles of subclinical rupture and repair driving rapid plaque growth. Although ruptured plaques have typical morphological features that can be reliably identified by intracoronary imaging, prospective studies have consistently shown low future major adverse cardiovascular event (MACE) rates attributable to such lesions. Thus, novel strategies to improve plaque risk-stratification are urgently required. Plaque structural stress (PSS) is known to be higher in patients presenting with MI, with elevated levels thought to promote rupture. In this study baseline non-culprit plaque features were determined in 170 patients undergoing three-vessel virtual-histology intravascular ultrasound (VH-IVUS) imaging. MACE was associated with lesions that had plaque burden (PB) ≥70% and minimal luminal area (MLA) ≤4mm². However, absolute event rates for the highest-risk lesions identified by VH-IVUS remained <10%. However, PSS was increased at specific plaque regions in plaques responsible for MACE versus matched controls, including PB≥70% and thin-cap fibroatheroma. Furthermore, PSS improved the ability of VH-IVUS to predict MACE in plaques with PB≥70% and MLA≤4mm². Finally, we show that plaques responsible for MACE had larger superficial calcium inclusions, which acted to increase PSS. Our results suggest that integration of PSS with existing plaque imaging may improve our ability to identify those plaques at highest risk of rupture and subsequent adverse clinical events.
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FIGURE LEGENDS

Figure 1. Calculation of plaque structural stress

(A) Virtual-histology intravascular ultrasound image identifying plaque components as white (dense calcium), red (necrotic core), green (fibrous) and light green (fibrofatty). (B-C) Segmented plaque model (B) complete with magnified imaged of fine mesh (C) demonstrating the basis for finite element analysis. (D) Final band plot of plaque structural stress (PSS) identifying regions of high stress within the plaque.

Figure 2. Time-to-event curves for major adverse cardiovascular event rates according to baseline plaque characteristics

(A-C) Kaplan-Meier curves for MACE rates in non-culprit lesions based on virtual-histology (VH) intravascular ultrasound features including plaque burden (PB) ≥70% (A), minimal luminal area (MLA) ≤4mm² (B), thin-cap fibroatheroma (VH-TCFA)(C).

Factors compared using adjusted Log-Rank test

Figure 3. Longitudinal profiles of plaque burden and structural stress

(A-B) Relationship between longitudinal plaque burden (PB) and plaque structural stress (PSS) within two coronary plaques. The plaque responsible for a major adverse cardiovascular event (MACE)(A) displays increased PSS associated with high PB, while the non-MACE plaque (B) exhibits low PSS despite PB being high.

Figure 4. Time-to-event curves illustrating the incremental benefit of plaque structural stress calculations within high-risk plaques
(A-C) Kaplan-Meier curves illustrating the incremental predictive benefit of plaque structural stress (PSS) calculations in lesions with plaque burden ≥70% (A), minimal luminal area (MLA) ≤4mm² (B) and virtual-histology thin-cap fibroatheroma (VH-TCFA)(C).

Factors compared using adjusted Log-Rank test

Figure 5. Effect of plaque composition on plaque structural stress

Two band plots (A and C) of the same virtual-histology intravascular ultrasound image, one with all plaque components (A and B) and one with superficial dense calcium and necrotic core inclusions removed for illustrative purposes (C and D). Plaque structural stress (PSS) is reduced by up to a third and the overall region of high stress (red colored areas on band plot) altered, after removal of these superficial structures.
Table 1.
Baseline patient clinical characteristics

<table>
<thead>
<tr>
<th></th>
<th>Overall (n=170)</th>
<th>MACE (n=28)</th>
<th>Non-MACE (n=142)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs</td>
<td>62.3 (10.4)</td>
<td>61.9 (12.2)</td>
<td>62.4 (10.1)</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>131 (77)</td>
<td>21 (75)</td>
<td>110 (78)</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>87 (51)</td>
<td>14 (50)</td>
<td>73 (51)</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>23 (14)</td>
<td>5 (18)</td>
<td>18 (13)</td>
</tr>
<tr>
<td>Smoker, n (%)</td>
<td>35 (21)</td>
<td>3 (11)</td>
<td>32 (23)</td>
</tr>
<tr>
<td>Previous MI, n (%)</td>
<td>18 (11)</td>
<td>4 (14)</td>
<td>14 (10)</td>
</tr>
<tr>
<td>ACS presentation, n (%)</td>
<td>70 (41)</td>
<td>9 (32)</td>
<td>61 (43)</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.4 [3.8-5.3]</td>
<td>4.4 [3.5-5.8]</td>
<td>4.4 [3.9-5.3]</td>
</tr>
<tr>
<td>LDL, mmol/L</td>
<td>2.3 [1.7-2.7]</td>
<td>2.2 [1.4-2.8]</td>
<td>2.3 [1.8-2.7]</td>
</tr>
<tr>
<td>HDL, mmol/L</td>
<td>1.1 [0.9-1.3]</td>
<td>1.1 [0.9-1.3]</td>
<td>1.1 [0.9-1.3]</td>
</tr>
<tr>
<td>Serum creatinine, mmol/L</td>
<td>88.5 (19.9)</td>
<td>89.8 (19.5)</td>
<td>88.2 (20.1)</td>
</tr>
<tr>
<td>FRS, %</td>
<td>20.5 (12.6)</td>
<td>19.4 (12.2)</td>
<td>20.8 (12.7)</td>
</tr>
</tbody>
</table>

ACS, acute coronary syndrome; FRS, Framingham Risk Score for 10-year estimated risk of total cardiovascular disease; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MACE, major adverse cardiovascular events; MI, myocardial infarction

Data are presented as mean (SD) or median [25th-75th percentile], as appropriate.
Table 2

Baseline grey-scale and virtual-histology intravascular ultrasound characteristics for non-culprit lesions

<table>
<thead>
<tr>
<th></th>
<th>All NCL (n=931)</th>
<th>MACE NCL† (n=22)</th>
<th>Non-MACE NCL‡ (n=909)</th>
<th>PSS NCL controls # (n=22)</th>
<th>p-value † vs. ‡</th>
<th>p value † vs. #</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gray-scale IVUS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MLA (mm²)</td>
<td>5.62 [3.58-8.58]</td>
<td>3.00 [2.20-3.79]</td>
<td>5.70 [3.62-8.61]</td>
<td>3.16 [2.30-4.21]</td>
<td>&lt;0.001</td>
<td>0.50</td>
</tr>
<tr>
<td>MLA≤4mm² (%)</td>
<td>27.0</td>
<td>77.3</td>
<td>27.6</td>
<td>81.8</td>
<td>&lt;0.001</td>
<td>1.00</td>
</tr>
<tr>
<td>Luminal volume, (mm³)</td>
<td>53.1 [24.6-125.5]</td>
<td>93.0 [75.0-250]</td>
<td>51.9 [24.5-124.7]</td>
<td>124.0 [68.0-301.0]</td>
<td>&lt;0.001</td>
<td>0.93</td>
</tr>
<tr>
<td>Plaque volume (mm³)</td>
<td>50.9 [21.1-129.5]</td>
<td>187.0 [107.5-344.5]</td>
<td>50.1 [20.9-126.4]</td>
<td>216.0 [96.0-359.0]</td>
<td>&lt;0.001</td>
<td>0.98</td>
</tr>
<tr>
<td>Plaque burden (%)</td>
<td>48.1 [44.2-53.4]</td>
<td>58.0 [56.0-61.0]</td>
<td>48.0 [44.1-53.2]</td>
<td>57.7 [52.5-61.1]</td>
<td>&lt;0.001</td>
<td>0.50</td>
</tr>
<tr>
<td>PB ≥70% (%)</td>
<td>20.2</td>
<td>81.8</td>
<td>18.7</td>
<td>86.4</td>
<td>&lt;0.001</td>
<td>0.77</td>
</tr>
<tr>
<td>RI &gt;1.05 (%)</td>
<td>27.3</td>
<td>63.6</td>
<td>26.5</td>
<td>68.2</td>
<td>&lt;0.001</td>
<td>1.00</td>
</tr>
<tr>
<td><strong>Virtual-histology IVUS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibrous tissue volume (mm³)</td>
<td>15.4 [6.0-44.3]</td>
<td>63.6 [35.9-111.1]</td>
<td>18.3 [6.6-50.0]</td>
<td>80.2 [30.4-111.9]</td>
<td>&lt;0.001</td>
<td>0.98</td>
</tr>
<tr>
<td>Fibrofatty tissue volume (mm³)</td>
<td>2.9 [0.9-9.5]</td>
<td>13.6 [7.5-39.1]</td>
<td>3.3 [1.0-10.7]</td>
<td>14.7 [4.9-26.2]</td>
<td>&lt;0.001</td>
<td>0.90</td>
</tr>
<tr>
<td>Necrotic core volume (mm³)</td>
<td>5.3 [1.8-17.3]</td>
<td>19.6 [10.4-39.1]</td>
<td>5.8 [1.9-19.8]</td>
<td>29.4 [10.6-60.0]</td>
<td>0.001</td>
<td>0.85</td>
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<tr>
<td>Dense calcium volume (mm³)</td>
<td>2.0 [0.5-6.7]</td>
<td>5.5 [2.1-17.3]</td>
<td>2.1 [0.5-7.6]</td>
<td>14.1 [3.6-26.4]</td>
<td>0.003</td>
<td>0.55</td>
</tr>
<tr>
<td>Fibrous tissue (%)</td>
<td>58.9 [50.0-66.5]</td>
<td>53.4 [45.5-63.8]</td>
<td>59.0 [50.4-66.4]</td>
<td>55.8 [47.9-63.6]</td>
<td>0.31</td>
<td>0.87</td>
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<tr>
<td>Fibrofatty tissue (%)</td>
<td>10.0 [6.0-16.6]</td>
<td>12.0 [7.1-23.2]</td>
<td>10.3 [6.2-16.6]</td>
<td>12.4 [8.9-19.4]</td>
<td>0.16</td>
<td>0.79</td>
</tr>
<tr>
<td>Dense calcium (%)</td>
<td>7.2 [3.5-13.1]</td>
<td>7.0 [2.1-16.9]</td>
<td>7.1 [3.4-12.9]</td>
<td>7.0 [4.7-14.4]</td>
<td>0.40</td>
<td>0.61</td>
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<tr>
<td>VH classification, (%)</td>
<td></td>
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<td></td>
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<tr>
<td>TCFA</td>
<td>60.3</td>
<td>72.7</td>
<td>60.0</td>
<td>77.3</td>
<td>0.56</td>
<td>0.74</td>
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<tr>
<td>ThCFA</td>
<td>15.7</td>
<td>13.6</td>
<td>15.7</td>
<td>9.1</td>
<td>9.1</td>
<td>9.1</td>
</tr>
<tr>
<td>FCa</td>
<td>9.9</td>
<td>9.1</td>
<td>9.9</td>
<td>4.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PIT</td>
<td>14.2</td>
<td>4.5</td>
<td>14.4</td>
<td>9.1</td>
<td></td>
<td></td>
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</tbody>
</table>
FCa, fibrocalcific; MACE, major adverse cardiovascular events; MLA, minimal luminal area; NCL, non-culprit lesion; PB, plaque burden; PIT, pathological intimal thickening; PSS, plaque structural stress; PSS NCL controls, plaque structural stress non-culprit lesion control group; RI, remodeling index; TCFA, thin-cap fibroatheroma; ThCFA, thick-cap fibroatheroma

Data are compared using linear mixed effect models to account for clustering of plaques within patients. Matched data are compared using paired t-test or McNemar’s test, as appropriate.

Data are presented as median [25\textsuperscript{th}-75\textsuperscript{th} percentiles].
Table 3.

Plaque structural stress in lesions responsible for major adverse cardiovascular events and controls

<table>
<thead>
<tr>
<th></th>
<th>PSS</th>
<th>Variation of PSS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MACE</td>
<td>Non-MACE</td>
</tr>
<tr>
<td></td>
<td>(n=22)</td>
<td>(n=22)</td>
</tr>
<tr>
<td><strong>PB ≥70%</strong></td>
<td>13.9 ± 0.66</td>
<td>10.2 ± 0.24</td>
</tr>
<tr>
<td><strong>MLA ≤4mm²</strong></td>
<td>13.3 ± 0.35</td>
<td>10.7 ± 0.20</td>
</tr>
<tr>
<td><strong>VH-TCFA</strong></td>
<td>14.0 ± 0.28</td>
<td>11.6 ± 0.12</td>
</tr>
<tr>
<td><strong>Other plaque regions</strong></td>
<td>12.4 ± 0.14</td>
<td>12.1 ± 0.18</td>
</tr>
</tbody>
</table>

*MACE, major adverse cardiovascular events; MLA, minimal luminal area; PB, plaque burden; VH-TCFA, virtual-histology thin-cap fibroatheroma.*

Comparisons between groups are performed using linear mixed-effects models.

Data are presented as mean ± SEM.
Figure 1. Calculation of plaque structural stress
(A) Virtual-histology intravascular ultrasound image identifying plaque components as white (dense calcium), red (necrotic core), green (fibrous) and light green (fibrofatty). (B-C) Segmented plaque model (B) complete with magnified imaged of fine mesh (C) demonstrating the basis for finite element analysis. (D) Final band plot of plaque structural stress (PSS) identifying regions of high stress within the plaque.
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(A-C) Kaplan-Meier curves for MACE rates in non-culprit lesions based on virtual-histology (VH) intravascular ultrasound features including plaque burden (PB) ≥70% (A), minimal luminal area (MLA) ≤4mm² (B) and thin-cap fibroatheroma (VH-TCFA)(C).
Factors compared using adjusted Log-Rank test
Figure 3. Longitudinal profiles of plaque burden and structural stress
(A-B) Relationship between plaque burden (PB) and plaque structural stress (PSS) longitudinally within two coronary plaques. The plaque responsible for a major adverse cardiovascular event (MACE) (A) displays increased PSS associated with high PB, while in contrast the non-MACE plaque (B) exhibits low PSS despite PB being high.
Figure 4. Time-to-event curves illustrating the incremental benefit of plaque structural stress calculations within high-risk plaques

(A-C) Kaplan-Meier curves illustrating the incremental predictive benefit of plaque structural stress (PSS) calculation in lesions with plaque burden ≥70% (A), minimal luminal area (MLA) ≤4mm² (B) and virtual-histology thin-cap fibroatheroma (VH-TCFA) (C).

Factors compared using adjusted Log-Rank test
Figure 5. Effect of plaque composition on plaque structural stress
Two band plots (A and C) of the same virtual-histology intravascular ultrasound image, one with all plaque components (A and B) and one with superficial dense calcium and necrotic core inclusions removed for illustrative purposes (C and D). Plaque structural stress (PSS) is reduced by up to a third and the overall region of high stress (red colored areas on band plot) altered, simply by removal of these superficial structures.