Coronary Endothelium-Dependent Vasoreactivity and Atheroma Volume in Subjects with Stable, Minimal Angiographic Disease versus Non-ST Segment Elevation Myocardial Infarction: An Intravascular Ultrasound Study

Puri et al: Coronary Plaque Burden and Endothelial Function

Rishi Puri,1,2 MBBS; Stephen J. Nicholls,1,3 MBBS, PhD; Steven E. Nissen,2 MD; Danielle M. Brennan,4 MS; Jordan Andrews,4 BS; Gary Y. Liew,1 MBBS, PhD; Adam J. Nelson,1 MBBS; Angelo Carbone,1 MMedSc; Barbara Copus,4 RN; E. Murat Tuzcu,2 MD; John F. Beltrame,1 BMBS, PhD; Stephen G. Worthley,1,5 MBBS, PhD; Matthew I. Worthley,1,5 MBBS, PhD

1Discipline of Medicine, University of Adelaide, South Australia; 2Department of Cardiovascular Medicine, Cleveland Clinic, Ohio; 3South Australian Health and Medical Research Institute, University of Adelaide, South Australia; 4C5Research, Cleveland Clinic, Ohio; 5Cardiovascular Investigation Unit, Royal Adelaide Hospital, South Australia

Correspondence to:
Associate Professor Matthew I. Worthley
Department of Medicine, Cardiovascular Research Centre
Royal Adelaide Hospital
Level 6, Theatre Block
North Terrace, Adelaide, SA, Australia (5000)
F: +61 8 2222454
P: +61 8 82224000
E: matthew.worthley@adelaide.edu.au

DOI: 10.1161/CIRCIMAGING.113.000460

Journal Subject Codes: [150], [29], [95], [135]
Abstract

**Background**—Epicardial plaque burden and endothelial function are recognized predictors of coronary events. We aimed to investigate mechanistic relationships between atheroma volume and endothelial function in patients with non-ST-segment elevation myocardial infarction (NSTEMI) utilizing intravascular ultrasound (IVUS).

**Methods and Results**—In coronary vessels of patients with near normal or minimal angiographic disease (n=23) and NSTEMI (n=24), IVUS-derived measures [percent atheroma volume (PAV)], arterial remodeling index (RI) and segmental lumen volumes (SLV) were performed in contiguous 5-mm epicardial segments. Repeat IVUS imaging was performed following consecutive 5-minute intracoronary (IC) infusions (vehicle solution, 0.30 μg/min and 0.60 μg/min IC salbutamol) to measure changes in SLV (endothelium-dependent function). Male gender, diabetes, smoking, higher triglycerides and lower HDL-C were more prevalent in the NSTEMI group. Patients with NSTEMI demonstrated greater segmental PAV (40.4±12 vs 27.5±14%, p<0.001), RI [1.2 (1.0, 1.5) vs 1.0 (0.9, 1.0), p<0.001] and displayed less endothelium-dependent vasomotion (%change SLV: 2.1±0.89 vs 5.1±0.89%, p=0.02) compared to patients with minimal angiographic disease. No significant difference in endothelial function between both groups was observed when controlling for plaque burden. Multivariate analysis for change in SLV identified PAV (β=-0.18, p=0.0004), high sensitivity C-reactive protein >2mg/L (β=-3.1, p=0.03), diabetes (β=-6.9, p<0.0001), LDL-C levels (β=-0.04, p=0.01) and smoking (β=-3.2, p=0.01) as independent associates.

**Conclusions**—Although coronary endothelial vasoreactivity is blunted in the setting of NSTEMI, this is a reflection of the greater volume of atherosclerosis and cardiovascular risk factors. Thus the relationship between coronary endothelium-dependent vasomotor reactivity and atheroma volume remains constant irrespective of the nature of the clinical presentation.

**Key Words:** plaque burden; endothelial function; intravascular ultrasound; salbutamol; adrenergic receptor; NSTEMI
The dynamic interplay between coronary endothelial dysfunction and epicardial plaque burden is thought to play a pivotal role in the pathogenesis of acute coronary syndromes. The degree of endothelial dysfunction has been found to be greater in the setting of acute coronary syndromes, compared to a stable clinical presentation. As a result, our traditional understanding is that enhanced vessel wall reactivity in unstable patients is activated, at least in part, by systemic inflammatory mechanisms, in concert with local plaque-related features.

Several investigators have reported the association of plaque burden with disease progression and incident coronary events. Similarly, the prognostic significance of coronary arterial vasoreactivity has also been well described, whereby endothelial dysfunction, shown to occur in a segmental fashion, independently associated with future cardiovascular events. A better understanding of the relationship between plaque burden and corresponding segmental vasomotor reactivity may be important in elucidating mechanisms underlying plaque progression and instability.

In a recent intravascular ultrasound (IVUS) study that validated intracoronary (IC) salbutamol (selective beta2-adrenoreceptor agonist) as a novel endothelium-dependent vasomotor stimulus, an exploratory sub-analysis revealed a significant inverse relationship between segmental plaque burden and corresponding endothelium-dependent lumen vasoreactivity. As such, the chief aim of this study was to utilize IVUS to further explore this relationship in the setting of non ST-segment elevation myocardial infarction (NSTEMI), and to compare and contrast these findings in stable patients with near normal or no significant angiographic coronary disease. Our primary hypothesis was that for each unit measure of IVUS-derived coronary atheroma volume, NSTEMI patients would display a difference in endothelial function compared with stable subjects with near normal coronary arteries.
Methods

Study subjects

We enrolled forty-seven patients (aged ≥ 18 yrs) referred for a clinically indicated coronary angiogram. Our ‘near normal’ cohort was defined by those patients with normal, or minimally detectable (<30% visual angiographic stenosis) coronary disease throughout the entire epicardial tree, and who were troponin T negative. This group comprised of consecutive patients undergoing coronary angiography for investigation of chest pain/chest pain equivalent syndromes, and were felt to represent a population being as close to ‘normal’ as deemed ethically possible undergoing an invasive study. The chosen vessel for IC provocation and imaging in this group of patients was invariably the longest vessel containing numerous side-branches (for ease of anatomic matching) and minimal tortuosity, for more stable IVUS imaging. In parallel, a separate group of twenty-three consecutive patients admitted to hospital with chest discomfort in concert with a significant elevation in troponin T with/without ST-segment depression/T-wave inversion, were classified as NSTEMI, and were considered for study enrolment. In these patients, the invasive research protocol was performed in a non-critically diseased (< 30% angiographic stenosis), non-culprit (‘study’) vessel, prior to percutaneous coronary intervention within the culprit vessel, adopting the same angiographic inclusion/exclusion criteria for selection of the study vessel as the stable, near-normal cohort. Following informed consent, vasoactive medications were held for at least 24 hrs prior to the invasive study. In the NSTEMI group, we only included patients who were pain free following hospitalisation and initial medical stabilization, and had no form of nitrate therapy in the 12-hours prior to their angiogram. All procedures were performed in the morning prior to an overnight fast. Exclusion criteria included significant valvular heart disease, left ventricular dysfunction (known ejection fraction ≤ 40%),
prior percutaneous or surgical revascularization, acute coronary syndrome within the preceding 4
weeks (in the near normal cohort), known coronary spasm, severe obstructive lung disease,
creatinine clearance \( \leq 60 \text{ mL/min} \), \( \beta \)-blocker use in the preceding 24 hrs, or the use of short or
long acting \( \beta_2 \) agonists within the previous 12 hrs. This study was approved by the Royal
Adelaide Hospital Human Research Ethics Committee.

**Cardiac catheterization and intravascular imaging protocols**

Coronary angiography was performed via a standard 6-French technique. Intravenous heparin
(70 IU/kg) was administered for the research protocol. A 0.014-inch coronary guide wire was
placed into the study vessel within its mid-segment away from major side-branches. This wire
was also used to deliver a 2.5-French 40-MHz Atlantis Pro IVUS catheter (Boston Scientific,
Natick, MA, USA) into the study artery. This was undertaken without pre-treatment with IC
nitroglycerin. If percutaneous coronary intervention was to be performed to a culprit lesion, this
was done immediately following the invasive research protocol. All IC infusions were
administered through an infusion pump at 2 mL/min via the coronary guiding catheter for a
period of 5-minutes. Following 3-minutes of IC infusion (of either vehicle solution or
salbutamol), the IVUS catheter was then moved from within the guiding catheter into the distal
conduit vessel and images were acquired during automated catheter withdrawal at 0.5 mm/sec.

Our previous validation study showed that repeated, consecutive, IC vehicle infusions over 5-
minutes during IC instrumentation with IVUS has no significant impact on changes in lumen
measurements over time \(^{11}\). The IVUS images were recorded on a DVD for off-line analysis.

**Coronary infusion and endothelial function testing protocols**

The infusion protocols that were performed for the validation of IC salbutamol as an
endothelium-dependent coronary vasomotor stimulus have been previously described in detail \(^{11}\).
Intracoronary salbutamol was chosen over IC acetyl choline (ACh). In patients with NSTEMI undergoing repeated IVUS-imaging without pre-treatment with IC nitroglycerin, there was concern regarding the possibility of inducing clinically significant coronary spasm with an ACh-based protocol. Moreover, a series of in vivo human observations have implicated coronary β2-adrenoceptor stimulation to cause nitric-oxide (NO)-mediated peripheral and coronary arterial vasomotor responses. However, underlying mechanisms for β2-adrenergic endothelial NO-release appear distinct to those following ACh stimulation. It is likely that β2-adrenergic signalling may involve similar actions upon adenylate cyclase to endothelial prostaglandins, direct stimulation of endothelial NO-synthase, and/or potassium channel activation to induce NO-synthase. For the purpose of further exploring the relationship between epicardial plaque burden and segmental β2-adrenergic mediated endothelium-dependent vasoreactivity, our endothelial function testing protocol involved following baseline IC 5% dextrose (vehicle) infusion] sequential 5-minute infusions of IC salbutamol at the doses of 0.30 µg/min and 0.60 µg/min respectively (Figure 1D).

Data acquisition and analysis

Analysis of IVUS data was performed by the Atherosclerosis Imaging Core Laboratory, Cleveland Clinic, according to prior experience and published guidelines. Technicians were blinded to clinical details and imaging sequence. Proximal and distal fiduciary markers (anatomical side-branches) were chosen to define the overall region of vessel to be analysed, as well as for segment matching. Cross-sectional images were selected every 30 frames (0.5-mm apart). Frames that precluded complete lumen or vessel wall planimetry were excluded from analysis, as were segments that involved branch points. Each IVUS pullback was divided into 5-mm segments comprising of 11 frames taken at 10 evenly spaced cross-sectional (0.5-mm)
intervals (Figure 1A-C). Given the known segmental heterogeneity of coronary vasomotor reactivity, each segment was thus analysed separately as an individual entity, with appropriately utilized statistical methods. Matching frames of anatomical side-branches from the baseline and post-salbutamol stimulation IVUS runs were co-registered to ensure accurate segment matching between runs. Leading edges of the lumen and external elastic membrane (EEM) were manually planimetered. Percent atheroma volume (PAV) was calculated to determine segmental plaque burden:

\[
\text{PAV} = \sum \left( \frac{(\text{EEM}_{\text{area}} - \text{Lumen}_{\text{area}})}{\sum \text{EEM}_{\text{area}}} \right) \times 100
\]

Segmental lumen volumes (SLV) were calculated as the summation of lumen area in each measured image. As some frames were technically inadequate for complete IVUS analysis, the SLV for each 5-mm segment was normalised to account for differences in the number of analysable frames within each pre-defined segment, as previously described:

\[
\text{SLV}_{\text{normalised}} = \sum \left( \frac{\text{Lumen}_{\text{area}}}{\text{number of analysable images in segment}} \right) \times 100
\]

Segmental remodeling and eccentricity indices were also calculated, as previously described. Briefly, segmental remodeling indices (RI) were determined by calculating the average segmental EEM\text{area} and dividing this by a reference EEM\text{area} taken from either a proximal or distal reference point located within 10-mm from the index segment with the least plaque burden, or before a major branch point. Segmental eccentricity indices (EI) were determined by calculating the average of all EI’s of each analysable frame within a coronary segment (EI= ratio of maximal to minimal plaque thickness). All measurements were performed by a single analyst blinded to the specific infusion sequence. Intra- and inter-observer variability analysis was performed following planimetry of lumen and plaque areas from 20 randomly selected IVUS
frames by two-independent observers and by one observer at two time points separated by 1 week.

**Observer variability**

For coronary lumen measurements, the intra-observer coefficient of variation was 1.1%, and inter-observer coefficient of variation was 2.6%. For plaque measurements, the intra-observer coefficient of variation was 1.8%, and the inter-observer coefficient of variation was 3.8%.

**Statistical analysis**

Data are expressed as mean ± standard deviation (SD) or median and 25\(^{\text{th}}\) and 75\(^{\text{th}}\) percentiles, as appropriate, for continuous data. Categorical data are presented as a percent of non-missing data. Comparisons between the near normal and NSTEMI groups were made using Student’s t-test for continuous data (or Wilcoxon-rank sum for non-normally distributed data) and chi-square tests for categorical data. Absolute and percent change in SLV was calculated for each segment and summarized across PAV tertiles. Least-square means were calculated within each tertile for change in SLV parameters using mixed models (via a variance component structure), accounting for multiple segment measurements within a patient, and controlling for baseline SLV. A separate subgroup analysis was performed comparing absolute and percent change in SLV in patients with hsCRP <2 and ≥2 mg/L across PAV tertiles. Tests for trend across PAV tertiles were calculated for the near normal and NSTEMI groups, as well as baseline hsCRP levels. Multivariable mixed models were created to identify independent predictors of change in segmental lumen volume. All variables that were univariately associated with change in lumen volume (p value < 0.20) were considered for multivariable adjustment. Variables were then kept in the final model if they reached statistical significance (p value < 0.05). Further adjustment was made for near normal/NSTEMI categorization of patients and baseline lumen volume.
Correlations between segmental PAV and endothelial function were computed using the Spearman-rank calculation. All analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC). P values < 0.05 were considered statistically significant.

Results

Clinical and IVUS data

Table 1 summarizes clinical, biochemical, angiographic and IVUS characteristics of the two patients groups. The NSTEMI cohort comprised of significantly more males, diabetic patients and a trend towards more hypertensive individuals. This group also demonstrated lower serum high-density lipoprotein cholesterol (HDL-C), higher triglyceride levels and a trend towards increased high sensitivity C-reactive protein (hsCRP) levels. All patients with a NSTEMI underwent successful percutaneous coronary intervention and stent insertion to the culprit lesion after undertaking the experimental protocol within the non-culprit vessel. Despite similar angiographic inclusion/exclusion criteria between patient groups, the NSTEMI group demonstrated greater PAV (40.4 ± 12 vs. 27.5 ± 14%, p < 0.001), smaller baseline lumen volume (78.4 ± 30 vs. 93.1 ± 37 mm³, p < 0.001), but similar external elastic membrane volume (134 ± 47 vs. 131 ± 49 mm³, p = 0.59), compared with the near normal group. The NSTEMI group also demonstrated segments with a higher remodeling index [1.2 (1.0, 1.5) vs. 1.0 (0.9, 1.0), p < 0.001] and lower eccentricity index [4.3 (3.0, 6.2) vs. 9.0 (6.4, 14), p < 0.001], consistent with a more concentric atherosclerotic plaque, in association with more expansive remodeling of the vessel wall.
Dose-response analysis: Near normal vs. NSTEMI not stratified for IVUS-derived plaque burden

Two patients in the near normal group experienced transient coronary spasm during instrumentation, prior to IC infusions, which responded promptly to administration of IC nitroglycerin. These subjects did not proceed to the study protocol and hence data was not acquired. Successive doses of IC salbutamol exerted no significant change from baseline upon blood pressure or heart rate.

Figure 2 illustrates segmental endothelium-dependent vasomotor reactivity to IC salbutamol, according to clinical presentation, in a dose-response manner. Following baseline IC vehicle infusion, patients in the near normal group displayed greater increases in SLV to both the 0.30 and 0.60 μg/min doses of IC salbutamol (% change SLV: 5.10±0.89 and 6.02±1.53%, p<0.001 from baseline respectively; absolute change SLV: 3.74±0.73 and 4.1±1.3 mm³, p<0.001 and p=0.002 from baseline respectively) compared to the NSTEMI group at each of the 0.30 and 0.60 μg/min doses respectively (% change SLV of 2.05±0.89 and 3.21±1.12, p=0.02, p=0.005 from baseline respectively; absolute change SLV: 1.2±0.73 and 1.9±0.97 mm³, p=0.11 and 0.05 from baseline respectively). The degree of vasomotor response observed following the 0.30 μg/min dose of IC salbutamol was significantly less in the NSTEMI group compared with the near normal group (differences in % change SLV: p=0.02; differences in absolute change SLV: p=0.01).

There was a significant, inverse correlation between segmental PAV and endothelial function (Spearman’s correlation coefficients and non-parametric p-value): Absolute change in SLV vs. PAV -0.26 (-0.36, -0.16), p<0.001; % change in SLV vs. PAV -0.21(-0.31, -0.11), p<0.001.
Lumen response: Near normal vs. NSTEMI controlled for IVUS-derived plaque burden

Figure 3 describes the interplay between endothelium-dependent lumen vasoreactivity and plaque burden in both near normal and NSTEMI cohorts, controlled and stratified according to common tertiles of plaque burden that were derived from the total number of coronary segments in the entire cohort. Tertiles 1, 2 and 3 of segmental plaque burden corresponded to PAV values of 6.01-26.59%, 26.6-40.6 % and 40.75-74.63% respectively, common to both clinical groups. Stratification was undertaken in order to accurately define lumen responses in each clinical group per common unit of plaque burden. An additional reason was the significant baseline differences observed for plaque burden and vessel remodeling between the near normal and NSTEMI clinical groups. Absolute and percent changes in lumen response to both salbutamol doses are presented in Figure 3.

Our results show that within each tertile of plaque burden, there were no significant differences in endothelium-dependent luminal responses between the near normal and NSTEMI groups. However, a consistent finding was the inverse relationship between the burden of segmental epicardial atherosclerosis and corresponding lumen response. Irrespective of the nature of coronary syndrome, segments containing the lowest tertile of plaque exhibited the greatest degree of vasodilatation (measured as absolute or percent change from baseline). On the contrary, segments containing the highest tertile of plaque burden exhibited the least degree of epicardial vasomotion, and occasionally vasoconstriction.

Multivariable predictors of segmental endothelium-dependent coronary vasomotor responses

Table 2 describes the multivariable analysis for predictors of change in SLV. The considered univariate factors that associated with a change in SLV (p value < 0.20) that were entered into the multivariable model included the nature of clinical presentation (near normal vs. NSTEMI),
age, hsCRP levels ≥ 2 mg/L, gender, total cholesterol levels, LDL-C levels, HDL-C levels, triglyceride levels, hypertension, diabetes mellitus, smoking status and PAV. An hsCRP level of ≥ 2 mg/L was used to dichotomize patients with higher levels of systemic subclinical inflammation.24 The multivariable analysis revealed that higher segmental plaque burden was independently associated with lower degrees of endothelial function (p=0.0004). Similar significant, independent relationships were found in active smokers (p=0.01), diabetic patients (p<0.0001), higher LDL-C levels (p=0.01), patients with an hsCRP levels ≥ 2mg/L (0.03), increasing age (p<0.0001) and greater baseline SLV (p<0.0001) per se. After controlling for these factors, clinical status (near normal vs. NSTEMI) was not significantly associated with a change in SLV (p=0.91).

**Associations between inflammation, atheroma burden and vascular reactivity**

A further subgroup analysis was undertaken to explore the association between inflammation, coronary atheroma burden and endothelium-dependent coronary vasomotion. Patients were dichotomized into 2 groups; hsCRP <2 mg/L and hsCRP ≥2 mg/L 24, with median CRP levels in each group being 1.1 (0.5, 1.5) and 4.4 (2.9, 11) respectively. Those with elevated hsCRP levels (≥2 mg/L) demonstrated greater PAV (35.3±15 vs. 30.2±14%, p=0.002), larger external elastic membrane volume (136.0±48 vs. 123.7±48 mm³, p=0.026), but similar baseline lumen volume volumes (134±47 vs. 131±49 mm³, p=0.59), compared to those with less systemic inflammation (hsCRP <2 mg/L). Segmental remodeling indices were similar between both groups [1.1 (1.0, 1.3) vs. 1.0 (0.9, 1.3), p=0.60]. Without stratification for the degree of plaque burden, coronary segments in patients with higher levels of systemic inflammation displayed less endothelium-dependent coronary vasomotion, than segments from individuals with less systemic inflammation (% change SLV: 2.51±0.73 vs. 6.52±1.2%, p=0.005; absolute change SLV:
1.67±0.59 vs. 4.63±0.98 mm³, p=0.01). In the lowest PAV tertile, the degree of systemic inflammation failed to impart any effect on endothelium-dependent luminal response (Figure 4). However, in the presence of greater amounts of plaque, systemic inflammation appeared to influence lumen response. In patients with hsCRP levels ≥2 mg/L, blunted, and even constrictive lumen responses were observed (for both relative and absolute changes in SLV, p-value for trend across all PAV tertiles <0.001). Yet, preservation of lumen responses was observed in those individuals with hsCRP levels ≥2 mg/L (for both relative and absolute changes in SLV, p-values for trend across PAV tertiles were 0.36 and 0.27 respectively) (Figure 4). A trend was observed regarding the interaction between coronary atheroma volume (tertiles of PAV) and hsCRP (<2 versus ≥2 mg/L) upon endothelium-dependent coronary vasoreactivity (change in SLV) (p-value for interaction = 0.09).

Discussion

We show for the first time that the volume of coronary atheroma significantly associates with endothelium-dependent lumen vasoreactivity, regardless of the nature of clinical syndrome. With the higher image resolution provided by IVUS, we found that the magnitude of endothelium-dependent lumen responses did not significantly differ according to clinical presentation, when the degree of atheroma volume was controlled equally across clinical groups. Endothelium-dependent lumen vasoreactivity related inversely with coronary atheroma volume, with similar associations found with a number of other systemic cardiovascular risk factors, but not with the nature of clinical presentation. Of particular note, was the observation of an inverse relationship between endothelium-dependent lumen vasoreactivity and subclinical systemic inflammation, characterized by hsCRP levels ≥2 mg/L. Subgroup analysis of the association between the degree
of systemic inflammation, plaque burden and lumen vasoreactivity revealed a possible interaction between hsCRP levels ≥2 mg/L and greater atheroma volumes, in mediating endothelium-dependent lumen vasoreactivity. These observations provide a mechanistic understanding of why patients with acute coronary syndromes frequently display greater degrees of conduit segment endothelial dysfunction. These data also provide intriguing insight into the dynamic interplay between coronary atheroma volume, systemic inflammation and subsequent vasomotor reactivity, and how the interaction between these components may mediate plaque instability, and subsequent clinical risk.

Following the notion that patients with unstable coronary disease display enhanced coronary vasoreactivity \(^2\), we anticipated that the magnitude of lumen vasoreactivity to an endothelium-dependent stimulus, per unit of coronary atheroma volume, would be greater in the setting of a NSTEMI than compared to a stable, near normal patient cohort. This suspicion stemmed from prior observations of greater lumen vasoreactivity in unstable patients with evidence of systemic inflammation \(^3,4\). However, our study findings are at partial odds with these prior observations, and challenge prior mechanistic appraisals of why epicardial lumen vasoreactivity has been shown to be impaired in the setting of an acute coronary syndrome. Given that prior evaluations of lumen vasoreactivity utilized coronary angiography, it is likely that the extent of atheroma volume in these vessels was greatly underestimated \(^25\). Furthermore, angiography did not permit previous investigators to conduct a detailed appraisal of the differences in magnitudes of lumen responses, stratified for the degree of atheroma volume, in the way IVUS has enabled us to do in this study. Despite adoption of the same angiographic inclusion/exclusion criteria for identifying the study vessel, our findings of more severe diffuse disease demonstrated in NSTEMI patients, compared to patients with stable coronary syndromes,
are in agreement with prior observations\textsuperscript{26,27}. Our data therefore suggests that the greater IVUS-
derived coronary atheroma volume demonstrated in patients with NSTEMI, in conjunction with
the influence of systemic risk factors, impacts upon the overall magnitude of observed lumen
vasoreactivity, rather than purely the nature of the clinical syndrome \textit{per se}.

The enhanced resolution of IVUS also allowed for a unique appraisal for potential
interactions between systemic inflammation and atheroma volume in mediating coronary lumen
vasoreactivity. While systemic inflammation did not affect vasomotor responses in the setting of
low plaque burden, higher levels of systemic inflammation significantly associated with
attenuated coronary vasomotor reactivity, and even vasoconstriction, in segments with higher
plaque burden. These observations suggest a possible interaction between circulating mediators
of inflammation and coronary atheroma in mediating coronary vasomotor reactivity. While
statin-mediated CRP-lowering was previously found to independently mediate the reduced rate
of coronary atheroma progression in a trial that employed serial IVUS\textsuperscript{28}, our data lends
mechanistic support as to the systemic benefit accrued from statins when prescribed to
individuals with CRP levels $\geq 2$ mg/L\textsuperscript{24}. Inflammation reduction with statins might have not only
improved NO bioavailability and subsequently improved endothelial function\textsuperscript{29,30}, but lipid-
lowering \textit{per se} reduces the inflammatory content of atheroma\textsuperscript{31,32}, with a resultant plaque
‘stabilizing’ effect\textsuperscript{33}. While some consider the prognostic role of hsCRP levels to be equivalent
to serum cholesterol levels\textsuperscript{34}, further studies are required to assess whether hsCRP levels, used
in concert with measured coronary atheroma volume, further improves coronary risk prediction.

Our observations of a mechanistic link between coronary structure, and corresponding
vessel function, may have implications for promoting the direct evaluation of the coronary
vasculature as a means to further stratify coronary risk. Moreover, this approach also avoids
uncertainties of extrapolating measurements from remote vascular beds to events known to arise from within the coronary tree. Complementary to the findings from IVUS studies, that have shown consistent associations between the baseline volumetric extent of coronary plaque and incident clinical events, semi-quantitative measures of coronary plaque burden with non-invasive imaging similarly associates with cardiovascular mortality. Moreover, these observations augmented the prediction of mortality beyond traditional methods of quantifying cardiovascular risk. In addition, the recent demonstration of a non-invasive means to evaluate vasomotor responses to isometric handgrip, further outlined the potential clinical utility of non-invasive coronary imaging for clinical risk-stratification. However, as it stands, there is currently no hard evidence to support the use of non-invasive coronary imaging as a means to better predict coronary risk. Ultimately, supportive data from large-scale clinical trials will be needed to justify the use of direct atherosclerosis imaging as an adjunct tool to further risk-stratify individuals in routine clinical practice. Nevertheless, our observations of a direct relationship between coronary atheroma volume with segmental endothelium-dependent vasoreactivity, independent to other systemic risk factors and the nature of clinical presentation, might suggest that simply measuring plaque burden may be sufficient as a novel risk-stratification tool. Although it remains to be seen whether the incorporation of vasomotor reactivity testing during direct coronary imaging will provide further incremental patient and lesion-specific prognostic capabilities.

Several limitations of this study should be noted. Due to the invasive nature of our experimental protocol and possibility of coronary spasm/constriction during simultaneous IC salbutamol infusion and IVUS interrogation, we evaluated non-culprit vessels with mild angiographic disease. Hence, these findings cannot be extrapolated to culprit segments.
containing critical stenoses, where platelet rich thrombus may also impact on focal vascular reactivity. However a large natural history study showed that IVUS-derived plaque burden in non-culprit vessels was an independent predictor of half of the future coronary events observed in the study, underscoring the systemic nature of the disease process and importance of non-culprit vessel pathology following a NSTEMI. Nearly half of the ‘near normal’ population comprised of females, some of whom are known to present with cardiac syndrome X, with such patients possibly having an atypical relationship between plaque burden and vasomotor reactivity. However on multivariable analysis, female sex was not found to independently associate with endothelial function. The reference group from which we drew comparisons were not entirely ‘normal’, in the sense that overt coronary atherosclerosis was still detectable on IVUS, despite appearing negligible on coronary angiography. However, this represented a population being as close to ‘normal’ as deemed ethically possible undergoing an invasive coronary evaluation. Direct vasodilator responses to intracoronary nitroglycerin injection were not evaluated. This would have allowed us to test if smooth muscle cell dysfunction, rather than impairment in NO-dependent function, contributed to blunted vessel wall reactivity in a number of segments. Salbutamol, however, has minimal direct smooth muscle cell dilating properties, as shown in our previous validation study. Plaque composition was not assessed in this study. Although current techniques of assessing plaque composition via interrogation of the radiofrequency backscatter signal currently possess certain limitations, its evaluation might have yielded added mechanistic insight into the possibility of atheroma phenotype mediating segmental lumen vasoreactivity.
Conclusions

Irrespective of the nature of clinical presentation, the magnitude of segmental lumen vasoreactivity was not significantly different, when controlled for by the degree of coronary atheroma volume. Rather, coronary lumen vasoreactivity appeared primarily dependent upon the volume of atheroma, established cardiovascular risk factors and the presence of subclinical inflammation. Systemic inflammation appears to play a role in mediating coronary endothelium-dependent lumen reactivity, in the presence of a greater, but not lesser, burden of coronary atheroma. This suggests a possible interaction between inflammation and plaque in determining coronary vasomotor reactivity. These findings outline the functional significance of coronary atheroma volume in vivo, thereby providing further mechanistic validation of its future potential clinical role as an imaging biomarker of coronary risk.

Sources of Funding

Dr. Puri is supported by a Postgraduate Medical Research Scholarship from the National Health & Medical Research Council (565579) and jointly funded by the National Heart Foundation of Australia (PC0804045) and Dawes Scholarships (Hanson Institute). Equipment funding for this study was obtained from a Cardiovascular Lipid Grant, Pfizer Australia Pty Ltd (CB21.08).

A/Prof M.I. Worthley is a SA Health Early to Mid Career Practitioner Fellow

Acknowledgments

We are grateful for the support received from the nurses, technicians and radiography staff of the Cardiovascular Investigation Unit of the Royal Adelaide Hospital, along with the technical expertise of the Atherosclerosis Imaging Core Laboratory, Cleveland Clinic.
Disclosures

None.

References


34. Ridker PM, Kastelein JJ, Genest J, Koenig W. C-reactive protein and cholesterol are equally strong predictors of cardiovascular risk and both are important for quality clinical care. *Eur Heart J.* 2013;34:1258-1261.


Table 1. Clinical, biochemical, angiographic and ultrasonic characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Near normal cohort</th>
<th>NSTEMI cohort</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 24 patients</td>
<td>N = 23 patients</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N = 193 segments</td>
<td>N = 193 segments</td>
<td></td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>58±12</td>
<td>59±12</td>
<td>0.93</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>10 (42)</td>
<td>19 (83)</td>
<td>0.004</td>
</tr>
<tr>
<td>*Smoker, n (%)</td>
<td>10 (42)</td>
<td>15 (65)</td>
<td>0.11</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>3 (13)</td>
<td>6 (26)</td>
<td>0.29</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>10 (42)</td>
<td>16 (70)</td>
<td>0.05</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>177±46</td>
<td>161±42</td>
<td>0.22</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>113±40</td>
<td>104±35</td>
<td>0.46</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>49±13</td>
<td>35±11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>66 (44,102)</td>
<td>97 (89,133)</td>
<td>0.009</td>
</tr>
<tr>
<td>hsCRP (mg/L)</td>
<td>2.4 (1.6,4.6)</td>
<td>4.0 (2.2,12)</td>
<td>0.07</td>
</tr>
<tr>
<td>Statin use</td>
<td>9 (38)</td>
<td>13 (59)</td>
<td>0.14</td>
</tr>
<tr>
<td>Troponin T (ng/mL)</td>
<td>0</td>
<td>1.7±3.6</td>
<td>N/A</td>
</tr>
<tr>
<td>Non-culprit study artery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAD, n (%)</td>
<td>21 (88)</td>
<td>11 (48)</td>
<td>0.004</td>
</tr>
<tr>
<td>LCx, n (%)</td>
<td>2 (8)</td>
<td>9 (39)</td>
<td>0.01</td>
</tr>
<tr>
<td>RCA, n (%)</td>
<td>1 (4)</td>
<td>3 (13)</td>
<td>0.28</td>
</tr>
<tr>
<td>Segments per patient</td>
<td>8 (6, 8)</td>
<td>8 (7, 10)</td>
<td>0.66</td>
</tr>
<tr>
<td>PAV</td>
<td>27.5±14</td>
<td>40.4±12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SLV (mm³)</td>
<td>93.1±37</td>
<td>78.4±30</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>EEM (mm³)</td>
<td>131±49</td>
<td>134±47</td>
<td>0.59</td>
</tr>
<tr>
<td>RI</td>
<td>1.0 (0.9, 1.0)</td>
<td>1.2 (1.0, 1.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>EI</td>
<td>9.0 (6.4, 14)</td>
<td>4.3 (3.0, 6.2)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data expressed as mean±SD or median and interquartile range when appropriate

*Definition of smoking was taken as current or within 4 weeks

LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol
hsCRP, high sensitivity C-Reactive protein
LAD, left anterior descending artery; LCx, left circumflex artery; RCA, right coronary artery
PAV, percent atheroma volume; TAV, total atheroma volume; SLV, segmental lumen volume; EEM, external elastic membrane; RI, remodeling index; EI, eccentricity index
N/A = not applicable, as by definition, all stable patients were tested negative for cardiac Troponin T levels
Table 2. Multivariable analysis for change in segmental lumen volume

<table>
<thead>
<tr>
<th>Variable</th>
<th>Beta-coefficient (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Near normal presentation (vs. NSTEMI)</td>
<td>-0.15 (-2.9, 2.6)</td>
<td>0.91</td>
</tr>
<tr>
<td>Baseline SLV (mm³)</td>
<td>-0.12 (-0.15, -0.08)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age (years)</td>
<td>-0.24 (-0.34, -0.13)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>hsCRP ≥2 mg/L (vs. hsCRP &lt;2 mg/L)</td>
<td>-3.1 (-5.9, -0.29)</td>
<td>0.03</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>-6.9 (-10.0, -3.9)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.0 (-1.5, 3.6)</td>
<td>0.43</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>-0.05 (-0.08, -0.01)</td>
<td>0.01</td>
</tr>
<tr>
<td>Smoker</td>
<td>-3.2 (-5.8, -0.64)</td>
<td>0.01</td>
</tr>
<tr>
<td>PAV</td>
<td>-0.18 (-0.27, -0.08)</td>
<td>0.0004</td>
</tr>
</tbody>
</table>

NSTEMI, non-ST segment elevation acute coronary syndrome; SLV, segmental lumen volume; LDL-C, low-density lipoprotein cholesterol; PAV, percent atheroma volume.
Figure Legends

Figure 1. Methodology for IVUS analysis of contiguous 5-mm coronary segments and intracoronary infusion and imaging protocol.

Methodology of segmental volumetric lumen and plaque analysis using IVUS. [A] The IVUS pullback was divided into 5-mm segments (denoted by red lines on longitudinal view), with each plaque and lumen volumes calculated (adapted from Puri R et al. Eur Heart J. 2012;33:495-04). [B] Methodology for tracing lumen and EEM on IVUS. Shaded area equates to atheroma area. [C] An example is shown of a segmental cross-section with low plaque burden (taken from a patient with near normal coronary angiography) and associated vasodilatation following intracoronary salbutamol. In contrast, a cross-section with higher plaque burden (taken from a NSTEMI patient) is shown to undergo vasoconstriction following intracoronary salbutamol. [D] Coronary endothelial function testing protocol.

Figure 2. Dose-response curves: Near normal vs. NSTEMI

Segmental epicardial endothelium-dependent vasoreactivity in near normal and NSTEMI patients. Figure 3A shows the % change in SLV to intracoronary salbutamol at 0.30 and 0.60 μg/min doses. *p<0.001 vs. to baseline, #p=0.02 vs. NSTEMI group. †p=0.02 vs. baseline, ‡p=0.005 vs. baseline. Figure 3B shows the absolute change in SLV to intracoronary salbutamol at 0.30 and 0.60 μg/min doses. *p<0.001 vs. baseline, #p=0.01 vs. NSTEMI group, †p=0.05 vs. baseline.

Figure 3. Lumen responses stratified according to clinical presentation and plaque burden
Segmental epicardial endothelium-dependent vasoreactivity (% change and absolute change in SLV) in all coronary segments stratified according to common tertiles of measured plaque burden (PAV) across both the near normal and NSTEMI groups, following the 0.30 μg/min salbutamol dose.

**Figure 4. Lumen responses stratified according to hsCRP levels and plaque burden**

Segmental epicardial endothelium-dependent vasoreactivity (% change and absolute change in SLV) in all coronary segments stratified according to common tertiles of measured plaque burden (PAV) across patients with hsCRP levels <2 mg/L vs. ≥2 mg/L, following the 0.30 μg/min salbutamol dose.
<table>
<thead>
<tr>
<th>PAV Tertile (%)</th>
<th>N=128</th>
<th>N=129</th>
<th>N=129</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1: (6.01-26.59)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2: (26.6-40.6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T3: (40.75-74.63)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Salbutamol 0.30 ug/min

- Near Normal P (trend) <0.001
- NSTEMI P (trend) = 0.007

%Δ Change in SLV

- T1
- T2
- T3

P=0.98

Absolute Δ in SLV (mm)

- T1
- T2
- T3

P=0.53
P=0.98

Circulation
Cardiovascular Imaging
Journal of the American Heart Association
### PAV Tertile (%)

<table>
<thead>
<tr>
<th>T1: (6.01-26.59)</th>
<th>T2: (26.6-40.6)</th>
<th>T3: (40.75-74.63)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=128</td>
<td>N=129</td>
<td>N=129</td>
</tr>
</tbody>
</table>

**Salbutamol 0.30 ug/min**

- **% Δ in SLV**
  - T1
  - T2
  - T3

- **P-values**
  - T1: 0.49
  - T2: 0.004
  - T3: 0.077

**hsCRP**
- hsCRP < 2 P (trend) = 0.36
- hsCRP ≥ 2 P (trend) = 0.001

**Absolute Δ in SLV (mm)**

- **T1**
- **T2**
- **T3**

- **P-values**
  - T1: 0.75
  - T2: 0.017
  - T3: 0.13

---

Circulation
Cardiovascular Imaging
Journal of the American Heart Association
Coronary Endothelium-Dependent Vasoreactivity and Atheroma Volume in Subjects with Stable, Minimal Angiographic Disease versus Non-ST Segment Elevation Myocardial Infarction: An Intravascular Ultrasound Study


Circ Cardiovasc Imaging. published online June 27, 2013;
Circulation: Cardiovascular Imaging is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2013 American Heart Association, Inc. All rights reserved.
Print ISSN: 1941-9651. Online ISSN: 1942-0080

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circimaging.ahajournals.org/content/early/2013/06/27/CIRCIMAGING.113.000460

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation: Cardiovascular Imaging can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation: Cardiovascular Imaging is online at:
http://circimaging.ahajournals.org/subscriptions/