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Relationship between plasma inflammatory markers and plaque fibrous cap thickness determined by intravascular optical coherence tomography

Q-X Li,1,2 Q-Q Fu,1 S-W Shi,1,2 Y-F Wang,1,2 J-J Xie,1 X Yu,1 X Cheng,1 Y-H Liao1

ABSTRACT
Objective The purpose of this study was to evaluate the relationship between human plaque fibrous cap thickness detected by intravascular optical coherence tomography (OCT) and the plasma levels of inflammatory factors in patients with coronary artery disease (CAD).

Methods and Results OCT was used to measure the fibrous cap thickness of coronary artery atherosclerotic plaques in patients with acute myocardial infarction (AMI), unstable angina pectoris (UAP) and stable angina pectoris (SAP). Plasma levels of inflammatory factors including highly sensitive C-reactive protein (hs-CRP), IL-18 and tumour necrosis factor alpha (TNFα) were detected by ELISA, and peripheral white blood cell (WBC) counts were performed. The results demonstrated that the plasma levels of inflammatory factors and WBC count were correlated inversely with fibrous cap thickness (r = -0.775 for hs-CRP, r = -0.593 for IL-18, r = -0.60 for TNFα and r = -0.356 for WBC count). Patients with cap thickness less than 65 μm (defined to be thin cap fibroatheromas; TCFA) had higher plasma levels of inflammatory factors as well as WBC counts than those with thicker fibrous caps. Receiver operator characteristic (ROC) curves for hs-CRP, IL-18, TNFα and WBC count, which displayed the capability of prediction about TCFA, showed the area under the curves were 0.95, 0.86, 0.79 and 0.70 (p<0.05), respectively. ROC curve analysis confirmed that an hs-CRP cut-off at 1.66 mg/l would detect TCFA with a sensitivity of 96% and a specificity of 90%, and was the strongest independent predictor of TCFA.

Conclusion There is an inverse linear correlation between fibrous cap thickness and plasma levels of inflammatory markers. The plasma hs-CRP concentration is the strongest independent predictor of TCFA.

One indication of a vulnerable plaque has been identified to be a thin cap fibroatheroma (TCFA) with a fibrous cap less than 65 μm thick, with or without previous plaque rupture.1 Data from large-scale, population-based studies have demonstrated that an increase in circulating levels of numerous inflammation biomarkers including cytokines, adhesion molecules and acute-phase reactants could predict future cardiovascular events.2 Although the link between inflammation biomarkers and clinical cardiovascular events is strong, there were still gaps in the knowledge about their correlation and causation.

Intravascular optical coherence tomography (OCT) is a recently developed optical imaging technique that provides high-resolution, cross-sectional images of tissue in situ.3–5 The resolution of OCT (≈10 μm) is appropriate for measuring a cap thickness less than 65 μm thus identifying a TCFA potential plaque, suggesting that this technology is well suited for identifying vulnerable plaques in patients. However, we are not able to perform OCT on every patient for the evaluation of plaque stability in routine clinical practice. A broad goal is to find and employ an easy method to screen patients for vulnerable plaque. The purpose of this study was to evaluate the correlation between fibrous cap thickness detected by OCT and plasma levels of inflammatory factors in patients with coronary artery disease (CAD) as an easy identifier.

METHODS
Subjects
Forty-six patients confirmed by coronary arteriography were enrolled in this study from November 2006 to July 2008 at the Department of Cardiology of the Affiliated Hospital to Jining Medical College. Based on the World Health Organization (WHO) criteria, there were 12 patients with acute myocardial infarction (AMI), 25 patients with unstable angina pectoris (UAP) and 11 patients with stable angina pectoris (SAP). Based on medical history, physical examinations and laboratory workups, patients were excluded if they had significant left main coronary artery disease, congestive heart failure, renal insufficiency with baseline serum creatinine greater than 1.8 mg/dl (>153 μmol/l), an intercurrent infection or other inflammatory diseases within the 3 months before, required emergency or primary percutaneous coronary intervention, or had extremely tortuous or heavily calcified vessels. No patients were treated with anti-inflammatory drugs such as non-steroidal anti-inflammatory drugs, steroids, etc. None had collagen disease, thromboembolism and disseminated intravascular coagulation, advanced liver disease, malignant disease, valvular heart disease, atrial fibrillation or was using a pacemaker. All demographic and clinical data were collected prospectively. Peripheral white blood cell (WBC) counts were performed and analysed at a single laboratory.

The study was approved by the Ethics Committee, and all patients provided written informed consent before participation. The subjects who were examined with OCT provided additional written informed consent specific to the OCT study.

Measurement of plasma inflammatory factors
Five millilitres EDTA-treated peripheral blood was obtained from all subjects, in the SAP group in the...
morning before breakfast, in the UAP group in the morning following hospital admission and in the AMI group after admission but before emergency intervention. The blood samples were immediately centrifuged at 3000 rpm for 10 minutes, and the plasma was obtained and stored at −80°C until biomarker detection testing. Plasma levels of highly sensitive C-reactive protein (hs-CRP), IL-18 and tumour necrosis factor alpha (TNFα) were measured by commercially available ELISA kits, according to the protocols of the manufacturer (Bender Medsystems, Vienna, Austria and Biosource, Camarillo, USA, CRP kit ref BMS288INST and lot no 28466013; IL-18 kit ref BMS267/2 and lot no 27722006 and TNFα kit ref KHC3011 and lot no CH 070602A).

OCT imaging studies

The location of the culprit lesion in the coronary tree was determined by analysis of angiography and the electrocardiographic changes (ischaemic ST-segment changes, T-wave inversions and/or pathological Q wave) at the onset of chest pain, and the identified culprit lesion was then visualised using the M2 OCT system (LightLab Imaging, Westford, Massachusetts, USA). In patients with SAP, the tightest lesion on coronary angiogram was selected, whereas a lesion with evidence of plaque rupture with or without local thrombus was considered to be the culprit lesion in patients with UAP and AMI.

OCT pullback images were acquired at 15 frames per second and digitally archived. All patients were given intravenous 2000 IU heparin before the OCT imaging procedure. A 6Fr guiding catheter was engaged into the coronary artery and nitroglycerin (200 μg) was administered through the guiding catheter. In order to remove blood from the field of view to acquire clear images, proximal occlusion with a balloon catheter (Helios; Avantec Vascular Corp, Sunnyvale, California, USA) and continuous flushing of heparinised saline were performed. A 0.016-inch OCT imaging catheter (ImageWire; LightLab Imaging) was introduced and advanced to the distal end of the culprit lesion through the occlusion balloon catheter central lumen. During pullback image acquisition, the occlusion balloon was inflated to 0.4–0.6 atm and heparinised saline was infused at 0.5 ml/s. The entire length of the culprit coronary artery was imaged with an automatic pullback device moving at 1–1.5 mm/s.

The quantitative analysis was performed with proprietary LightLab OCT software. An average of three measurements was taken in each pullback image by three reviewers. The lipid content of a plaque was quantified simply as the number of quadrants with lipid pools that were identified on the cross-sectional OCT image, and the highest number of lipid quadrants was used for analysis for each plaque. For each individual plaque, the thinnest part of the fibrous cap thickness of the atherosclerotic plaque was measured three times in the pullback. TCFA was defined as a lipid-rich plaque (lipid identified as signal poor and attenuating) of more than two quadrants of vessel lumen with a fibrous cap (identified as signal rich, or brightly reflecting, with low attenuation) thickness measuring 65 μm or less.

Statistical analysis

Continuous data were expressed as mean (SD). The plasma levels of inflammatory factors and WBC count were normally distributed, when stratified according to clinical demographics or plaque characteristics, the resulting data subsets were still normally distributed. Therefore Mann–Whitney and Kruskal–Wallis tests were used for the analyses. The correlation between plasma levels of inflammatory factors, WBC count and the fibrous cap thickness was analysed by Pearson correlation analysis and expressed as correlation coefficient (r), and p<0.05 was required for statistical significance. Natural logarithmic (Ln) transformation was used for correlation analysis as it could normalise the distribution better. Associations between hs-CRP, IL-18, TNFα and WBC count as independent variables, and TCFA as the dependent variable were evaluated using a logistic regression model. Receiver operating characteristic (ROC) curves were constructed comparing the true positive rate (sensitivity) with the false positive rate (1-specificity) of hs-CRP, IL-18, TNFα and WBC count, and a model with all of the parameters for predicting TCFA. We used multiple logistic regression models to detect the combined variant to predict TCFA, or find the only significant independent predictor. All analyses were performed using SPSS version 12.0. A p<0.05 was required for statistical significance.

RESULTS

A total of 46 culprit plaques was analysed. Fibrous cap thickness obtained from 46 culprit plaques and inflammatory factors data were obtained in 46 CAD patients, of which 22 (47.8%) were categorised as TCFA.

Baseline characteristics

The average age of the cohort was 59 years (range 41–81), and 50 (65.2%) were men. The average concentrations of hs-CRP, IL-18, TNFα were 1.90 mg/l, 95.90 μg/l and 44.68 μg/l, respectively. Thirty-five (76.1%) patients presented after an acute coronary syndrome (ACS). Patients with ACS were treated medically with fibrinolysis (for ST-segment elevation myocardial infarction) or antithrombotic therapy and imaged a mean of 3 days from initial symptom onset. The clinical demographics of the patients in relation to the concentrations of the inflammatory factors (hs-CRP, IL-18 and TNFα) and WBC counts are presented in table 1. There were no significant differences in the concentrations of the inflammatory factors between all demographic categories except the diagnosis categories. The concentration of hs-CRP was significantly higher in patients with an ACS compared with patients with SAP (2.24 mg/l (SD 0.80) for ACS vs 0.80 mg/l (SD 0.54) for SAP, p<0.001). There were similar trends of distribution in IL-18, TNFα and WBC counts between ACS and SAP (table 1).

Assessment of artery atherosclerotic plaque stability by OCT

Pullback images of the normal coronary artery lumen, displaying clear differentiation of the intima, media and adventitia, with homogenous sonic properties (figure 1A), were obtained from the distal end of the culprit lesion as well as proximal and distal reference segments. As fibrous cap thickness is considered to be the most suitable predictor of plaque stability, a fibrous cap thickness of less than 65 μm was defined to be an unstable plaque. In this study, we used fibrous cap thickness measured in OCT images as the most important evaluation parameter. In CAD patients, the OCT study demonstrated many features including various cap thicknesses in diseased arteries, irregular layers, massive atherosclerotic plaques underneath the intima, thinning fibrous caps and previously ruptured intima (figure 1B, C and D).

Inflammatory factors concentration, WBC count and plaque characteristics

The relationship between the concentrations of the inflammatory factors (hs-CRP, IL-18 and TNFα), WBC count and plaque
lipid content are shown in table 2. The concentrations of the inflammatory factors with lipid-rich plaque were higher than those with non-lipid-rich plaques (hs-CRP 2.19 mg/l (SD 0.83) vs 0.86 mg/l (SD 0.63), p < 0.001; IL-18 104.85 μg/l (SD 55.02) vs 10.94 μg/l (SD 16.97), p < 0.001; TNFα 52.05 μg/l (SD 19.17) vs 19.84 μg/l (SD 7.96), p < 0.001; WBC count 9.19 × 10^9/l (SD 32.84) vs 7.85 × 10^9/l (SD 9.01), p = 0.03).

With increasing plaque lipid content (the highest number of quadrants with lipid pools displaying lipid-rich plaque appearance), the concentrations of inflammatory factors seemed to have a trend towards higher levels. There was no relationship between inflammatory factor concentrations and the presence of calcification or thrombus formation. Although we could find a trend towards higher inflammatory factor levels in plaque with evidence of fibrous cap rupture, there was no statistical significance in IL-18 (p = 0.2496) and TNFα (p = 0.0822), except hs-CRP (p = 0.0255) and WBC count (p = 0.0246).

There was a significant inverse linear relationship between fibrous cap thickness (natural logarithm of fibrous cap thickness) and plasma levels of inflammatory factors as shown in figure 2. The correlation coefficients (Pearson r) were −0.775 (p < 0.001) for fibrous cap thickness/hs-CRP, −0.595 (p < 0.001) for fibrous cap thickness/IL-18, −0.60 (p < 0.001) for fibrous cap thickness/TNFα and −0.356 (p < 0.05) for fibrous cap thickness/WBC count, respectively. These results suggest that there were significant correlations between the coronary artery atherosclerotic plaque fibrous cap thickness revealed by OCT and the blood levels of inflammatory factors.

**Plasma levels of inflammatory factors and TCFA**

When plaques were classified as TCFA, univariate analysis demonstrated that plasma levels of hs-CRP, IL-18, and TNFα were significantly related to TCFA and the WBC count was similar (figure 3A). Plaques that were categorised as TCFA had higher concentrations of hs-CRP, IL-18, TNFα and WBC counts compared with plaques that were determined not to be TCFA.

Logistic regression analysis with hs-CRP, IL-18, TNFα and WBC count as independent variables showed that all of the four parameters independently predicted the probability of TCFA. Odds ratios in each parameter were 33.86 (p = 0.01) for hs-CRP, 1.02 (p < 0.05) for IL-18, 1.02 (p < 0.05) for TNFα and 1.24 (p < 0.05) for WBC count.

Finally, ROC curves for the concentrations of hs-CRP, IL-18, TNFα and WBC count were computed for the prediction of TCFA (figure 3B). The area under the curve for hs-CRP was 0.95 (p < 0.001), for IL-18 0.86 (p < 0.001), for TNFα 0.79 (p < 0.001) and for WBC count 0.70 (p < 0.05). As the independent parameters for predicting the probability of TCFA, the hs-CRP concentration of 1.66 mg/l would detect TCFA with a sensitivity of 96% and a specificity of 90%, the IL-18 concentration was 113.59 μg/l (sensitivity 66%, specificity 90%), the TNFα concentration was 56.60 μg/l (sensitivity 50%, specificity 90%) and the WBC count was 11.26 × 10^9/l (sensitivity 35%, specificity 90%). ROC curve analysis confirmed that an hs-CRP cut-off at 1.66 mg/l was the best independent predictor of TCFA. In multivariate logistic regression analysis, hs-CRP was the only significant independent predictor and a proper combined variate for predicting TCFA was not found.

**DISCUSSION**

Over the past decade, insights into the prediction of vulnerable coronary plaques have generated advances in the understanding of CAD, including plaque morphology and circulating inflammatory markers.11
The fibrous cap thickness is an important index of plaque morphology when it comes to vulnerable plaques. Near the rupture site in those plaques that have been studied post-rupture, the fibrous cap thickness is on average 23 μm (SD 19), with 95% of the caps measuring less than 64 μm.1 Beyond showing all the features revealed by intravascular ultrasonography, OCT can also provide additional details about the structure of the coronary artery and atherosclerosis in question.

<table>
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<th>Number</th>
<th>hs-CRP (mg/l)</th>
<th>IL-18 (μg/l)</th>
<th>TNFα (μg/l)</th>
<th>WBC count (10^9/l)</th>
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<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
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<td>4</td>
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<td>100.14 (39.44)</td>
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<td>9 (19.57)</td>
<td>2.53 (0.65)</td>
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<td>107.88 (31.70)</td>
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Values are expressed as mean (SD); p value is significant at <0.05. hs-CRP, highly sensitive C-reactive protein; TNFα, tumour necrosis factor alpha; WBC, white blood cell.
and better differentiate lipid-rich plaques, fibrous plaques and intima proliferation.\textsuperscript{7,8} Therefore, we used OCT imaging to assess many features of CAD, including the extremely variable thickness of the diseased coronary artery wall, mixed composition atherosclerosis lesions presenting with differing layers, disorganised coronary artery wall structure with relatively thick fibrous cap in stable plaque, and thinning fibrous cap with previously ruptured intima in unstable plaque. These observations demonstrated the value of OCT in assessing atherosclerotic plaque stability.

Inflammation plays a critical role in the progression of atherosclerotic plaques from stable plaque to unstable plaque.\textsuperscript{12} Studies have demonstrated that increased circulating levels of numerous markers of inflammation, including hs-CRP, TNF\textsubscript{z} and IL-18, could predict future cardiovascular events.\textsuperscript{5,13} Tearney et al\textsuperscript{14} reported that cap macrophage density, which was quantified morphometrically by immunoperoxidase staining with CD68 and compared with the standard deviation of the OCT signal intensity at corresponding locations, had a high degree of positive correlation with OCT intensity ($r = 0.84, p<0.001$). Raffel et al\textsuperscript{15} recently provided the first in-vivo data that macrophage density correlated with WBC count, and both parameters could independently and particularly in combination predict the presence of TCFA. Therefore, there appears to be a link between plaque stability and inflammatory reactions. Consequently, our current study focused on screening peripheral blood inflammation markers and WBC count to predict the instability of coronary artery atherosclerotic plaques, so as to find an easy method to differentiate between stable and unstable plaques by a non-invasive approach. This in-vivo study investigated the correlating relationships between the important indices of plaque morphology and the four circulating inflammatory markers. Our findings demonstrated that there was an inverse linear correlation between fibrous cap thickness and plasma levels of inflammatory markers as well as WBC count; moreover, plasma hs-CRP, IL-18, TNF\textsubscript{z} and WBC count could independently predict the presence of TCFA.

The current study showed that, with the increasing severity of clinical events, as the stability of coronary artery atherosclerotic plaques progressively decreased, plasma hs-CRP, IL-18 and TNF\textsubscript{z} levels as well as WBC counts increased, and atherosclerotic fibrous caps became thinner. The predominant inflammatory factor was hs-CRP. Corresponding with the plaque morphology detected by OCT, we found that the concentration of hs-CRP increased significantly in the condition of fibrous cap thickness less than 65 \(\mu\)m, large amounts of accumulation of lipids in the plaques and previous ruptures of the intima. There was a significant inverse linear relationship between the plasma levels of hs-CRP and fibrous cap thickness (natural logarithm of fibrous cap thickness), which was the best marker to reflect plaque stability, and hs-CRP was also an independent predictor of TCFA (odds ratios 33.86, $p = 0.01$). ROC curve analysis confirmed that an hs-CRP cut-off at 1.66 mg/l would detect TCFA with a sensitivity of 96\% and a specificity of 90\%, and was the strongest independent predictor of TCFA. Meanwhile, in multivariate logistic regression analysis, hs-CRP was the only significant independent predictor of TCFA. Therefore, although IL-18, TNF\textsubscript{z} and WBC count could also show similar characteristics, their predictive value was weak compared with hs-CRP. In future, screening of CAD patients, testing blood for levels of hs-CRP can potentially provide guidance on treatment by indicating the risk of vulnerable plaques.

Current limitations of this study include the use of the occlusion balloon approach, which was used to provide a blood-free field for intravascular OCT, and could result in transient ischaemia, even occasionally inducing ventricular fibrillation, which might not be tolerated by some patients during the OCT operational procedure. The sample size in this study was also relatively small and a large-scale study is warranted. Moreover, we only evaluated plaque stability in the culprit vessel but not in coronary artery disease

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**Figure 2** Correlation between cap thickness and the levels of inflammatory markers. Scatter plots of (A) highly sensitive C-reactive protein (hs-CRP) concentrations, (B) IL-18 concentrations and (C) tumour necrosis factor alpha (TNF\textsubscript{z}) concentrations. (D) Peripheral white blood cell (WBC) count to the logarithm of plaque fibrous cap thickness. Pearson’s correlation coefficient ($r$) and the p value are depicted in the insert.
three vessels. There is much precise work to be done to validate further the value of these plasma markers in predicting coronary artery atherosclerotic plaque instability.

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Competing interests None.

Ethics approval The study was approved by the Ethics Committee.

Patient consent Obtained.

Provenance and peer review Not commissioned; externally peer reviewed.

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