Relationship Between a Systemic Inflammatory Marker, Plaque Inflammation, and Plaque Characteristics Determined by Intravascular Optical Coherence Tomography

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Objective—The purpose of this study was to evaluate the relationships between the peripheral white blood cell (WBC) count, local plaque fibrous cap macrophage density, and the morphological features and presence of thin-cap fibroatheromas (TCFA) identified by optical coherence tomography (OCT).

Methods and Results—OCT was performed in patients undergoing catheterization. Images were analyzed using validated criteria for plaque characteristics. Baseline WBC count correlated with macrophage density ($r=0.483$, $P<0.001$). Both parameters were associated with lipid-rich plaque and correlated inversely with plaque fibrous cap thickness ($r=-0.547$ for macrophage density and $-0.423$ for WBC count, $P<0.015$). Plaques classified as TCFA had a higher median macrophage density than non-TCFA plaques (7.4 versus 4.9, $P<0.001$). Patients with TCFA had a higher WBC count compared with those without TCFA (11.0 versus 7.9, $P=0.007$). Receiver operator curves for WBC count, macrophage density, and these combined parameters for prediction of TCFA showed the area under the curves were 0.88, 0.91, and 0.97 ($P<0.001$), respectively.

Conclusion—This study provides the first in vivo data linking the peripheral WBC count, plaque fibrous cap macrophage density, and the characteristics and presence of TCFA. Macrophage density correlated with the WBC count, and both parameters independently and particularly in combination predict the presence of TCFA. (Arterioscler Thromb Vasc Biol. 2007;27:1820-1827.)

Key Words: optical coherence tomography • atherosclerosis • thin-cap fibroatheroma • leukocytes • macrophage

Histopathologic studies on subjects with sudden cardiac death suggest that most acute coronary events occur as a result of occlusive thrombus formation after disruption of a thin-cap fibroatheroma (TCFA), a so-called vulnerable plaque.1 These lesions are characterized by a thin fibrous cap, which overlies a large necrotic lipid core. Inflammation both locally within each plaque and systemically appears to play a critical role in this process. Leukocytes are an integral component of this inflammatory response. There is extensive ex vivo and in vitro histopathologic and biochemical evidence linking local macrophage infiltration with plaque characteristics and vulnerability.1-3 Histologically, disrupted TCFA demonstrate intense macrophage infiltration localized within the thin fibrous cap.4 The degree of macrophage infiltration is proportional to the degree of attenuation of the thin fibrous cap.5 The peripheral white blood cell (WBC) count, widely used in clinical practice as a marker of systemic inflammation, has been shown in numerous population based studies to be an independent predictor of cardiovascular events, both in healthy individuals free of CHD at baseline and in patients with stable and unstable coronary disease,6,7 thus providing an epidemiological link between systemic leukocytosis and the presence of unstable vulnerable plaque and plaque disruption. However, there is no direct in vivo data exploring the association between the peripheral WBC count, local plaque macrophage content, and the characteristics and presence of vulnerable plaque.

Optical coherence tomography (OCT) is an optical analog of intravascular ultrasound (IVUS) providing high resolution (~10 μm) cross-sectional images of the arterial wall.8 Using histological controls, the OCT characteristics for various components of coronary plaque including fibrous cap thickness and fibrous cap macrophage density have been validated.9-11 The aim of this study was to evaluate for the first time in vivo the relationships between the peripheral WBC count, local plaque fibrous cap macrophage density, and the constituents and presence of TCFA identified by OCT.
Methods

Study Population
A total of 43 patients undergoing coronary angiography for an acute coronary syndrome (ACS) defined as ST-elevation myocardial infarction (STEMI) or non-ST-elevation ACS (NSTEACS) and stable angina pectoris (SAP) who had an identifiable culprit lesion in a native coronary artery were enrolled into the study. The median age of the cohort was 59 years (range 40 to 77), and 39 (86.7%) were male. Subjects were excluded if they had significant left main coronary artery disease, congestive heart failure, renal insufficiency with baseline serum creatinine ≥1.8 mg/dL (≥133 μmol/L), an intercurrent infection or other inflammatory disease, required emergency or primary percutaneous coronary intervention, or had extremely tortuous or heavily calcified vessels. All demographic and clinical data were collected prospectively. Peripheral WBC counts were performed at baseline and analyzed at a single laboratory. The clinical data were collected prospectively. Peripheral WBC counts were performed at baseline and analyzed at a single laboratory. The Partners Institutional Review board approved the study, and all patients provided written informed consent before participation.

Lesion Identification
The culprit lesion for each patient was determined using coronary angiography and corroborated with information from the patient’s ECG, nuclear or echocardiographic stress test, and ventriculogram. In addition, within the same vessel, angiographically mild or moderate lesions (30% to 70% stenosis) that were remote from the culprit site were imaged.

OCT Image Acquisition
Imaging was performed before any percutaneous coronary intervention. The technique of intravascular OCT imaging has been previously described. Before introduction into the body, the Z offset was adjusted to calibrate the imaging catheter to the fiducial marks on the OCT monitor and the intensity of the sheath reflection was used to calibrate detection sensitivity. After administration of intracoronary nitroglycerin (100 to 200 μg), the 3.2F OCT catheter was advanced through a 7F catheter over a 0.014-inch guide wire under fluoroscopic guidance to the culprit or remote site. Images were obtained at 4 frames/s during intermittent saline flush (6 to 10 mL) through the guiding catheter to transiently displace blood. Images were acquired at the center of each plaque and at its proximal and distal segments and stored digitally for subsequent analysis. OCT imaging required an additional 10 minutes for each lesion.

OCT Image Analysis
All OCT images were analyzed by 2 independent investigators using previously validated criteria for OCT plaque characterization. Images with significant signal attenuation that precluded satisfactory evaluation of plaque morphology were excluded from the analysis.

Lipid containing plaques imaged with OCT characteristically show diffusely bordered, signal-poor regions (lipid pools/core) with overlying signal-rich bands, corresponding to fibrous caps. The lipid content of a plaque was semiquantified as the number of quadrants with lipid pools identified on the cross-sectional OCT image. For each plaque, the cross-sectional image with the highest number of lipid quadrants was used for analysis. Histopathologic studies show that TCFA with evidence of rupture have a large lipid core occupying >34% plaque area and a fibrous cap thickness <65 μm. Therefore if lipid was present in ≥2 quadrants within a plaque, it was considered a lipid-rich plaque. We have previously demonstrated using histological controls a sensitivity and specificity of 92% and 94%, respectively, for the detection of lipid-rich plaques using this OCT system.

For all images of culprit plaque with an OCT-determined lipid pool, the overlying fibrous cap thickness was measured at its thinnest part. The average of 3 measurements was taken for each image. For each individual culprit plaque, the thinnest fibrous cap thickness measurement obtained from the 3 imaging locations was used for subsequent analysis. TCFA were defined as lipid-rich plaque with a fibrous cap thickness measuring <65 μm.

The presence of plaque disruption, calcium, or thrombus was also noted. A thrombus was defined as an irregular mass protruding into the lumen that had a measured dimension ≥250 μm.

Quantitative Analysis of Fibrous Cap Macrophage Density
Fibrous cap macrophage density was evaluated in all plaques with a lipid pool using a previously validated technique. Using this technique we have demonstrated that the OCT-derived macrophage density of plaque fibrous caps correlated strongly (Pearson’s correlation coefficient r=0.84, P<0.0001) with macrophage density quantified histomorphometrically by CD68 immunoperoxidase staining in the corresponding histological samples. For each image the cap was outlined using automatic bimodal histogram segmentation with the threshold set at the nadir of the bimodal histogram distribution computed from the pixel values within the plaque. Measurement of macrophage content within these regions of interest was performed on raw OCT data. Median filtering was performed with a 3×3 square kernel to remove speckle noise (IPLab Spectrum 3.1, Scancoimaging). The normalized standard deviation (NSD) was then measured for each pixel within each cap using a 125 μm² window centered at the pixel location:

\[
NSD(x,y) = \frac{\sigma(x,y)}{S_{max}-S_{min}} \times 100
\]

Figure 1. OCT images of coronary plaque from two subjects. (A) A thin-cap fibroatheroma with associated thrombus (arrowhead) from a subject with an acute coronary syndrome. The image demonstrates a lipid rich plaque (lipid pools denoted by L) with a thin fibrous cap (arrow) measuring 43 μm. There is a high OCT signal with significant signal heterogeneity within the fibrous cap consistent with high macrophage content. The macrophage content derived from the raw OCT signal NSD was 6% and the subject’s peripheral WBC count was 9.0. (B) A fibroatheroma from another subject demonstrating a lipid pool (L) involving only one quadrant underlying a homogenous signal-poor thick fibrous cap suggestive of a low macrophage density. The macrophage density derived from the raw OCT signal NSD was 3% and the subject’s peripheral WBC count was 5.4. WBC = white blood cell count (10³/μL).
Where $NSD(x,y)$ was the normalized standard deviation of the OCT signal at pixel location $(x,y)$, $S_{max}$ was the maximum OCT image value, and $S_{min}$ was the minimum OCT image value. Pixels within the $(125 \times 125) \mu m^2$ window that did not overlap with the segmented cap were excluded. For each image, macrophage density was assessed as the average of the NSD values within the segmented/ROI of the cap (mean NSD). For each individual plaque the value of the highest macrophage density obtained from the 3 sampling sites was used for subsequent data analysis.

**Statistical Analysis**

Continuous variables were reported as medians with interquartile ranges (IQR) unless otherwise stated. WBC count was treated both as a continuous and a categorical variable defined as low (<25th percentile), intermediate (25th to 75th percentile), and high (>75th percentile) WBC counts. Both macrophage density and WBC count were normally distributed. However, when stratified according to clinical demographics or plaque characteristics, the resulting data subsets were not normally distributed. Therefore Mann–Whitney and Kruskal–Wallis tests were used for these analyses. The chi-squared test was used for analysis of categorical variables. Correlation between continuous variables was estimated using Pearson correlation coefficient. As the distribution of fibrous cap thickness was skewed to the right, natural logarithmic (Ln) transformation, which normalized the distribution, was used for correlation analysis. An analysis of covariance was used to determine the influence of diagnosis on the relationship between macrophage density, FCT, and the WBC count.

**Results**

A total of 56 plaques were analyzed. Of these, 34 (60.7%) were culprit plaques and 22 (39.3%) remote plaques. Macrophage and WBC count data were obtained in all cases. Fibrous cap thickness was obtained in 34 culprit plaques, of which 19 (56%) were categorized as TCFA.

**Baseline Characteristics**

The median age of the cohort was 59 years (range 40 to 77), and 39 (86.7%) were male. The median WBC count was 9.1 (range 4.7 to 16.1). 32 (74.4%) patients presented after an ACS (18 with STEMI and 14 with NSTEACS). Patients with ACS were treated medically with fibrinolysis (for STEMI) or antithrom-

**TABLE 1. Baseline Clinical Demographics, Macrophage Density, and WBC Count**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Number (%) (n=43)</th>
<th>Macrophage Density</th>
<th>WBC Count (10^9 cells/L)</th>
<th>P Value</th>
<th>Median (IQR)</th>
<th>P Value</th>
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<td>&lt;65</td>
<td>39 (69.6)</td>
<td>6.48 (2.59)</td>
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<td>≥65</td>
<td>17 (30.4)</td>
<td>5.72 (2.05)</td>
<td>9.40 (3.60)</td>
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<td></td>
<td></td>
</tr>
<tr>
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<tr>
<td>Male</td>
<td>37 (86)</td>
<td>5.94 (2.63)</td>
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<td>8.80 (3.50)</td>
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<td>Female</td>
<td>6 (14)</td>
<td>6.60 (2.86)</td>
<td>10.30 (4.48)</td>
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<td>ACS</td>
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<td>6.13 (2.56)</td>
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<td>SAP</td>
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<td>Yes</td>
<td>31 (72.1)</td>
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<td>10 (23.3)</td>
<td>5.44 (2.11)</td>
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<td>Yes</td>
<td>26 (60.5)</td>
<td>5.90 (2.58)</td>
<td>0.502</td>
<td>8.65 (3.25)</td>
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<td>9.60 (3.65)</td>
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<tr>
<td>Yes</td>
<td>9 (20.9)</td>
<td>5.94 (2.21)</td>
<td>0.607</td>
<td>6.80 (5.40)</td>
<td>0.105</td>
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<td>6.05 (2.64)</td>
<td>9.15 (3.08)</td>
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<td>Lesion site (n=56)</td>
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<td>Culprit</td>
<td>35</td>
<td>6.0 (2.52)</td>
<td>0.246</td>
<td>9.2 (3.1)</td>
<td>0.691</td>
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<td>Remote</td>
<td>21</td>
<td>5.2 (3.46)</td>
<td>9 (3.9)</td>
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</table>

WBC indicates white blood cell; IQR, interquartile range; ACS, acute coronary syndrome; SAP, stable angina pectoris; MI, myocardial infarction. P value significant at <0.05.
brotic therapy and imaged a mean of 3 days from initial symptom onset. The clinical demographics of the patients in relation to fibrous cap macrophage density and WBC count are presented in Table 1. There were no significant differences in macrophage density and WBC count between all demographic categories except the diagnosis. Median WBC count was significantly higher in patients with an ACS compared with patients with SAP (9.50, IQR 3.63 for ACS versus 8.10, IQR 3.4 for SAP, \(P=0.03\)). There was a trend toward a higher macrophage density in patients with ACS; however, this was not statistically significant.

### Macrophage Density of Fibrous Cap and Plaque Characteristics

The associations between fibrous-cap macrophage density and plaque lipid content are summarized in Table 2. The macrophage density of the fibrous cap of lipid-rich plaque was higher compared with non–lipid-rich plaques (6.10, IQR 2.59 versus 4.62, IQR 2.96, \(P=0.049\)). With increasing plaque lipid content (number of quadrants involved) there was a trend toward a higher fibrous-cap macrophage content. There was no relationship between plaque macrophage content and the presence of calcification or thrombus formation, and a trend toward a higher macrophage density in plaque with evidence of fibrous cap rupture (Table 2).

The correlation between fibrous cap thickness and fibrous cap macrophage density is shown in Figure 2A. There was a significant inverse linear relationship (natural logarithm of fibrous cap thickness) demonstrating an inverse linear relationship between plaque fibrous cap thickness and fibrous cap macrophage density (Pearson \(r=-0.547, P=0.001\)). Using a cut point of 65 \(\mu\)m for fibrous cap thickness, a higher median macrophage density was seen in both groups, although it appeared stronger in culprit plaque: 7.35 (IQR1.65) for fibrous caps \(>65\) \(\mu\)m compared with \(\leq 65\) \(\mu\)m (\(P<0.001\)).

### White Blood Cell Count and Plaque Characteristics

The associations between WBC count and plaque lipid content are summarized in Table 2. The WBC count in subjects who had lipid-rich plaques was significantly higher compared with those with non–lipid-rich plaques (9.40, IQR 2.80 versus 7.0, IQR 3.20, \(P=0.008\)). There was no association between the peripheral WBC count and the presence of plaque calcification, thrombus, or rupture (Table 2).

The correlations between WBC count, fibrous cap macrophage density, and fibrous cap thickness are shown in Figure 2B and 2C.

There was a significant linear relationship between WBC count and plaque fibrous cap macrophage density (Pearson \(r=0.483, P<0.001\)). Given the association between patient diagnosis (ACS and SAP) and WBC count (Table 1), we tested using an analysis of covariance whether this linear relationship, with additional terms for interaction between diagnosis and macrophage density, depended on the diagnosis. There was no significant variation in either the slope or the intercept between the group with ACS and the group with SAP. When culprit and remote plaques were considered separately, the linear relationship between WBC count and macrophage density was seen in both groups, although it appeared stronger in culprit plaque: \(r=0.517, P=0.002\) for culprit plaque and \(r=0.463, P=0.035\) for remote plaque.

WBC count also correlated with fibrous cap thickness (natural logarithm of fibrous cap thickness) demonstrating an inverse linear relationship (\(r=-0.423, P=0.013\) independent of the diagnosis (Figure 2B).

### Macrophage Density, WBC Count, and TCFA

When plaques were classified as TCFA, univariate analysis using variables which included age, sex, dyslipidemia, dia-

| TABLE 2. OCT Plaque Characteristics, Macrophage Density, and WBC Count |
|-------------------|-------------------|------------------|-------------------|
|                   | Macrophage Density | WBC Count (10^9 cells/L) |
|                   | Number | Median (IQR) | \(P\) Value | Median (IQR) | \(P\) Value |
| Lipid rich plaque | Yes     | 45 | 6.10 (2.59) | 0.049 | 9.40 (2.80) | 0.008 |
|                   | No      | 11 | 4.62 (2.96) |     | 7.0 (3.20)  |     |
| Lipid quadrants   |        |    |        |        |        |        |
|                   | 1       | 10 | 4.80 (3.19) | 0.066 | 7.25 (3.35) | 0.063 |
|                   | 2       | 19 | 5.19 (2.27) |     | 8.90 (3.90) |     |
|                   | 3       | 15 | 6.87 (1.64) |     | 10.20 (2.10) |     |
|                   | 4       | 11 | 7.29 (2.55) |     | 9.60 (2.80)  |     |
|                   | 1 vs 4  |     |        | 0.05 | 0.036 |        |
| Calcium           | Yes     | 8  | 6.01 (2.49) | 0.774 | 9.05 (3.35) | 0.497 |
|                   | No      | 48 | 5.91 (2.86) |     | 9.15 (3.60)  |     |
| Thrombus          | Yes     | 11 | 5.94 (3.45) | 0.781 | 8.50 (1.50)  | 0.288 |
|                   | No      | 45 | 6.00 (2.67) |     | 9.40 (3.70)  |     |
| Rupture           | Yes     | 10 | 7.06 (3.08) | 0.404 | 10.30 (3.03) | 0.161 |

WBC indicates white blood cell; IQR, interquartile range. \(P\) value significant at <0.05.
betes, hypertension, smoking history, macrophage density, and WBC count demonstrated that only macrophage density and WBC count were significantly related to TCFA (Figure 3). Plaques that were categorized as TCFA had a higher macrophage density than plaques that were not TCFA (7.4, IQR 1.84 versus 4.99, IQR 1.85; P < 0.001), and patients with culprit plaque that were TCFA had a higher median WBC compared with those with culprit plaque that were not TCFA (11.0, IQR 2.6 versus 7.9, IQR 2.5 respectively; P = 0.007). When the WBC count was categorized into low (<25th percentile), medium (25th to 75th percentile), and high (>75th percentile) groups, the frequency of TCFA increased significantly from patients with a low WBC count to those with a high WBC count (Figure 4A). Logistic regression analysis with both macrophage density and WBC count as independent variables showed that both parameters independently predicted the probability of TCFA. Odds ratios per unit change in each parameter was 5.5 (P = 0.028) for macrophage density and 3.3 (P = 0.024) for WBC count.

Finally, receiver operator curves (ROC) for macrophage density, WBC count, and a logistic regression probability model with both parameters were computed for the prediction of TCFA (Figure 4B). The area under the ROC curve (± standard error) for macrophage density was 0.91 ± 0.05 (P < 0.001), for WBC count was 0.88 ± 0.06 (P = 0.001), and the probability model with both parameters was 0.97 ± 0.03 (P < 0.001). Using a combination of a WBC count of 9.7 and fibrous cap macrophage density of 5.7 would detect TCFA with a sensitivity of 95% and a specificity of 94%.

**Discussion**

This is the first in vivo study to evaluate the relationships between the important characteristics of vulnerable plaque, their macrophage concentration, and the peripheral WBC count. The findings corroborate those of the many histomorphological studies which have formed the foundation on which our current paradigm of the pathophysiology of ACS is based.1,3,4,17–20 By demonstrating the relationship between the WBC count and the biological activity and morphological characteristics of individual plaque it also provides a direct in vivo link that connects the levels of systemic proinflammatory markers with their ability to predict the risk of cardiovascular events.21

The inflammatory activity of an individual plaque dictates its structural morphology, and these together determine its vulnerability to rupture and thrombosis.22–24 Macrophages are an integral functional component and a marker of this inflammatory activity. They contribute to the growth of the necrotic lipid core and the attenuation of the overlying fibrous cap: the hallmark features of a TCFA. Fibrous cap thickness and integrity are the critical features of plaque vulnerability. They depend for the most part on the balance between synthesis and degradation of the interstitial collagen within the matrix of the fibrous cap. Plaque macrophages by expressing a variety of matrix-degrading enzymes and cytokines tip this balance toward collagen breakdown, favoring attenuation and weakening of the fibrous cap. Histopathologic studies on coronary and aortic plaque ex vivo have demonstrated that plaque fibrous cap macrophage density was correlated positively with lipid content5,25 and correlated negatively with plaque fibrous cap thickness.5 Autopsy data on coronary plaques from victims of sudden cardiac death showed that the macrophage density in the fibrous cap of TCFA were significantly higher than in stable plaques (non-TCFA), especially if the TCFA demonstrated evidence of rupture.1,3,4 This study extends these findings to patients with
acute and stable coronary disease. It demonstrates a significantly higher macrophage density in plaque that are lipid-rich (>2 quadrants). It also shows that, independent of presentation, as the density of macrophages within the fibrous cap increases the fibrous cap thickness diminishes. In addition, macrophage density of plaques classified as TCFA were significantly higher than plaques that were not TCFA. There was also a nonsignificant trend toward a higher macrophage density in fibrous caps of plaque that showed evidence of cap rupture. The method of discrete OCT image acquisition, however, probably underestimated the incidence of rupture in our cohort thereby influencing this result.

There is now a large body of evidence supporting the role of systemic inflammation in the atherosclerotic process.26-27 Histomorphological and imaging studies have demonstrated multiple vulnerable and ruptured plaques remote from the culprit site in the coronary and noncoronary vasculature.3,28-32 These are supported by numerous studies demonstrating the ability of proinflammatory biomarkers in predicting future cardiovascular events. The WBC count is a robust, simple, and clinically widely available marker of systemic inflammation. A number of studies have shown that the peripheral WBC count, independent of other traditional risk factors, strongly predicts future cardiovascular events.6,7,33 In addition to macrophages, many of the other leukocyte subpopulations including lymphocytes, mast cells, and neutrophils have been both demonstrated within vulnerable atheromatous plaque and pathophysiological studies have established mechanistic links between these cells and the atherosclerotic process.27,34 However, there is little data relating the peripheral WBC count with the features of local plaque inflammation and vulnerability. Avanzas et al showed that the peripheral WBC count along with high-sensitivity C-reactive protein levels were related to the presence of multiple complex stenosis defined by contrast angiography in patients with acute35 and stable36 coronary disease. Using OCT imaging to delineate detailed plaque morphology we have now demonstrated the

![Figure 3](image1.png)

**Figure 3.** Box plots of (A) macrophage density of the fibrous cap of plaque and (B) the patients WBC count based on the classification of plaque into TCFA. Plaques categorized as TCFA demonstrated a significantly higher fibrous cap macrophage density and these patients had higher peripheral WBC counts. Values for macrophage density and WBC count are presented as a median with an interquartile range. TCFA was defined as lipid rich plaque (>2 quadrants of lipid) with a fibrous cap thickness <65µm. TCFA = thin-cap fibroatheroma, WBC = white blood cell count (10⁹cells/L).

![Figure 4](image2.png)

**Figure 4.** (A) Frequency of TCFA in relation to baseline WBC count, categorized as low (≤25th percentile, <7.45), intermediate (25th to 75th percentiles, 7.45 to 11.0), and high (75th percentile, 11.0). (B) Receiver operator curves for prediction of TCFA. TCFA was defined as lipid rich plaque (>2 quadrants of lipid) with a fibrous cap <65µm. TCFA = thin-cap fibroatheroma, WBC = white blood cell count (10⁹cells/L).
association between the peripheral WBC count and the important features of plaque vulnerability. Firstly, the WBC count correlated positively with plaque fibrous cap macrophage density. This relationship was independent of a patient’s presentation suggesting that irrespective of an individual’s clinical stability, a higher systemic inflammatory state reflected by high levels of a proinflammatory biomarker like the WBC count may, by virtue of being associated with plaque instability, still confer significant risk of future atherothrombotic events. This concurs with the epidemiological data from studies that demonstrate the ability of high WBC counts to predict cardiovascular events not only in subjects with ACS, but also in both stable vascular patients and subjects without known cardiovascular disease. 6,7,33 In addition the correlation between the WBC count and macrophage density was present in both culprit and remote plaques supporting the concept of the multicentric nature of the inflammatory process. Next, a higher WBC count was related to the presence of lipid-rich plaque and correlated negatively with fibrous cap thickness. Finally, patients who had plaque classified as TCFA had higher peripheral WBC counts than those who did not have TCFA. Subjects who had a WBC count within the 25th percentile for the group had no culprit plaque that were TCFA, whereas those who were in the upper quartile had 89% of all TCFA. Taken together these results are consistent with the findings of the prospective studies that show a graded increase in cardiovascular risk as the peripheral WBC count rises.6,7

Another interesting finding from this study is that the macrophage density and WBC count predict the presence of TCFA independently of each other with odds ratios of 5.5 and 3.0, respectively (P<0.05 for both). Further, in combination, they do so with greater precision. This would suggest that the peripheral WBC count might be representative of being more than just a marker of the biological activity of a plaque and adds further weight to the hypothesis that systemic inflammation is an important contributing factor to the vulnerability of an individual plaque. Future studies that involve the assessment of plaque vulnerability, therefore, will clearly need to include not only local plaque morphological and biological characteristics but also systemic proinflammatory biomarkers like the WBC count.

**Study Limitations**

There are some limitations to this study. Although consecutive subjects who were eligible and consented to the study were enrolled, excluding patients who were unstable, had renal impairment, or calcified vessels may have resulted in a selection bias. However, the enrollment criteria used are common to all studies that require invasive imaging and are necessary for subject safety. Blood leads to significant attenuation of the emitted infrared light. Therefore a blood-free zone is required for OCT imaging. This was achieved through intermittent saline flushes through the coronary guide catheter resulting in short image acquisition times. In addition, the low frame rates precluded catheter withdrawal while imaging. This enabled cross-sectional imaging only at discrete locations precluding comprehensive evaluation of the entire vessel. As a result only a limited part of the total plaque burden of the lesion could be sampled. Although careful catheter position using orthogonal angiographic views was undertaken, it is possible we did not image the exact center and shoulder regions on a plaque. It is also possible that we failed to image an area of importance such as an exact rupture site within a culprit lesion where fibrous cap attenuation and macrophage density would be at its greatest. Poor OCT image quality attributable to inadequate saline purging resulted in exclusion of some images from analysis, and this may have introduced an inadvertent selection bias. The limited penetration depth (2 to 3 mm) constitutes another limitation of OCT, because it does not enable visualization of the entire plaque or measurements such as plaque volume or the percentage area occupied by the entire lipid pool. However, because the most important morphological determinants of plaque vulnerability are superficial, this region of greatest interest was within the imaging range of the OCT system. Further despite the potential limitations of the OCT system, our results achieved a high level of significance. A second generation OCT technology, Optical Frequency Domain Imaging, is currently undergoing development and can provide more than 2 orders of magnitude improvement in image acquisition speed while not compromising resolution or image contrast. This development will facilitate continuous imaging of long segments of vessel wall, eliminating many of the technical limitations of the present study. With the resolution of the OCT system, the preprocessing steps used, and the range of sizes of macrophages, individual macrophages are not resolved. The computed macrophage density (NSD) originates from the reflectivity differences between the macrophages and surrounding cap matrix. When validated against histological controls this measurement demonstrated a significant correlation with immunohistochemical CD68 staining for macrophages. We evaluated only fibrous cap macrophage content. Lymphocytes, neutrophils, and other leukocyte subpopulations have been demonstrated in coronary plaque, however macrophages are pathophysiologically the most important and the predominant inflammatory cell population within the fibrous cap of vulnerable plaque.

We used only the total peripheral WBC count and therefore could not evaluate the associations of the various leukocyte subpopulations in the peripheral blood with vulnerable plaque characteristics. Further, we did not use other proinflammatory biomarkers such as high sensitivity C-reactive protein, interleukin (IL)-6, or lipoprotein associated phospholipase A2. The use of the WBC count and high sensitivity C-reactive protein level in combination have been shown in some studies to be a stronger risk predictor than either alone.33,37 It would, therefore, have been interesting to see if the addition of other biomarkers would have strengthened the associations already demonstrated with the WBC count in this study.

Imaging and assessments were performed only at a single time point. Prospective studies with follow-up will need to be done to confirm the robustness of the findings of this study over time and to explore their clinical relevance.

**Conclusions**

This study provides the first in vivo data demonstrating the association between the peripheral WBC count a marker of systemic inflammation, plaque fibrous cap macrophage density a marker of local plaque inflammation, and the characteristics and presence of vulnerable TCFA. The findings are in agreement with our current understanding of the pathophysiology of atherothrombosis and adds further validation for the role of inflamma-
tion in this process. It also demonstrates the capability of OCT as an imaging modality in defining the critical features of plaque vulnerability and its potential use, along with systemic biomarkers like the WBC count, in the quest to identify and study in vivo the natural history of vulnerable plaque.

Sources of Funding
O. Christopher Raffel was supported by the White-Parsons fellowship grant awarded by the National Heart Foundation of New Zealand.

Disclosures
G.J. Tearney and B.E. Brown received grant funding and honoraria from Terumo Medical Corporation.

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Arterioscler Thromb Vasc Biol. 2007;27:1820-1827; originally published online May 31, 2007;
doi: 10.1161/ATVBAHA.107.145987

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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