Plaque Neovascularization Is Increased in Ruptured Atherosclerotic Lesions of Human Aorta
Implications for Plaque Vulnerability

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Background—Growth of atherosclerotic plaques is accompanied by neovascularization from vasa vasorum microvessels extending through the tunica media into the base of the plaque and by lumen-derived microvessels through the fibrous cap. Microvessels are associated with plaque hemorrhage and may play a role in plaque rupture. Accordingly, we tested this hypothesis by investigating whether microvessels in the tunica media, the base of the plaque, and the fibrous cap are increased in ruptured atherosclerotic plaques in human aorta.

Methods and Results—Microvessels, defined as CD34-positive tubuloluminal capillaries recognized in cross-sectional and longitudinal profiles, were quantified in 269 advanced human plaques by bicolor immunohistochemistry. Macrophages/T lymphocytes and smooth muscle cells were defined as CD68/CD3-positive and α-actin–positive cells. Total microvessel density was increased in ruptured plaques when compared with nonruptured plaques ($P <$0.0001). Furthermore, microvessel density was increased in lesions with severe macrophage infiltration at the fibrous cap ($P <$0.0001) and at the shoulders of the plaque ($P <$0.0001). In addition, microvessel density was also increased in lesions with intraplaque hemorrhage ($P <$0.04) and in thin-cap fibroatheromas ($P <$0.038). Logistic regression analysis identified plaque base microvessel density ($P <$0.003) as an independent correlate to plaque rupture.

Conclusions—Thus, neovascularization as manifested by the localized appearance of microvessels is increased in ruptured plaques in the human aorta. Furthermore, microvessel density is increased in lesions with inflammation, with intraplaque hemorrhage, and in thin-cap fibroatheromas. Microvessels at the base of the plaque are independently correlated with plaque rupture, suggesting a contributory role for neovascularization in the process of plaque rupture. (Circulation. 2004;110:2032-2038.)

Key Words: atherosclerosis ■ plaque ■ inflammation ■ aorta

Nourishment of normal blood vessels is accomplished by oxygen diffusion from the lumen of the vessel or from adventitial vasa vasorum.¹ When vessel wall thickness exceeds the effective diffusion distance of oxygen, vasa vasorum proliferate in the inner layers of the vessel wall, where they are normally absent. Vasa vasorum are present in most arteries, including the aorta and coronary, carotid, and femoral arteries.¹ Pathological neovascularization of the vessel wall is a consistent feature of atherosclerotic plaque development and progression of the disease.²,³ Furthermore, microvessels play a role in plaque hemorrhage associated with the development of symptoms in cerebrovascular disease.⁴,⁵ In addition, microvessels are increased in coronary lesions from patients with acute myocardial infarction, suggesting a potential role for microvessels in plaque rupture.⁶,⁷ Histological features associated with plaque rupture include a large lipid core, thin fibrous cap, and increased inflammation.⁸ Furthermore, rupture of the internal elastic lamina (IEL) is also seen in complex plaques.⁹ Recently, microvessel-related intraplaque hemorrhage has been associated with lipid-core expansion through the accumulation of free cholesterol from erythrocyte membranes.¹⁰ In addition, intraplaque hemorrhage is a potent stimulus for macrophage activation and foam cell formation, thereby increasing plaque inflammation.¹¹ Therefore, microvessels may play a role in plaque rupture.

The present study was designed to test the hypothesis that microvessel density is increased in atherosclerotic lesions with plaque rupture and to investigate independent histological features associated with plaque rupture.

Methods

Full-thickness aortic wall histological sections from 269 lesions were taken sequentially at autopsy from 24 male patients (mean ± SD age,
61±16 years; range, 29 to 89 years). The aorta was slit open longitudinally, and the intima was washed with saline and then examined visually. On gross examination, aortas had diffuse atherosclerotic lesions with variably distanced spaces between plaques. No aneurysms were observed. A 20-cm aortic segment from the lower thoracic aorta extending into the abdominal aorta above the renal arteries was selected. Individual atherosclerotic plaques raised above the surface with a long axis >0.75 cm were studied. A 1.0-cm long by 0.5-cm wide sample with an edge of normal tissue was obtained for each plaque. The minimum distance between plaques was 0.5 cm. A total of 12 serial sections from each specimen were cut and stained by (1) hematoxylin and eosin (n=3); (2) elastic trichrome method (n=3); (3) double-labeled immunohistochemicals for endothelial cells and inflammatory cells (macrophages/T lymphocytes) with CD34 and CD68/CD3, respectively (n=3); and (4) double-labeled immunohistochemicals for endothelial cells and smooth muscle cells with CD34 and α-actin, respectively (n=3). The average value (total numbers divided by 3) is reported for each stain.

Immunohistochemistry

Labeling was performed on formalin-fixed, paraffin-embedded, 4-μm tissue sections on polylysine-coated–plus glass slides. Tissue sections were deparaffinized and then pretreated with sodium citrate–antigen retrieval at 5-minute intervals up to a total of 15 minutes. After endogenous peroxidase activity was blocked with 10% H2O2 in methanol, sections were subjected to double labeling. Primary monoclonal mouse anti-human antibodies of the IgG1 class (DAKO Corp) included the CD34 endothelial clone Qbend-10 at 1:30 dilution, CD68 macrophage clone KP-1 at 1:100 dilution/CD3 (DAKO Corp) included the CD34 endothelial clone Qbend-10 at 1:50 dilution. Affinity-purified, secondary anti-mouse IgG antibodies with Western blot confirmation (Vector) and red (alkaline phosphatase SK 5100, Vector) chromogen contrasting with smooth muscle cells surrounding neovessels in brown chromogen stained with α-actin.

Specificity of all antibodies was confirmed by routine positive and negative controls for each stain in human tonsil and bowel tissue to exclude nonspecific binding of the primary antibody.

Morphometry

Neovascularization

Microvessels were defined as tubuloluminal CD34-positive capillaries recognized in cross-sectional and longitudinal profiles as identified by double immunohistochemistry with CD34/CD68–CD3 in the intima and CD34/smooth muscle actin in the tunica media with a 40× magnification objective. Microvessel density was calculated by taking the total number of microvessels and dividing by plaque area (mm²). Quantification was regionally tabulated for 3 contiguous, nonoverlapping transmural sites for each individual section, including (1) microvessels located within the intima media, (2) microvessels located at the base of the plaque, and (3) microvessels located at the fibrous cap and shoulders. Because we encountered a highly variable quantity of adventitial tissue in these sections, we opted to exclude adventitial measurements from the analysis. Finally, total microvessel density (sites 1, 2, and 3 combined divided by plaque area) is also reported.

Inflammatory cells were defined as CD68/CD3-positive mononuclear round cells per high-power field with the 40× magnification objective. Inflammation was tabulated for 2 different regions of the atherosclerotic lesion including the fibrous cap and plaque shoulders. Severity of inflammation was scored as 0 (<5 cells), 1 (6 to 25 cells), and 2 (>25 cells).

Rupture of the IEL and other measurements, including minimum fibrous cap thickness, total plaque area, and lipid-core area, were quantified by nonautomated ocular micrometry and computerized planimetry, as previously reported. Data reported in this study are from a new set of aortas not included in our previous publication.

Histological Classification

Lesions with fibrous-cap rupture were defined as plaques with discontinuation of the fibrous cap associated with hemorrhage or thrombus, as shown in Figure 2A. Lesions without fibrous cap rupture were defined by using a modified version of the American Heart Association classification as fibrocalcific (class Vb and Vc) and lipid-rich (class IV and Va) plaques. Fibrocalcific plaques are usually devoid of a lipid core. Therefore, no fibrous cap could be consistently identified. Therefore, for neovessel comparisons with
lipid-rich and ruptured plaques in the fibrous cap/shoulder category, the corresponding luminal part of fibrocalcific plaques was evaluated and included in the analysis.

Lesions With Plaque Hemorrhage
Hemorrhage was defined as red blood cell extravasation within the plaque, as shown in Figure 2B. Serial sectioning was performed in every case of plaque hemorrhage to exclude fibrous cap rupture as the cause of hemorrhage. With these criteria, plaque hemorrhage was observed only in lipid-rich plaques.

Lesions With Thin, Fibrous Cap
Fibrous cap thickness ≤60 μm was defined as thin-cap fibroatheromas, as previously reported, and was observed only in lipid-rich plaques.

Lesions With Inflammation
Mild (score 0) inflammation was compared with moderate (score 1) and severe (score 2) inflammation within the fibrous cap and shoulder regions of the plaque, as described earlier.

Statistical Analysis
Data are presented as mean±SEM. For 2-group comparisons, gaussian-distribution samples were compared by the 2-tailed Student t test, preceded by Levene F test for equality of variances. Nongaussian-distribution samples were compared by the Mann-Whitney nonparametric test. For multiple comparisons, 1-way ANOVA was used. Discrete variables were compared with the χ2 test. The following variables were used in the analysis: plaque rupture and cap thickness ≤60 μm (dichotomous variables); cap inflammation and shoulder inflammation score (ordinal variables with values of 0, 1, or 2); media neovessel density; plaque-base neovessel density; cap/shoulder neovessel density; total neovessel density; plaque area; and lipid area (continuous variables). Plaque rupture was the outcome variable. To identify independent correlates with plaque rupture, univariate analysis consisting of cross tabulations of each variable by plaque rupture was performed. Continuous variables were categorized on the basis of quartiles for cross tabulation. Bivariate correlation coefficients between variables were also computed to identify collinearity. When the correlation coefficient between 2 variables was >0.6, only 1 was selected in the final model. The selection was based on the results of the univariate analysis and taking into account the significance level. Variables exhibiting statistical significance in the univariate logistic regression were chosen and then used in a multiple logistic regression model. SPSS 12.0 software was used for the analysis. Probability values <0.05 were considered significant.

Results
Lesions With Fibrous-Cap Rupture
Neovessel density in ruptured and nonruptured plaques is shown in Figure 3. Total neovessel density was increased in ruptured plaques when compared with nonruptured plaques (P=0.0001). Segmental analysis showed increased neovessel density at the tunica media (P=0.002) and at the base of the plaque (P=0.001) and a similar density in fibrous cap/shoulders (P=NS).

Lesions Without Fibrous-Cap Rupture
Neovessel densities in fibrocalcific and lipid-rich plaques were compared with those of ruptured plaques, as shown in Figure 4. Total neovessel density was lower in fibrocalcific plaques when compared with lipid-rich and ruptured plaques (P=0.0001). Segmental analysis showed decreased neovessel density in fibrocalcific plaques when compared with lipid-rich and ruptured plaques in all areas studied (P=0.0001). Neovessel density in the tunica media and at the fibrous cap was similar between lipid-rich and ruptured plaques (P=NS).
TABLE 1. Neovessel Density in Lipid-Rich Plaques With Hemorrhage and Thin, Fibrous Cap

<table>
<thead>
<tr>
<th>Neovessel Location</th>
<th>Hemorrhage (n=44)</th>
<th>No Hemorrhage (n=108)</th>
<th>P</th>
<th>Thin Cap (n=34)</th>
<th>No Thin Cap (n=118)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tunica media density</td>
<td>26±4.15</td>
<td>19±1.35</td>
<td>0.058</td>
<td>30±5.59</td>
<td>18±1.07</td>
<td>0.037</td>
</tr>
<tr>
<td>Plaque base density</td>
<td>2.1±0.41</td>
<td>0.8±0.05</td>
<td>0.004</td>
<td>1.8±0.46</td>
<td>1.0±0.10</td>
<td>0.08</td>
</tr>
<tr>
<td>Cap-shoulder density</td>
<td>0.5±0.16</td>
<td>0.2±0.08</td>
<td>0.036</td>
<td>0.3±0.08</td>
<td>0.3±0.13</td>
<td>0.99</td>
</tr>
<tr>
<td>Total neovessel density</td>
<td>29±4.7</td>
<td>20±1.5</td>
<td>0.042</td>
<td>32±6.1</td>
<td>19±1.3</td>
<td>0.038</td>
</tr>
</tbody>
</table>

However, at the base of the plaque, neovessel density was higher in ruptured plaques when compared with lipid-rich plaques (P=0.0001).

Lesions With Plaque Hemorrhage
Plaque hemorrhage without fibrous-cap rupture was seen only in lipid-rich lesions. Therefore, comparisons were performed within this category only (Table 1). Plaque base, cap/shoulder, and total neovessel densities were higher in lipid-rich lesions with hemorrhage when compared with lipid-rich lesions without hemorrhage (P<0.05). A tendency toward higher neovessel density in the tunica media was also observed (P=0.058).

Lesions With Thin, Fibrous Cap
Intact thin fibrous caps were present only in lipid-rich lesions. Therefore, comparisons were performed within this category only (Table 1). Total neovessel density was higher in thin-cap when compared with non-thin cap lesions (P=0.038; Table 1).

Lesions With Inflammation
Total neovessel density was lowest in lesions with mild inflammation, moderate in lesions with moderate inflammation, and highest in lesions with severe inflammation, as quantified at the fibrous cap and plaque shoulders (P=0.0001; Table 2). Finally, rupture of the IEL, lipid area, and inflammatory scores were higher and cap thicknesses lower in ruptured plaques when compared with lipid-rich and fibrocalcific plaques (P=0.0001; Table 3).

Correlates of Plaque Rupture
Univariate analysis identified significant variables as shown in Table 4. Furthermore, bivariate correlations identified collinearity in 5 pairs of variables, as follows: (1) Media neovessel density was correlated with total neovessel density (r=0.996); (2) plaque-base neovessel density was correlated with total neovessel density (r=0.711); (3) plaque-base neovessel density was correlated with media neovessel density (r=0.659); (4) lipid area was correlated with plaque area (r=0.853); and (5) cap inflammation was correlated with shoulder inflammation (r=0.644).

Of these 5 pairs of variables, plaque base neovessel density, lipid area, and cap inflammation exhibited more powerful statistical correlation with plaque rupture when compared with their colinear variables in the univariate analysis (as shown in Table 4) and were included in the final model, along with cap thickness ≤60 μm and rupture of the IEL. The final model had a good fit, as indicated by the Nagelkerke r² value of 0.69, a sensitivity of 94%, a specificity of 85%, and 92% overall correct prediction. All correlates included in the final model were statistically significant as shown in Table 4: A cap thickness ≤60 μm resulted in a significantly higher probability of plaque rupture than did cap thickness >60 μm (odds ratio, 23.4; P<0.001); rupture of the IEL resulted in a significantly higher probability of plaque rupture than nonrupture of the IEL (odds ratio, 13.7; P<0.001); a higher cap inflammation score increased the odds of plaque rupture >3-fold (odds ratio, 3.12; P=0.002), as did high microvessel density at the base of the plaque (odds ratio, 1.47; P=0.003) and large lipid area (odds ratio, 1.15; P=0.037).

Discussion
In this study of atherosclerotic neovascularization, we compared microvessel density in ruptured and nonruptured human plaques. Systematic quantification by computerized planimetry and ocular micrometry in bicolor contrasting immunostained sections documented increased microvessel density in ruptured plaques. Microvessel density was also increased in lipid-rich and ruptured plaques when compared with fibrocalcific lesions. Furthermore, lesions with intraplaque hemorrhage and a thin, fibrous cap also

TABLE 2. Neovessel Density in Advanced Lesions Evaluated by the Degree of Plaque Inflammation

<table>
<thead>
<tr>
<th>Neovessel Location</th>
<th>Mild (n=74)</th>
<th>Moderate (n=119)</th>
<th>Severe (n=76)</th>
<th>P</th>
<th>Mild (n=47)</th>
<th>Moderate (n=81)</th>
<th>Severe (n=141)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tunica media density</td>
<td>12.9±1.3</td>
<td>20.9±1.9</td>
<td>22.6±1.6</td>
<td>0.001</td>
<td>9.7±1.05</td>
<td>19.6±2.2</td>
<td>22.1±1.4</td>
<td>0.0001</td>
</tr>
<tr>
<td>Plaque base density</td>
<td>0.66±0.08</td>
<td>1.3±0.16</td>
<td>1.8±0.22</td>
<td>0.0001</td>
<td>0.49±0.13</td>
<td>1.05±0.12</td>
<td>1.6±0.17</td>
<td>0.0001</td>
</tr>
<tr>
<td>Cap-shoulder density</td>
<td>0.11±0.05</td>
<td>0.30±0.1</td>
<td>0.34±0.1</td>
<td>0.22</td>
<td>0.01±0.01</td>
<td>0.26±0.11</td>
<td>0.35±0.08</td>
<td>0.61</td>
</tr>
<tr>
<td>Total neovessel density</td>
<td>13.7±1.4</td>
<td>23.9±2.2</td>
<td>24.7±1.9</td>
<td>0.0001</td>
<td>10.2±1.2</td>
<td>20.9±2.4</td>
<td>24.1±1.7</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Mild indicates <5 cells per high-power field (HPF); moderate, 6 to 25 cells per HPF; and severe, >25 cells per HPF.
displayed higher microvessel density. In addition, microvessel density was low in lesions with mild inflammation and increasingly higher in lesions with moderate and severe inflammation. Finally, multiple regression analysis identified microvessels at the base of the plaque as an independent correlate of plaque rupture, along with established variables including a thin cap, inflammation, lipid area, and rupture of the IEL.

Our understanding of the role of neovascularization in atherosclerotic plaques is evolving and may include several processes. As part of the cellular inflammatory response to injury, microvessels contribute to the phase of repair. Once phagocytosis has removed the injurious agent, nonleukocytic mesenchymal elements such as neovascularization play an important role in repair.16 In pathological conditions, neovascularization varies from a transient contribution to healing (wound granulation tissue that regresses) to a permanent contribution for tissue regeneration. If the injurious agent persists, various patterns of chronic inflammation can develop. Newly formed microvessels coexist with osteoclast-like foreign-body giant cells (engulfing cholesterol crystals), necrosis, immune cells, and macrophages localizing an expanding granulomatous-like reaction, which characterizes complex atherosclerosis within the arterial wall.17 Recently, inhibition of angiogenesis by endostatin reduced plaque growth by 70% to 85%, suggesting a role for microvessels in progression of the disease.18

The origin of atherosclerotic neovascularization was elucidated by Kumamoto et al,19 who established communication with adventitial vasa vasorum in 97% of human coronary plaque microvasculature. The relation between microvessels, inflammation, and lipid-core expansion in advanced atherosclerosis is also evolving. Microvessels facilitate blood-derived inflammatory cells to penetrate through the vessel wall, increasing macrophage infiltration. Furthermore, inflammation also increases microangiogenic activity, amplifying macrophage recruitment.20 This study identified an incremental relation between neovascularization and inflammation, supporting a synergistic association in advanced disease. However, the association between neovascularization and plaque rupture

### TABLE 3. Histological Characteristics of Fibrocalcific, Lipid-Rich, and Ruptured Plaques

<table>
<thead>
<tr>
<th>Classification</th>
<th>No. (%)</th>
<th>Plaque Area, mm²</th>
<th>Lipid Area, mm²</th>
<th>Cap Thickness, µm</th>
<th>Ruptured IEL, No. (%)</th>
<th>Inflammation Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrocalcific plaques</td>
<td>42 (16)</td>
<td>10.4±0.88</td>
<td>2.4±0.38</td>
<td>...</td>
<td>26 (62)</td>
<td>0.1±0.07 0.3±0.08</td>
</tr>
<tr>
<td>Lipid-rich plaques</td>
<td>152 (66)</td>
<td>9.4±0.48</td>
<td>3.5±0.28</td>
<td>28±20</td>
<td>95 (63)</td>
<td>1.0±0.06 1.5±0.05</td>
</tr>
<tr>
<td>Ruptured plaques</td>
<td>75 (28)</td>
<td>12.7±0.77</td>
<td>6.2±0.44</td>
<td>33±2.0</td>
<td>68 (91)</td>
<td>1.5±0.06 1.7±0.06</td>
</tr>
<tr>
<td>Total and P value</td>
<td>269 (100)</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001 0.0001</td>
</tr>
</tbody>
</table>

Abbreviations are as defined in text.

### TABLE 4. Univariate and Multivariate Analysis to Identify Correlates of Plaque Rupture

<table>
<thead>
<tr>
<th></th>
<th>P</th>
<th>Odds Ratio</th>
<th>95% Confidence Intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Univariate analysis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Media neovessel density</td>
<td>0.087</td>
<td>1.01</td>
<td>0.99 1.028</td>
</tr>
<tr>
<td>Plaque base neovessel density</td>
<td>&lt;0.001</td>
<td>1.42</td>
<td>1.17 1.72</td>
</tr>
<tr>
<td>Cap/shoulder neovessel density</td>
<td>0.27</td>
<td>1.17</td>
<td>0.89 1.5</td>
</tr>
<tr>
<td>Total neovessel density</td>
<td>0.048</td>
<td>1.01</td>
<td>1 1.03</td>
</tr>
<tr>
<td>Rupture of IEL</td>
<td>&lt;0.001</td>
<td>5.86</td>
<td>2.55 13.44</td>
</tr>
<tr>
<td>Plaque area</td>
<td>&lt;0.001</td>
<td>1.09</td>
<td>1.05 1.15</td>
</tr>
<tr>
<td>Lipid area</td>
<td>&lt;0.001</td>
<td>1.29</td>
<td>1.18 1.41</td>
</tr>
<tr>
<td>Cap thickness &lt;60 µm</td>
<td>&lt;0.001</td>
<td>36.92</td>
<td>16.55 82.36</td>
</tr>
<tr>
<td>Cap inflammation score</td>
<td>&lt;0.001</td>
<td>4.82</td>
<td>3.02 7.69</td>
</tr>
<tr>
<td>Shoulder inflammation score</td>
<td>&lt;0.001</td>
<td>2.91</td>
<td>1.83 4.62</td>
</tr>
<tr>
<td><strong>Multivariate analysis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cap thickness &lt;60 µm</td>
<td>&lt;0.001</td>
<td>23.5</td>
<td>9.3 58.9</td>
</tr>
<tr>
<td>Rupture of IEL</td>
<td>&lt;0.001</td>
<td>13.7</td>
<td>4.02 46.9</td>
</tr>
<tr>
<td>Cap inflammation score</td>
<td>0.002</td>
<td>3.12</td>
<td>1.51 6.45</td>
</tr>
<tr>
<td>Plaque base neovessel density</td>
<td>0.003</td>
<td>1.47</td>
<td>1.14 1.9</td>
</tr>
<tr>
<td>Lipid area</td>
<td>0.037</td>
<td>1.15</td>
<td>1.01 1.32</td>
</tr>
</tbody>
</table>

Abbreviations are as defined in text.
may be mechanistically or casually related. Microvessels are traditionally increased in large plaques. Therefore, increased microvessel density in ruptured plaques may be a reflection of plaque size. Nevertheless, microvessel density was independently associated with plaque rupture. Furthermore, large, fibrocalcific plaques exhibited the lowest microvessel density. In addition, recent studies suggest that microvessel leakage may contribute to lipid-core expansion preceding plaque rupture.21 As a result, some evidence for a mechanistic association is emerging. With the advent of potent imaging technology (magnetic resonance imaging or ultrasound-directed microbubble imaging), neovascularization may constitute a suitable target to completely elucidate this issue.22,23

Previous reports have studied plaque neovascularization in advanced atherosclerosis in carotid endarterectomy specimens.24,25 However, these studies did not evaluate neovascularization across the vessel wall. Our study in aortic sections identified neovessel density within the plaque to be lower than that within the tunica media. Nevertheless, only neovessels at the base of the plaque were independently associated with plaque rupture. Furthermore, correlations between macrophage infiltration in high-risk zones, lipid area and minimum cap thickness (in microns), are also needed to address independent relations between microvessel density and plaque rupture. Thus, our results expand knowledge in providing a careful evaluation of neovessel density across the vessel wall, with a segmental correlation between microvessel density and plaque rupture, independent of traditional features of vulnerability.

Finally, the low microvessel density in fibrocalcific plaques requires further investigation. These lesions exhibited a large plaque area with a very minimal amount of lipid. This observation may be important in the understanding of plaque stabilization, but more studies are needed to elucidate this issue.26

Study Limitations

The abdominal aorta may be considered avascular tissue. Nevertheless, previous studies have shown vasa supplying the abdominal aorta arising from the origin of lumbar and mesenteric arteries.27 Data are reported as the number of neovessels per plaque area. Medial area may have been included when reporting medial neovessel density. Nevertheless, data were collected for plaque area only and may limit the interpretation of the results. In addition, coronary lesions were not investigated in this study. Previous extensive experience in aortic disease influenced our decision to use aortic instead of coronary plaques. As a result, the interpretation of results should be maintained in the setting of aortic disease. Finally, despite the fact that CD34 immunostaining is specific for endothelial cells in proliferating vessels, it cannot differentiate between microcapillaries and microlymphatics. Lymphangiogenesis from large-vessel origin (thoracic duct) may be identified with specific markers.28,29 Therefore, differentiation between microvessels and microlymphatics requires further investigation.

Conclusion

Vessel-wall and plaque microvessels are increased in ruptured atherosclerotic plaques, suggesting a link between microvessels and plaque instability. Future prospective studies evaluating plaque microvessels in vivo are needed to completely elucidate this issue.

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