The correlation of carotid plaque pathohistologic features and neurological symptoms: A meta-analysis of observational studies

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Objective: To investigate the association between the pathological features of carotid plaque and ipsilateral symptoms of cerebral ischemia. Materials and Methods: We sought to identify clinical observational studies comparing the incidence of pathological features between symptomatic and asymptomatic carotid plaques in terms of ulceration, thrombosis and intraplaque hemorrhage (IPH), published between 1996 and 2006. A search on PubMed was supplemented by a review of bibliographies of relevant articles and lists of references in it. Odds ratios (OR) for the presence of each feature as a role in the pathogenesis of neurological events were calculated and combined by a meta-analysis. Results: We integrated 16 clinical studies totaling 2839 plaques harvested at endarterectomy procedures. The reported incidence for each feature was highly variable. The methods in defining ulceration, thrombosis and IPH were very heterogeneous. The time intervals between the latest onset of ischemic symptoms and pathological examinations varied greatly. More considerations should be given to the uniformity in definition as well as the control of confounders and time intervals in future studies.

Conclusions: Ulcerated carotid plaque is significantly correlated with the production of neurological events, whereas thrombosis fails to correlate with ischemic symptoms because of the presence of substantial heterogeneity. The association between IPH and clinical presentations is less clear.

Key words: Asymptomatic carotid stenosis, endarterectomy, symptomatic carotid stenosis, pathology, meta-analysis
selectively those who are at great risk.

Nevertheless, the causal relationship of pathological features (particularly for surface thrombus and IPH) to a history of ipsilateral brain ischemia before CEA was reported conflicting. Conclusive data by means of a quantitative synthesis were also not available.

This systematic review was designed to utilize quantitative methods for combining information across different studies over a span of 11 years. It was undertaken to determine whether a correlation could be established between pathological features of carotid plaque and clinical presentations. Also, the effect sizes were estimated and possible sources of heterogeneity in results across studies were identified and explained.

**Materials and Methods**

**Search strategy**

We sought observational studies in English, published between January 1996 and September 2006, inclusive. (1) We identified studies by comprehensive text-words and MeSH-based electronic searches of Medline (Entrez PubMed NIH); (2) Electronic search terms included: carotid stenosis/plaque, pathology, histology, ulcerated, or ulceration, thrombosis or thrombus, Intra-plaque hemorrhage or intramural hemorrhage; (3) The abstract of each article was carefully reviewed to detect appropriate publication; (4) Perusal of all reference lists of all relevant articles containing data that concerned were checked for further leads; (5) Additionally, we did a manual search of the following journals: Stroke and Journal of Vascular Surgery.

**Inclusion criteria**

Studies were included if: (1) specimens were harvested at endarterectomy and the pathological features were characterized; (2) they compared the incidence of pathological features between symptomatic and asymptomatic patients; (3) basic data regarding the frequency of ulceration, thrombosis and IPH were provided in both groups.

Generally, there were two methods used for defining: microscopic (histological) examination and macroscopic (gross) inspection. Specimens should be removed en bloc in order to avoid misleading gross findings. Histological features were evaluated by examining sections. Patients or plaques were defined as symptomatic if ipsilateral symptoms of cerebral ischemia were present within the last six months, such as transient ischemic attack (TIA), amaurosis fugax (AFX) or stroke. Asymptomatic plaques were from patients without symptoms or from the side without symptoms in those undergoing bilateral operations.

**Exclusion criteria**

Studies that met one of the following items were excluded: (1) animal studies, case reports, editorials, letters and reviews; (2) highly selected studies (symptomatic patients only); (3) failed to characterize the pathological features; (4) nonatherosclerotic carotid stenosis (e.g., fibromuscular dysplasia, postradiotherapy); (5) restenosis reports (e.g., occurring after endarterectomy or carotid angioplasty and stenting). Multiple publications from the same project were eliminated from the meta-analysis and the one that was most appropriate to this review was recruited.

**Data extraction**

The following general descriptive information was extracted from each study; (1) number, age and sex of participants; plaque number; (2) time intervals between the latest onset of ischemic symptoms and pathological examinations; (3) methods of evaluation: macroscopy or microscopy; (4) numbers of plaques with ulceration, thrombosis and IPH assigned to each group; (5) risk factors for atherosclerosis.

The authors (P, G, F, L and Z, Q, C.) selected the studies to be included in the review, extracted the data independently and cross-checked them, with disagreement resolved by consensus.

**Statistical analysis**

We calculated the odds ratio (OR) in each study. The statistical validity of aggregating the studies was assessed with a χ² for heterogeneity by means of a standard fixed-effect model of overview (P<0.1 suggests that the assumption of homogeneity was violated). A random-effect model was performed if an apparent trend for heterogeneity was observed. If the value of OR was more than 1, the risk of a particular feature was greater within symptomatic groups.

We performed sensitivity analysis to test the results under varied conditions such as the exclusion of studies that contributed the largest number of plaques and as a group. The meta-analysis was performed using RevMan (Version 4.2.8). Potential publication bias was assessed using an inverted funnel plot for asymmetry. Egger's and Begg's methods were used to test publication bias objectively (Version 9.0, STATA). A cut-off P value of 0.1 was used as the positive threshold bias to assess the power of statistical test.

**Results**

Thirty-six potentially relevant studies were identified. We excluded seven that were validation studies comparing carotid plaque imaging with histology[6-12] five that failed to provide sufficient data in each group;[13-17] five that were highly selected studies;[18-22] three studies in which ulcerated plaques were identified by angiography in vivo.[23-25] The remaining 16 studies included 2769 patients totaling 2839 carotid plaques [Table 1]. The ratio of males to
females was 2.2:1. The average age was 68.5 years. The number of carotid plaques in the studies ranged from 19 to 1008 (median, 78). There were 1699 symptomatic plaques and 1140 asymptomatic plaques (ratio, 1.5:1) altogether. Associated risk factors were equally distributed in eight studies[26,27,29,34,36,37,40,41] and not stated in the remaining. Five studies evaluated plaques from groups with equal severity of carotid stenosis,[26,27,29,33,37] The presenting symptoms were further sub-typed into transient symptoms and prior stroke in six studies.[28,31,33,37,38,41] The time intervals between the latest onset of symptoms and surgery were available from 13 studies,[26,31,33-39] with great variations. Plaque specimens were evaluated in five studies by macroscopy,[28,31,34,35,39] and in 11 by microscopy or a combination of both. For macroscopy, the basis for evaluation was in situ observation and photography after arteriotomy.[28,31] We, along with Lovett’s review,[42] found that histological methods including position, number, stains and frequency of section varied considerably. A transversal microscopic evaluation was performed among all the included studies.

There were three studies specifying the details about the interference of severity of stenosis with plaque morphology.[28,31,38] They all found that the frequency of plaque ulceration was independent of the degree of ICA stenosis. As for IPH, Park et al indicated that hemorrhage occurred more frequently in the highly stenotic lesions.[33] In Ballotta’s study, the frequency of IPH also appeared increased with the extent of disease, but the difference did not reach significance.[31] The presence of thrombus was unaffected by the degree of stenosis in Fisher’s study.[36]

### Ulceration
Among 10 studies,[26-29,31,32,34,35,38,40] a significantly higher incidence of ulceration was found in the symptomatic group compared with asymptomatic [Figure 1A]. Because the two largest studies that contributed approximately 70.8% of the total number of plaques dominated the results of the overview,[28,31] we performed a meta-analysis (exclusion of the two studies) to test the robustness of the result. The strength of association was even stronger and the sensitivity analysis did not substantially alter the point estimates of the results. No visual asymmetry could be observed in Funnel plotting [Figure 1B]. Formal statistical testing did not indicate evidence of publication bias.

#### Table 1: Summary of 16 clinical studies

<table>
<thead>
<tr>
<th>References</th>
<th>Year</th>
<th>Micro†</th>
<th>Macro‡</th>
<th>Case no.</th>
<th>Plaque no.</th>
<th>Symptoms</th>
<th>Pathological features</th>
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<tbody>
<tr>
<td>Can[26]</td>
<td>1996</td>
<td>+</td>
<td>+</td>
<td>44</td>
<td>44</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Bassiouy[27]</td>
<td>1997</td>
<td>+</td>
<td>+</td>
<td>99</td>
<td>99</td>
<td>19</td>
<td>25</td>
</tr>
<tr>
<td>Park[28]</td>
<td>1998</td>
<td>+</td>
<td>...</td>
<td>1008</td>
<td>1008</td>
<td>59</td>
<td>40</td>
</tr>
<tr>
<td>McCarthy[29]</td>
<td>1999</td>
<td>+</td>
<td>...</td>
<td>28</td>
<td>28</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>Montauban[30]</td>
<td>1999</td>
<td>+</td>
<td>...</td>
<td>47</td>
<td>47</td>
<td>33</td>
<td>14</td>
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<td>Ballotta[31]</td>
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<td>+</td>
<td>...</td>
<td>401</td>
<td>457</td>
<td>289</td>
<td>168</td>
</tr>
<tr>
<td>Kim[32]</td>
<td>2000</td>
<td>+</td>
<td>+</td>
<td>55</td>
<td>55</td>
<td>38</td>
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<td>71</td>
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<td>25</td>
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<td>Malatt[34]</td>
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<td>+</td>
<td>22</td>
<td>22</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td>Stork[35]</td>
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<td>...</td>
<td>109</td>
<td>109</td>
<td>71</td>
<td>38</td>
</tr>
<tr>
<td>Mile[36]</td>
<td>2003</td>
<td>+</td>
<td>...</td>
<td>272</td>
<td>281</td>
<td>133</td>
<td>148</td>
</tr>
<tr>
<td>Spagnoli[37]</td>
<td>2004</td>
<td>+</td>
<td>...</td>
<td>269</td>
<td>269</td>
<td>187</td>
<td>82</td>
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<td>Fisher[38]</td>
<td>2005</td>
<td>+</td>
<td>...</td>
<td>208</td>
<td>208</td>
<td>80</td>
<td>128</td>
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<tr>
<td>Tziadas[39]</td>
<td>2005</td>
<td>+</td>
<td>...</td>
<td>18</td>
<td>19</td>
<td>11</td>
<td>8</td>
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<tr>
<td>Krupinski[40]</td>
<td>2006</td>
<td>+</td>
<td>+</td>
<td>38</td>
<td>38</td>
<td>29</td>
<td>9</td>
</tr>
<tr>
<td>Turu[41]</td>
<td>2006</td>
<td>+</td>
<td>+</td>
<td>84</td>
<td>84</td>
<td>55</td>
<td>29</td>
</tr>
</tbody>
</table>

†Micro, microscopic examination, ‡Macro, macroscopic inspection, **IPH, intraplaque hemorrhage. ND, No data

**Figure 1:** A. Pooled estimate of ORs and 95% CI for ulcerated carotid plaques (via a fixed-effect model). B. Assessment of publication bias using the funnel plot. No asymmetry was observed.
Six studies\cite{26,27,35-38} were identified focusing on the characterization of surface thrombosis. The reported incidence had been highly variable (7.1-72.7%, median 20.7%). Thrombosis was defined diversely. Microscopically, Spagnoli et al defined a thrombotti
cally active plaque as the presence of an acute thrombus constituted of pla
et or fibrin on the plaque surface. In this context, thrombosis was divided into two categories: thrombus associated with plaque rupture and super-facial erosion.\cite{37} The former category, rather than erosion, was significantly correlated with the presence of ischemic symptoms. Fisher et al stressed that the criteria for the presence of thrombosis required a substantial-sized lesion. In this study, thrombus was considered present only when the lumen surface was covered by thrombotic material larger than 340 x 110 \(\mu\)m.\cite{38} Macroscopically, thrombus was defined as that adherent to the plaque surface and/or propagating into the arterial lumen.\cite{35} Of note, two studies indicated that fresh specimens were rinsed to remove surface blood.\cite{27,36} No definitions were specified in one study.\cite{27} We began with a fixed-effect model for our analysis. The magnitudes of overall differences between two groups were of statistical significance [Figure 2A]. However, homogeneity test indicated an extreme trend for heterogeneity among studies. Estimated effect sizes were then combined via a random-effect model and the differences between the two groups lost significance [Figure 2B].

**Discussion**

**Ulceration**

The first result of this review is the demonstration of a strong association between plaque ulceration and an initial presentation with TIA, AFx or stroke. As early as the 1970s, a strong association was found between plaque ulceration and clinical presentations.\cite{43-44} This correlation was reproduced in subsequent studies\cite{28,31,45,47} except two.\cite{26,46} It was further verified in our meta-analysis.

It should be noted that the reported incidence has been highly variable in the literature (9.1-89.1%, median 65.3%), partly explained by the diverse methods in defining. Pathologically, such ulcers have been previously defined as plaque surface defects of at least 1 mm or more than 560 \(\mu\)m in diameter and depth.\cite{48-50} Usually, gross ulceration was considered as plaque surface irregularity

**Intraplaque hemorrhage**

Pathologic evaluation was investigated in four studies\cite{26,27,35,38} by macroscopy and in seven\cite{26,27,29,30,33,36,41} by microscopy. The incidence of IPH had been reported highly variable (18.2-87.2%, median 39.3%). The methods in defining IPH ranged remarkably, from visual inspection of gross sections,\cite{28,31} microscopic evidence of red blood cells,\cite{26,29,38} histological evidence of hemosiderins,\cite{33,36} to a combination of all degenerated blood constituents.\cite{26} For macroscopy, IPH was usually characterized by its immediate recognition or on transecting the plaques at the time of arteriotomy.\cite{26,28,31} Studies in which light microscopy was performed, had been based on a broad definition of hemorrhage. Definitions were not specified in three studies.\cite{27,30,41} The magnitudes of overall difference between two groups failed to yield statistical significance. Likewise, insignificant differences were reproduced in sub-group analyses with respect to macroscopy and microscopy [Figure 3].

All the results are summarized in Table 2.
at the time of arteriotomy. Pinholes of less than 1 mm or smooth invaginations with no evidence of disruption of fibrous cap were not considered.\textsuperscript{[28,31]} In contrast, the definitions of microscopic ulceration tended to be more variable. In Carr’s study, plaques with evidence of rupture were classified separately from those with intimal disruption without rupture. Thus few plaques were identified with microscopic ulceration and no significant difference was found between two groups.\textsuperscript{[26]} Fisher \textit{et al} described a relatively low incidence (21.6\%) compared with other studies, because the presence of histological ulceration required a substantial size (560 \(\mu\)m or more).\textsuperscript{[38]} Currently, the correlation between ulceration and degree of stenosis remains a controversial matter.\textsuperscript{[28,31,38,45]} There has been no evidence that the pathogenetic role of ulceration might differ between patients with transient ischemia and those with prior stroke.

**Thrombosis**

Thrombosis, frequently secondary to disruption of carotid plaques, appears to be important for the production of ischemic symptoms. A large clinicopathologic series of CEA specimens has demonstrated that thrombosis associated with plaque rupture was one of the major determinants of ischemic stroke.\textsuperscript{[27]} Further evidence could be found that luminal thrombosis was the main source of downstream cerebral microemboli as detected by transcranial Doppler monitoring.\textsuperscript{[51]} Unfortunately, we observed an extreme tendency for heterogeneity across studies. An insignificant estimator was observed by combing effects via a random-effect model.

The occurrence of major heterogeneity across studies was partly due to the lack of consensus and consistency in defining surface thrombosis. Differences in the identification of thrombus could account for variable pathogenetic role in the symptom production. Thrombosis could be defined either visually or microscopically. Gross assessment was usually susceptible to subjective judgment. Some misleading findings may also be introduced by rinsing to remove surface blood. Thus, it otherwise explained the fact that reported incidence of thrombus varied widely from one to another. Second, some\textsuperscript{[37,38,52]} noted that the low incidence of thrombus was associated with a longer time interval between the onset of symptoms and pathological examination. Symptomatic carotid stenosis is usually defined by the occurrence of neurological symptoms related to the side of stenosis within the last six months prior surgery. However, Spagnoli \textit{et al} even included some patients with a time interval of 30 months.\textsuperscript{[37]} Variable time intervals appeared to account for some of the heterogeneity. Finally, factors such as long-term preoperative administration of aspirin and use of heparin during surgery could modulate the development of thrombosis as well.\textsuperscript{[36]}

On the other hand, there was some evidence that thrombosis was less important in the production of symptoms. It was found that the presence of thrombosis was strongly associated with ulceration and IPH.\textsuperscript{[21,38]} Ulceration represents an important substrate from which thrombus may later arise. Thrombus hence sometimes failed to serve as a separate entity per se. This could potentially prevent it from being an independent risk factor for neurological events.

Additionally, there were two studies in which symptoms were sub-typed into transient ischemia and prior stroke. Fisher \textit{et al} found that thrombus was similarly observed between TIA and stroke groups,\textsuperscript{[18]} whereas in Spagnoli’s report, the incidence of thrombosis in major stroke group was significantly higher than that in TIA group (\(P<0.001\)).\textsuperscript{[37]} The pathogenetic role of thrombus appeared varying over the types of ischemic symptoms. It suggested neurological symptoms should be stratified in future studies instead of a simple combination of AFX, TIA or stroke or the findings might be overinterpreted.

**IPH**

There has been considerable emphasis on the role of IPH in the pathogenesis of brain ischemia following extensive clinicopathologic analysis. Unfortunately, previous studies presented a mass of conflicting results.\textsuperscript{[46,47,53-56]} Our meta-analysis suggested a null correlation. Sub-category analysis in terms of the methods of definition was performed and an insignificant correlation was observed in each group.

Effect might be invalidated by the heterogeneous methods of definitions. Tiny or small hemorrhage may be overlooked grossly. Microscopy tended to be more sensitive for detecting IPH than macroscopy.\textsuperscript{[26]} Diverse histological methods of definition could be found across studies as well. Therefore, a uniform and accurate method for definitions is necessary. Second, insignificant effect

<table>
<thead>
<tr>
<th>Types of studies combined</th>
<th>N</th>
<th>Odd ratios</th>
<th>95% confidence interval</th>
<th>(P) value</th>
<th>Test for heterogeneity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulceration</td>
<td>10</td>
<td>2.32*</td>
<td>1.90-2.83</td>
<td>&lt;0.00001</td>
<td>0.62</td>
</tr>
<tr>
<td>Thrombosis</td>
<td>6</td>
<td>2.02*</td>
<td>1.45-2.81</td>
<td>&lt;0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>Thrombosis</td>
<td>6</td>
<td>1.57*</td>
<td>0.68-3.64</td>
<td>0.29</td>
<td>0.0001</td>
</tr>
<tr>
<td>Intraplaque hemorrhage</td>
<td>11</td>
<td>1.09*</td>
<td>0.81-1.32</td>
<td>0.35</td>
<td>0.10</td>
</tr>
<tr>
<td>microscopic studies</td>
<td>7</td>
<td>1.18*</td>
<td>0.83-1.68</td>
<td>0.36</td>
<td>0.03</td>
</tr>
<tr>
<td>macroscopic studies</td>
<td>4</td>
<td>1.06*</td>
<td>0.85-1.32</td>
<td>0.60</td>
<td>0.68</td>
</tr>
</tbody>
</table>

*Values were calculated with a fixed-effect model. *Values were calculated with a random-effect model.
could be partly explained by the variable intervals between the latest onset of ischemia and pathological examinations. Lasby et al.\(^{[34]}\) suggested a short interval (2.9 weeks) as a key factor in establishing the positive correlation between acute hemorrhages and clinical manifestations. However, our review included studies with intervals ranging from within several weeks\(^{[26,30]}\) to six months.\(^{[27,36]}\) Acute or fresh IPH could be stabilized by a process of healing or turning into an avascular structure (calcified or fibrous tissues) as time goes by. Therefore, the true number of IPH could be underestimated in symptomatic groups. Finally, as previous studies suggested that IPH occurred more frequently with high-grade stenosis,\(^{[28,57,58]}\) carotid artery severity, this confounder that related to both the presence of IPH and the neurological symptoms under study, could potentially distort results. To correct for the confounder, it is recommended that equal severity in both groups should be assumed in the subsequent studies. However, details were specified in only four studies out of 11.\(^{[26,27,29,33]}\)

Likewise, there was some evidence that the pathogenetic role of IPH might differ between patients with transient events and those with permanent neurological deficits.\(^{[28,31]}\) Basic data were provided in only four studies.\(^{[28,31,33,44]}\) A summation calculation for them suggested the incidence of IPH was not significantly different between asymptomatic and transiently symptomatic groups (32.5% vs. 31.2%, \(P=0.632\)). Nevertheless, there was a salient trend of more IPH in the prior stroke group, compared with asymptomatic group (43.8% vs. 32.5%; \(P=0.003\)).

Currently, improved technology and resolution have resulted in the capacity to quantify plaque components.\(^{[59,60]}\) Pixel analysis at ultrasonography and local temperature probes as well as \(^{[11]}\) Indium-platelet scintigraphy is able to help in identifying vulnerable plaques.\(^{[61-63]}\) Also, high-resolution MRI could not only identify, but also stage intraplaque hemorrhage with good accuracy and reproducibility in advanced atherosclerotic carotid plaques.\(^{[12]}\) Therefore, a better understanding of its role in plaque stability and evolution would be achieved.\(^{[64]}\) Moreover, current techniques provide an opportunity to prospectively examine the relationship between plaque features and subsequent cerebrovascular events.\(^{[65]}\) Early interventions aimed to prevent imminent neurological events might be more justifiable.

Generally, there were three methodological issues that could influence the strength of association including diverse methods of definitions, variable time interval and the unequal degree of carotid stenosis. One limitation of this meta-analysis may be the heterogeneity of the different studies as far as the populations studied were concerned. Statistical validity of our meta-analysis could be potentially attenuated by the variation in the criteria used in defining the pathologic features. Individual studies were mostly hospital-based, which could also potentially prevent the inference of our results to the general population. Second, the age, size, number and location of IPH as well as the size of ulceration and thrombosis could influence the strength of association. Nevertheless, we could not draw a valid estimate because few studies provided specific data. Finally, our study was based on a qualitative evaluation. A number of quantitative reports have surfaced in the past decade.\(^{[30,46,65]}\) Similarly, an estimator was not permitted to be made mainly because of disparate methods in quantifying and insufficient details regarding these features.

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References

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