The vulnerable plaque: From plaque instability towards thrombus instability
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Total burden of intraplaque hemorrhage in coronary arteries relates to the use of coumarin-type anticoagulants but not platelet aggregation Inhibitors

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Submitted
Chapter 4

Abstract

Background
Intraplaque hemorrhage (IPH) is a crucial factor in progression and destabilization of an atherosclerotic plaque. Anti-thromboembolic drugs are widely used as prophylactic treatment against arterial and venous thrombotic diseases, but a major complication is bleeding. We investigated the association between exposure to anti-thromboembolic therapy and IPH in post mortem coronary arteries.

Materials and methods
Coronary arteries with postmortem angiographically confirmed extensive atherosclerosis were obtained at autopsy from patients who had received oral anticoagulants (n=10), platelet aggregation inhibitors (n=10) or no anti-thrombotic drugs (n=10) before death. Coronary arteries were cut at 3-mm interval and all plaque containing segments were immunohistochemically screened for IPH and microvessels. These data were related to overall plaque composition and the use of anti-thromboembolic therapies.

Results
IPH was found in 483 out of 904 (53%) coronary segments with advanced atherosclerotic plaques, and more frequently in patients on oral anticoagulants (174/284, 61%) than in patients on anti-platelets (198/376, 53%) or without therapy (111/244, 46%) (P=0.02 and P=0.001, respectively). Also, intraplaque microvascular leakage was more frequently observed in patients on anticoagulants than in non-treated patients (P=0.03). Finally, the IPH appeared to be larger in plaques of patients on anti-coagulant treatment (P<0.001). Density of intraplaque microvessels was highest in plaques of patients on platelet inhibitors (P<0.05), but this was not associated with increased hemorrhagic burden.

Conclusion
Prophylactic therapy with oral coumarin-type anticoagulants appears to be associated with a higher hemorrhagic burden in atherosclerotic coronary arteries, which may lead to increase in plaque volume over time, in this selected subgroup of patients.
Introduction

Intracoronary thrombosis initiated by disruption of an atherosclerotic plaque is the most important trigger for the onset of acute coronary syndromes\(^1,2\). Many plaques that are prone to develop thrombotic complications can be histologically defined as lesions with a thin fibrous cap, a high inflammatory burden and a large necrotic core. Intraplaque hemorrhage (IPH) can play a crucial role in the development of such “high risk” plaques\(^3-7\). Such hemorrhages can lead to abrupt enlargement of the liponecrotic core of a plaque by accumulation of free cholesterol that is released from erythrocyte membranes\(^5,6\). Moreover, IPH further contributes to inflammatory activity inside plaques by facilitating the recruitment and activation of inflammatory cells as well as by oxygen radicals in lesions\(^8,9\).

Both arterial and venous types of thrombosis are associated with high morbidity and mortality, especially within the elderly population. Arterial thrombotic vessel occlusion, which is initially a platelet driven process followed by activation of the coagulation cascade, underlies various acute vascular events such as myocardial infarction, stroke and limb ischemia\(^10\). In the venous system, the coagulation system is believed to play an important role in thrombus formation, leading to fibrin rich thrombi underlying deep venous thrombosis\(^11\). Consequently, platelet aggregation inhibitors and anticoagulants are widely used by patients who are at high risk to develop these arterial and venous thrombotic diseases. Clinical studies showed that these prophylactic treatment strategies significantly reduce fatal thrombotic and thromboembolic events\(^12-14\), but their safety in terms of sometimes life threatening side effects such as major hemorrhages is a matter of great concern\(^15\). Major hemorrhages in most cases affect the gastrointestinal tract, soft tissues, and/or urinary tract\(^16\). The incidence of intracranial bleeding is much lower, but this complication often has a fatal outcome\(^16-18\).

Preliminary studies in symptomatic atherosclerotic plaques have also demonstrated a positive association between the use of anticoagulant drugs and presence of IPH in symptomatic atherosclerotic plaques that were studied in endarterectomy specimens of patients with TIA and/or stroke\(^19\).

Patients with extensive coronary atherosclerosis, especially in the elderly population with a high rate of cardiovascular co-morbidity, frequently use oral platelet aggregation inhibitors or anticoagulant therapy or both. These drugs could initiate the onset of IPH or aggravate the extent of IPH in advanced vascularized plaques throughout their coronary arteries, also in pre-symptomatic stages, and thus contribute significantly to the progression of the coronary plaque burden. In order to test this hypothesis, we systematically investigated the incidence and severity of IPH in coronary arteries of patients who were treated with either oral anticoagulants or with platelet aggregation inhibitors.
For this purpose we studied the entire epicardial coronary artery tractus of 30 autopsied patients who were selected for the presence of atherosclerosis in all the three epicardial coronary arteries using histology and immunohistochemistry. In these patients the hemorrhagic burden of coronary plaques and histopathological plaque characteristics were related to anti-thromboembolic medication profiles, which was classified as either anti-platelet therapy, anticoagulant therapy or no anti-thromboembolic medication.

Materials and methods

Patient materials
The study is based on 78 out of a total of 648 patients who were autopsied at the department of Pathology of the Academic Medical Center Amsterdam in the period between 2008 and 2010, and of whom all the three epicardial coronary arteries showed presence of atherosclerotic plaques on postmortem coronary angiograms. Clinical data were collected from hospital electronic patient record. For the enrollment of patients in this study we applied the following inclusion criteria: 1) availability of information on anti-thromboembolic medication, 2) patients age > 50 years. Exclusion criteria were: sepsis, previous PTCA treatment and treatment with immunosuppressive drugs or chemotherapy. Following this approach, we identified 18 patients treated with coumarine-type anticoagulants (acenocoumarol or fenprocoumon), but no platelet aggregation inhibitors; 11 patients treated with platelet aggregation inhibitors (acetylsalicylic acid and / or clopidogrel), but no anticoagulants, and 19 patients who used neither anticoagulation nor anti-platelet therapy. Of each group, 10 patients were randomly selected for this study. In addition, one patient who received a double-treatment with both anticoagulant and platelet aggregation inhibitor was not included for the final analysis. This study was performed in accordance with the Helsinki Declaration and met the criteria of the code of proper use human tissue that is used in the Netherlands.

Tissue processing
After fixation in buffered formalin and decalcification in ethylene diamine tetra acetic acid (EDTA), the coronary arteries were transversely cut at 3 mm intervals throughout the three major epicardial coronary arteries (left anterior descending, left circumflex, and right coronary artery). To get insight of total hemorrhagic burden of the entire coronary system, all the segments containing macroscopically visible atherosclerotic lesions were collected and embedded in paraffin. Sections of 5μm were stained with hematoxylin and eosin (H&E) and Elastic van Gieson (EvG) stains for histological classification. Perls stain was performed to visualize iron.
Classification of atherosclerotic lesions

Early lesions are described as focal aggregations of lipid-laden macrophages or smooth muscle cells with disperse extracellular lipid, but without a well-formed lipid core or calcifications. Advanced lesions with fibrous cap overlying a lipid core were classified as fibrolipid plaques; lesions that were mostly calcified were classified as calcified plaques; and lesions that consisted mainly of fibrous tissue were classified as fibrotic plaques. Plaque disruption was defined as the presence of intraluminal thrombus superimposed on rupture of the fibrous cap (plaque rupture) or due to erosion of the superficial endothelial layer (plaque erosion).

Immunohistochemical stains

Glycophorin A (GFA) is present on the membranes of erythrocytes, including membrane fragments that remain present in the tissue long after the onset of a bleeding. Therefore, immunohistochemical stains were performed to visualize IPH with use of the monoclonal antibodies against glycophorin A (dilution 1:200; Dako, Glustrop, Denmark)\(^{21}\). Microvessels and microvascular leakage was identified by means of immunostaining against von Willebrand factor (vWF; 1:50, Dako)\(^{22}\).

Paraffin tissue sections were dewaxed in xylene and rehydrated in graded alcohols. Endogenous peroxidase activity was blocked with 0.3% \(\text{H}_2\text{O}_2\) in methanol for 20 min. Sections were subsequently digested with 0.25% pepsin dissolved in 10mM HCl for 10 min at 37\(^\circ\)C prior to vWF antibody labeling. No pre-treatment was performed for anti-glycophorin A staining. The tissue sections were subsequently incubated with primary antibodies for 1 hr at room temperature; and thereafter with appropriate anti-mouse or anti-rabbit HRP-labeled polymers (Immunologic, Duiven, The Netherlands) for 30 min; HRP-activity was visualized using DAB+ (Dako) for 8 min.

Intraplaque hemorrhage (IPH)

IPH were classified into 3 groups: (1) recent IPH: extravascular localization of intact (fresh) erythrocytes (>10 cells / field of view at 400x magnification) on GFA immunostained sections, (2) old IPH: presence of iron deposits (Perls stain) and / or erythrocyte fragments (GFA immunostain)\(^{21}\), and (3) ongoing IPH: simultaneous presence of both recent and old hemorrhage in one section. The severity of IPH was semiquantitatively graded as: 1) small: hemorrhages of not more than 10-50 erythrocytes; 2) large: microscopic hemorrhages>50 cells, clusters of erythroctic fragments or macroscopically visible IPH in the plaque.
Intraplaque microvessels
Microvessels were visualized by means of anti-vWF immunostain and the total number was counted per plaque section. Microvessel leakage was defined as diffuse immune staining of vWF around microvessels in plaque as previously described\textsuperscript{5,22}. Histomorphology and immunostains of all coronary segments were evaluated by two observers independently.

Statistics
Statistical analysis was performed with the Statistical Package for Social Sciences software (SPSS 18 for windows, SPSS inc, Chicago, IL). Categorical data were expressed as percentages and evaluated with Chi-square test. Continuous data were expressed as mean ± SD or median with interquartile range. Normally distributed data were analyzed with student’s t-test. Mann-Whitney and Kruskal-Wallis tests were used for not-normally distributed data. Logistic regression analysis was used to test the independent association of medications with presence of IPH. Tissue sections of atherosclerotic plaques were pooled and analyzed among the three groups of patients. Furthermore, the percentages or medians of IPH parameters for each individual patient were calculated and a comparison was made between the patients who were treated with either anticoagulants or platelet aggregation inhibitors and those without use of any anti-thromboembolic drugs. P value of ≤0.05 was considered statistically significant.

Results
Clinical characteristics of patients
The clinical characteristics of the 30 selected patients are presented in table 1. Hypertension, statin and beta-blocker use were more frequently found in the patients treated with anticoagulants or platelet aggregation inhibitors than in patients without anti-thromboembolic medication (P<0.05 for all). Only statin use appeared to be associated with increased risk on intraplaque hemorrhage (P=0.01, OR: 1.80, CI: 1.15-2.81).

Histological classification of atherosclerotic coronary artery segments
A total of 1059 coronary segments with macroscopically visible atherosclerosis were investigated (length of these tissue samples in total amounts to circa 3 meters). Histology of 66 samples could not be evaluated due to artifacts during tissue processing (n=60) or presence of large plaque ruptures (n=6). Of the remaining 993 tissue samples, 904 (91%) contained advanced atherosclerotic plaques, classified as either fibrolipid (164), calcified (290) or fibrotic (450) plaques. Overall, advanced
Table 1. Patient clinical characteristics

<table>
<thead>
<tr>
<th></th>
<th>No medicine</th>
<th>Anticoagulants</th>
<th>Antiplatelets</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(N=10)</td>
<td>(N=10)</td>
<td>(N=10)</td>
<td></td>
</tr>
<tr>
<td>M/F</td>
<td>6/4</td>
<td>7/3</td>
<td>8/2</td>
<td>0.62</td>
</tr>
<tr>
<td>Age</td>
<td>69.6±11</td>
<td>74.7 ± 8</td>
<td>69.6 ±6.8</td>
<td>0.34</td>
</tr>
<tr>
<td>(mean ± SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>2</td>
<td>7</td>
<td>7</td>
<td>0.02</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>0.24</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>0.27</td>
</tr>
<tr>
<td>Smoking</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0.36</td>
</tr>
<tr>
<td>MI</td>
<td>5</td>
<td>2</td>
<td>4</td>
<td>0.37</td>
</tr>
<tr>
<td>Statins</td>
<td>1</td>
<td>5</td>
<td>9</td>
<td>0.002</td>
</tr>
<tr>
<td>Beta-blocker</td>
<td>0</td>
<td>5</td>
<td>4</td>
<td>0.04</td>
</tr>
<tr>
<td>ACE-inhibitor</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>0.84</td>
</tr>
</tbody>
</table>

M/F: male / female
MI: myocardial infarction
ACE inhibitor: angiotensin-converting-enzyme inhibitor
types of plaque morphology was observed more frequently in plaques of patients who used anticoagulants (94% advanced vs 6 early lesions) or antiplatelet therapy (98% vs 2%) than in those without anti-thromboembolic therapy (82% vs 20%).

Intraplaque hemorrhage and coronary plaque composition

Presence of IPH was observed in 499 of 993 atherosclerotic plaque segments (50%), which represents the overall hemorrhagic burden in the coronary arteries of all the 30 patients under investigation. Of these, 72 / 993 (7%) showed recent hemorrhage (Fig. 1 A-C), 188 / 993 (19%) had old IPH (Fig. 1 D and E) and 239 / 993 (24%) showed both recent and old bleeding (ongoing bleeding).

IPH was observed in only 18% (n=16/89) of the early lesions, but appeared to be a common feature in advanced types of atherosclerotic plaques (483/904, 53%; P<0.001, table 2). Since IPH was more frequently observed in advanced than early lesions, we evaluated the occurrence of IPH and its potential association with anti-thromboembolic therapy in early and advanced lesions separately. Among the different types of advanced plaques, IPH was more frequently observed in fibrolipid (105/164, 64%) and in calcified plaques (168/290, 58%) than in fibrous plaques (207/450, 46%) (P<0.05 for both types of lesions vs fibrous plaques).

**Figure 1.** Intraplaque hemorrhage and leakage of microvessels

A – H&E stain of small hemorrhage (x400 magnification), extravascular location of 10-50 erythrocytes
B – H&E stain of a large hemorrhage (x200 magnification), extravascular location of more than 50 erythrocytes
C – Macroscopic view of a large intraplaque hemorrhage
D – Perls stain (x400 magnification) identifies hemosiderin deposit (blue pigments), indicating old hemorrhage
E – Immunostain for Glycophorin A (x400 magnification), showing erythrocyte fragments in a necrotic core, which indicates old hemorrhage
F – Immunostain for von Willebrand factor (vWF) (x400 magnification), leakage of microvessels is identified by presence of diffuse perivascular vWF deposits
Relationship between anti-thromboembolic drugs and presence of IPH
All advanced plaque samples were pooled into 3 groups: plaques samples of patients treated with platelet aggregation inhibitors (n=376), plaques of patients on oral anticoagulants (n=284) and those of patients without any anti-thromboembolic therapy (n=244). The incidence of IPH appeared to be higher in plaques of patients treated with anticoagulants (174/284, 61%) than in plaques of patients treated with platelet aggregation inhibitors (198/376, 53%) (P=0.02, OR: 1.74; CI: 1.08-2.79) or in plaques of patients without anti-thromboembolic drugs (111/244, 46%) (P=0.001, OR: 2.55, CI: 1.43-4.54, see table 2). Plaques of patients treated with anticoagulants (89/284, 31%) more often showed ongoing hemorrhages in advanced plaque segments than plaques of patients without anti-thromboembolic therapy (44/244, 18%) (P<0.001, OR: 3.62, CI: 2.03-6.45); in contrast, platelet aggregation inhibitors was not associated with ongoing IPH (102/376, 27%) (P=0.32, table 2). Moreover, the severity of IPH, which was semi-quantitatively assessed in immunostained sections, appeared highest in plaque samples of patients treated with anticoagulants among the three groups of patients (P<0.001 for both, table 2).

Fragile microvessels are leaky and prone to rupture, and have been identified as the most important predictor for IPH\textsuperscript{23}. The numbers of such intraplaque microvessels per plaque section was significantly higher in plaque samples of patients treated with platelet aggregation inhibitor (median: 21 / plaque section, IQR: 9-41) than those of patients on anticoagulant therapy (median: 17 / plaque section, IQR: 6-34) or patients without any anti-thromboembolic drugs (median: 11 / plaque section, IQR: 5-23) (P=0.012 and P<0.001, respectively). On the other hand, the incidence of microvascular leakage was higher in patients treated with anticoagulants (74%) than non-treated patients (56%, P=0.03, OR: 2.01, CI: 1.22-3.32); in contrast, use of platelet aggregation inhibitor was not related to an increased risk on microvascular leakage (59%, P=0.81).

Compared with advanced lesions, only few microvessels were observed in the early atherosclerotic lesions. There was no difference in microvascular density of early plaques among the three groups of patients: no drugs: median 3 vessels / plaque section, interquartile range 0-11; anticoagulants: median 0 vessels/ plaque section, interquartile range 0-8; and anti-platelet: 0 vessels / plaque section, interquartile range 0-13. No association was observed between occurrence of IPH or microvascular leakage and the use of anti-coagulant therapy (P=0.62 ) and antiplatelet therapy (P=0.29) in early lesions.
Table 2. Intraplaque hemorrhage of pooled advanced plaque segments.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>No medicine</th>
<th>Anticoagulants</th>
<th>P1</th>
<th>Platelets inhibitors</th>
<th>P2</th>
</tr>
</thead>
<tbody>
<tr>
<td>(993 samples)</td>
<td>(305 samples)</td>
<td>(303 samples)</td>
<td>Anticoagulants</td>
<td>(385 samples)</td>
<td>Platelets inhibitors</td>
<td></td>
</tr>
<tr>
<td>Advanced lesions</td>
<td>904 (91%)</td>
<td>244 (80%)</td>
<td>284 (94%)</td>
<td>0.03</td>
<td>376 (98%)</td>
<td>0.13</td>
</tr>
<tr>
<td>Total IPH</td>
<td>483 (53%)</td>
<td>111 (46%)</td>
<td>174 (61%)</td>
<td>0.001</td>
<td>198 (53%)</td>
<td>0.16</td>
</tr>
<tr>
<td>Recent IPH</td>
<td>70 (8%)</td>
<td>23 (9%)</td>
<td>21 (7%)</td>
<td>0.60</td>
<td>26 (7%)</td>
<td>0.29</td>
</tr>
<tr>
<td>Old IPH</td>
<td>178 (20%)</td>
<td>44 (18%)</td>
<td>64 (23%)</td>
<td>0.25</td>
<td>70 (19%)</td>
<td>0.07</td>
</tr>
<tr>
<td>Ongoing IPH</td>
<td>235 (26%)</td>
<td>44 (18%)</td>
<td>89 (31%)</td>
<td>&lt;0.001</td>
<td>102 (27%)</td>
<td>0.32</td>
</tr>
<tr>
<td>Large IPH</td>
<td>110 (12%)</td>
<td>14 (6%)</td>
<td>48 (17%)</td>
<td>&lt;0.001</td>
<td>48 (13%)</td>
<td>0.62</td>
</tr>
<tr>
<td>Microvessels</td>
<td>16; 7-34</td>
<td>11; 5-23</td>
<td>17; 6-34</td>
<td>0.001</td>
<td>21; 9-41</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Leakage</td>
<td>552/834 (63%)</td>
<td>127/226 (56%)</td>
<td>180/245 (74%)</td>
<td>0.03</td>
<td>215/363 (59%)</td>
<td>0.81</td>
</tr>
</tbody>
</table>

Table 2. Intraplaque hemorrhage of pooled advanced plaque segments
The patient treated with both anti-coagulants and platelet aggregation inhibitors is not included for statistical analysis.
Presence and extent of IPH evaluated in individual patients
In addition to analysis of the pooled plaque sample analysis, we also studied atherosclerotic plaque IPH on the individual patient level. The presence of IPH in advanced lesions in individual patients varied from 13% to 96% of plaque segments in each individual patient. This huge variation was not related with any of the classical risk factors for atherosclerosis. When analyzed on patient level, the incidence of IPH (recent, old or ongoing) differed not significantly among the three groups of patients (P>0.1 for all), although the extent of IPH tended to be more severe in coronary plaques of patients on anticoagulant treatment than those without anti-thromboembolic medicines (P=0.09). Intraplaque microvessel density was highest in patients treated with platelet aggregation inhibitors, which differed significantly from those without therapy (P=0.01), but not from those treated with anticoagulant therapy (P=0.35).

Discussion
In the present study, we evaluated histopathologically the influence of anti-thromboembolic drugs (anticoagulants and platelet aggregation inhibitors) on the total hemorrhagic burden (number and severity of IPH) in the entire coronary system of older patients (>50 years of age) with trivascular coronary artery disease. We found that the use of oral coumarin-type anticoagulants but not of antiplatelet agents is associated with a significantly higher burden of coronary IPH when compared with the hemorrhagic burden of patients who received no anti-thromboembolic treatment. Moreover, anticoagulant treatment appeared to be associated with larger IPH, and also with a simultaneous presence of recent and old hemorrhage in one and the same plaque (repeated hemorrhages) in the same plaque.
Several earlier studies have demonstrated that intraplaque hemorrhage is not only a common feature in advanced atherosclerotic plaques as confirmed in our study, but also plays an important role in the process of plaque growth and plaque destabilization which ultimately may lead to clinical ischemic events. Therefore, the use of anticoagulants in patients with advanced coronary atherosclerosis could be additionally detrimental in terms of disease progression due to aggravating the extent and severity of IPH.

Oral anticoagulant therapy and IPH
Until now, only few studies have been performed to evaluate the relationship between prophylactic anti-thromboembolic treatment and the risk on IPH. In 1971, Jorgensen et al. focused for the first time on this issue and showed that in acute cardiac death
patients, there was no increase of IPH in non-ruptured culprit lesions of patients treated with anticoagulants (n=5) versus non treated patients (n=11) 25. These findings were confirmed by Fisher et al. who demonstrated that in symptomatic carotid plaques (n=40), IPH was not related to use of anticoagulant medications 26. However, both of the studies were limited by their small sample sizes. The largest histopathological study on this issue was performed recently by Derksen et al. 19. With use of 794 carotid endarterectomy samples obtained from stroke patients, the authors demonstrated that coumarin-type anticoagulant treatment is an independent predictor for the presence of IPH in the carotid atherosclerotic plaques which apparently gave rise to symptoms. Our study further endorses these effects of anticoagulant therapy by demonstrating a higher incidence and more extensive IPH and also in non culprit (symptomless) plaques in the coronary arteries of coumarin treated patients. Oral administration of anticoagulants affects on all coronary plaques, rather than acting solely on a single culprit plaque.

Platelet aggregation inhibitors and IPH
With use of carotid endarterectomy samples, Ernst et al. evaluated the effect of platelet aggregation inhibitors on IPH in symptomatic carotid plaques.27 The authors showed a high incidence of IPH in their study samples, but IPH could not be related to the use of these antiplatelet agents. Similar results were obtained from the study performed by Derksen et al. who demonstrated that the use of platelet inhibitors is not associated with increased risk on IPH in carotid or in femoral endarterectomy samples.19 In line with these previous studies, we also find that antiplatelet therapy is not associated with a higher incidence of IPH in coronary atherosclerotic plaques. With use of 154 carotid endarterectomy samples, AbuRahma et al. demonstrated more severe and repeated bleeding in symptomatic carotid plaques of patients on anti-platelet treatment compared with the group of non treated patients.28 In contrast to these findings, we show that antiplatelet therapy appear to be not related with repeated process of IPH (i.e. simultaneous presence of fresh and old) in non-culprit coronary atherosclerotic lesions. Differences in study materials may underlie these discrepancies: AbuRahma et al. focused on IPH in culprit carotid lesions; in contrast, we investigated hemorrhagic burden throughout entire coronary arteries which composed mainly of non-culprit coronary plaques. In the current study, only 4 patients on antiplatelet therapy suffered from myocardial infarction before their death; and the culprit lesions in 2 of these patients showed disruption of fibrous cap which were excluded from final assessment (see method section). On the remaining 2 culprit lesions, no statistical analysis was performed. Furthermore, differences in hemodynamics between carotids
Anti-thromboembolic therapy and intraplaque hemorrhage

and coronary arteries may also contribute to difference in plaque composition and eventual IPH. Interestingly, we found that the densities of intraplaque microvessels in patients on platelet inhibitor treatment were higher than in non treated patients. Microvessels are considered to be the viable source of IPH, but in contrast to anticoagulants, platelet aggregation inhibitors did not increase the IPH burden in our study.

**Conclusion**

The use of oral anticoagulants as a preventive therapy against thromboembolic diseases appeared to be associated with an increased intraplaque hemorrhagic burden in the coronary artery system of patients with severe coronary atherosclerosis. We suggest that these effects could lead to a significant larger plaque volume over time and eventually also onset of clinical symptom in the selected subgroup of patients.
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19. Derksen WJ, Peeters W, Tersteeg C, de Vries JP, de Kleijn DP, Moll FL, van der Wal AC, Pasterkamp G, Vink A. Age and coumarin-type anticoagulation are associated with the occurrence of intraplaque hemorrhage, while statins are associated less with intraplaque hemorrhage: A large histopathological study in carotid and femoral plaques. Atherosclerosis. 2011;214:139-143


