Artery-Related Differences in Atherosclerosis Expression
Implications for Atherogenesis and Dynamics in Intima-Media Thickness

Søren Dalager, MD, PhD; William P. Paaske, MD, DrMedSci; Ingrid Bayer Kristensen, MD; Jacob Marsvin Laurberg, MD, PhD; Erling Falk, MD, DrMedSci

Background and Purpose—Information about the expression of atherosclerosis in different arteries is important. The impact of cardiovascular risk factors is artery-related, and the assessment of arterial structure and function in peripheral arteries are increasingly used as surrogate markers for coronary atherosclerosis and the risk of developing heart attack.

Methods—In an autopsy study, we analyzed the coronary, carotid and superficial femoral arteries from 100 individuals (70 men; 20 to 82 years of age) of which 27 died from coronary atherosclerosis. Microscopic sections (n=4756) were analyzed blindly using a modification of the histological classification endorsed by the American Heart Association (AHA).

Results—We found distinct artery-dependent patterns of atherosclerosis with a high prevalence of foam cell lesions and lipid core plaques in the carotid arteries. The femoral arteries were least affected by atherosclerosis, foam cell lesions were rare, and the development of advanced atherosclerosis was strongly age-dependent and dominated by fibrous plaques. Plaques were most common in the left anterior descending coronary artery and the carotid bifurcation. In coronary (versus noncoronary) death, lipid core plaques were more prevalent in all arteries.

Conclusions—The initiation, speed of development, and phenotypic expression of atherosclerotic plaques are artery-related. Foam cell lesions are frequent in the carotid arteries, probably explaining the dynamics in carotid intima-media thickness. Atherosclerosis develops slowly in femoral arteries, and severe atherosclerosis is dominated by fibrous plaques. The higher prevalence of lipid core plaques in all arteries in coronary death indicates a systemically more vulnerable expression of atherosclerosis in these individuals. (Stroke. 2007;38:2698-2705.)

Key Words: atherosclerosis • carotid atherosclerosis • coronary atherosclerosis • pathology • peripheral vascular disease

Information about the expression of and the relationship between atherosclerosis in different arteries is important for several reasons. The classic cardiovascular risk factors have different impact in different arterial territories. Cholesterol is particularly important in ischemic heart disease, hypertension in ischemic stroke, and smoking and diabetes in intermittent claudication. Moreover, some arteries, eg, the internal mammary artery, are relatively spared from atherosclerosis. Better understanding of the nature and reasons for these differences are important in prevention and treatment of atherosclerosis and its complications.

In spite of the differences, measures of atherosclerosis in peripheral arteries serve as fast and continuous end points in clinical testing of drugs assumed to have antiatherosclerotic properties, and are used in the assessment of the overall atherosclerotic burden and cardiovascular risk. Examples of these measures include the carotid artery intima-media thickness (IMT), and the ankle-brachial blood pressure index.

Comparative and descriptive pathological studies of atherosclerosis in different arteries are numerous but belong to an era without any widely accepted histological classification of atherosclerosis. Many of these studies were not performed on histological sections, but on gross specimens with more emphasis on disease severity than composition.

Since then, the American Heart Association (AHA) classification of atherosclerosis has emerged. Although debated and subsequently updated, it provides a useful framework for atherosclerosis description. Our aims were: (1) to characterize and compare the type of atherosclerosis within different arteries using the AHA classification; and (2) to analyze the relation with age, gender and cause of death.

Materials and Methods

Individuals
Clinically important atherosclerosis-susceptible segments were obtained from 6 locations: the proximal parts of the left anterior descending (LAD) and the right coronary artery (RCA), both carotid arteries, and both superficial femoral arteries. The carotid arteries and the superficial femoral arteries were examined over a large part of their lengths, eg, the carotid artery segments included the distal...
part of the common carotid artery, the bifurcation (bulb region), and the proximal part of the internal carotid artery (Figure 1). The segments were prospectively collected from 100 autopsies at the Institute of Forensic Medicine, University of Aarhus, Denmark. The autopsies were performed from February 1996 to March 1999, and the study was approved by the Regional Research Ethics Committee and The Danish Data Protection Agency. The individuals underwent postmortem examination if death occurred unexpectedly outside the hospital or under unclear circumstances. Data on pre-mortem risk factors were unavailable. Only individuals with a postmortem interval 4 days were included. The mean age of the 100 individuals was 47.1 years (mean SD; range: 20 to 82 years), and 30 were women. In 49 cases the cause of death was natural (27 coronary deaths, 22 other natural deaths) whereas 51 individuals had unnatural deaths (7 suicides, 42 accidents, 2 homicides).

**Tissue Processing**

The coronary arteries were cut open longitudinally as part of the forensic examination. The artery segments were fixed in 4% phosphate buffered formalin for 24 hours and decalcified (unless 45 years of age) in 10% formic acid for 24 hours. Cross-sectioning was performed at 4-mm intervals and the resulting sections were embedded in paraffin, yielding 48 paraffin blocks from each subject (4756 paraffin blocks in total; 44 sections were unavailable, primarily from the distal part of the internal carotid arteries). All tissue sections were cut at 4-μm thicknesses, mounted on Superfrost + glass slides, and stained with hematoxylin and eosin (HE).

**Histological Grading of Arterial Disease**

Histological evaluation was performed using a light microscope (Olympus BX51) equipped with polarization filters and an integrated eyepiece graticule. The sections were graded in random order blinded to subject data according to a slightly modified AHA classification (Table 1). The modifications consisted of an addition of chronic occlusion (type IX lesion) and omission of the “acute” type VI lesion (surface defect, hematoma, or thrombosis). Instead, these lesions were graded according to the underlying “chronic” atherosclerotic plaque (eg, a type V lesion with rupture and thrombosis was graded as type V, not type VI). Fibrosis (hyalinization) was detected by the characteristic birefringence of collagen when the HE-stained sections were viewed under polarized light. Histological examples are given in Figure 2. For each artery segment, the plaque burden was calculated as the number of sections with plaque (AHA type IV) divided by the total number of sections in that artery segment (range: 0 to 1).

**Statistics**

Intercooled STATA 9.2 for Windows (Statacorp LP) was used for statistical analysis. The sections were pooled according to localization and further stratified by age, gender and cause of death. Lesion sections were cut at 4-μm thicknesses, mounted on Superfrost + glass slides, and stained with hematoxylin and eosin (HE).

<table>
<thead>
<tr>
<th>Type</th>
<th>Modified AHA Classification of Atherosclerotic Lesions*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal intima or intimal thickening</td>
</tr>
<tr>
<td>1</td>
<td>Foam cell lesions (intimal xanthoma)</td>
</tr>
<tr>
<td>2</td>
<td>Intermediate lesion (pathological intimal thickening)</td>
</tr>
<tr>
<td>3</td>
<td>Lipid core plaque (fibrous cap atheroma)</td>
</tr>
<tr>
<td>4</td>
<td>Complicated plaque</td>
</tr>
<tr>
<td>5</td>
<td>Calcified plaque (fibrocalcific plaque)</td>
</tr>
<tr>
<td>6</td>
<td>Fibrous plaque (fibrocalcific plaque)</td>
</tr>
<tr>
<td>7</td>
<td>Chronic occlusion (total occlusion)</td>
</tr>
<tr>
<td></td>
<td>Characteristics</td>
</tr>
<tr>
<td></td>
<td>No foam cells</td>
</tr>
<tr>
<td></td>
<td>Single isolated foam cells</td>
</tr>
<tr>
<td></td>
<td>Pools of extracellular lipid with no or only few cholesterol crystals</td>
</tr>
<tr>
<td></td>
<td>Extracellular lipid core, also called necrotic core</td>
</tr>
<tr>
<td></td>
<td>Surface defect, hematoma, or thrombosis</td>
</tr>
<tr>
<td></td>
<td>≥50% of plaque area calcium</td>
</tr>
<tr>
<td></td>
<td>Fibrous plaque without a lipid core</td>
</tr>
<tr>
<td></td>
<td>Chronically occluded artery</td>
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<tr>
<td></td>
<td>Definition</td>
</tr>
<tr>
<td></td>
<td>No foam cells, may exhibit minor intimal thickening or lymphocyte infiltration</td>
</tr>
<tr>
<td></td>
<td>Single isolated foam cells</td>
</tr>
<tr>
<td></td>
<td>Multiple foam cells (≥2 layers)</td>
</tr>
<tr>
<td></td>
<td>Minor accumulations of structure- and colorless material displacing the normal structural components of the intima</td>
</tr>
<tr>
<td></td>
<td>Colorless cavity without extracellular matrix and containing spindle-shaped empty spaces (cholesterol crystals)</td>
</tr>
<tr>
<td></td>
<td>+ fibrosis</td>
</tr>
<tr>
<td></td>
<td>+ Hyalinization (birefringent fibres)</td>
</tr>
<tr>
<td></td>
<td>Surface defect, plaque hemorrhage, or luminal thrombus</td>
</tr>
<tr>
<td></td>
<td>≥50% of plaque area calcium, with or without lipid core</td>
</tr>
<tr>
<td></td>
<td>Fibrous plaque with hyalinization without any lipid core (unless the core has undergone complete calcification)</td>
</tr>
<tr>
<td></td>
<td>Artery occluded by plaque and connective tissue, no fresh thrombus</td>
</tr>
</tbody>
</table>

*Based on the updated17 histological classification of atherosclerotic lesions endorsed by the AHA. Type VI was graded as the underlying lesion without complications. Alternative terms proposed by Virmani et al16 are shown in parentheses.
types at the different localizations are presented as percentage distribution. Because of the descriptive and exploratory nature of the study, the distribution of lesion types is presented without calculations of statistics. The correlation between plaque burdens in the different arteries was calculated using Spearman rank correlation test. Inter- and intraobserver reproducibility of the grading of AHA lesion types is presented as percentage agreement and kappa (κ) values.

Results
Sections from all individuals were pooled (n=4756), and the percentage distribution of lesion types at each location is shown in Figure 3 (tabulated percentages are given in supplemental Table I, available online at http://stroke.ahajournals.org). Type I and II lesions were combined as foam cell lesions, and type IV and V lesions were combined as lipid core plaques.

Sections from the coronary arteries had the highest prevalence of atherosclerotic plaques (LAD 52%; RCA 43%), with lipid core plaques being more frequent than fibrous plaques. Plaques were more often fibrous (type VIII) in the RCA (42% of plaques versus 36% of LAD plaques). Foam cell lesions and intermediate lesions were rare (9% in both arteries). Although many of the coronary sections contained intima without foam cells or plaques, the coronary intima was diffusely thickened compared with that of other arteries. The distribution of lesion types was almost similar when comparing right and left sided arteries in the carotid and femoral beds. Therefore, percentages are presented below as mean values of right and left. Overall, the carotid arteries had fewer plaques (30%) and much more foam cell lesions and intermediate lesions (37%) than the coronaries, being diseased in a distinct pattern. The common carotid arteries were dominated by foam cell lesions (58%) with almost all plaques (22%) being lipid-rich (90% of plaques).

In the bifurcation region, the fraction of sections with plaque was actually higher than in the coronary arteries (55%) and 70% of the plaques were lipid core plaques. The internal carotid artery was dominated by normal or thickened intima without foam cells (62%); plaques were rare (17%). The femoral arteries were dominated by normal or thickened intima without foam cells (74%) in a binary pattern with some plaques (20%). Foam cell lesions and intermediate lesions were few (6%) and more than half (52%) of the femoral plaques were fibrous type VIII lesions. The proximal part of the superficial femoral artery immediately after the bifurcation was most plaque-prone.

Influence of Age
As the carotid arteries were diseased in a distinct pattern, the common carotid artery, the bifurcation and the internal carotid artery were treated as separate arteries. The age-stratified percentage distributions of lesions in the right- and
left-sided carotid and femoral arteries were similar and therefore combined. The age-stratified distribution of atherosclerotic lesions is illustrated in Figure 4 (tabulated percentages are given in supplemental Table II, available online at http://stroke.ahajournals.org). There was an increase in atherosclerosis severity with age in all arterial beds with acceleration in severity from the third to the fourth decade. Coronary and carotid plaques were observed from the third decade (LAD 22-year-old male, coronary death; RCA and carotid bifurcation 28-year-old male, noncoronary death) whereas femoral artery plaques did not occur until 34 years of age (male, noncoronary death).

**Influence of Gender**

We included more men (n=70) than women (n=30); their mean age did not differ (men 47.8±15.1 versus women 45.4±11.5 years; \( P=0.43 \)), and they were equally likely to have died from coronary causes (18 [26%] men and 9 [30%] women; \( P=0.66 \)). There were no qualitative difference between men and women, ie, the pattern was the same, but the disease was more advanced in men with the exception of the carotid bifurcation and the internal carotid artery (supplemental Table II).

**Influence of Cause of Death**

Twenty-seven individuals died of coronary atherosclerosis. Their mean age (coronary death: 47.2±12.0 versus noncoronary death: 47.1±14.9; \( P=0.97 \)) and gender distribution (see above) were not different from those dying from noncoronary causes. Disease was more advanced in all arteries, and notably lipid core plaques were much more common, especially in the carotid bifurcation (supplemental Table II).
Correlations Between Plaque Burden in Different Arteries
Within individuals, plaque burden in one artery correlated with plaque burden in another (Table 2; $P<0.002$ for all correlations). The correlations were strongest when comparing right- and left-sided arteries from the same arterial beds ($\rho=0.77$ to 0.78).

Reproducibility Studies
A subset of 100 randomly selected sections were graded 1 year later by the same observer (S.D.) and another observer (J.M.L.) to determine the intra- and interobserver agreement of the grading. The intraobserver percentage agreement was 83% with a $\kappa$ value of 0.77 (95% CI: 0.68 to 0.87) indicating substantial agreement, whereas the interobserver percentage agreement was 67% with a $\kappa$ value of 0.57 (95% CI: 0.47 to 0.66) indicating moderate agreement.

Discussion
The most important finding in our study was the artery-related differences in initiation, speed of development, and phenotypic expression of atherosclerotic plaques.

Carotid IMT: An Ultrasound Measure of Foam Cells?
The predominance of foam cell lesions in the common carotid artery is interesting because this artery is the preferred site for measurements of intima-media thickness. The reversibility of foam cell lesions may explain why the IMT can decrease in response to treatment, eg, with statins. A decrease in thickness would be difficult to explain if the thickening was fibrous, but if the bulk of the thickness was accounted for by foam cells a decrease is much more likely. Numerous clinical studies have shown that the dynamic changes in IMT follow favorable changes in risk factors and, therefore, are used as a

Figure 4. Percentage distribution of AHA lesion types in the different arteries stratified by age decade. Types are ordered from bottom (type 0) to the top (type IX) of bars.
Some authors state that pre-existing intimal thickening, not foam cell lesions, is the precursor of atherosclerotic plaque. Our study design does not allow us to elaborate further on this, but the paucity of foam cell lesions in the femoral artery indicate that foam cells play a lesser role in plaque formation in that artery. Atherosclerosis is a multifactorial disease resulting in heterogeneous plaques. Different pathways probably exist, the dominating one being determined by localization and individual factors. Still, the pathways are not exclusive, eg, the femoral arteries may also exhibit rupture of a lipid core with subsequent thrombosis (Figure 2F).

The key to the influence of localization may be found in hemodynamic differences and the underlying arterial wall structure. The influence of hemodynamics is best understood and explains some of the propensity for plaque formation in the carotid bifurcation and the proximal LAD. A potential influence of the site-specific arterial wall structure is more hypothetical. The common carotid artery is an elastic artery, just as the aorta which is also known to be prone to foam cell formation. The proximal parts of the coronary arteries and the carotid bifurcation are situated in a transitional zone between elastic and muscular artery types. These artery segments develop foam cell lesions and lipid core plaque at an early age. In the coronary arteries foam cell lesions are common already in childhood and adolescence. The internal carotid artery and superficial femoral arteries are muscular arteries and exhibit a remarkably similar pattern, albeit foam cell lesions are more common in the internal carotid artery which becomes truly muscular at a varying distance (0.5 to 2 cm) from the bifurcation. Whatever mechanisms are at play, the hypothetical influence of arterial wall structure deserves further study.

More Vulnerable Atherosclerosis in Coronary Death Individuals?
Plaques were more prevalent in individuals dying from coronary causes, not only in the coronary arteries and the carotid bifurcation, but also at sites normally spared from atherosclerosis indicating a generally more aggressive form of disease. This was particularly evident in the femoral arteries (supplemental Table II), which is interesting given the strong epidemiological association between peripheral arterial disease and coronary death. It is commonly accepted that lipid-rich plaques carry a higher risk of complications. Lipid core plaques were relatively more common in all arteries in coronary death, especially in the carotid arteries. This could point toward a systemically more lipid-rich plaque phenotype in some atherosclerosis-prone individuals.

The high prevalence of advanced fibrotic atherosclerotic plaques in the RCA is somewhat conflicting with previous findings, reporting LAD as the predilection site. On the
other hand, it has been shown that proximal culprit lesions in RCA are more common in sudden cardiac death victims than among myocardial infarct cases surviving into the hospital. RCA culprit arteries may carry a higher risk of brady-asystolic cardiac arrest because the arterial supply to the sinus node and atioventricular node usually comes from RCA. In forensic materials such as ours, cases of coronary death are almost invariably sudden out-of-hospital deaths. Thus, the discrepancy may be related to this inherent selection. We found an almost equal prevalence of mature plaques in the LAD and the RCA in coronary death individuals. Conversely, the plaque prevalence was higher in the LAD in individuals dying from other causes (supplemental Table II).

Plaque Burden Correlations
The correlations between plaque burdens in arteries are well in line with the findings in other autopsy studies where correlations are also strongest between bilateral arteries and different branches within the same arterial territory. The strongest correlations have been reported between the different cerebral artery branches ($r=0.9$), whereas the correlations between other arteries, such as between the coronary arteries, are less strong ($r=0.7$ to 0.8). Spearman $\rho$ is equivalent with Pearson $r$, and even though the older data are primarily based on grading of gross specimens, our correlations are remarkably similar.

The strength of the interarterial correlations appears promising, but it is important to remember that materials covering wider age ranges (both including young individuals with sparse atherosclerosis and older individuals with extensive atherosclerosis) are more likely to show strong correlations than materials from narrow age ranges.

Limitations
We illustrated the age, gender, and cause of death related patterns by pooling of several sections from the same individuals because our categorical data did not permit calculation of “mean AHA types.” Subsequent statistical analyses of age, gender, and cause of death differences would be inappropriate because the sample-size (and subsequently statistical power) would be artificially increased. Our findings must therefore be interpreted as descriptive.

We used the AHA classification which has been criticized by some of the leading atherosclerosis researchers for its assumption of an orderly, linear pattern of lesion progression, unclear distinction between lesion types, and for being difficult to remember with its combination of roman numerals and letter codes. As discussed above, we agree that lesions do not always follow the same linear progression pattern, and we did experience problems with distinction between lesion types (moderate interobserver reproducibility). These problems were primarily caused by heterogeneous expression of atherosclerosis. On a group basis, reproducibility was excellent, as shown by the similar lesion distribution in right- and left-sided arteries.

Conclusions
Our analyses indicate differences in initiation, speed of progression, and phenotypic expression of atherosclerotic plaques in different arteries depending on localization and individual factors. Foam cell lesions play an important role in progression of carotid atherosclerosis, and they are the most frequently encountered lesions at the preferred sites for measurements of carotid IMT. The reversibility of foam cell lesions, therefore, provides an explanation for the dynamics in carotid IMT. Femoral atherosclerosis was less advanced; more fibrous and foam cell lesions were rare. These phenotypic differences may relate to underlying differences in hemodynamics and arterial wall structure. Plaques in all analyzed arteries were more lipid-rich in coronary (versus noncoronary) death, indicating a systemically more vulnerable expression of atherosclerosis in persons dying of coronary atherosclerosis.

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Disclosures
Dr Falk is on the Scientific Advisory board of the High-Risk Plaque Initiative, BG Medicine, Waltham, Mass.

References


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