Atherosclerotic plaque formation is a chronic process starting early in life. Luminal narrowing by atherosclerosis is determined by gradual plaque growth and arterial remodeling. Plaque accumulation can be compensated for by expansive remodeling of the vessel wall (1). Failure to enlarge and even constrictive remodeling also frequently occur (2).

Examples of acute manifestations of atherosclerosis are acute myocardial infarction and unstable angina pectoris. Rupture of the plaque surface and subsequent luminal thrombus formation is probably the most important mechanism underlying these acute syndromes (3). The risk of plaque rupture depends on plaque composition rather than on plaque size (3). Histopathologic data have led to the concept that a soft lipid-rich core, a thin cap and inflammation in cap and shoulders of the plaque make a plaque vulnerable for rupture (3,4).

Atherosclerotic lesions preferentially develop in lesion-prone areas near bifurcations (determined by local flow-related factors), but the gradual growth of plaques is predominantly determined by global or systemic factors such as gender, age, hypercholesterolemia and hypertension. However, it is unknown to what extent arterial remodeling and plaque vulnerability are determined by systemic factors.

The femoral artery is a long (30 cm) artery prone to develop atherosclerotic disease, with only minor tapering, which makes this artery ideally suited to study not only plaque morphology but also arterial remodeling. In the present study we assessed whether the presence of plaque-related features (size, remodeling, vulnerability) in one femoral artery was associated with their presence in the contralateral artery obtained from the identical individual. A role for systemic factors was assumed if a phenomenon was equally present in both arteries.

**METHODS**

**Human femoral arteries.** Plaque size, arterial remodeling and plaque vulnerability were assessed in bilateral femoral arteries. We assumed that right and left femoral arteries are relatively similar before being influenced by potential systemic factors. Ninety-six femoral arteries of 48 donated corpses (history of cardiovascular disease unknown) were pressure fixed in situ within 24 h after death using formal-
dehydrate 4% (pressure: age + 100 mm Hg). The femoral arteries were divided in 1-cm segments (n = 2,688). Each 1-cm segment was cut in two 0.5-cm segments. The matching cutting faces were used for morphometric analysis and (immuno)histochemical staining, respectively (5). Of every two 0.5-cm segments obtained from a 1-cm segment, one 0.5-cm segment was stained with Lawson’s elastic tissue stain and studied under magnification. Staining of the entire 0.5-cm segment avoided cutting artifacts attributable to histologic sectioning before analysis. The macroscopic images of the cross-sections were recorded on sVHS videotape with a 3CCD video camera for analysis of geometry. The cross-sections recorded on videotape were analyzed with a digital video analyzer. Subsequently, the lumen area and the area encompassed by the internal elastic lamina (IEL) area were measured. Plaque area was calculated as IEL area minus lumen area. Cross-sectional narrowing was defined as (plaque area/IEL area) × 100%. To avoid inclusion of arteries without variation in plaque size, arteries with minor atherosclerosis were excluded. If one or both arteries of an individual did not reveal cross-sections with cross-sectional narrowing of at least 30% (arbitrary limit), this individual was excluded from the study (n = 6). The 84 arteries of the remaining 42 individuals (27 men and 15 women, average age 83 ± 8 years) were used to calculate the average arterial plaque size and to correlate plaque area and IEL area.

**Plaque size.** Average arterial plaque size was defined as the average value of all plaque areas in an artery. Before calculation of the average arterial plaque size, all measured values of an artery were adjusted to correct for differences in arterial size among individuals. A correction factor was calculated for each individual as follows: (average IEL area of all arteries of all individuals)/(average IEL area of both arteries of one individual). Between right and left arteries, the correlation in average plaque size was studied.

**Arterial remodeling.** For each artery, regression analysis was performed to assess the relation between plaque area and IEL area (a measure of structural expansive remodeling) (1,6). Arteries were categorized as with or without a significant correlation between plaque and IEL area. The presence or absence of a significant correlation between plaque and IEL area was compared between right and left arteries.

**Plaque vulnerability.** In 30 randomly selected individuals the presence of histopathologic features related to vulnerable plaques (a large atheroma and inflammation) was determined in right and left femoral arteries. We have reported on the CD68 staining of 36 of these arteries (18 pairs) (5). Staining was performed in the 0.5-cm segments that matched the segments used for morphometric analysis. Of each artery, six arterial segments were selected for additional (immuno)histochemical staining. Of each artery, the segments that fulfilled the following criteria were selected for additional staining: smallest lumen area, largest lumen area, smallest plaque area, largest plaque area, smallest IEL area and largest IEL area. Thirty-one segments selected according to the six categories appeared to be identical for two categories. Thus, a total of 329 segments obtained from 60 arteries of 30 individuals were selected for staining.

Segments were embedded in paraffin, sectioned at 5 μm thickness and mounted on different microscopic slides. Serial sections were stained. To detect collagen, sections were stained with picrosirius red. A mouse antihuman CD68 monoclonal antibody (Dakopatts, Glostrup, Denmark) was used to visualize the presence of macrophages. To make the CD68 epitope accessible for the anti-CD68 monoclonal antibody, the transverse cross-sections were boiled in sodium citrate buffer (10 mM, pH 6.0) for 15 min. An antibody against CD45RO (Dakopatts) was used to detect T-lymphocytes. Immunohistochemical detection of the preferred epitopes was performed according to the indirect horseradish peroxidase and alkaline phosphatase technique for CD68 and CD45RO, respectively.

The percentage atheroma of the total area of the plaque was visually estimated using picrosirius red staining and polarized light. Two groups were considered on the basis of percentage of atheroma in the plaque: lipid-rich core occupying < or ≥40% of total plaque area (4). Thrombus formation is most likely to occur because of inflammation of the cap and cap rupture near the shoulder of the plaque (3,7). Therefore, analysis of inflammatory cells focused on these regions within the plaque. Staining of CD68 (or CD45RO) was considered positive if clusters of >10 stained cells were observed. Presence of superficial inflammation was defined as staining of CD68 and/or CD45RO in cap and/or shoulder. All stained cross-sections were analyzed independently by two observers. If the two observers disagreed on classification, a third observer analyzed the cross-section. Consensus was reached in all cases. After the boiling procedure for the CD68 staining, the immunohistochemical staining of nine cross-sections (from four individuals) could not be analyzed because of artifacts. These four individuals were excluded for the analysis of inflammatory cells.

**Statistics.** The correlation between right and left arteries of continuous variables was calculated by linear regression analysis. Kappa statistics were used to calculate whether presence of a categorized variable in an artery was associated with its presence in the (contralateral) artery obtained from the same individual. Measured values are presented as mean ± standard deviation. A value of p < 0.05 was considered statistically significant.

**RESULTS**

A total of 2,373 segments were obtained from 84 arteries of 42 individuals (28 ± 5 per artery). The mean measured...
lumen area, IEL area, plaque area and cross-sectional narrowing were $21.3 \pm 10.3 \text{ mm}^2$, $33.6 \pm 12.8 \text{ mm}^2$, $12.3 \pm 6.5 \text{ mm}^2$ and $37.5 \pm 15.9\%$, respectively.

**Plaque size.** Average plaque size in the right femoral artery correlated strongly with average plaque size in the left femoral artery ($r^2 = 0.5$, $p < 0.001$, Fig. 1).

**Arterial remodeling.** The relation between plaque area and IEL area is considered to represent expansive remodeling (1,6). In 25 of 42 individuals, a significant correlation between plaque area and IEL area was found in both femoral arteries. In 7 of 42 individuals, no significant correlation between plaque area and IEL area was found in both arteries. Thus, in 32 of 42 individuals (76%) the presence or absence of a significant correlation between plaque area and IEL area was similar in the right and left artery (Table 1, kappa $= 0.42$, $p = 0.007$). Figure 2A shows an example of an individual with a significant increase of IEL area in response to plaque formation in both arteries. Figure 2B shows an example of an individual without enlargement of the IEL area in response to plaque formation in both arteries.

Within the individual, the slopes of the correlation between plaque area and IEL area appeared to be concordant in the right and left femoral artery. Linear regression analysis revealed a correlation between the slopes of the right and left arteries ($y = 0.6x + 0.3$, $r^2 = 0.2$, $p = 0.002$).

**Lipid-rich plaques.** The number of plaques with a lipid-rich core that occupied $\geq 40\%$ of the total plaque (Fig. 3), irrespective of the presence of inflammation, was compared between left and right femoral arteries. A total of 90 plaques with a lipid-rich core were detected (per artery: $1.5 \pm 1.3$, median 1, range 0 to 5; per individual: $2.2 \pm 2.5$, median 2.5, range 0 to 7). There was concordance in the number of plaques with a lipid-rich core between right and left arteries (kappa $0.60$, $p = 0.001$, Table 2).

**Inflammation.** The presence of inflammation in cap and shoulders (Fig. 3), irrespective of atheroma size, was compared between left and right femoral arteries. A total of 137 plaques with inflammation were observed (per artery: $2.6 \pm 1.5$, median 3, range 0 to 6; per individual: $4.6 \pm 2.9$, median 4.5, range 0 to 12). There was no concordance in number of plaques with inflammation between right and left femoral arteries (kappa $0.067$, $p = 0.61$, Table 3).

**DISCUSSION**

In the present study we investigated whether atherosclerotic remodeling and plaque vulnerability are systemically deter-
mined. For this purpose, plaque-related features were compared between right and left femoral arteries. The principal findings of this study are: 1) average plaque size in the right femoral artery correlated with average plaque size in the left artery; 2) in 32 of 42 individuals (76%) similarity concerning the presence or absence of a significant correlation between plaque area and IEL area was observed between right and left femoral arteries; 3) there was concordance between both arteries concerning the number of lipid-rich plaques; and 4) the extent of inflammation in one femoral artery was not concordant with the extent in the contralateral artery.

**Table 2. Number of Plaques in Which Lipid-Rich Core Occupies ≥40% of Total Plaque Area in Right and Left Femoral Arteries**

<table>
<thead>
<tr>
<th>Left Femoral Artery</th>
<th>0–1</th>
<th>2–6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right Femoral Artery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–1</td>
<td>13 (43%)</td>
<td>3 (10%)</td>
</tr>
<tr>
<td>2–6</td>
<td>3 (10%)</td>
<td>11 (37%)</td>
</tr>
</tbody>
</table>

Agreement 80%, kappa = 0.60 (p = 0.001).

**Arterial remodeling.** It is unclear whether systemic factors play a role in the mechanisms underlying de novo atherosclerotic remodeling. The present data show that in the majority of the individuals, right and left femoral arteries had similar expansive remodeling in response to plaque formation. This result suggests a role for systemic factors.

The mechanisms involved in remodeling of de novo atherosclerotic arteries are largely unknown. It has been postulated that increased shear stress due to encroachment of the plaque into the lumen underlies expansive remodeling (1). Systemic factors that impair the natural endothelial response to alterations in shear stress might lead to failure of the compensatory enlargement response or even to shrinkage of the artery. Evidence for a role of systemic factors in arterial remodeling has been provided in recent studies, where constrictive remodeling in culprit lesions was found to be associated with systemic factors such as smoking status (9,10) and insulin-treated diabetes mellitus (11). In both animal (12) and clinical (10) studies, hypercholesterolemia has been associated with expansive remodeling. Genetic factors might also play a role.

**Table 3. Number of Plaques With Superficial Inflammation in Right and Left Femoral Arteries**

<table>
<thead>
<tr>
<th>Left Femoral Artery</th>
<th>0–1</th>
<th>2–4</th>
<th>5–6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right Femoral Artery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–1</td>
<td>2 (8%)</td>
<td>8 (31%)</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>2–4</td>
<td>2 (8%)</td>
<td>9 (35%)</td>
<td>2 (8%)</td>
</tr>
<tr>
<td>5–6</td>
<td>0 (0%)</td>
<td>1 (4%)</td>
<td>1 (4%)</td>
</tr>
</tbody>
</table>

Agreement 46%, kappa = 0.067 (p = 0.61). Superficial inflammation: staining of CD68 and/or CD45RO in cap and/or shoulder.
the plaque have been shown in a rabbit model (15). In this model, a long-term reduction of serum low density lipoprotein cholesterol resulted in a decrease of the area of macrophages plus extracellular lipid deposits in favor of collagen.

**PLAQUE INFLAMMATION.** Inflammation of the cap is considered an important mechanism underlying cap destruction (3). Evidence for a role of inflammation in plaque rupture has been demonstrated by colocalization of inflammation and plaque rupture sites (7). No association was observed in the extent of inflammation between right and left femoral arteries. This could be interpreted as a nonhomogeneous distribution of inflammation in atherosclerotic arteries. The fact that inflammation in atherosclerotic lesions seems to be present with local preference suggests that plaque inflammation is locally affected. Evidence for local immunologic activation has been provided by the demonstration of activated T lymphocytes and macrophages (7) and extensive expression of human leucocyte antigen class II molecules in the atherosclerotic plaque (16). Local antigens could be autoantigens (17) or antigens of infectious agents, like Chlamydia pneumoniae (18).

Systemic markers of inflammation, such as C-reactive protein (CRP), have been associated with atherosclerosis and its thrombotic complications (19). A systemically determined risk marker such as CRP may reflect cumulation of all inflammation in the arterial system, independent of the distribution in the circulation. The association between systemic risk markers and a thrombotic event is then a matter of chance; the more inflammation present, the more plaques may rupture with subsequent thrombotic occlusion of the lumen.

The present results suggest that plaque inflammation is locally affected and that arterial remodeling is influenced by systemic factors. These observations seem to contradict the results of a previous study of our group. In a postmortem histology study an association between local arterial remodeling and the vulnerability of the atherosclerotic plaque was demonstrated (20). Moreover, intravascular ultrasound studies showed a correlation between culprit lesion remodeling and the clinical course of coronary artery disease (21,22) and target lesion revascularization after nonstent intervention (23). However, in these previous studies arterial remodeling was studied locally, whereas in the present study systematic arterial remodeling along the artery was studied. Therefore, the present observation does not exclude an association between local plaque composition and arterial remodeling.

**Study limitations.** The present study was performed in femoral arteries. It is unknown whether the present results can be extrapolated to the coronary arteries. The femoral artery hardly tapers (6) and has no major side branches, which makes it ideal to study geometric remodeling. Results of studies of atherosclerotic remodeling in the short arterial segments of coronary arteries, however, are consistent with findings in the femoral artery (2,20,22,24,25). The prevalence of inflammation of atherosclerotic plaques was also found to be comparable for coronary and femoral arteries (5).

The age of the studied population was high, and it is unknown whether the present results can be extrapolated to younger individuals. Previously, however, we demonstrated (6) that the prevalence of the different modes of arterial remodeling in a high-age patient group was comparable with a lower age population suffering from claudication.

**Summary.** These results suggest that not only plaque burden, but also vascular remodeling and lipid deposition in plaques, are influenced by systemic factors. The nonhomogeneous distribution of inflammation in atherosclerotic arteries supports the hypothesis that plaque inflammation is locally affected. These data suggest that the development of rupture-prone plaques is a complicated process that is influenced by both systemic and local factors.

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