Arterial Neovascularization and Inflammation in Vulnerable Patients

Early and Late Signs of Symptomatic Atherosclerosis

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Background—Atherosclerosis is complicated by cardiovascular events such as myocardial infarction, stroke, or peripheral arterial occlusive disease. Inflammation and pathological neovascularization are thought to precipitate plaque rupture or erosion, both causes of arterial thrombosis and cardiovascular events. We tested the hypothesis that arterial inflammation and angiogenic events are increased throughout the arterial tree in vulnerable patients, ie, in patients who suffered from cardiovascular events, compared with patients who never suffered from complications of atherosclerosis.

Methods and Results—In a postmortem study, we quantified the inflammatory infiltrate and microvascular network in the arterial wall of iliac, carotid, and renal arteries. Tissue microarray technology was adapted to investigate full-thickness arterial sectors. We compared 22 patients with symptomatic atherosclerosis with 27 patients who never had suffered from any cardiovascular event. The absolute intimal macrophage content was 2- to 4-fold higher in vulnerable patients at all 3 arterial sites analyzed ($P<0.05$). Patients with symptomatic atherosclerosis had a denser network of vasa vasorum than patients with asymptomatic disease (33 ± 2 versus 25 ± 2 adventitial microvessels per 1 mm^2; $P=0.008$). Hyperplasia of vasa vasorum was an early and macrophage infiltration was a late sign of symptomatic atherosclerosis.

Conclusions—High intimal macrophage content and a hyperplastic network of vasa vasorum characterize vulnerable patients suffering from symptomatic atherosclerosis. These changes are uniformly present in different arterial beds and support the concept of symptomatic atherosclerosis as a panarterial disease. (***Circulation*. 2004;110:2843-2850.**)

Key Words: arteriosclerosis ■ inflammation ■ angiogenesis, pathological ■ tissue microarray

Atherosclerosis is an inflammatory lipid storage disease of large and medium-sized arteries[1–3] complicated by cardiovascular events. These are most commonly the result of sudden arterial thrombosis in the heart, brain, legs, and other organs. Plaque rupture and erosion are causes of an acute arterial thrombosis.[4,5] Atherosclerotic plaques that are prone to precipitate acute thrombotic occlusions are vulnerable plaques.[6] Recently, the term "vulnerable patient" has been coined to emphasize that not all patients with atherosclerotic lesions will develop symptomatic, complicated atherosclerotic disease.[4,7] This study was designed to test the hypothesis that specific morphological changes of the arterial wall identify vulnerable patients and are present at all sites of the arterial tree. Active inflammation has been defined as a major criterion of vulnerable plaques.[4] Ectopic neovascularization in the intima and media is a hallmark of advanced atherosclerotic lesions.[8] These pathological microvessels are prone to rupture, and intraplaque hemorrhage has recently been shown to accelerate plaque formation.[9] In a histopathological postmortem analysis, we morphometrically quantified macrophage content and the intramural microvascular network of the carotid, renal, and iliac arteries by applying tissue microarray technology.[10] We also assessed plaque burden and arterial wall dimensions. These histomorphological findings were linked to the clinical history of the patients by focusing on cardiovascular events and risk factors.

Methods

Patients and Tissue Specimens

All investigations were approved by the institutional ethics review board and were performed in accordance with institutional guidelines. Between December 2002 and April 2003, 49 consecutive patients who died at our hospital (Department of Medicine, University Hospital Bruderholz, Bruderholz, Switzerland; autopsy rate >75%) and whose autopsy was performed within 36 hours were prospectively and nonselectively included in this study. Intact 0.5-cm-long ring segments of the left common iliac artery (2 cm distal of the aortic bifurcation), left common carotid artery (2 cm proximal of the carotid bifurcation), and left renal artery (2 cm from the aortic origin) were removed and immediately fixed in 4% phosphate buffered formalin. In 11 patients who died with known or suspected coronary heart disease, a postmortem angiography was

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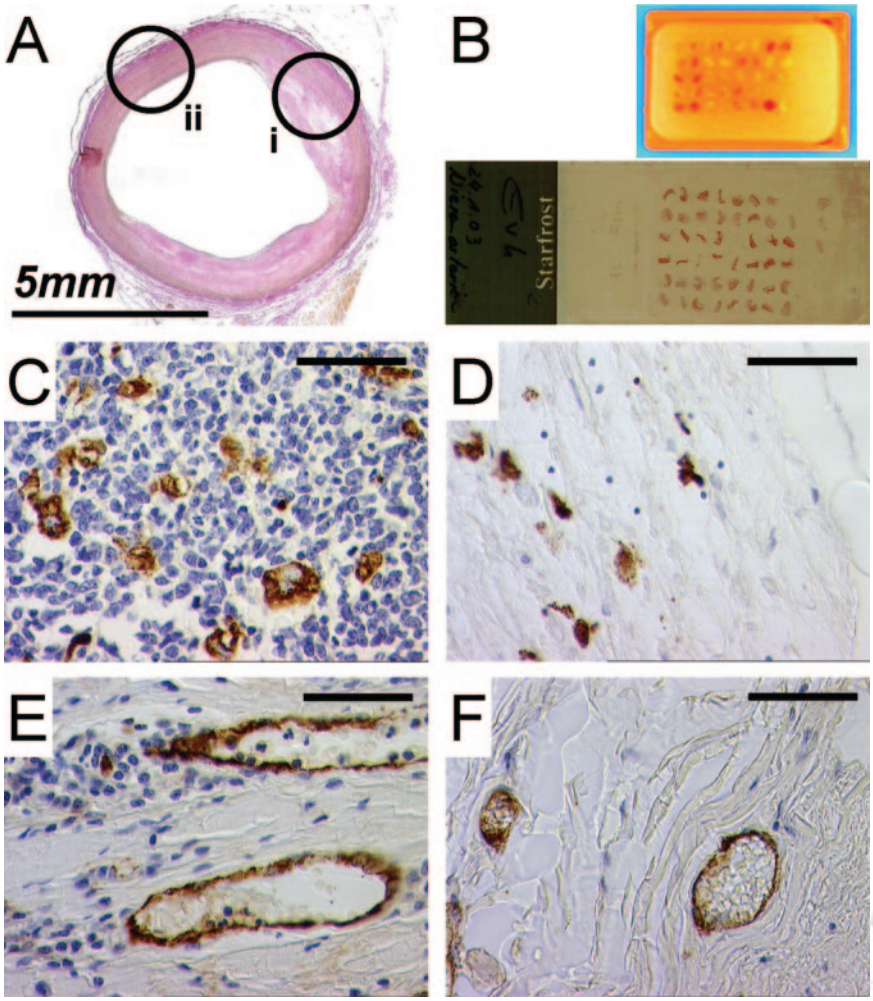

Figure 1. Arterial tissue microarrays for histomorphological analysis of atherosclerosis. A, Arterial ring segment of left carotid artery displaying atheroma in its most-affected sector (i) and normal intima in its best-preserved sector (ii). B, Recipient paraffin block containing 54 cylindrical arterial tissue specimens (top) and histological slide with arterial tissue samples from 20 to 30 patients (bottom). C, E, One of several control tissues incorporated in microarray. Tonsils were used as control tissue and stained for CD68-positive cells (C) and von Willebrand factor–positive blood vessels (E). D, CD68-positive monocytes/macrophages. F, Microvessels in arterial wall. Scale bars (C through F) represent 50 μm.

routinely performed. Therefore, the coronary arteries that were not equally treated in all patients were excluded from this analysis. The patient's clinical record was retrospectively reviewed for cardiovascular events or risk factors. If a history of cardiovascular events was present, the patient was assigned to the group with symptomatic atherosclerosis. Cardiovascular events were defined as myocardial infarction, angina pectoris with signs of myocardial ischemia, cerebrovascular ischemic stroke, transient ischemic attack, peripheral arterial occlusive disease, symptomatic aortic aneurysm, or any arterial revascularization procedure to treat atherosclerosis. On average, symptomatic atherosclerosis was diagnosed 4.8±1 years before death.

Histological Processing and Tissue Microarrays

Before embedding in paraffin, all arterial tissues were briefly decalcified to remove excessive calcium. For histological examination, complete arterial ring sections were stained by Elastica–van Gieson's stain. A total of 147 arterial rings were examined histologically. The most-affected and the best-preserved arterial sectors were typed according to the American Heart Association (AHA) consensus report.[11–13] This histopathological score of atherosclerotic lesions distinguishes 6 major plaque types: 1=intimal thickening, 2=fatty streaks, 3=intermediate lesions, 4=atheroma, 5=fibroatheroma or calcified plaques, and 6=complicated lesions characterized by rupture, thrombus, or hematoma. Normal arterial sectors free of atherosclerotic lesions were scored as type 0. The interobserver variability of plaque typing was determined by correlating the independent assessment of 2 investigators (M.K., B.C.B.), both blinded to the clinical condition of the patient at the time of scoring. Tissue microarray technology[10] was adapted to analyze full-thickness arterial wall sectors (Figure 1). For each arterial ring, the most-affected and the best-preserved arterial sectors were harvested (Figure 1A). Depending on the arterial wall dimensions, either 2- or 4-mm-thick tissue cylinders were punched out and transferred to a recipient paraffin block prepared to accommodate cylinders of different sizes (Figure 1B) with a homemade, semiautomated tissue microarrayer. From the 49 patients, a total of 294 arterial wall sectors were incorporated into the tissue microarrays. A relevant number of control tissues such as skin, tonsils (Figure 1C and 1E), liver, and kidney were incorporated into each array block.

Immunohistochemistry of Tissue Microarray Sections

Sections of the microarray block (6 μm thick) were cut and transferred to a glass slide. Sections were air dried at 60°C for 45 minutes. Paraffin was removed by xylol, sections were hydrated, and endogenous peroxidase activity was blocked by 2% H_20_2. For antigen retrieval, slides were incubated for 2 minutes in 10 mmol/L citrate buffer with a steam presser chamber. Macrophages were identified with an antibody against CD68 (Figure 1C and 1D); endothelial cells were identified with an antibody against von Willebrand factor (Figure 1E and 1F). Slides were rinsed in PBS and incubated with the first antibody: mouse anti-human CD68 (M-0876, 1:200) or mouse anti-human von Willebrand factor (M-0616, 1:10, DAKO, Glostrup). After 30 minutes at room temperature, the slides were washed twice and incubated with a peroxidase-conjugated goat anti-mouse antiserum (DAKO Envision). After 30 minutes' incubation at room temperature, slides were washed twice and incubated with diaminobenzidine as a substrate. Hemalaun was used for counterstaining. The sections were dehydrated and embedded (Pertex, Medite).

TABLE 1. Patient Characteristics

	No Cardiovascular Events (n=27)	Cardiovascular Events (n=22)	*P*
Male sex, n (%)	13 (48)	11 (50)	0.90
Age, y	74±14	79±9	0.09
Cardiovascular risk factors			
Diabetes mellitus, n (%)	1 (4)	10 (45)	<0.001†
Body mass index, kg/m^2	23±6	28±6	0.006†
Hypercholesterolemia, n (%)	2 (7)	7 (32)	0.03†
Arterial hypertension, n (%)	6 (22)	11 (50)	0.04†
Smoking, n (%)	5 (18)	7 (32)	0.35
History of cardiovascular disease, n (%)			
Coronary heart disease	0 (0)	21 (95)	
Cerebrovascular disease	0 (0)	8 (36)	
Arterial occlusive disease	0 (0)	8 (36)	
Autopsy (hours after death)	20±12	24±9	0.15
Infection at death, no. (%)*	11 (41)	10 (45)	0.80

Values are mean±SD when appropriate.

*Infection at death was defined by the presence of ≥2 of the following criteria: Body temperature >38°C, C-reactive protein >50 mg/L, neutrophils (band forms) >10%, and positive blood cultures.

†$P<0.05$.

Quantitative Morphometric Analysis of Wall Dimensions, Leukocyte Infiltrates, and Vasa Vasorum

The arterial rings were examined microscopically (Leitz DMRB). Digital pictures (Hitachi HVC20 M/L, Hitachi Ltd) were calibrated (DISKUS, Hilgers) and morphometrically analyzed (AnalySIS, Soft Imaging System GmbH). The luminal circumference was measured, and the circular luminal area (mm^2) and average luminal radius (mm) were calculated. At 8 randomly selected sites, intimal and medial thicknesses were measured, and the average value for each arterial wall layer was calculated. The intimal area was calculated according to the following formula: area (mm^2)=[$\pi\times$(luminal radius+intimal thickness)^2]−($\pi\times$luminal radius^2). The tissue microarray blocks contained either 54 (2-mm cylinders) or 25 (4-mm cylinders) arterial tissue samples. Each tissue sample was linked to the individual patient's anonymous clinical data record. From each arterial sector, a series of 15 to 20 digital pictures was taken covering the intima, media, and adventitia. The stained cells and vascular profiles per picture area (72 000 μm^2) were counted, and the average number was normalized to 1 mm^2. Absolute intimal macrophage content was calculated for each individual arterial ring by multiplying the intimal cell count per 1 mm^2 with the intimal area (mm^2).

Statistical Analysis

The different variables obtained in the 2 groups of patients with and without cardiovascular events were compared by use of the Mann-Whitney *U* test. The presence of cardiovascular risk factors in the 2 groups of patients and the fraction of plaques with ectopic neovascularization were compared by the χ^2 test.[14] Tests were performed with SPSS 11.0 software (SPSS Inc). Unless otherwise stated, values are given as mean±SEM. Values of $P<0.05$ were considered to indicate a significant difference between the 2 groups of patients.

Results

Clinical Characteristics of Patients With Symptomatic Atherosclerosis

Of 49 patients, 22 (45%) suffered from symptomatic atherosclerosis. Of these patients with active disease, 95% had coronary heart disease, 36% had cerebrovascular disease, and 36% had peripheral arterial occlusive disease. In 56% of the patients, cardiovascular events occurred in >1 organ system. Of 49 patients, 27 (55%) never had complications of atherosclerosis. Of these patients, 75% had asymptomatic atherosclerosis; ie, they had overt atheroma (AHA type 4 or greater plaque) at ≥1 of the arterial sites analyzed, but they had never suffered from cardiovascular events. The clinical characteristics of the 2 patient groups are summarized in Table 1. Among the known cardiovascular risk factors, diabetes mellitus, hypercholesterolemia, and arterial hypertension were significantly higher in the group with symptomatic atherosclerosis. Vulnerable patients had a higher body mass index. Smoking was more common in the group with active disease, but this difference did not reach statistical significance. By definition, cardiovascular diseases such as coronary heart disease, cerebrovascular disease, and peripheral arterial occlusive disease were present exclusively in the group with symptomatic atherosclerosis. The occurrence of severe infections before death was similar in both groups. Infections such as urinary tract infections or pneumonia had no effect on the mural leukocyte infiltration (data not shown).

Plaque Burden in Vulnerable Patients

Atherosclerotic lesions were typed according to the AHA consensus report.[13] Two independent investigators examined 294 arterial sectors, and the interobserver correlation for plaque scoring was high ($r=0.97$; $P<0.001$). We first tested the hypothesis that vulnerable patients would have a higher plaque burden in all 3 arterial beds examined compared with patients who never suffered from complications of atherosclerosis. As expected, patients who suffered from cardiovascular events during their lifetime had a higher plaque burden compared with patients who never suffered from cardiovascular complications (Table 2). At all arterial sites examined, both in the severely affected and well-preserved sectors, plaque burden as assessed by the AHA consensus report was

TABLE 2. Plaque Burden in Patients With and Without Cardiovascular Events

	No Cardiovascular Events (n=27)	Cardiovascular Events (n=22)	P
Left common iliac artery			
AHA plaque type, best-preserved sector	2 (2–2)	3 (2–4.5)	0.01†
AHA plaque type, most-affected sector	4 (3–5)	5 (5–6)	0.008†
Left common carotid artery			
AHA plaque type, best-preserved sector	2 (1–2)	2 (1–2)	0.44
AHA plaque type, most-affected sector	3 (2–3)	3 (3–5)	0.03†
Left renal artery			
AHA plaque type, best-preserved sector	0 (0–2)	1 (0–2)	0.17
AHA plaque type, most-affected sector	2 (2–3)	3 (2–4.25)	0.02†
Frequency distribution of atherosclerotic plaques, n (%)			
AHA plaque type 0*	23 (14)	9 (7)	
AHA plaque type 1	19 (12)	14 (10)	
AHA plaque type 2	57 (35)	41 (31)	
AHA plaque type 3	33 (20)	18 (14)	
AHA plaque type 4	14 (9)	13 (10)	
AHA plaque type 5	14 (9)	23 (17)	
AHA plaque type 6	2 (1)	14 (11)	

Values are median (interquartile range) when appropriate.
†$P<0.05$.
*Normal arterial sectors.

higher in patients with symptomatic atherosclerosis than in patients free of cardiovascular events (Table 2). This difference was more pronounced at the most-affected than at the best-preserved arterial sector analyzed. For patients with and those without cardiovascular events, the iliac artery was the most affected blood vessel, and the renal artery had the mildest lesions.

Panarterial Morphological Changes in Vulnerable Patients

We next tested which of the morphological signs analyzed were different at all 3 arterial sites in vulnerable patients. Vulnerable patients had an increased intimal area with preserved luminal area (Table 3). Tissue microarray sections stained for blood vessels and macrophages were used to

TABLE 3. Vascular Dimensions of the 3 Elastic Arteries

	No Cardiovascular Events (n=27)	Cardiovascular Events (n=22)	P
Left common iliac artery			
Intimal thickness, mm	0.54±0.33	0.90±0.72	0.02*
Intimal area, mm^2	14.2±11.6	28.4±26.7	0.02*
Media thickness, mm	0.46±0.08	0.40±0.14	0.01*
Lumen, mm^2	46.7±26	47.4±28.4	0.97
Left common carotid artery			
Intimal thickness, mm	0.22±0.14	0.39±0.27	0.02*
Intimal area, mm^2	3.4±3.5	7.2±6.1	0.01*
Media thickness, mm	0.68±0.19	0.62±0.14	0.10
Lumen, mm^2	17.0±8.3	19.7±6.4	0.24
Left renal artery			
Intimal thickness, mm	0.16±0.10	0.30±0.34	0.2
Intimal area, mm^2	1.7±1.5	4.0±4.7	0.08
Media thickness, mm	0.41±0.07	0.40±0.09	0.92
Lumen, mm^2	7.3±4.4	11.9±16.5	0.31

Values are mean±SD.
*$P<0.05$.

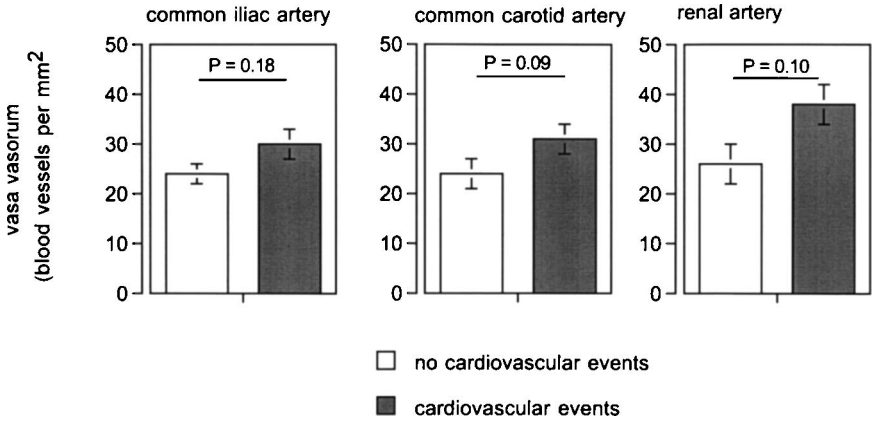

Figure 2. Vasa vasorum in symptomatic and asymptomatic patients. Microvessel density of vasa vasorum (blood vessels per 1 mm^2 adventitia) was determined in iliac, carotid, and renal arteries. For each arterial site, 2 groups of patients were compared by Mann-Whitney *U* test.

quantify the arterial microvascular network and inflammatory infiltrate. The microvascular network of vasa vasorum in the adventitial layer was hyperplastic in patients with cardiovascular events (Figure 2). When the average value of adventitial microvessel density per patient was calculated, vulnerable patients had significantly more blood vessels per area compared with asymptomatic patients (33±2 compared with 25±2 blood vessels per 1 mm^2; $P=0.008$) Diabetes mellitus is known to cause proliferative microangiopathy in the retina and kidney.[15] We tested whether vulnerable patients with diabetes would have a denser microvascular network in the adventitia compared with patients without diabetes. Vulnerable patients with diabetes had an adventitial microvessel density of 37±3 compared with 29±2 blood vessels per 1 mm^2 in vulnerable patients without diabetes ($P=0.04$). In vulnerable patients, pathological neovascularization, ie, ectopic microvessels in the intima and media, was found in 60%, 37%, and 33% of the arterial sectors of the iliac, carotid, and renal arteries, respectively. In patients free of cardiovascular events, pathological neovascularization was found in 38%, 25%, and 0% of the arterial sectors of the iliac, carotid, and renal arteries. Plaque burden, intimal thickening with preserved arterial lumen, hyperplasia of vasa vasorum, and ectopic neovascularization were increased in vulnerable patients at all arterial sites analyzed.

In contrast, the intimal macrophage infiltration, determined as the number of macrophages per 1 mm^2, was not uniformly increased in patients with symptomatic atherosclerosis (Figure 3, top). However, when the absolute cross-sectional intimal macrophage content was calculated by multiplying the macrophage number per 1 mm^2 with the intimal area (mm^2), the differences between vulnerable and asymptomatic patients became significant at all arterial sites analyzed (Figure 3, bottom). In summary, the absolute intimal macrophage content but not the number of macrophages per 1 mm^2 discriminated between patients with symptomatic and asymptomatic atherosclerosis.

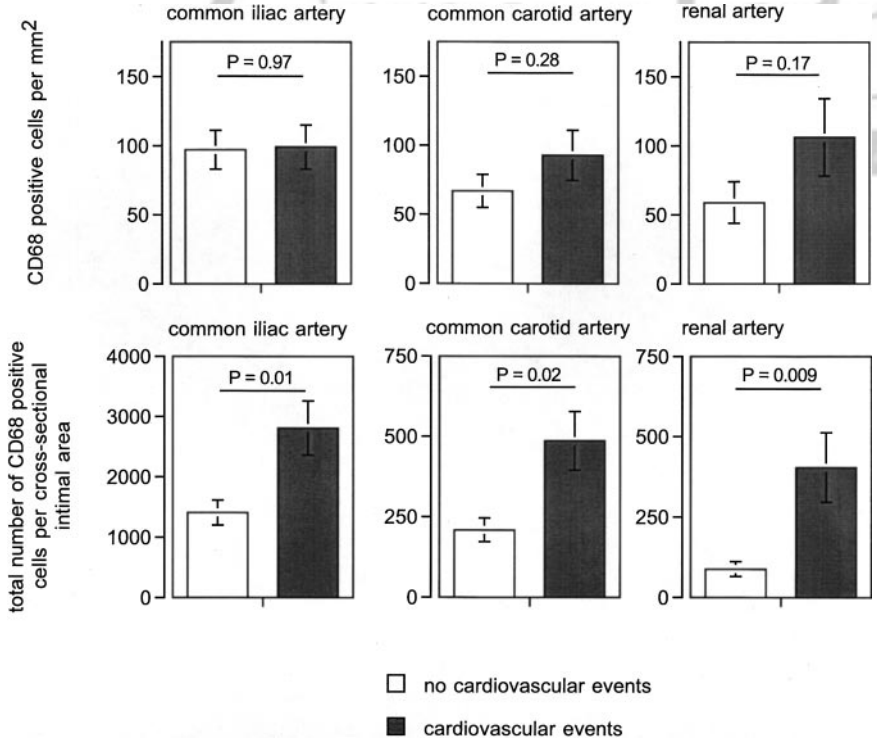

Figure 3. Arterial inflammation in symptomatic and asymptomatic patients. Top, Macrophage density (cells per 1 mm^2 intima); bottom, intimal macrophage content (cells per cross-sectional intimal area). Macrophage density and content were determined in intima of iliac, carotid, and renal arteries. For each arterial site, 2 groups of patients were compared by Mann-Whitney *U* test.

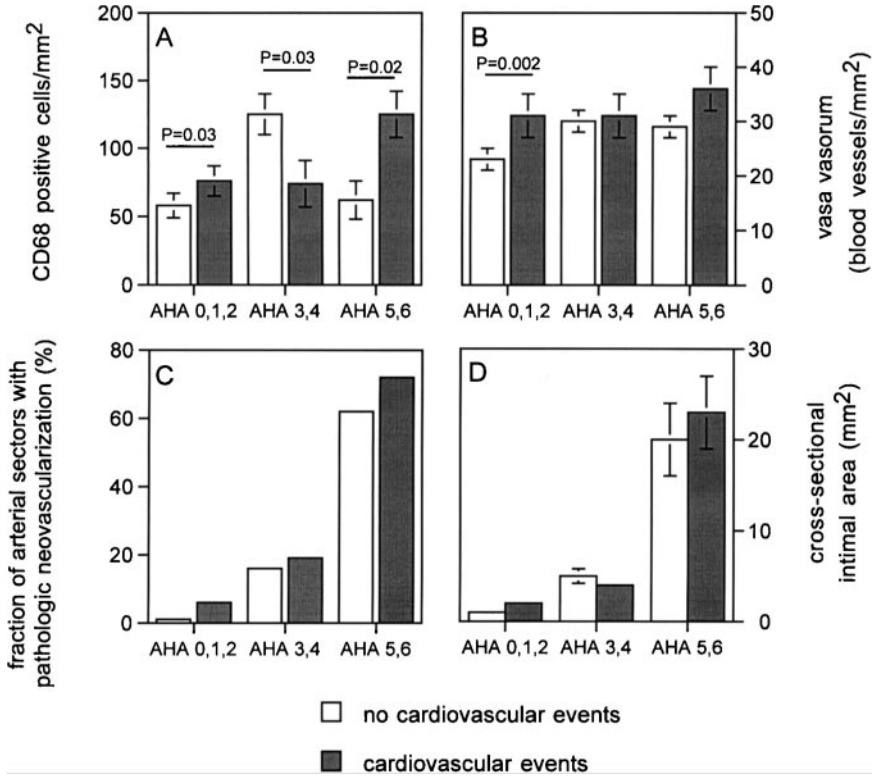

Figure 4. Intimal thickness, neovascularization, and inflammation in plaques of equal severity. All arterial sectors from 3 different arterial sites were included in this analysis. A, Monocyte/macrophage infiltration is sign of advanced, severe lesions in vulnerable patients. Asymptomatic patients have dense macrophage infiltrate at intermediate stages of the disease. B, Adventitial microvessel (vasa vasorum) density is significantly higher in symptomatic patients at early stages of atherosclerotic plaque formation. C, Ectopic neovascularization is sign of advanced lesions but does not discriminate between symptomatic and asymptomatic patients. D, Cross-sectional intimal area increases with plaque severity. Values are mean±SEM. *$P<0.05$.

Early and Late Morphological Signs of Symptomatic Atherosclerosis

The panarterial changes found in vulnerable patients who suffered from complicated atherosclerosis could be the consequence of increased plaque burden in these patients. To rule out this hypothesis, we grouped all the arterial sectors into categories according to plaque type (Table 2, bottom). Plaques of equal severity were compared between patients with and without cardiovascular events. Figure 4 shows macrophage infiltration, the adventitial microvascular network, pathological neovascularization, and the intimal area (D) in early (AHA types 0, 1, and 2), intermediate (AHA types 3 and 4), and advanced (AHA types 5 and 6) atherosclerotic lesions. Macrophage infiltration was high in late-stage lesions (AHA type 5 and 6 plaques) of patients with symptomatic atherosclerosis (Figure 4A). Unexpectedly, asymptomatic patients showed a dense macrophage infiltrate at intermediate stages of the disease (AHA type 3 and 4 plaques). In arterial sectors free of atherosclerosis and in mildly affected arterial sectors (AHA type 0, 1, and 2 plaques), a hyperplastic network of vasa vasorum was discriminating most clearly between symptomatic and asymptomatic patients (Figure 4B). Ectopic neovascularization was more pronounced in more advanced atherosclerotic lesions but did not discriminate between symptomatic and asymptomatic patients (Figure 4C). As expected, arterial rings with advanced plaques had the largest intimal cross-sectional area (Figure 4D). The fraction of plaques with ectopic neovascularization correlated closely with the average cross-sectional intimal area ($r=0.99$; $P<0.001$). Therefore, plaque neovascularization is not an independent predictor of plaque rupture but rather reflects intimal growth.

Discussion

Several pathological studies have been performed to define advanced or vulnerable atherosclerotic lesions. However, either they focused exclusively on the organ affected by the acute complication of atherosclerosis,[9,16,17] or they were limited by the lack of correlation with the clinical history of the investigated patients.[18–20] Here, we present a quantitative analysis of angiogenic and inflammatory events in the arterial tree of vulnerable patients, ie, patients who suffered from cardiovascular events during their lifetime.

We report that arteries from vulnerable patients are characterized by intimal thickening with a preserved luminal diameter, by a high absolute intimal macrophage content, by a hyperplastic network of vasa vasorum, and by ectopic neovascularization. These vascular changes are not confined to the organ affected by cardiovascular events but are uniformly found at all arterial sites investigated in this study.

We identified 2 distinct angiogenic events evolving in symptomatic atherosclerosis: ectopic neovascularization of plaques and a hyperplasia of vasa vasorum. Our findings suggest that different mechanisms are involved in triggering these 2 events. Ectopic neovascularization seems to reflect primarily an adaptive response of the arterial wall to an increased nutritional demand, as it may occur in the course of intimal thickening. In contrast, hyperplasia of vasa vasorum as a sign of symptomatic atherosclerosis was observed in very early lesions in which intimal thickening was negligible and the arterial dimensions were virtually normal. We found that vulnerable patients with diabetes mellitus had a significantly denser network of vasa vasorum than vulnerable patients without diabetes. In addition to hypoxia and nutrient depletion induced by plaque growth, the dense microvascular

network of vasa vasorum in diabetic patients may further facilitate plaque neovascularization in advanced lesions. In the course of diabetes mellitus, excessive angiogenesis is involved in the pathogenesis of retinopathy and nephropathy.[15] Interestingly, the degree of capillary hyperplasia observed in diabetic nephropathy[21] is similar to the 20% to 30% increase in adventitial microvessels observed in our study. In a murine model of atherosclerosis, antiangiogenic treatment inhibited plaque progression.[22] Although our findings are descriptive, they suggest that such an approach may be successful in preventing symptomatic atherosclerosis in humans, particularly in patients with diabetes. Inflammatory infiltrates have been described in atherosclerotic lesions.[23,24] Active inflammation has recently been defined as a major criterion of vulnerable plaques.[4] In our study, the intimal macrophage density (cells per 1 mm^2) did not unequivocally identify vulnerable patients. Macrophages may play a dual role in the pathogenesis of atherosclerosis; we found dense macrophage infiltrations in advanced lesions (AHA type 5 and 6 plaques) from vulnerable patients, and we identified a substantial number of macrophages per 1 mm^2 in intermediate lesions (AHA type 3 and 4 plaques) from asymptomatic patients. In advanced stages of atherosclerosis, inflammation could further destabilize atherosclerotic plaques.[25] On the other hand, at intermediate stages of atherosclerosis, inflammation may have a beneficial effect in the maintenance of arterial integrity.

A variety of noninvasive imaging techniques are available for the diagnosis of atherosclerosis. They are based on luminographic, tomographic, or scintigraphic methods. On the basis of our results, luminographic approaches like conventional angiography are not suitable for identifying vulnerable patients. Tomography can detect calcified coronary arteries and large, lipid-rich plaques in the aorta.[26–28] Tomographic methods may be suitable to visualize intimal thickening, pathological neovascularization, and the hyperplastic network of vasa vasorum. In vivo labeling of apoptotic macrophages has been successfully used to detect atherosclerotic lesions in a rabbit model of the disease.[29] Our findings of a significant increase of absolute macrophage numbers per total intimal area favor a scintigraphic, integrating approach for measuring relevant inflammation in vulnerable patients.

Our study may be limited by the advanced age of the study population. However, the selection of 3 rather mildly affected arterial segments—the common carotid, renal, and common iliac arteries—guaranteed the investigation of a significant number of early lesions, allowing a valid analysis of arterial changes at all stages of the disease in both patient groups.

Tissue microarrays have been developed to validate the pathogenic and prognostic role of cancer genes in different tumors. This technology allows an unbiased, systematic, and cost-efficient analysis of immunohistochemical stainings and in situ hybridizations in a large number of samples under controlled conditions. We successfully used tissue microarrays as a novel approach for the histoproteomic investigation of an anatomically more complex tissue such as arteries from patients with atherosclerosis. Gene expression profiling or comprehensive genomic linkage analyses continue to reveal a broad range of candidate molecules putatively participating in the pathogenesis of human atherosclerosis. For each of these molecules, its precise role can now be validated on a large number of arterial tissues obtained from patients at different stages of the disease.

Over the past years, there has been controversy about a reliable and reproducible histopathological classification of atherosclerosis.[17,30] We find that the AHA consensus report[13] provides a valid instrument for classifying atherosclerotic lesions in humans. It enables the reproducible histopathological classification of atherosclerotic lesions and clearly identifies vulnerable patients.

In conclusion, our study indicates that the in vivo assessment of arterial intimal thickness, intimal macrophage content, and the intramural microvascular network at several sites of the arterial tree may improve the identification of vulnerable patients. The panarterial changes of symptomatic atherosclerosis justify a systemic approach to treat or prevent complicated disease.

Acknowledgments

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