Calcification of Coronary Intima and Media: Immunohistochemistry, Backscatter Imaging, and X-Ray Analysis in Renal and Nonrenal Patients

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Coronary calcification is a potent predictor of cardiac events. In patients with chronic renal disease, both prevalence and intensity of coronary calcification are increased. It has remained uncertain whether it is the intima of the coronaries or the media that is calcified and whether the morphologic details of calcified plaques differ between renal and nonrenal patients. Autopsy samples of coronaries were obtained from standard sites in 23 renal and 23 age- and gender-matched nonuremic patients. Specimens were examined using light and electron microscopy, immunohistochemistry, backscatter imaging, and x-ray analysis. In coronaries, calcified plaques occupied a similar proportion of the intima area in renal *versus* nonrenal patients (17.3 ± 11.9 *versus* 18.1 ± 11.9%) but occupied a significantly higher proportion of the media (16.6 ± 10.6 *versus* 3.8 ± 2.31%). Expression of the proteins osteocalcin, C-reactive protein, TGF-β, and collagen IV was significantly more intensive around coronary plaques of renal compared with nonrenal patients. The non–plaque-bearing intima of renal patients showed minimal staining for fetuin, but fetuin staining was seen surrounding calcified plaques. In addition, more pronounced deposition of C5b-9 was found around coronary plaques of renal patients, and glycophorin deposition pointed to more past intraplaque hemorrhage in renal patients. Calcification by electron backscatter analysis is more intense in the coronary media, but not if the intima is more intense in renal compared with nonrenal patients. A more marked inflammatory response in renal patients is suggested by more frequent presence and greater intensity of markers of inflammation.

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In renal patients, coronary atherosclerosis (as well as atherosclerosis in other vascular territories) is more prevalent and cardiovascular events also are more frequent. Little information is available, however, concerning the underlying mechanisms of plaque progression and rupture in patients with renal dysfunction. Because cardiovascular disease is the main cause of death in renal patients, the problem is of obvious clinical importance (1,2).

Definite proof for the concept of accelerated atherogenesis in renal disease meanwhile has been provided by animal experiments (3,4). Epidemiologic data document an excessive rate of coronary events in patients with renal dysfunction. Furthermore, studies that have used electron beam computed tomography (EBCT) have shown accelerated deposition of calcium (Ca) even in the coronaries of patients whose renal function was only slightly impaired (5). In uremic patients who had come to autopsy, staging of coronary plaques according to Stary (6,19) documented more frequent calcification of plaques, yet, in contrast to nonrenal patients, the composition of coronary plaques and the expression of potential target molecules that are involved in the pathogenesis of coronary plaque have not been studied in patients (7–10). Histologic investigations that addressed underlying pathomechanisms of vascular disease in humans with renal failure so far have been restricted to peripheral arteries (11,12).

Recently, micro-inflammation was identified in nonrenal (13) as well as renal (14) patients with coronary heart disease. This finding possibly is linked to the generation of reactive oxygen species. Complement activation also has been proposed to participate in both the initiation and the progression of atherosclerosis (15). This hypothesis is plausible because activated complement components, particularly the membrane attack complex (C5b-9), have been identified in atherosclerotic plaques of nonrenal patients (16). In addition, novel pathomechanisms of vascular calcification in uremia have been documented (17,18), but the detailed morphology of calcified coronary plaques, which is important in the clinic for the interpretation of EBCT or multislice CT, have not been studied in renal patients.

The aim of our study was to assess extent and location (intima *versus* media) of calcifications in the coronary artery comparing renal patients with matched nonrenal control subjects. An additional aim was to investigate whether molecules that are involved in the inflammatory response are present

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Table 1. Clinical data[a]

Data	Nonrenal Patients (n = 23)	Renal Patients (n = 23)
Age	64.9 ± 17.5	65.5 ± 10.2
Gender (m:f)	10:13	11:12
Body weight (kg)	73 ± 8.5	65 ± 10.4
Stage of renal failure		
proteinuria		10
CAPD		3
hemodialysis		10
Some clinical data		
hypertension[b]	11	23
history of smoking	2	1
myocardial infarction[c]	2	5
peripheral arterial occlusive disease[c]	2	4
stroke[c]	1	2

[a]CAPD, continuous ambulatory peritoneal dialysis.
[b]More than 140/90 mmHg or antihypertensive medication.
[c]Clinical diagnosis.

more abundantly within and around plaques of renal as opposed to nonrenal patients.

Materials and Methods

Patients

Between January 2000 and December 2003, all available consecutive patients who had chronic renal disease (n = 23) and had come to autopsy at the Department of Pathology of the University Heidelberg were included in the study. Patients with diabetes were excluded. Ten patients had been on dialysis, three of whom died after a failed graft, and the others were in preterminal renal failure. Information concerning coronary risk factors (*e.g.*, hypertension, nicotine abuse) was obtained from hospital records. Clinical data are listed in Table 1. Renal patients were compared with 23 nonrenal patients with established coronary atherosclerosis of comparable age and gender. The first 2 cm of the coronary ramus interventricularis anterior were sampled.

Semiquantitative Evaluation of Coronary Arteries

Hematoxylin- and eosin-stained paraffin sections of the coronary arteries were investigated using light microscopy (×25 magnification). The

Table 2. Categorization of coronary atherosclerosis according to Stary

Category	Description	Nonrenal Patients (n = 23)	Renal Patients (n = 23)
Type I (initial lesion)	Protein accumulation	—	—
Type II (fatty streak)	All type I changes plus lipoprotein accumulation in smooth muscle cells, still no tissue damage	—	—
Type III (preatheroma)	All type II changes plus multiple deposits of pooled extracellular lipids; microscopic evidence of tissue damage and disorder	5	2
Type IV (atheroma)	All type II changes plus confluent mass of extracellular lipid with massive structural damage to intima	7	3
Type V (fibroatheroma)	All type IV changes plus development of marked collagen and smooth muscle cell increase (cap) above the lipid core	6	7
Type VI (complicated plaque)	All type V changes plus a thrombotic deposit and/or hemorrhage and/or erosion or fissure	—	—
Type VII (calcified plaque)	Any advanced lesion type composed predominantly of calcium; substantial structural deformity	3	17[a]
Type VIII (fibrous plaque)	Any advanced lesion type composed predominantly of collagen; lipid may be absent	—	—

[a]$P < 0.05$ *versus* nonrenal patients.

Table 3. Vessel geometry (intima and media of coronary arteries)[a]

Parameter	Nonrenal Patients (n = 23)	Renal Patients (n = 23)
Media thickness (mm)		
no plaque	0.36 ± 0.035	0.38 ± 0.06
calcified plaque	0.32 ± 0.56	0.39 ± 0.04
Intima thickness (mm)		
no plaque	0.72 ± 0.53	0.72 ± 0.53
calcified plaque	1.28 ± 0.65	1.09 ± 0.25
Media area (mm^2)		
no plaque	3.7 ± 0.49	4.8 ± 1.16
calcified plaque	3.44 ± 0.53	4.23 ± 0.5
Intima area (mm^2)		
no plaque	3.25 ± 0.34	4.08 ± 2.5
calcified plaque	4.5 ± 1.8	4.2 ± 1.3
Plaque area (mm^2)		
no plaque	0.9 ± 0.4	1.5 ± 0.7
calcified plaque	0.6 ± 0.08	1.4 ± 0.4
Lumen area (mm^2)		
no plaque	3.57 ± 1.69[b]	6.9 ± 2.1
calcified plaque	2.7 ± 0.6[c]	7.2 ± 2.4
Lumen area/lumen + intima area		
no plaque	0.67 ± 0.06	0.48 ± 0.05
calcified plaque	0.6 ± 0.08	0.4 ± 0.05

[a]ANOVA control *versus* uremia $P < 0.05$.
$P < 0.05$: [b]control *versus* renal, [c]control calcified *versus* renal calcified.

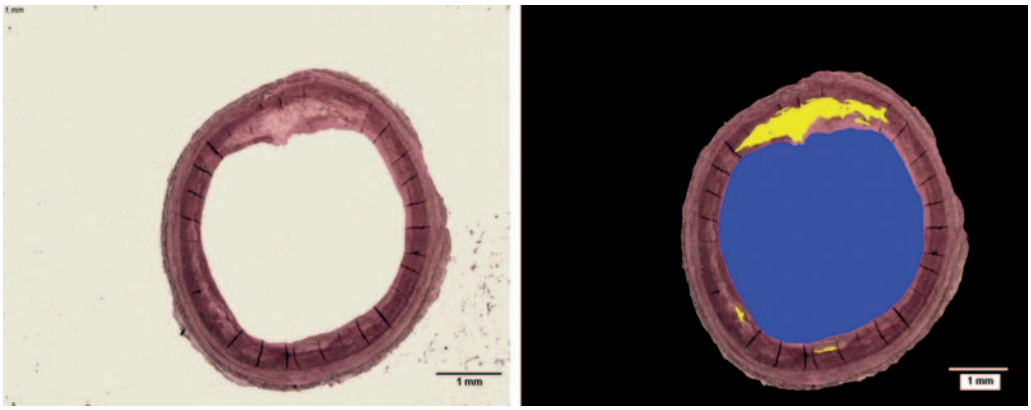

Figure 1. Quantitative evaluation of coronary arteries. Paraffin sections, planimetry, and semiquantitative image analysis.

coronary lesions were scored according to Stary (19) (Table 2). To evaluate calcification and elastic tissue in coronary arteries, we investigated Kossa and Elastica van Giesson stained paraffin sections as well (6).

Quantitative Evaluation of Coronary Arteries

Intima and media thickness and lumen and plaque area were determined on paraffin sections (×25 magnification) using planimetry and a semiquantitative image analyzing system (IBAS II; Kontron Co. Eching, Germany).

Immunohistologic Investigation

Specimens of the coronary (first 2 cm of the coronary ramus interventricularis anterior) were shock-frozen, sectioned, and examined immunohistochemically as described elsewhere (6). Two investigators who were blinded with respect to the diagnosis used a semiquantitative scoring system for the analysis (light microscopy; ×200 magnification). The mean calculated concordance for the scores between the two investigators was between $\kappa = 0.75$ and 0.82. As described previously (20), the intensity of staining was ranked on an arbitrary scale: Grade 0, no staining; grade 1, faintly positive staining; grade 2, positive staining involving up to 50% of the field of view; grade 3, positive staining involving >50%; grade 4, positive staining of all structures within the field of view.

Antibodies against proteins with a potential role in the pathogenesis of atherosclerosis were used to characterize the cellular infiltrate and

Table 4. Linear multiple regression between age, gender, and hypertension to parameters[a]

Parameter	β	95% CI	P
Media thickness			
age	0.179	0.216 to 1.00	0.003
gender	0.068	0.009 to 0.071	0.666
hypertension	0.190	0.048 to 0.189	0.234
Intima thickness			
age	0.068	0.022 to 0.014	0.658
gender	0.041	0.303 to 0.233	0.792
hypertension	0.302	0.011 to 0.693	0.057
Media area			
age	0.204	0.01 to 0.054	0.181
gender	0.084	0.609 to 0.346	0.581
hypertension	0.191	0.238 to 1.025	0.215
Intima area			
age	0.102	0.01 to 0.054	0.512
gender	0.1	0.609 to 0.346	0.524
hypertension	0.1	0.238 to 1.025	0.523
Plaque area			
age	0.308	0.002 to 0.041	0.032
gender	0.075	0.216 to 0.37	0.598
hypertension	0.274	0.014 to 0.762	0.058
Lumen area			
age	0.183	0.178 to 0.041	0.214
gender	0.035	1.437 to 1.827	0.811
hypertension	0.358	0.440 to 4.758	0.019
Lumen area/lumen + intima			
age	0.07	0.005 to 0.008	0.638
gender	0.055	0.077 to 0.111	0.715
hypertension	0.366	0.275 to −0.27	0.018
CRP intima			
age	0.097	0.025 to 0.013	0.525
gender	0.019	0.299 to 0.265	0.904
hypertension	0.262	0.058 to 0.687	0.096
calcification	0.335	0.513 to −0.097	0.005
renal insufficiency	0.596	0.336 to 0.753	0.0001
CRP media			
age	0.114	0.008 to 0.108	0.429
gender	0.127	0.275 to 0.108	0.385
hypertension	0.389	0.083 to 0.59	0.01
age	0.345	0.389 to −0.064	0.007
gender	0.499	0.166 to 0.492	0.0001
PTX3 intima			
age	0.086	0.008 to 0.016	0.538
gender	0.417	0.441 to −0.085	0.005
hypertension	0.313	0.025 to 0.496	0.031
PTX3 media			
age	0.264	0.022 to 0.002	0.86
gender	0.0	0.175 to 0.175	1
hypertension	0.182	0.094 to 0.368	0.238
Fetuin A intima			
age	0.019	0.031 to 0.035	0.9
gender	0.122	0.691 to 0.291	0.416
hypertension	0.303	1.303 to −0.004	0.049
calcification	0.498	0.481 to 1.147	0.0001
renal insufficiency	0.582	1.288 to −0.62	0.0001

Table 4. Continued

Parameter	β	95% CI	*P*
Fetuin A media			
age	0.049	0.038 to 0.027	0.738
gender	0.171	0.770 to 0.203	0.246
hypertension	0.308	1.314 to −0.027	0.041
calcification	0.3	0.099 to 0.891	0.016
renal insufficiency	0.564	1.333 to −0.538	0.0001
HIF-1α intima			
age	0.007	0.022 to 0.021	0.962
gender	0.091	0.411 to 0.218	0.541
hypertension	0.379	0.112 to 0.944	0.014
HIF-1α media			
age	0.119	0.01 to 0.025	0.422
gender	0.035	0.23 to 0.291	0.814
hypertension	0.315	0.018 to 0.706	0.04
C5b-9 intima			
age	0.001	0.041 to 0.041	0.993
gender	0.004	0.605 to 0.622	0.978
hypertension	0.107	0.54 to 1.083	0.503
C5b-9 media			
age	0.028	0.032 to 0.027	0.854
gender	0.09	0.31 to 0.558	0.567
hypertension	0.184	0.910 to 0.239	0.245
Collagen IV intima			
age	0.111	0.02 to 0.009	0.466
gender	0.195	0.36 to 0.081	0.208
hypertension	0.2	0.103 to 0.48	0.2
Collagen IV media			
age	0.109	0.018 to 0.009	0.488
gender	0.044	0.221 to 0.167	0.78
hypertension	0.116	0.163 to 0.35	0.467
TGF-β intima			
age	0.142	0.022 to 0.008	0.34
gender	0.007	0.215 to 0.225	0.963
hypertension	0.342	0.04 to 0.623	0.027
TGF-β media			
age	0.148	0.024 to 0.008	0.327
gender	0.011	0.25 to 0.233	0.943
hypertension	0.346	0.042 to 0.677	0.027
ET-1 intima			
age	0.109	0.021 to 0.01	0.468
gender	0.033	0.254 to 0.204	0.829
hypertension	0.361	0.055 to 0.657	0.022
ET-1 media			
age	0.111	0.025 to 0.012	0.482
gender	0.012	0.265 to 0.286	0.937
hypertension	0.168	0.173 to 0.552	0.298
vWF intima			
age	0.116	0.018 to 0.042	0.423
gender	0.051	0.367 to 0.52	0.729
hypertension	0.349	0.108 to 1.281	0.021
vWF media			
age	0.001	0.03 to 0.03	0.993
gender	0.034	0.492 to 0.392	0.822
hypertension	0.365	0.127 to 1.297	0.018

Table 4. Continued

Parameter	β	95% CI	P
eNOS intima			
age	0.242	0.015 to 0.001	0.095
gender	0.039	0.136 to 0.104	0.789
hypertension	0.4	0.06 to 0.378	0.008
eNOS media			
age	0.014	0.01 to 0.01	0.925
gender	0.09	0.193 to 0.105	0.556
hypertension	0.307	0 to 0.395	0.05
Glycophorin A intima			
age	0.013	0.102 to 0.093	0.93
gender	0.083	1.852 to 1.054	0.582
hypertension	0.337	0.215 to 4.059	0.03
CD68 intima			
age	0.054	0.094 to 0.134	0.728
gender	0.148	0.909 to 2.518	0.349
hypertension	0.140	1.262 to 3.246	0.379
CD68 media			
age	0.083	0.585 to 0.333	0.582
gender	0.046	7.923 to 5.835	0.761
hypertension	0.354	1.737 to 19.837	0.021
Intima calcium			
age	0.036	0.444 to 0.358	0.828
gender	0.033	5.526 to 6.727	0.844
hypertension	0.019	8.295 to 7.438	0.913
Intima phosphorus			
age	0.177	0.338 to 0.103	0.288
gender	0.057	2.774 to 3.918	0.731
hypertension	0.103	3.134 to 5.89	0.54
Media calcium			
age	0.061	0.429 to 0.289	0.695
gender	0.215	9.173 to 1.793	0.181
hypertension	0.295	0.596 to 13.485	0.072
Media phosphorus			
age	0.051	0.09 to 0.123	0.759
gender	0.118	2.185 to 1.046	0.479
hypertension	0.127	1.365 to 2.991	0.454
CRP *in situ* hybridization intima			
age	0.115	0.058 to 0.25	0.423
gender	0.184	0.246 to 1.049	0.216
hypertension	0.111	1.193 to 0.606	0.510
calcification	0.527	0.538 to 1.76	0.001
renal insufficiency	0.333	0.002 to 1.454	0.049
CRP *in situ* hybridization media			
age	0.056	0.058 to 0.25	0.704
gender	0.072	0.246 to 1.049	0.630
hypertension	0.017	1.193 to 0.606	0.919
calcification	0.517	0.538 to 1.76	0.001
renal insufficiency	0.33	0.002 to 1.454	0.056

[a]CI, confidence interval; CRP, C-reactive protein; eNOS, endothelial nitric oxide synthase; ET-1, endothelin-1; HIF-1α, hypoxia-inducible factor-1α; vWF, von Willebrand factor.

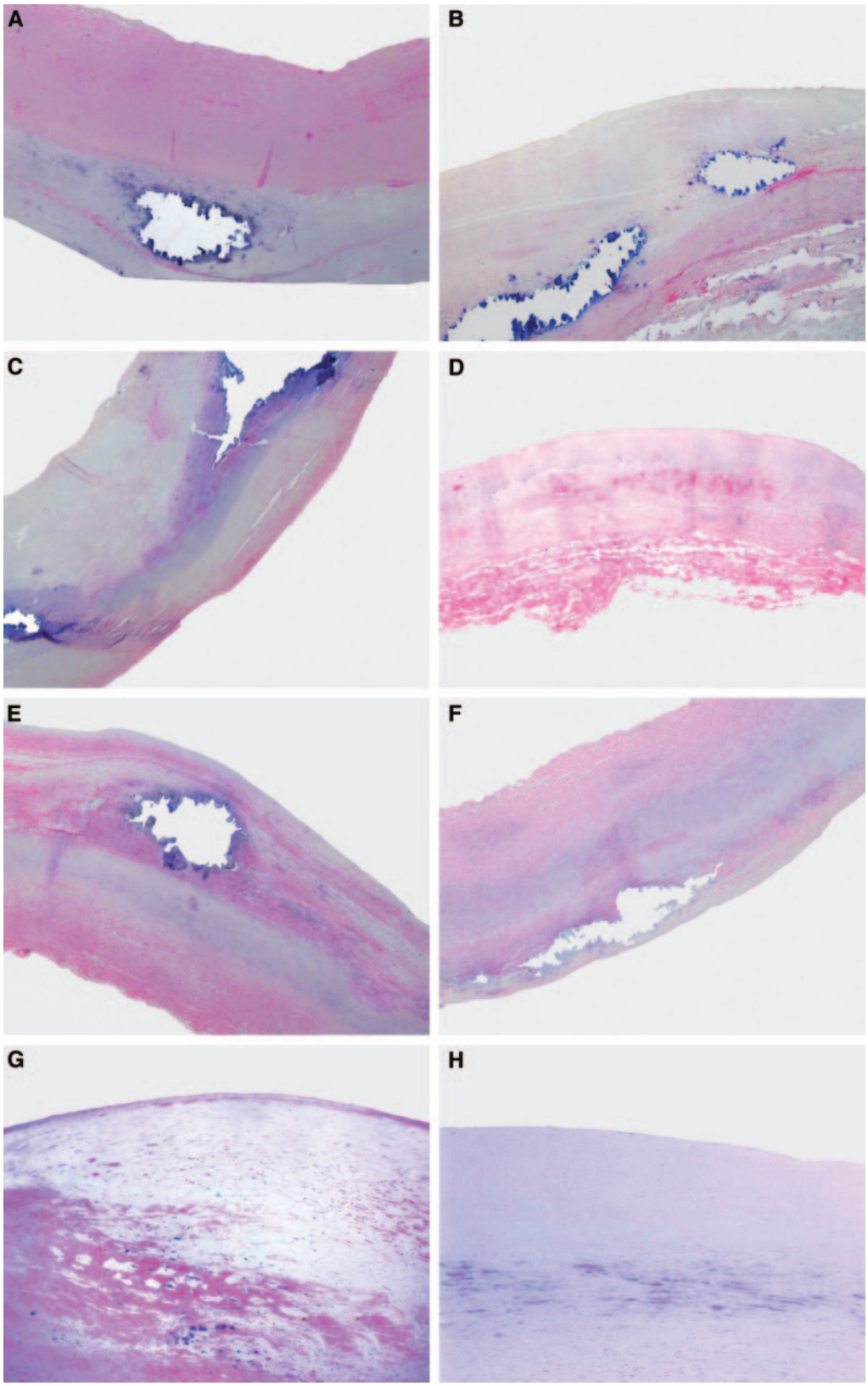

Figure 2. Staining for TGF-β, endothelin-1 (ET-1), collagen IV, glycophorin A, and hypoxia-inducible factor-1α (HIF-1α). (A and B) Markedly increased staining for TGF-β in a representative example of the coronary intima and media of a renal artery (A) as compared with the coronary wall of a nonrenal patient (B). (C and D) Increased staining for ET-1 in the intima and media, especially enhanced around calcified plaques in the coronary of a renal (C) compared with the coronary of a nonrenal patient (D). (E and F) Increased staining for collagen IV in the coronary intima and media of a renal (E) compared with a nonrenal patient (F). (G and H) Increased staining for glycophorin A in the coronary wall of a renal (G) compared with a nonrenal patient (H). Magnification, $\times$200.

Table 5. Logistic regression analysis between the variable dialysis and age, gender, and hypertension

Dialysis	β	95% CI	P
Age	0.895	0.818 to 0.981	0.017
Gender	1.667	0.505 to 5.498	0.402
Calcification	1	0.306 to 3.268	1

the presence of proteins of potential relevance for plaque formation and progression. For further details concerning the method, see the Supplemental Appendix, available online.

In Situ *Hybridization*

Nonradioactive *in situ* hybridization using endothelin-1 (ET-1) sense and antisense probes was carried out using 11 samples per patient group, as explained by Yasojima *et al.* (21).

Backscatter Imaging and X-Ray Analysis

Chemical analyses were carried out using x-ray analysis (Leo 440 Scanning electron microscope equipped with a Si-Li detector; Oxford Instruments GmbH, Wiesbaden Nordenstadt, Germany) at the Institute of Mineralogy, University of Heidelberg. Samples were enriched with uranium (U) for better discrimination of the vessel layers. Backscatter electron images of the entire thin section were obtained before chemical analysis was carried out to find the optimum orientation for the sample profiles. After selection of the position for the measurements, two parallel-line profile measurements were analyzed using an analytical scan of 30×50 μm.

Major elements in the obtained spectra were carbon (C), U, oxygen (O), chloride (Cl), Ca, and phosphorus (P). Ca and P were the elements of interest for this study. The relative proportion of the elements in the investigated area field was measured in percentage. For avoidance of sampling errors, great care was taken to check whether corresponding structures were present in the consecutive sections that were used for the respective stains.

Statistical Analyses

For each patient group, renal and nonrenal, data are means ± SD by one-way ANOVA, followed by unpaired *t* test or Mann-Whitney *U* test. The results were considered significant at $P < 0.05$. The difference between renal and nonrenal patients was tested using Duncan multiple-range test. The results were considered significant at $P < 0.05$. The two-sided 95% confidence intervals of the effects (β) age, gender, and hypertension were tested using the linear multiple regression program (SPSS 14; SPSS, Chicago, IL) and the parameter dialysis using the logistic regression analysis program.

Results

Patients

Age, gender distribution, and body weight were comparable in renal patients and control subjects (Table 1).

Stary Classification of Coronary Plaques

In a significantly higher proportion of renal patients, "calcified lesions" were the most frequent plaque category according to Stary (Table 2). Thirteen of 23 renal patients were on dialysis, and they had more calcified plaques. In contrast, in nonrenal patients, the most frequent category was "atheroma" and "fibroatheroma."

Vessel Geometry

There was no significant difference of intima or media thickness in non–plaque-bearing segments of coronaries between renal and nonrenal patients (Table 3, Figure 1). It is important to note, however, that the lumen area was significantly greater in renal patients, indicating outward remodeling. The size of the calcified plaques did not differ, although, again, the average vessel lumen even in the plaque-bearing coronary segments of renal patients was greater compared with that of control subjects. Media thickness was less at young and plaque area greater at older age (multiple linear regression analysis; Tables 4 and 5).

Immunohistological Analysis

Markers of Inflammation. Scores for C-reactive protein (CRP) were significantly higher in the noncalcified intima (and media) of coronaries from renal patients compared with that of control patients. There was only faint staining for pentraxin 3, however. A representative example of CRP staining is shown in Figure 2. As shown in Table 5, this was paralleled by significantly higher scores for CD68 (macrophages) in the noncalcified as well as calcified areas of intima and media from renal compared with nonrenal patients. Conversely, the scores for the anti-inflammatory protein fetuin A were significantly lower in the noncalcified as well as calcified intima and media of renal compared with nonrenal patients. Detailed analysis showed that in renal patients, the intact media showed virtually no staining for fetuin, whereas thin bands that showed intense staining were observed along the contours of calcified plaques.

Markers of Hypoxia and of Oxidative Stress. Scores for hypoxia-inducible factor-1α (HIF-1α) were significantly higher in the intima but not the media of coronaries of renal compared with control patients. This finding is illustrated by the representative examples in Figure 2. Scores for nitrotyrosine and endothelial nitric oxide synthase were not different between the groups.

Complement. Scores for C5b-9 were particularly high in calcified areas of the intima and media of coronaries and significantly higher in renal compared with nonrenal patients.

Matrix. Scores for collagen IV were significantly higher in the noncalcified areas of the intima (and media) of renal compared with nonrenal patients. Scores for matrix metalloproteinase 1 and 2 were low and not significantly different between the

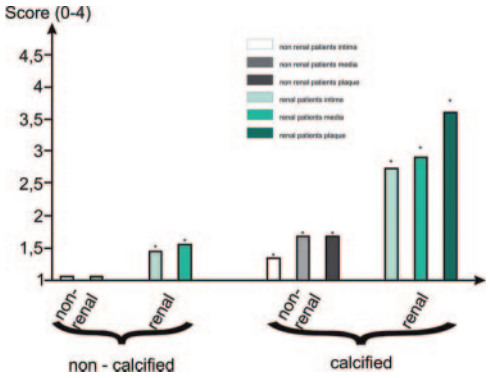

Figure 3. Expression of C-reactive protein (CRP) mRNA by nonradioactive *in situ* hybridization showed significantly marked expression in vessel walls of renal patients compared with nonrenal patients in calcified but also in noncalcified parts of arteries.

groups. Scores for TGF-β were significantly and markedly higher in the noncalcified (but not in the calcified) segments of the intima (and media) of the coronaries of renal as compared with control patients. A representative example is shown in Figure 2.

Markers of Endothelial Cell Dysfunction. Scores for ET-1 were significantly higher in the calcified segments of intima and media in coronaries of renal compared with nonrenal patients (Figure 2). Scores for von Willebrand factor (vWF) were elevated similarly in the two groups (Table 6).

Glycophorin Deposition. Deposits of erythrocyte membrane–derived material (glycophorin A) were significantly more pronounced in the intima of renal patients compared with nonrenal control subjects, pointing to past intraplaque hemorrhage.

Markers of Calcification. No differences were found for the scores of osteoprotegerin, osteopontin, sialo bone protein, and aggrecan. In contrast, the scores for osteocalcin in noncalcified and calcified segments of the intima (but not of the media; data not shown) were significantly higher in renal compared with nonrenal patients.

Staining with the following antibodies showed no significant differences between renal and nonrenal patients: CD15, CD45-R0, C4, Flt-1, vascular endothelial growth factor, HLA-DR, IL-6, leukocyte common antigen, myelo histiocyte antigen, and TNF-α.

By multiple linear regression analysis, hypertension was correlated with higher expression in the media of CRP, HIF-1α, TGF-β, vWF and more CD68-positive cells and with higher expression in the intima of TGF-β, ET-1, vWF, endothelial nitric oxide synthase, and glycophorin A. Expression of CRP was less in calcified plaques and was more pronounced in uremia. Ex-

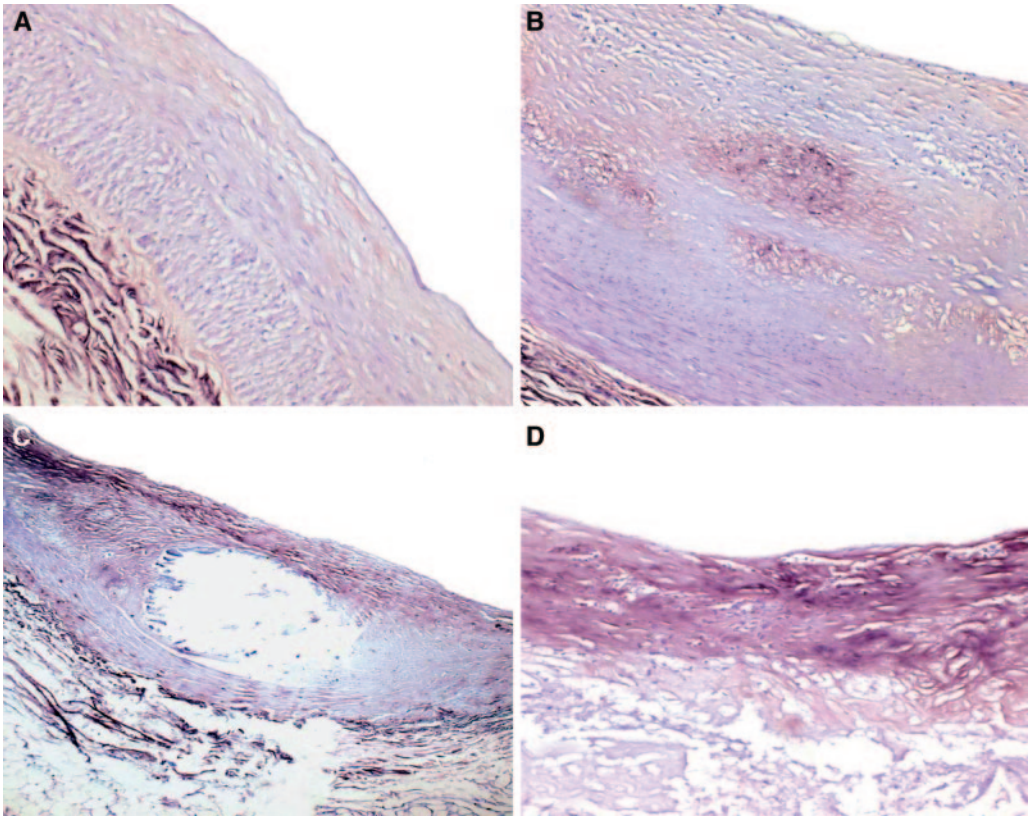

Figure 4. (A) Artery wall of a nonrenal patient with no expression for CRP mRNA. (B) Artery wall of a nonrenal patient with slightly increased CRP mRNA expression around plaque formation. (C) Artery wall of a renal patient with marked expression of CRP mRNA in the intima and around calcified plaque. (D) Artery wall of a renal patient with marked expression of CRP mRNA in the intima. Magnifications: ×100 in A, B, and D; ×40 in C.

Table 6. Immunohistologic scores of coronary arteries

Parameter	Nonrenal ($n = 11$)	Nonrenal, Calcified ($n = 13$)	Uremia ($n = 12$)	Uremia, Calcified ($n = 12$)	P (ANOVA)
CRP					
intima	0.2 ± 0.12	0.23 ± 0.16	0.53 ± 0.14[a]	0.3 ± 0.09	<0.005
media	0.27 ± 0.08	0.38 ± 0.19	0.59 ± 0.42[a]	0.38 ± 0.01	<0.05
PTX3					
intima	0.19 ± 0.09	0.43 ± 0.27	0.53 ± 0.27	0.53 ± 0.27	<0.05
media	0.33 ± 0.06	0.41 ± 0.2	0.6 ± 0.26	0.6 ± 0.26	<0.05
Fetuin A					
intima	0.95 ± 0.5	2.03 ± 0.7	0.53 ± 0.1	0.77 ± 0.5[b]	<0.001
media	1.43 ± 0.5	2.2 ± 0.3	1.1 ± 0.2	1.3 ± 0.6[b]	<0.05
HIF-1α					
intima	0.63 ± 0.5	0.38 ± 0.34	1.34 ± 0.47[a]	0.86 ± 0.38	<0.05
media	0.93 ± 0.22	0.90 ± 0.4	1.42 ± 0.61	1.28 ± 0.38	NS
C5b-9					
intima	0.31 ± 0.1	1.9 ± 0.1[c]	0.46 ± 0.3	2.53 ± 0.1[d]	<0.05
media	0.04 ± 0.05	0.2 ± 0.04[c]	0.2 ± 0.2	0.6 ± 0.1[d]	<0.05
Collagen IV					
intima	0.3 ± 0.1	0.4 ± 0.44	0.91 ± 0.3[a]	0.3 ± 0.19	<0.05
media	0.4 ± 0.15	0.4 ± 0.3	0.77 ± 0.12[a]	0.29 ± 0.21	<0.05
TGF-β					
intima	0.06 ± 0.01	0.06 ± 0.008	0.49 ± 0.26[a]	0.2 ± 0.14[b]	<0.05
media	0.06 ± 0.01	0.06 ± 0.008	0.49 ± 0.26[a]	0.2 ± 0.14[b]	<0.05
ET-1					
intima	0.03 ± 0.01[a,c]	0.59 ± 0.2	0.3 ± 0.1[d]	0.89 ± 0.2	<0.05
media	0.05 ± 0.1[a,c]	0.99 ± 0.3	0.5 ± 0.3	0.94 ± 0.2	<0.05
vWF					
intima	0.94 ± 0.6	1.42 ± 0.2	2.02 ± 0.5[a]	1.96 ± 0.9	<0.001
media	0.89 ± 0.3	1.8 ± 0.2	1.7 ± 0.4[a]	1.9 ± 0.4	<0.05
eNOS					
intima	0.08 ± 0.01[a]	0.1 ± 0.4	0.4 ± 0.02	0.22 ± 0.01	<0.05
media	0.02 ± 0.01[a,c]	0.2 ± 0.3	0.6 ± 0.3	0.2 ± 0.4	<0.05
Glycophorin A					
intima	0[a,c]	0.14 ± 0.01	0.82 ± 0.06	0.64 ± 0.03	<0.001
media	0	0	0	0	NS
CD68					
intima	0[b]	2.15 ± 1.05[c]	0.8 ± 0.2	5.75 ± 3.32[a]	<0.05
media	1.1 ± 0.2[b]	2.21 ± 0.75[c]	7.5 ± 4.8	25.08 ± 4.89[a]	<0.05

$P < 0.05$: [a]control *versus* renal, [b]control calcified *versus* renal calcified, [c]control *versus* calcified controls, [d]renal *versus* calcified renal.

pression of fetuin A was more pronounced in calcified plaques and was less in uremia.

Expression of CRP mRNA by Nonradioactive In Situ *Hybridization*

Significantly ($P < 0.05$) higher scores (0 through 4) for CRP mRNA in smooth muscle–like cells and macrophages of intima (0.44 ± 0.07) and in the media (0.55 ± 0.07) were found in renal compared with nonrenal patients (intima 0.05 ± 0.01; media 0.05 ± 0.1). In calcified vessels, expression of CRP mRNA also was significantly higher, especially around the plaques, in renal (intima 1.73 ± 0.9; media 1.9 ± 1; around plaque 2.64 ± 1.2) compared with nonrenal patients (intima 0.33 ± 0.05; media 0.67 ± 0.7; around plaques 0.667 ± 0.5; Figures 3 and 4).

By multiple linear regression analysis, the scores for CRP mRNA were higher in calcified plaques. In uremia, the scores for CRP mRNA were higher in the intima but not in the media.

Backscatter Images and X-Ray

The x-ray analysis showed a significantly higher relative proportion (% area) of Ca and P in the media (area as well as a higher content pro unit volume) of calcified coronaries of renal patients compared with nonrenal patients. The proportion of

Table 7. Calcium and phosphorous in calcified and non-calcified areas of the coronary (% of area)

Parameter	Intima		Media	
	Calcium	Phosphorous	Calcium	Phosphorous
Nonrenal ($n = 9$)	0.76 ± 0.63	0.56 ± 0.53	0.37 ± 0.37	0.15 ± 0.07
Nonrenal, calcified ($n = 11$)	17.27 ± 11.83[a]	8.27 ± 5.1[a]	6.2 ± 0.51[a]	3.27 ± 0.17[a]
Renal ($n = 12$)	0.91 ± 0.44	0.61 ± 0.46	0.75 ± 0.64	0.53 ± 0.46
Renal, calcified ($n = 11$)	19.1 ± 11.89[b]	9.27 ± 5.55[b]	15.02 ± 6.45[b,c]	4.46 ± 0.28[b]
P (ANOVA)	<0.05	<0.05	<0.05	<0.05

$P < 0.05$: [a]control *versus* calcified controls, [b]renal *versus* calcified renal, [c]control calcified *versus* renal calcified.

calcified area and the Ca content of the intima were not different between renal patients and nonrenal patients (Table 7; Figures 5 and 6).

Discussion

The salient feature of our study is the somewhat surprising documentation that the proportion of the coronary artery that was occupied by plaques was not higher in patients with advanced renal disease compared with nonrenal patients with coronary heart disease, but the plaques were strikingly different with respect to morphology, calcification, and inflammatory character. X-ray analysis showed that compared with nonrenal patients, the calcified portions of the intima and media of renal patients contained more Ca (relative to background Ca content of noncalcified intima or media). The proportion of the media but not the intima that was occupied by calcified plaques was significantly higher in renal patients. Such heavy calcification of the coronary media is of note, because in a previous study of this laboratory (6), no calcification of the media was reported. In this study, however, only a short segment of the most proximal part of the coronary was studied, whereas our analysis concerned more distal parts of the coronary as well, an important point in view of the distance from the ostium as an independent determinant of coronary plaque composition (22). The Ca deposits were highly enriched in P. X-ray analysis documented that the deposits were composed of hydroxyapatite. The hypothesis that renal patients have a greater propensity for calcification is supported by the finding of higher scores for osteocalcin and lower scores for the calcification inhibitor fetuin A in and around calcified plaques.

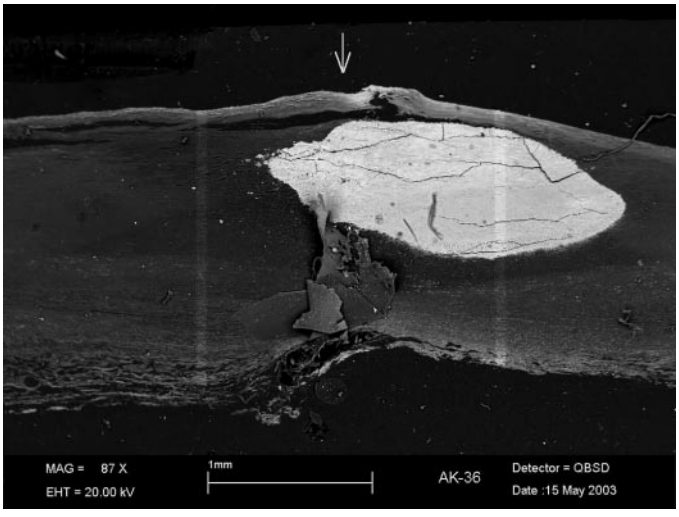

Figure 5. Backscatter imaging of a scan through the coronary vessel wall of a renal patient. Note the calcified plaque's encroaching on the intima and media.

The inflammatory character of the plaques was documented by the higher scores for macrophage infiltration and for CRP by *in situ* hybridization. The finding indicates deposition of circulating CRP; staining for the locally produced proinflammatory molecule (23) pentraxin 3 was only faint. The scores for fetuin A were low, except immediately around plaques; fetuin not only is an inhibitor of calcification (24) but also has anti-inflammatory properties (25). The serum fetuin concentration of dialysis patients is inversely related to that of CRP (26). Moe *et al.* (17) found increased staining for fetuin A in epigastric arteries of dialysis patients. It is uncertain whether this reflects differences between vessels or different patterns of calcification. In contrast to experimental studies (27) no evidence of increased nitrotyrosine (as a marker of oxidative stress) was found.

A potential role of activated complement in the atherogenesis of renal patients is supported by the documentation of more intense C5b-9 deposits in coronaries of renal patients. This increase was restricted to calcified plaques, however, so we cannot exclude passive absorption to existing plaques as an explanation for this observation (24). Evidence of endothelial cell dysfunction as a presumed early step in atherogenesis was provided by the finding of significantly increased ET-1 in intima and media as well as by more marked staining for vWF.

Notable by its absence was evidence of increased neoangiogenesis, which recently has been thought to trigger plaque rupture (28). Increased staining for neither vascular endothelial growth factor nor the flt-1 receptor was noted. Deposition of glycophorin A, an erythrocyte-specific marker, is considered as evidence of past intraplaque hemorrhage that resulted from neoangiogenesis (29,30). This marker was present more frequently and stained with higher intensity in the intima of renal compared with nonrenal patients, pointing to greater instability of plaques in renal patients. The discrepancy between no evidence of neoangiogenesis in the media and the presence of more frequent and intense hemorrhage in the intima is difficult to explain. Intimal bleeding might be the result of a hemorrhagic diathesis.

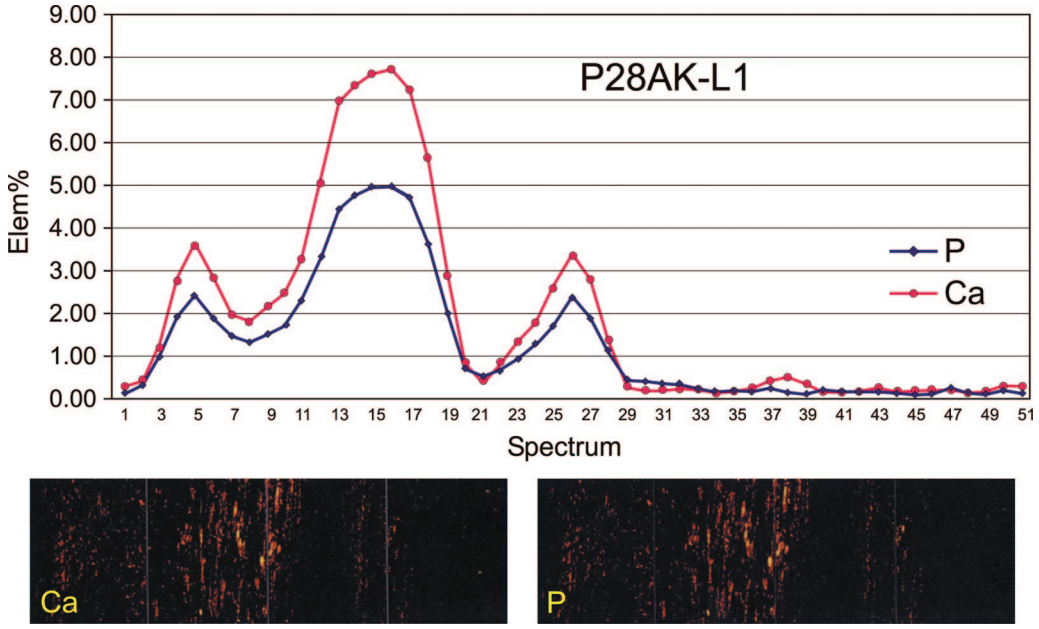

Figure 6. X-ray analysis documenting the relative distribution of the elements calcium and phosphorous in the form of hydroxyapatite in a plaque of the coronary intima and media of a renal patient.

Confirming previous results (6), we found larger coronary lumina in renal patients compared with control patients, suggesting the presence of outward coronary remodeling, similar to what is found in the carotid artery (31) even in early renal failure. The risk for coronary ischemia is thought to be lower in coronaries with outward as opposed to concentric remodeling, because vessels with larger lumen should accommodate higher coronary flow rates (6) if the driving force is unchanged.

The findings concerning the topography of coronary calcifications may be useful for the interpretation of coronary calcification by EBCT (32) or multislice CT in renal patients The methods do not distinguish between calcification of the intima and the media. The implications of medial calcification (Moenckeberg type) are different from those of calcification of intimal plaques (6,33). At any rate, the calcification score predicts coronary death (34,35).

In the past, it commonly was thought that calcified plaques were quiescent (36). This idea presumably should be revised, because Schmermund *et al.* (37) found calcified deposits in most patients who had coronary thrombosis as a result of plaque rupture. Huang *et al.* (38) found that calcification did not have an impact on biomechanical stress in human atherosclerotic lesions, because removal of Ca changed stress insignificantly. In contrast, a study on compressive stress relaxation found marked differences of stress relaxation between calcified and noncalcified plaques (38). It is probable that calcified plaques increase the risk for plaque rupture by imposing abnormal stress on the shoulder (*i.e.*, the transition between calcified plaque and intact endothelium).

Recent findings (12,25,39) suggest that vascular calcification to some extent replicates bone calcification, as indicated by the expression of osteoblast-specific proteins that are involved in osteogenesis. With immunohistochemistry, we analyzed staining for proteins that are involved in bone mineralization. Staining for osteopontin or osteoprotegerin expression was similar in renal and nonrenal patients. The same was true for osteocalcin, a vitamin K–dependent inhibitor of Ca salt precipitation (40,41), and for aggrecan, a protein that is expressed during the transformation of cartilage into bone (42).

It has been recognized increasingly that uremia is a state of microinflammation (43,44). Recent evidence suggests that elevated horse CRP concentrations predict cardiovascular death (45). This is the case in renal failure as well (14).

Our finding of increased deposition of CRP and more marked infiltration by CD68-positive macrophages in renal compared with nonrenal patients is consistent with a more pronounced inflammatory character of coronary lesions in renal patients. The trigger for such local inflammatory reactions is unknown, but in view of our previously advanced hypothesis (1), it is of note that the terminal component of complement system (*i.e.*, C5b-9) was found in higher concentrations in and particularly around coronary plaques of renal patients. Conjoint deposition of complement and CRP also has been described in nonrenal patients with coronary heart disease (46). Our observation suggests that this process is amplified in patients with chronic renal disease and might contribute to enhanced calcification. HIF-1α is a hypoxia sensor. The increased staining for HIF-1α in the intima and media of coronary arteries of renal patients is of potential interest. Whether the increase of HIF-1α is simply the consequence of anemia or plays a more direct role in the accelerated atherogenesis of chronic renal disease remains undecided.

Conclusion

In autopsy samples of renal patients, a group with known excessive cardiovascular risk (47), marked differences of the

characteristics of coronary plaques were found between renal and nonrenal patients with coronary heart disease. The salient differences concern intensity and topography of plaque calcification, more pronounced evidence of microinflammation and complement deposition, more intense vessel wall hemorrhage despite no evidence of increased neoangiogenesis, and diminished deposition of the anti-inflammatory agent and calcification inhibitor fetuin A.

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Disclosures

None.

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