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Arteriolar Lesions in Renal Transplant Biopsies

Prevalence, Progression, and Clinical Significance

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Arteriolar hyalinosis in kidney transplants is considered the histopathologic hallmark of chronic calcineurin inhibitor (CNI) toxicity. However, the lesion is not specific. We assessed prevalence, progression, and clinical significance of arteriolar lesions in 1239 renal transplant sequential protocol biopsy samples and 408 biopsy for cause samples in 526 patients. Associations between arteriolar lesions and presumed risk factors, concomitant histopathologic lesions, demographic factors, and graft function were evaluated. The frequency of arteriolar lesions was stable during the first 2 years after transplantation, and increased thereafter (14.8% at 6 months versus 48.6% at >2 years; $P < 0.0001$). We were unable to find associations with diabetes, hypertension, or CNI therapy. However, patients with early arteriolar lesions received grafts from older donors (mean ± SD age, 54.4 ± 13.4 years versus 43.1 ± 16.6 years; $P < 0.0001$), and had inferior graft function (estimated glomerular filtration rate 55 ± 21 mL/min versus 63 ± 24 mL/min at 6 weeks, 53 ± 19 mL/min versus 60 ± 23 mL/min at 1 year, and 49 ± 19 mL/min versus 59 ± 22 mL/min at 2 years; $P < 0.05$). Evaluation of late biopsy samples from patients not receiving CNI therapy revealed a high prevalence of AH without clear-cut identifiable underlying cause. Reproducibility of arteriolar lesions was at best moderate ($\kappa \leq 0.62$). Sampling error in sequential biopsy samples was frequent. In conclusion, in samples from sequential protocol biopsies and biopsies for cause in individual patients, arteriolar lesions in renal transplants not only increase over time without being specific for CNI toxicity but are affected by sampling error and limited reproducibility. ***(Am J Pathol 2012, 180:1852–1862; DOI: 10.1016/j.ajpath.2012.01.038)***

Evaluation of renal allograft biopsy samples includes acute as well as chronic alterations in glomeruli, tubulointerstitium, and vessels. One incompletely understood lesion is arteriolar hyalinosis (AH). Nevertheless, it is frequently considered the consequence of chronic calcineurin inhibitor (CNI) toxicity, and is regarded as an irreversible lesion that will eventually affect nearly every renal transplant with increasing duration of CNI therapy. A few other conditions also related to arteriolar lesions include diabetes, hypertension, and aging. The nodular pattern of AH, sometimes described as like a pearl necklace, in the media is believed to represent a more specific sequela of chronic CNI toxicity.[1–7] A possible (reversible) precursor of the nodular pattern is vacuolization of arteriolar smooth muscle cells (VSM), which may eventually be replaced by hyaline deposits.[7]

Despite the suggested causes, the specificity and reproducibility of AH have been questioned.[8,9] New grading systems have been proposed to improve reproducibility.[10,11] However, systematic analysis of reproducibility and specificity of the various patterns of arteriolar lesions in short- and long-term sequential biopsy samples is lacking. The objective of the present retrospective study in a large cohort of samples from sequential protocol biopsies (PB) and from biopsies for

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Table 1. Scoring of AH

Description	Histologic findings
Extension of AH	
No AH	No iAH or nAH in the biopsy sample
Focal AH	≤50% of arterioles in the biopsy sample affected
Diffuse AH	>50% of arterioles in the biopsy sample affected
Severity of iAH	
No iAH	No iAH in the biopsy sample
Segmental (mild) iAH	≤50% of arteriolar cross-section involved
Circumferential (severe) iAH	>50% of arteriolar cross-section involved
Severity of nAH	
No nAH	No nAH in the biopsy sample
Segmental (mild) nAH	≤50% of arteriolar cross-section involved
Circumferential (severe) nAH	>50% of arteriolar cross-section involved

AH, arteriolar hyalinosis; iAH, intimal pattern AH; nAH, nodular pattern AH.

cause (BFC) was to explore the evolution of arteriolar lesions over time. In an attempt to identify potential underlying causes of AH and VSM, we analyzed the associations between arteriolar lesions and clinical and histopathologic variables concurrently present in the biopsy samples.

Materials and Methods

Patient Biopsy Samples

The study was approved the by the Institutional Ethics Review Board of Hannover Medical School (Hannover, Germany). A total of 1599 biopsy samples from 490 patients who underwent transplantation at Hannover Medical School between 2000 and 2006 were re-evaluated for arteriolar lesions (Table 1). Of these biopsies, 1239 were PB serially performed at 6 weeks (PB1) after transplantation in 380 patients, at 3 months (PB2) in 420 patients, and at 6 months (PB3) in 439 patients. In the same group of patients, 372 BFC were performed before 6 months after transplantation in 240 patients, at 6 to 12 months in 38 patients, at 1 to 2 years in 59 patients, and after >2 years in 35 patients. In addition, we examined arteriolar lesions in late allograft BFC samples from 25 patients receiving CNI therapy at a mean of 49.8 months (range, 20.7 to 235 months) after transplantation and 11 patients not receiving CNI therapy at 221.1 months (range, 29.1 to 404.3 months) after transplantation. The 25 patients receiving CNI-based immunosuppression therapy underwent transplantation between 1987 and 2007, and the 11 patients in the CNI-free group underwent transplantation between 1977 and 2006. They had received azathioprine, mycophenolate mofetil, or sirolimus or everolimus and steroids as basic immunosuppression therapy. Four out of eleven patients received cyclosporine therapy for a short period after transplantation: one for <4 weeks (biopsy was performed 13 years later), one for 5 months (biopsy was performed 11 years later), and two for 3 months (biopsy was performed 6 and 14 years later, respectively).

Histopathology

Biopsy samples were processed routinely for paraffin embedding, and serial sections were stained with PAS and H&E. Histopathologic lesions were graded according to the Banff classification.[11,12] AH was graded quantitatively and qualitatively, and was further divided into a nodular pattern (nAH) with hyaline material between arteriolar smooth muscle cells and an intimal pattern (iAH) with hyaline material confined to the intima (Table 1). For scoring the various qualities of AH, the most severe lesion in a biopsy sample was graded. Presence of VSM was recorded without further grading.

Patient Characteristics

Two hundred twenty-one patients (42%) were women. Their mean ± SD age at transplantation was 48.4 ± 13.8 years. Four hundred thirty-three patients (88.4%) received their first transplant. Simultaneous pancreas-kidney transplantation was performed in 41 patients (7.8%). Grafts were from blood-related living donors in 37 cases (7%), and from non–blood-related living donors in 36 cases (6.8%). Induction therapy was via interleukin-2 receptor antibodies in 410 patients (77.9%), and antithymocyte globulin in 32 (6.1%). Standard maintenance immunosuppression therapy consisted of cyclosporine A and prednisolone in 178 patients (33.8%); 199 patients (37.8%) received triple immunosuppression with additional mycophenolate mofetil. In 149 patients (28.3%), alternative regimens including sirolimus or everolimus, or azathioprine and tacrolimus were used. Overall, maintenance immunosuppression included cyclosporine A in 425 patients (80.8%), tacrolimus in 59 (11.2%), mycophenolate mofetil in 279 (53.1%), sirolimus or everolimus in 27 (5.1%), azathioprine in 11 (2.1%), and prednisolone in 504 (95.8%). Subclinical and clinical borderline, acute tubulointerstitial, and vascular T-cell–mediated rejection episodes were treated using standard protocols as previously described.[13]

Statistical Analysis

From the total sample, κ values for intraobserver (V.S.-V.S.) reproducibility of findings were estimated using a representative selection of 150 biopsy samples, and for interobserver (V.S.-V.B.) reproducibility using 168 biopsy samples. Values are given as mean ± SD. The percentages of nominal data were tested using Fisher's exact test for two groups, and the χ^2 test for more than two groups. Continuous nonparametric data were tested using the Kruskal-Wallis and Mann-Whitney *U* tests. Multivariate logistic regression analysis was used to examine factors that significantly influence the primary outcome variable (patients with and without arteriolar lesions). The multivariate logistic regression model was built using variables with a value of $P < 0.05$ at univariate analyses. Thereafter, the multivariate model was narrowed using

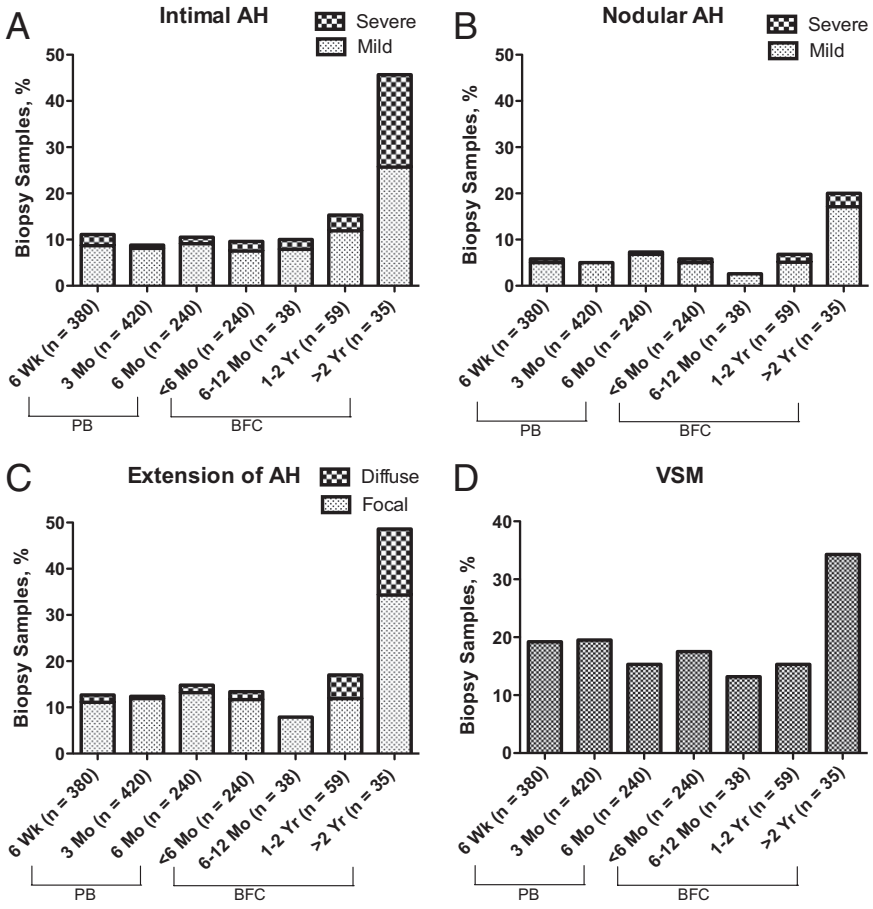

Figure 1. Prevalence, progression, and severity of arteriolar lesions as measured in intimal AH (**A**), nodular AH (**B**), extension of AH (**C**), and VSM (**D**). Prevalence of arteriolar lesions is stable during the first 2 years after transplantation, and increases in BFC later. Most lesions are mild and focal rather than severe. Numbers in parentheses represent the total number of biopsy samples at the respective time.

backward selection to determine significant explanatory variables. The two-sided type I error was set to 5%. The PASW Statistics 18 software system (SPSS, Inc, Chicago, IL) was used to perform calculations.

Results

Prevalence and Progression of Arteriolar Lesions in PB and BFC Samples

The frequency of iAH and nAH was stable in PB and BFC samples during the first 2 years after transplantation, and severity was mostly mild (Figure 1, A and B). Frequency and severity of both iAH and nAH increased in BFC at >2 years after transplantation (Figure 1, A and B). In the first 2 years after transplantation, <15% of the biopsy samples showed AH, primarily as a focal lesion (Figure 1C). VSM was more frequent than AH, also steady during the first 2 years (<20%), and increased to 34% in biopsy samples after the first 2 years (Figure 1D).

VMS has been considered a precursor lesion of nAH. However, neither nAH nor iAH were more frequent in PB2 or PB3 when VSM was present in a previous PB. Twenty-nine patients had iAH and/or nAH in a BFC at >2 years after transplantation; however, VSM in previous PB or BFC samples was observed in only five of these patients, which argues against an association between VSM and subsequent development of AH.

Reproducibility of Arteriolar Lesions

To assess whether AH lesions persist in sequential biopsy samples, we analyzed data only for patients with three available PB samples. Of 66 patients with iAH in one PB sample, 12 had the same finding in a second PB sample (9 in PB1 and PB3, 1 in PB1 and PB2, and 2 in PB2 and PB3); however, none had iAH in all three biopsy samples. Of 53 patients with nAH in one PB sample, only 3 had the same finding in another PB sample (1 each in PB1 and PB3, PB1 and PB2, and PB2 and PB3); only 1 patient had nAH in all three sequential biopsy samples. Thus, neither iAH nor nAH are consistently found in sequential biopsy samples from individual patients.

Intraobserver reproducibility was moderate (κ = 0.43 and 0.51) for presence of iAH and extension of AH (focal versus diffuse), and good (κ = 0.62 and 0.61) for presence of nAH and VSM. The overall reproducibility of AH (iAH and/or nAH) was moderate (κ = 0.53). Interobserver reproducibility was moderate (κ = 0.45) for presence of iAH, fair (κ = 0.26 and 0.34) for presence of nAH and VSM, and poor for determination of AH extension (focal versus diffuse) (κ = 0.197).

According to Banff criteria (at least seven glomeruli and one artery), 65.7% of all PB samples and 60.8% of all BFC samples were adequate. We assessed the prevalence of arteriolar lesions in relation to the adequacy of biopsy samples. In PB performed at 6 weeks and at 3

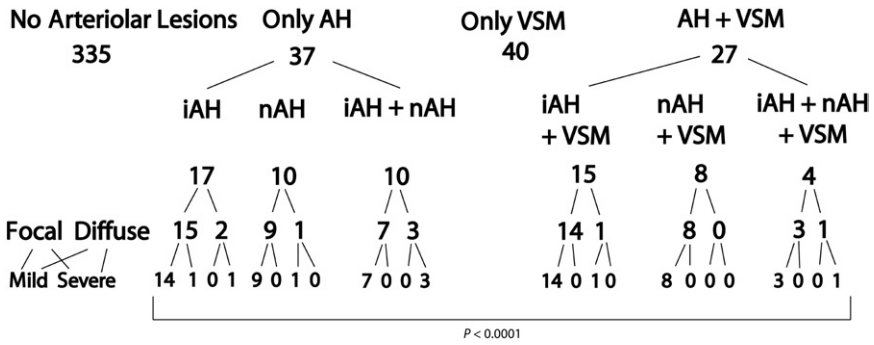

Figure 2. Combination of various arteriolar lesions in PB samples at 6 months ($n = 439$). The 6-month PB is given as an example to illustrate the multiplicity of possible combinations of various qualities of arteriolar lesions (iAH, nAH, and VSM), extension (focal or diffuse), and severity (severe or mild). The *P* value illustrates the significant association (χ^2 test) between extension and severity of AH. To simplify further analyses and to retain sufficient numbers per group, biopsy samples were merged into four superordinate histopathologic categories: no AH, no VSM; only VSM; only AH (nAH and/or iAH, severe or mild); and AH plus VSM.

months, no difference in prevalence of arteriolar lesions was observed between adequate and inadequate biopsy samples. Only in PB performed at 6 months was the percentage of inadequate biopsy samples significantly greater in samples without arteriolar lesions, compared with those with VSM ($P = 0.021$) and those with iAH and/or nAH ($P = 0.044$), which suggests that sample adequacy might marginally influence detection and, thus, prevalence of arteriolar lesions.

Arteriolar Lesions Are Associated with Each Other

In PB samples, VSM, iAH, and nAH correlated with each other: range, 0.121 to 0.380 for iAH and nAH; 0.173 to 0.218 for nAH and VSM; and 0.212 to 0.248 for iAH and VSM (all $P < 0.05$).

Quantitative and qualitative scoring of iAH, nAH, and VSM resulted in 24 possible combinations of type, severity, and extension of lesions within a biopsy sample (Figure 2, with PB at 6 months as an example). Extension (focal or diffuse) and severity (mild or severe) of AH were associated in PB samples at 6 weeks ($P = 0.021$), 6 months ($P < 0.0001$; Figure 2), and all PB samples considered together ($P < 0.0001$), and also in all BFC samples considered together ($P < 0.0001$) and all PB and BFC samples considered together ($P < 0.0001$). However, the small number of severe diffuse lesions precluded separate analysis of this group. To simplify further analyses and to retain sufficient numbers in each group, we merged the biopsy samples at each time point into four broader histopathologic categories: i) no AH and no VSM; ii) only VSM; iii) only AH (nAH and/or iAH, severe or mild); and iv) AH and VSM. All subsequent results concerning biopsy samples obtained >6 months after transplantation are available online (see Supplemental Tables S1–S3 at *http://ajp.amjpathol.org*).

Arteriolar Lesions are not Significantly Associated with CNI Therapy, Diabetes, or Hypertension

For each patient, mean trough levels of cyclosporine A and tacrolimus were calculated from the available measurements at definite intervals after transplantation (<2 weeks, 2 to 6 weeks, and between three PB). Comparisons of trough levels from each interval were made between patients with and without arteriolar lesions in the subsequent PB sample (Figure 3). Lower trough levels of cyclosporine A were observed in biopsy samples with only VSM compared with those without arteriolar lesions at <2 weeks after transplantation: mean ± SD,175 ± 78 ng/mL versus 205 ± 71 ng/mL ($P < 0.05$). This was the only significant difference in relation to CNI therapy. At >6 months after transplantation, no systematically documented CNI trough levels were available. For comparison of late BFC (>2 years) with and without CNI therapy, see *Prevalence of Arteriolar Lesions in Late BFC With and Without CNI Therapy*.

Insofar as arteriolar lesions and diabetes mellitus after transplantation, the only significant difference was observed in PB3. Patients with only VSM in the biopsy sample more often had diabetes mellitus type 2 at biopsy when compared with patients without arteriolar lesions (Table 2, and Table ST1).

Systolic and diastolic arterial blood pressure levels in patients with arteriolar lesions compared with patients without revealed no significant differences in PB (see Supplemental Figure S1 at *http://ajp.amjpathol.org*) and BFC (see Supplemental Table S2 at *http://ajp.amjpathol.org*). The number of anti-hypertensive drugs taken by each patient was not different between the four histopathologic groups of PB and BFC samples (not shown).

Prevalence of Arteriolar Lesions in Late BFC with and without CNI Therapy

To identify potential long-term effects of CNI on the development of arteriolar lesions, we additionally analyzed late BFC (>2 years) in patients with and without CNI therapy (see *Materials and Methods*). A total of 71 late BFC were examined, which included 35 patients from the PB collective with late BFC. Biopsy samples from patients who did not receive CNI therapy exhibited a high prevalence of both iAH and nAH, with a substantial percentage of severe and diffuse AH findings, whereas VSM was observed less frequently (Figure 4, A and B). Prevalence of iAH, nAH, and VSM was not significantly different between biopsy samples from patients with or without CNI therapy. Time from transplantation to biopsy was significantly longer in patients who did not receive CNI therapy: mean ± SD, 221.2 ± 118.9 months (range, 29.1 to 404.3 months) versus 49.8 ± 44.8 months (range, 20.7 to 235.9 months) ($P < 0.000$).

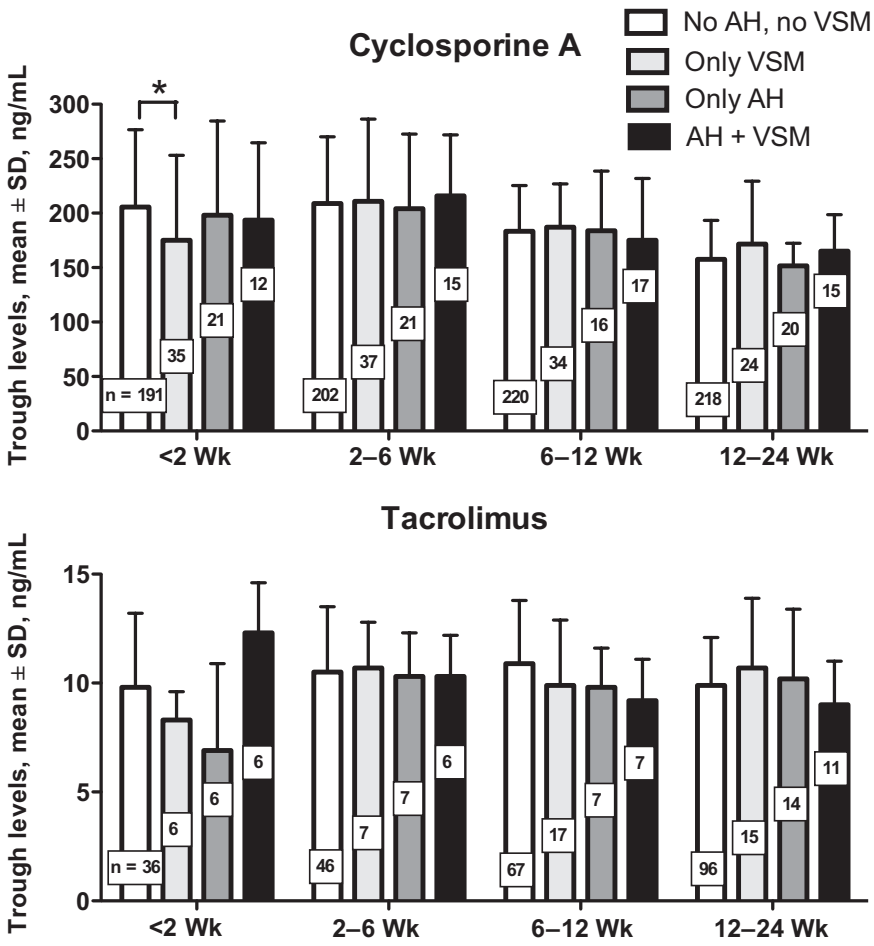

Figure 3. Cyclosporine A and tacrolimus trough levels. Trough levels of cyclosporine A and tacrolimus were determined for each patient at definite intervals after transplantation (<2 weeks and between PB). Each single measurement for each patient during the respective period determined the patient's mean trough level. We compared trough levels between patients with and without different arteriolar lesions in the subsequent PB. *$P < 0.05$, Mann-Whitney U-test versus no AH, no VSM.

Association of Arteriolar Lesions with Other Histopathologic Findings

Correlation of arteriolar lesions with other concomitant histopathologic findings in PB and BFC samples at <6 months revealed significant associations with arteriosclerosis (fibrous intimal thickening of arcuate and interlobular arteries) and glomerular mesangial matrix increase (Banff score ≥1) in PB1, PB3, and BFC samples at <6 months (Table 3). The same trend was observed in BFC samples at >2 years, but did not reach statistical significance (Table ST3). The numbers in the various AH lesion groups in BFC samples at >6 months after transplantation were small but revealed that transplant glomerulopathy (Banff index cg ≥1) was more

Table 2. Arteriolar Lesions and Diabetes in PB and BFC at ≤6 Months

	PB at 6 weeks (n = 375)				PB at 3 months (n = 411)			
Variable	No AH, No VSM (n = 274)	Only VSM (n = 50)	Only AH (n = 29)	AH + VSM (n = 22)	No AH, No VSM (n = 305)	Only VSM (n = 55)	Only AH (n = 24)	AH + VSM (n = 27)
Diabetes type 1	7 (2.6)	0	0	0	6 (2.0)	2 (3.6)	0	0
Diabetes type 2	29 (10.6)	6 (12)	1 (3.4)	2 (9.1)	38 (12.5)	9 (16.4)	0	5 (18.5)
	PB at 6 months (n = 428)				BFC at ≤6 months (n = 221)			
	No AH, No VSM (n = 326)	Only VSM (n = 38)	Only AH (n = 37)	AH + VSM (n = 27)	No AH, No VSM (n = 167)	Only VSM (n = 26)	Only AH (n = 16)	AH + VSM (n = 12)
Diabetes type 1	8 (2.5)	0	0	0	6 (3.6)	0	0	0
Diabetes type 2	45 (13.8)	11 (28.9)*	5 (13.5)	4 (14.8)	20 (12.0)	7 (26.9)	0	0

Data are expressed as No. of patients (%).
χ^2 test for each group vs No AH, no VSM.
*$P < 0.05$.

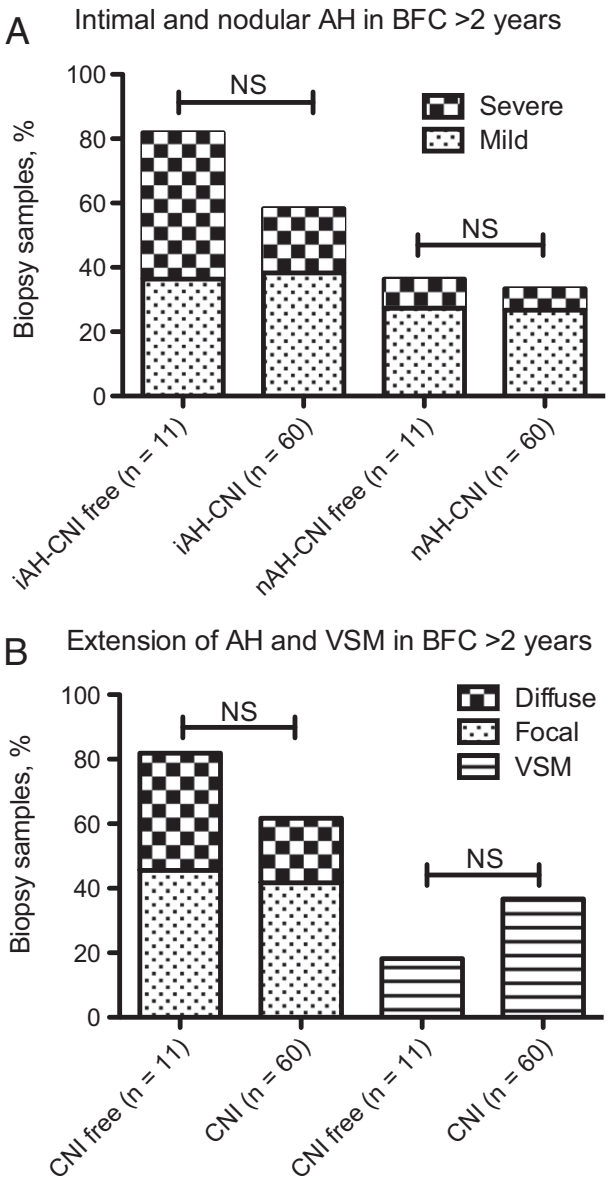

Figure 4. Prevalence of arteriolar lesions in late BFC samples with and without CNI therapy. Comparison between patients with and without CNI therapy (χ^2 test) in terms of iAH and nAH (**A**) and VSM (**B**). Total numbers of biopsy samples are given in parentheses.

frequent in biopsy samples with arteriolar lesions in BFC at 6 to 12 months (Table ST3).

Associations of Arteriolar Lesions with Donor or Recipient Factors

To assess relationships between arteriolar lesions and time-invariant recipient- or donor-dependent factors, data for patients who underwent sequential PB and BFC were analyzed, as grouped into the four histopathologic categories described. Patients with an arteriolar lesion in any biopsy sample qualified for inclusion in the relevant group. Data for patients who underwent only PB were analyzed separately from data for those who underwent PB plus BFC.

When considering all patients together, donor age was significantly higher in patients with arteriolar lesions compared with patients without such lesions (Figure 5A). This association between donor age and arteriolar lesions was also observed in separate analysis of patients who underwent only PB (Figure 5B) and patients who underwent both PB and BFC (Figure 5C).

Patients with arteriolar lesions less frequently underwent simultaneous kidney-pancreas transplantation compared with patients without arteriolar lesions: 2 of 93 (2.2%) versus 22 of 220 (10%) for all patients, and none of 39 versus 13 of 131 (13%) for patients who underwent only PB (both, $P < 0.05$). In line with this, patients with arteriolar lesions less frequently had diabetes mellitus type 1 before transplantation: 2 of 93 (2.2%) versus 26 of 215 (12.1%) for all patients, and none of 39 versus 17 of 127 (13.4%) for patients who underwent only PB (both, $P < 0.05$).

Patients who underwent additional BFC and with arteriolar lesions were older at transplantation (mean ± SD, 49.8 ± 14.2 years versus 45.6 ± 12.1 years; $P < 0.05$), and more often received a graft from a male donor: 58 of 91 (63.7%) versus 101 of 218 (46.3%) for all patients, and 36 of 53 (67.9%) versus 39 of 88 (44.3%) for patients with PB plus BFC (both, $P < 0.01$). The donor serum creatinine concentration was higher in the group with additional BFC (94 ± 40.9 μmol/L [$n = 39$] versus 71.7 ± 23.8 μmol/L [$n = 54$]; $P < 0.01$). No significant associations were observed for other examined variables including diabetes mellitus type 2 before transplantation, living or deceased donor, delayed graft function, and cold ischemia time.

Multivariate Analysis of Factors Associated with Arteriolar Lesions

Factors included in the multivariate analyses were acute tubular injury, arteriosclerosis, mesangial matrix increase in any of the PB or BFC samples, and donor age and sex. Donor serum creatinine concentration was not included because missing data would have significantly reduced the number of observations. Patients were grouped into those with any arteriolar lesion (AH or VSM) and those without these lesions. The first analysis included all patients with their findings in PB and BFC samples within the first 6 months after transplantation. Factors left over in the final model were donor age, donor male sex, and presence of arteriosclerosis or mesangial matrix increase, with a model sensitivity of 75% and specificity of 58%, and an area under the curve value of 71% in the receiver operating characteristic curve (Table 4).

Separate multivariate analysis of patients who underwent PB only and those who underwent PB plus BFC revealed similar trends for the identified factors. Donor age and arteriosclerosis were significant factors in both groups. Donor male sex was an additional significant factor in patients who underwent PB only, whereas mesangial matrix increase was included as a factor in patients who underwent PB and additional BFC (not shown).

Arteriolar Lesions and Graft Function

Renal function was assessed using the calculated glomerular filtration rate via the Cockroft-Gault formula.[14] At

Table 3. Arteriolar Lesions and Other Histopathologic Findings in PB and BFC at ≤6 Months

	PB at 6 weeks (n = 378)				PB 3 months (n = 418)			
Variable	No AH, No VSM (n = 277)	Only VSM (n = 50)	Only AH (n = 29)	AH + VSM (n = 22)	No AH, No VSM (n = 311)	Only VSM (n = 55)	Only AH (n = 25)	AH + VSM (n = 27)
Banff t ≥1	101 (36.5)	10 (20)*	7 (24.1)	4 (18.2)	117 (37.6)	17 (30.9)	10 (40)	7 (25.9)
Banff i ≥1	112 (40.4)	13 (26)	11 (37.9)	6 (27.3)	151 (48.6)	21 (38.2)	11 (44)	11 (40.7)
Banff g ≥1	12 (4.3)	0	0	0	9 (2.9)	1 (1.8)	1 (4.0)	0
Banff ptc ≥1	16 (10.6) (MV = 126)	2 (8) (MV = 25)	2 (14.3) (MV = 15)	0 (MV = 11)	23 (12.3) (MV = 124)	7 (21.9) (MV = 23)	3 (18.8) (MV = 9)	2 (14.3) (MV = 13)
Banff v ≥1	7 (2.5)	0	0	0	3 (1)	2 (3.6)	0	0
Banff ci ≥1	10 (3.6)	5 (10)	1 (3.4)	1 (4.5)	51 (16.4)	10 (18.2)	5 (20)	6 (22.2)
Banff ct ≥1	63 (22.7)	15 (30.0)	8 (27.6)	6 (27.3)	133 (42.8)	31 (56.4)	13 (52)	16 (59.3)
Banff c ≥1	9 (3.2)	4 (8.0)	1 (3.4)	1 (4.5)	51 (16.4)	10 (18.2)	5 (20)	7 (25.9)
Banff cv ≥1	1 (0.4)	0	1 (3.4)	0	5 (1.6)	0	0	0
Banff cg ≥1	0	0	0	0	2 (0.6)	0	0	0
Banff mm ≥1	19 (6.9)	7 (14.0)	8 (27.6)†	5 (22.7)*	33 (10.6)	12 (21.8)*	3 (12.0)	6 (22.2)
Banff c at 4 days ≥5%	14 (9.3) (MV = 126)	4 (15.4) (MV = 24)	2 (14.3) (MV = 15)	0 (MV = 11)	20 (10.8) (MV = 124)	4 (12.5) (MV = 23)	1 (6.3) (MV = 9)	1 (6.7) (MV = 13)
ATI	118 (42.6)	24 (48)	19 (65.5)*	14 (63.6)	141 (45.3)	28 (50.9)	13 (52)	8 (29.6)
ATI with isometric vacuolization	35 (12.6)	8 (16.0)	6 (20.7)	5 (22.7)	49 (15.8)	9 (16.4)	5 (20)	5 (18.5)
Nephrosclerosis (fibrous intimal thickening)	20 (8.2) (MV = 32)	5 (11.9) (MV = 8)	4 (15.4) (MV = 3)	6 (28.5)* (MV = 1)	32 (10.3) (MV = 1)	7 (12.7)	3 (12.0)	3 (11.1)
Nephrocalcinosis	19 (6.9)	4 (8.0)	0	2 (9.1)	32 (10.3)	6 (10.9)	4 (16.0)	2 (7.4)
Borderline changes	54 (19.5)	7 (14.0)	2 (6.9)	2 (9.1)	67 (21.5)	9 (16.4)	8 (32.0)	6 (22.2)
Banff a grade I/II	34 (12.3)	2 (4.0)	5 (17.2)	1 (4.5)	33 (10.6)	7 (12.7)	0	1 (3.7)
	PB at 6 months (n = 437)				BFC at ≤6 months (n = 234)			
	No AH, no VSM (n = 333)	Only VSM (n = 40)	Only AH (n = 37)	AH + VSM (n = 27)	No AH, no VSM (n = 174)	Only VSM (n = 28)	Only AH (n = 18)	AH + VSM (n = 14)
Banff t ≥1	81 (24.3)	11 (27.5)	7 (18.9)	7 (25.9)	67 (38.5)	5 (17.9)	6 (33.3)	4 (28.6)
Banff i ≥1	124 (37.2)	12 (30.0)	13 (35.1)	13 (48.1)	80 (46.0)	8 (28.6)	7 (38.9)	6 (42.9)
Banff g ≥1	8 (2.4)	0	2 (5.4)	1 (3.7)	12 (6.9)	2 (7.1)	0	1 (7.1)
Banff ptc ≥1	29 (14.4) (MV = 132)	6 (24.0) (MV = 15)	2 (8.7) (MV = 14)	2 (12.5) (MV = 11)	19 (28.8) (MV = 108)	2 (20) (MV = 18)	0 (MV = 7)	2 (40.0) (MV = 9)
Banff v ≥1	3 (0.9)	0	0	0	13 (7.5)	3 (10.7)	1 (5.6)	1 (7.1)
Banff ci ≥1	111 (33.3)	16 (40.0)	13 (35.1)	11 (40.7)	15 (8.6)	3 (10.7)	4 (22.2)	1 (7.1)
Banff ct ≥1	247 (74.2)	31 (77.5)	31 (83.8)	23 (85.2)	42 (24.1)	9 (32.1)	6 (33.3)	4 (28.8)
Banff c ≥1	122 (36.6)	16 (40.0)	15 (40.5)	12 (44.4)	13 (7.5)	3 (10.7)	4 (22.2)	2 (14.3)
Banff cv ≥1	7 (2.1)	1 (2.5)	2 (5.4)	2 (7.4)	0	1 (3.6)	0	0
Banff cg ≥1	2 (0.6)	0	0	2 (7.4)*	0	1 (3.6)	0	0
Banff mm ≥1	11.7 (39)	15 (6)	29.7 (11)†	29.6 (8)*	8 (14)	7.1 (2)	16.7 (3)	28.6 (4)*
Banff c at 4 days ≥5%	20 (9.9) (MV = 131)	2 (8.0) (MV = 15)	2 (8.7) (MV = 14)	1 (6.3) (MV = 11)	14 (21.2) (MV = 108)	2 (18.2) (MV = 17)	1 (9.1) (MV = 7)	0 (MV = 9)
ATI	43.5 (145)	40 (16)	54.1 (20)	33.3 (9)	60.9 (106)	57.1 (16)	50 (9)	71.4 (10)
ATI with isometric vacuolization	44 (13.2)	6 (15.0)	12 (32.4)†	3 (11.1)	32 (18.4)	6 (21.4)	5 (27.8)	4 (28.6)
Arteriosclerosis (fibrous intimal thickening)	51 (16.6) (MV = 26)	6 (16.7) (MV = 4)	13 (36.1)* (MV = 5)	9 (33.3)*	9 (5.2)	3 (10.7)	2 (11.1)	3 (21.4)*
Nephrocalcinosis	55 (16.5)	7 (17.5)	6 (16.2)	6 (22.2)	9 (5.2)	4 (14.3)	0	2 (14.3)
Borderline changes	46 (13.8)	5 (12.5)	5 (13.5)	6 (22.2)	29 (16.7)	2 (7.1)	4 (22.2)	3 (21.4)
Banff a grade I/II	24 (7.2)	3 (7.5)	1 (2.7)	1 (3.7)	41 (23.6)	5 (17.9)	2 (11.1)	2 (14.3)

Data are given as number of biopsy samples (%). χ^2 test for each group versus No AH, no VSM.
*$P < 0.05$, †$P < 0.01$.
ATI, acute tubular injury; cg, chronic transplant glomerulopathy; ci, interstitial fibrosis; ct, tubular atrophy; cv, chronic transplant vasculopathy; g, transplant glomerulitis; i, interstitial inflammation; mm, mesangial matrix increase; MV, number of missing values; ptc, peritubular capillaritis; t, tubulitis; v, vasculitis/intimal arteritis.

all three time points, ie, <6 weeks (best clearance during first 6 weeks) and at 1 and 2 years after transplantation, allograft function was significantly worse in patients with arteriolar lesions compared with those without such lesions (Figure 6A, all patient data considered together). When data for patients who underwent only PB were analyzed separately, a higher glomerular filtration rate was observed at 6 weeks in patients with VSM (Figure 6B). In the group who underwent additional BFC, patients with arteriolar lesions exhibited a glomerular filtration rate

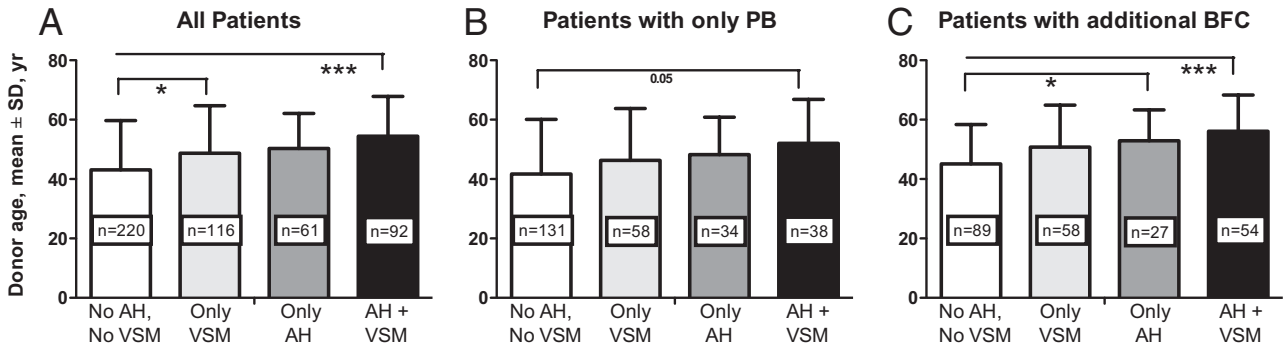

Figure 5. Arteriolar lesions and donor age for all patients (**A**), patients who underwent only PB (**B**), and patients who underwent PB plus BFC (**C**). $^{*}P < 0.05$, $^{***}P < 0.0001$, Mann-Whitney *U*-test versus no AH, no VSM.

significantly lower at 1 and 2 years after transplantation (Figure 6C).

Discussion

The present study examined the prevalence, clinicopathologic correlates, and significance of various qualities of arteriolar lesions in sequential PB and BFC samples in kidney transplants. Arteriolar lesions in early transplant renal biopsy samples most likely represent preexisting donor-derived factors, and occur more frequently in kidney grafts with impaired function. Although AH increases significantly over time and arteriolar lesions occur frequently in very late BFC samples from patients both with and without CNI therapy, they do not show any specific association with a particular pathomechanism such as CNI therapy, diabetes, or hypertension. Furthermore, various arteriolar lesions are related to each other and coexist in the same transplant. They are only moderately reproducible and significantly affected by sampling error, which makes their histologic assessment and interpretation even more difficult and of limited clinical usefulness. Thus, different arteriolar lesions are essentially a general feature of allograft injury, and do not carry reliable specificity for CNI toxicity.

An increase in arteriolar lesions over time after kidney transplantation has been described,[15–18] and it has become a kind of dogma in the literature that this represents chronic CNI toxicity. After the study by Nankivell et al[15] in 2003, the inevitable effects of CNI over time on AH, and the effects this has on long-term graft survival, have rarely been subjected to critical scrutiny in the literature in the last decade. It has been generally accepted that the nodular necklace-like deposition of hyaline material between arteriolar smooth muscle cells is a specific hallmark of vascular CNI toxicity; however, this is based on studies from the 1980s and 1990s, when drug concentrations tended to be much higher than current concentrations.[3]

However, most studies investigating the effect of CNI on the development of AH are limited by lack of controls because CNIs are so widely used. Only recently has the concept of vascular CNI toxicity been challenged: Stegall et al[18] examined the progression of various chronic lesions in renal transplants over 5 years. Snanoudj et al[19] placed special focus on arteriolar lesions in a smaller cohort of CNI-treated patients compared with patients who did not receive CNI therapy. Both studies showed convincingly that new-onset AH is not specific to CNI toxicity.

The present study offers valuable additional evidence to these aforementioned publications. With a focus on different patterns and quantities of arteriolar lesions, their prevalence and progression in a large number of

Table 4. Demographic and Histopathologic Variables Associated With Arteriolar Lesions

	Lesion		Univariate analysis			Multivariate analysis		
Variable	AH and VSM	No AH, No VSM	OR*	95% CI	*P* value	OR*	95% CI	*P* value
Donor age, years†	NA	NA	1.03	1,02–1.05	0.00	1.03	1.01–1.04	0.00
Arteriosclerosis	43.7 (18/270)	20.5 (45/220)	3.02	2,01–4.53	0.00	2.27	1.46–3.50	0.00
Mesangial matrix increase	39.3 (106/270)	19.1 (42/220)	2.74	1,81–4.15	0.00	2.06	1.32–3.21	0.00
Male donor	55.4 (148/267)	46,3 (101/218)	1.44	1.01–2.06	0.06	1.71	1.15–2.53	0.01
Acute tubular injury	77.0 (208/270)	70.5 (155/220)	1.41	0.94–2.11	0.12	NA	NA	NA

Data are given as percentage of patients (No. of patients).
*OR is given as mean ± SD: 51 ± 14 vs 46 ± 16 years.
†Increment for each year of life.
NA, not available; OR, odds ratio.

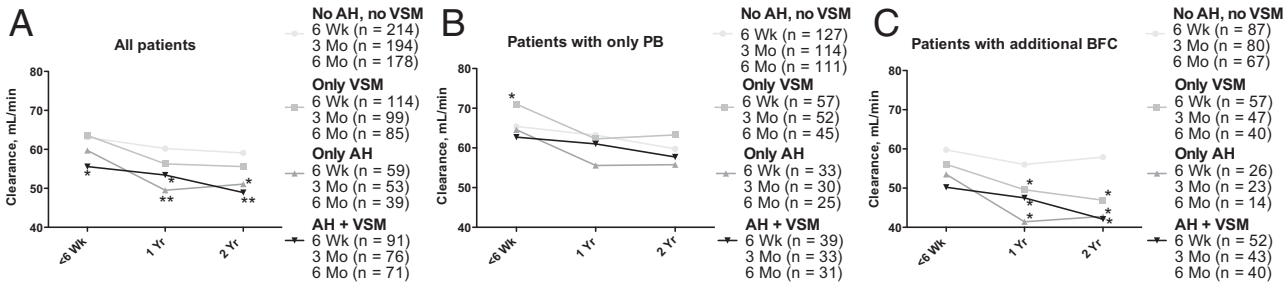

Figure 6. Arteriolar lesions and graft function for all patients (**A**), patients who underwent only PB (**B**), and patients who underwent PB plus BFC (**C**). The best clearance during the first 6 weeks is indicated by <6 Wk. Clearance (glomerular filtration rate) was assessed using the Cockroft-Gault formula. $^{*}P < 0.05$, Mann-Whitney *U*-test versus no AH, no VSM.

sequential early and late biopsy samples from individual patients was evaluated. The findings indicate unequivocally that sampling error and limited reproducibility are major problems in diagnostic interpretation of this lesion, two points that have not been evaluated in previous studies.

Of note, the diagnosis of AH was not found to be regularly reproduced in subsequent biopsy samples. There are several explanations for this: i) the diagnosis is poorly reproducible, as has been shown by us and by others,[10,20] ii) the diagnosis is missed owing to sampling error, or iii) arteriolar lesions are reversible. Sis et al[10]: and Mihatch et al[21] have pointed out that AH is difficult to reproduce and can be missed, in particular when it is focal and mild, as in the present study, in most cases. Reversibility of AH has been a matter of debate, but has not been conclusively shown in humans [22–25]. In their large prospective study of the natural history of chronic allograft nephropathy, Nankivell et al[15,16] concluded that AH was due to CNI toxicity when newly detected after previous biopsy samples without this lesion. However, sampling error may have impeded recognition of arteriolar lesions in earlier biopsy samples, thus challenging the assumption of new-onset hyalinosis in every case. In our series of patients who underwent three sequential PB, persistent presence of arteriolar lesions was observed only in a minority. This further indicates a significant sampling error in detection of these lesions. However, with increasing severity, the lesion is more likely to be (repeatedly) detected because more arterioles will be affected.

Insofar as CNI-related effects, Stegall et al[18] did not find significant differences in arteriolar lesions between CNI-treated patients and those who received a CNI-free immunosuppression protocol. Similarly, Snanoudj et al[19] reported AH, even in its nodular necklace-like pattern, in a substantial percentage of patients who never received CNI therapy, which is similar to our findings in very late biopsy samples from patients who received CNI therapy. In previous studies, several authors have raised concerns about the harm of chronic CNI therapy.[15,26,27] However, in a recent study that sought to identify specific causes for allograft loss, CNI toxicity alone was rarely the reason for graft failure.[28] Preliminary data from the DeKAF (Long-term Deterioration of Kidney Allograft Function) study showed that the pathologic diagnosis of presumed CNI toxicity was not associated with outcome.[29]

A relationship between AH and aging has been demonstrated, without consistent associations with arterial hypertension.[30–34] In an autopsy-based population survey study, AH was present in up to 8.7% of patients aged 40 to 59 years,[30] indicating that the frequency of AH in allografts during the first 2 years after transplantation in the present study generally reflects the average in a population. Previous studies confirm this view; in the study by Cosio et al,[35] AH was found more frequently in biopsy samples from older donors. Similar to our results, no clear-cut correlations with diabetes after transplantation, blood pressure levels, or the dosage of cyclosporine A at 1 year were found.[35] We observed arteriolar lesions in early transplant biopsy samples with arteriosclerosis and mesangial matrix increase, findings that are thought to be age-related or due to long-standing arterial hypertension, presumably in the donor. Accordingly, the presence of these biopsy findings was significantly associated with older donor age.

These results do not preclude a role of hypertension, diabetes mellitus, or CNI toxicity in the development of arteriolar lesions. We are aware of the limitations of a retrospective study, which cannot check for all influencing factors or establish causality between potential factors and the lesion. We found no association between arteriolar lesions and CNI blood concentrations or other features of CNI toxicity; however, systemic CNI drug concentration may not represent local effective intragraft drug accumulation and toxicity. Polymorphisms in the multiple-drug efflux transporter P-glycoprotein gene *ABCB1* and the cytochrome P450 isoenzyme CYP3A5 in tubular epithelial cells have been linked to a higher prevalence of CYP3A5 expression in biopsy samples with new-onset AH [36–38]. However, all studies have in common that controls with new-onset AH but without CNI therapy are lacking. AH has been demonstrated in native kidneys in patients receiving long-term CNI therapy for various indications including non-renal transplantation, and some of these patients may have had additional risk factors such as hypertension or diabetes mellitus.[39,40] However, in the present study, when compared with patients receiving CNI therapy, late biopsy samples from patients not receiving CNI therapy more frequently demonstrated arteriolar lesions, and time since transplantation was significantly longer. Thus, AH is apparently a function of time after transplantation, without specific or exclusive relation to hypertensive or diabetic vascular

damage or to CNI toxicity. It may be the result of cumulative burden of vascular injury to the allograft. Consequently, this lesion is not of specific diagnostic value, in particular because of its poor reproducibility and sampling error.

Rather more ambiguous are the relevance and causes of VSM. The lesion has been interpreted as the first structural CNI-induced damage to the small vessels.[1,41–43] In a recent study, VSM was more severe in biopsy samples with AH with typical signs of CNI toxicity.[44] However, all data analyzed were from patients receiving CNI therapy. In the present study, the presence of VSM was not associated with higher CNI trough levels, and patients who did not receive CNI therapy exhibited VSM lesions as well. Degeneration of smooth muscle cells and their replacement by hyaline material has been observed in early studies of arterial hypertension.[45,46] However, we could not find associations with blood pressure levels or the number of hypertensive drugs. In PB samples at 6 months after transplantation, patients with diabetes mellitus type 2 more often had VSM. Most likely, different types of injury on endothelium and smooth muscle cells may have an additive effect, as has been proposed from early studies in animal models.[47]

In summary, evaluation of AH and VSM in renal transplant biopsy samples is impaired by sampling error and limited reproducibility. Donor age is the major determinant for the presence of arteriolar lesions early in the course after transplantation. Over the long term, AH increases substantially without a demonstrable clear-cut relationship to one specific pathomechanism (eg, hypertension, diabetes, CNI therapy, or any other identifiable factor), which suggests that it represents a final common pathway to various arteriolar injuries.

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