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ORIGINAL ARTICLE

Morphologic Features and Clinical Impact of Arteritis Concurrent with Transplant Glomerulopathy

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Abstract Little is known about the morphology and clinical relevance of arteritis in renal allograft biopsies with transplant glomerulopathy. We retrospectively reviewed the morphologic findings and clinical course of 59 patients with cg, 16 of which featured concurrent arteritis (fibrosing intimal arteritis with luminal narrowing in 15, and acute intimal arteritis in 1 case). Fifteen out of the 16 cases with arteritis fulfilled the morphological diagnostic criteria for chronic active antibody-mediated rejection, and 11 cases with arteritis showed morphological evidence of concurrent, ongoing T-cell-mediated alloimmune response (acute T-cell-mediated rejection in 5, borderline changes in 6 cases). Further, the Banff grades of interstitial inflammation in scarred and nonscarred cortex, total cortical inflammation, and arterial luminal narrowing were significantly higher in biopsies with arteritis. By immunohistochemistry, T-lymphocyte predominance over macrophages was found in the intimal infiltrates in 14 out of 16 cases, and cytotoxic T-lymphocytes were identified among intimal mononuclears in 10 cases. Patients with arteritis demonstrated a significantly shorter renal survival (7.5 vs. 29 months). In conclusion, T-cell-mediated mechanisms could play a role in the development of arteritis concurrent with cg. However, this finding does not exclude the possibility that antibody-mediated rejection can also contribute to the evolution of the lesion. Importantly, the lesion carries negative prognostic value likely via severe arterial luminal narrowing.

Keywords Chronic antibody-mediated rejection · Graft survival · Kidney allograft · T-cell-mediated rejection · Arteritis

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Introduction

Two patterns of rejection arteritis are observed in kidney allograft biopsies. One is intimal arteritis, characterized by mononuclear cells beneath the endothelium, which can be seen in acute T-cell-mediated rejection (TCMR), acute antibody-mediated rejection (ABMR) or mixed TCMR/ABMR [1]. If the arteritis is T-cell-mediated, it is often associated with acute T-cell-mediated interstitial rejection, but it can also occur as an isolated vascular lesion. The imunophenotyping of intimal inflammatory infiltrates has revealed a mixture of CD3+ T-lymphocytes and CD68+ macrophages in both pathways of acute rejection [2–4]. The second pattern of rejection arteritis, characterized by fibrous intimal thickening, mononuclear cells and occasionally foam cells is the defining lesion of chronic active TCMR [5], but it can also be seen in biopsies with chronic ABMR [6].

Recent publications have concluded that acute rejection has a poorer prognosis if a vascular component is present and particularly if the intimal arteritis is antibody-mediated [7, 8]. However, the clinicopathological relevance of arteritis in chronic ABMR is unclear. To acquire more data on this topic, we have reviewed all our renal transplant biopsies with transplant glomerulopathy (corresponding Banff abbreviation: cg)

collected over a decade, with special emphasis on examination of the arteries. Cg is recognized as the most specific histological phenotype of chronic ABMR [1] and is strongly related to the de novo appearance of donor-specific IgG HLA antibodies [9]. Our main goal was to analyze the morphological characteristics and immunophenotype of the inflammatory cells of the vascular lesion in biopsies with cg, and its relationship to other morphological variables. The clinical impact of the arteritis on the graft survival and kidney function was also studied.

Material and Methods

Case Selection

We routinely evaluate renal transplant biopsies with light microscopy (hematoxylin and eosin, periodic acid-Schiff, trichrome, methenamine silver and elastic stainings) and immunofluorescence microscopy on frozen sections (complement 4d [C4d], C3, HLA-DR, IgG, IgA, and IgM); tissue sampling is performed for optional electron microscopy. Between 2001 and 2011, we investigated 755 indication biopsies from 442 recipients. Those biopsies were enrolled in the present study in which cg was identified either by light microscopy or by electron microscopy and were adequate. The adequacy criteria included ≥2 arteries and ≥7 nonscarred glomeruli for light microscopy, ≥ 4 glomeruli for immunofluorescence, and ≥1 glomerulus and ≥10 periglomerular peritubular capillaries for electron microscopy. Cg was defined at the ultrastructural level as thickening of the glomerular capillary wall in at least 3 loops in consequence of the widening of the subendothelial space by abnormal basement membrane material and the formation of a new layer(s) of basal lamina [10]. At least 3 peritubular capillaries with 5 to 6 circumferential basement membrane layers or 1 peritubular capillary with ≥7 layers was an indication of alloantibody-induced transplant peritubular capillaropathy [11, 12]. New-onset vascular changes were assessed by comparison with the implantation biopsies. Concurrent chronic calcineurin inhibitor toxicity and post-transplantation glomerulonephritis were diagnosed with regard to arteriolar peripheral nodular hyalinosis, and the characteristic light microscopic, immunofluorescent and electron microscopic features.

Re-Evaluation of the Enrolled Samples

The samples were scored and graded from 0 to 3 by 2 independent observers (D.D. and B.I.). In the event of disagreement, a consensus score was agreed on. Intimal arteritis, tubulitis, peritubular capillaritis, cg, interstitial fibrosis, tubular atrophy and arteriolar hyalinosis were scored according to the Banff criteria [13, 14]. Glomerulitis was defined as the presence of ≥5 leukocytes per glomerulus [15]. Interstitial infiltrates were scored in the nonscarred cortex, in the scarred cortex, and as total interstitial inflammation as described recently [16]. Chronic arterial changes were assigned as fibrosing intimal arteritis with mononuclears (second pattern of rejection arteritis described in the Introduction), intimal fibrosis (marker lesion of chronic ABMR), and intimal fibroelastosis (related to aging/hypertension). The arterial lesions were scored on the basis of the luminal narrowing, similarly as for fibrous intimal thickening in the Banff scheme [17]. Intimal fibroelastosis was defined as two layers or more of internal elastic lamina seen on elastic staining. The C4d positivity of peritubular capillaries was documented in the biopsy reports as negative (if no or only minimal staining was present) focal or diffuse, similarly as in the Banff recommendation [1]. The ultrastructural grading of peritubular capillary basement membrane multilayering was three-tired: mild (3–4 layers), moderate (5–6 layers) or severe (≥7 layers). Tubular HLA-DR expression, upregulated in acute TCMR [18–20], was assessed on the basis of whether it was observed in at least 4 tubular profiles or not (scores 1 or 0, respectively). Two groups were defined for statistical analysis: cg with arteritis and cg without arteritis.

Serial sections from formalin-fixed paraffin-embedded biopsies adjacent to the section in which the arteritis lesion was found were stained for CD3 (T-cells), and CD68 (macrophages). Non-adjacent sections were stained for CD8 and T-cell intracellular antigen-1 (TIA-1, marker of cytotoxic T-cells). For each biopsy, the intimal area of the inflamed arteries was measured with an eyepiece graticule, and the ratio of CD3+ cells to all inflammatory cells within the intima was calculated.

The clinical data were collected from the patients' records. All data were handled anonymously. Systematic serological testing for anti-HLA alloantibodies on the Luminex platform started in our medical center in 2011, and alloantibody data were therefore available for only 1 patient.

Statistics

Statistical analysis was performed with SPSS 20.0 software. Continuous variables were expressed as medians with interquartile range or mean±standard deviation or standard error, and categorical variables were presented as numbers of cases. The clinical and demographic comparisons of the two cohorts were performed with the independent samples *t*-test, the Mann–Whitney *U*-test and the chi-square test, where applicable. The Mann–Whitney test and the Kruskal-Wallis test were used in the comparison of arterial narrowing due to fibrosing intimal arteritis with mononuclears, intimal fibrosis, and intimal fibroelastosis. The problem of multiple comparisons was counteracted with the Bonferroni correction. Values of $p<0.05$ were considered significant. The morphological lesions were correlated according to Spearman's rank order.

Hierarchical clustering was used to explore [21] the class relationships among the lesions.

The Modification of Diet in Renal Disease equation served for estimation of the GFR [22]. To compare the estimated GFR means over time in the two groups, we compiled the results of estimated GFR measurements from 36 months pre-biopsy (or, if the transplantation was performed within 36 months before the biopsy, from the time of transplantation) to 24 months post-biopsy (or, if graft failure occurred within 24 months, to the point of graft failure). Since some data were missing from our data set, the two-way mixed ANOVA model was used with time as the repeated-measures (within-subject) factor and group as the between-subject factor. Pairwise comparisons were performed on estimated marginal means by taking into account the presence of interaction. P values were corrected by the Holm-Sidak method. The interval between the repeated measures was 3 months. Measurements performed within 1 month before or after the time of the enrolled biopsy were also included.

The graft survival was tested by medians of Kaplan-Meier curves. Graft failure was defined as the restarting of dialysis. Patient censoring was performed either at the end of the follow-up (October 2013) or because of death with a functioning graft or removal of the functioning graft for a clinical reason.

Results

Study Population; Demographic and Clinical Data

In the study period, 88 biopsies of 65 patients displayed cg; 20 recipients had>1 biopsy showing cg; in these 20 patients, the first biopsies in which cg was seen were analyzed. Six samples from 6 patients who underwent biopsy only once during the study period were not adequate and were excluded. Eventually, 59 biopsies from 59 patients were enrolled. The median time to biopsy was 58 months (interquartile range: 67), and the median age of the patients was 44 years (interquartile range: 23). At the time of the biopsy, two-thirds of the patients were in stage IV or V chronic kidney disease [23]. All but 2 recipients received a deceased donor kidney; all kidneys were matched for HLA (for the degree of matches see Table 1) and blood group antigens, and in all cases both donors and recipients were Caucasians. Induction immunosuppression consisted of anti-thymocyte globulin or interleukin-2 receptor blockers. 75 % of the patients were on a calcineurin inhibitor-based maintenance immunosuppressive regimen. Donor specific alloantibodies against HLA class II antigens were demonstrated in the tested patient. The demographic and clinical characteristics in the two study groups at the time of the biopsy did not differ significantly (Table 1).

Biopsy Findings

According to the Banff 2013 classification (1) 45 % of our cases met the histological criteria of C4d-negative chronic active ABMR (g+ptc≥2) while 48 % of our cases were compatible with C4d-positive chronic active ABMR (diffuse and focal positivity was present in 34 and 14 %, respectively). 7 % of the patients with cg were C4d-negative without at least moderate microvascular inflammation (g+ptc<2). Cg was identified by light and electron microscopy in 53 biopsies and exclusively by electron microscopy in 6 biopsies. Thirty-three biopsies also displayed transplant peritubular capillaropathy. Concurrent lesions included arteritis in 16 samples, Banff IA or IB acute TCMR in 13 samples, borderline changes in 19 samples, chronic calcineurin inhibitor toxicity in 22 samples, and glomerulonephritis in 8 samples. Five patients acquired serological positivity of hepatitis C virus infection before transplantation. None of them displayed glomerular immune complexes on electron microscopy. Further, C4d positivity and peritubular capillaropathy were observed in 3 of these 5 cases, C4d positivity in 1 case, and peritubular capillaropathy in 1 case, therefore the glomerular double contours in these patients were considered as the manifestations of chronic ABMR-mediated tissue injury; since the possibility of membranoproliferative glomerulonephritis induced by hepatitis C virus infection could be excluded.

Group 1: cg with Arteritis

This group comprised 16 biopsies. Nine cases involved C4d+ chronic active ABMR, 6 C4d- chronic active ABMR and 1 chronic inactive ABMR. The arteritis lesion was confined to the intima of small interlobular arteries, and consisted of mononuclear cells and occasionally foam cells. In 15 samples, the inflammatory cells were scattered throughout the fibrously thickened intima and were associated with severe (Fig. 1), moderate or mild luminal narrowing in 10, 4 and 1 samples, respectively (fibrosing intimal arteritis with mononuclears). The cell density within the inflamed intima was in the 50–500 CD3+ cells/mm^2 range. In the biopsy from a patient with documented noncompliance (index case 2), the infiltrate was accompanied by insuded fibrinoid material. The mean ratio of involved arteries per biopsy was 62 %.

Five cases were accompanied by concurrent acute interstitial cellular rejection and 6 cases by borderline changes. Tubular HLA-DR expression was positive in all cases with acute interstitial rejection, and in 5 cases with borderline changes.

The immunophenotyping of arteritis revealed the presence of CD3 positive, CD8/TIA-1 positive, and CD68 positive cells in 15, 10 and 5 cases, respectively (Table 2). Only T-cells were found in 11 cases, while in 5 cases macrophages could also be identified. CD68+ cells predominated over

Table 1 Demographic data and clinical characteristics of the recipients at the time of the biopsy procedure

	cg without arteritis (n=43)	cg with arteritis (n=16)	p
Recipient female gender (n)	13/43	6/16	0.755
Recipient age (years)	51 (IQR[a]=23)	41 (IQR=30.5)	0.831
Native kidney disease			
Glomerulonephritis	21	6	0.56
Polycystic kidney disease	7	4	0.468
Chronic pyelonephritis/vesicoureteral reflux	6	3	0.692
Hypertensive kidney disease	2	0	1
Diabetic nephropathy	2	0	1
Not determined	5	3	0.67
Previous Tx[b] (n patients with >1 Tx)	7	0	0.173
HLA mismatch			
0–1	15	8	0.371
2–4	25	7	0.368
5–6	3	1	1
Panel reactive antibodies			
0	22	8	0.315
>0	5	0	
No data	16	8	
Post-transplantion time (months)	57 (IQR=60)	60.5 (IQR=83.5)	0.959
Maintenance immunosuppression			
CNI[c]	33	11	0.522
MMF[d]	35	10	0.172
St.[e]	24	9	1
mTOR[f] inhibitor	13	4	0.759
eGFR[g] level (ml/min/1.73 m^2)	25.5 (IQR=49.20)	21 (IQR=18.3)	0.257
Proteinuria			
<3.5 g/24 h	36	11	0.277
≥3.5 g/24 h	7	5	

[a] Interquartile range

[b] Transplantation

[c] Calcineurin inhibitor

[d] Mycophenolate mofetil

[e] Steroid

[f] Mammalian target of rapamycin

[g] Estimated glomerular filtration rate

CD3+ cells in 2 cases. Five of the 10 samples with intimal cytotoxic T-cells displayed concurrent interstitial cellular rejection, and 3 of that 10 exhibited concurrent borderline changes and tubular HLA-DR expression. Biopsies with arteritis had significantly higher scores of interstitial infiltrates in the nonscarred cortex (p=0.038), total interstitial inflammation (p=0.018) and interstitial infiltrates in the scarred cortex (p=0.027) as compared with biopsies without arteritis (Table 3.). The grade of luminal narrowing was more severe in biopsies with fibrosing intimal arteritis with mononuclears (median=3, p<0.001) than in biopsies with arterial intimal fibrosis or fibroelastosis.

Group 2: cg without Arteritis

This group consisted of 43 samples. Eighteen cases were assigned to the category of C4d+chronic active ABMR, 22 cases to C4d- chronic active ABMR, and 3 cases to chronic inactive ABMR. Arterial intimal fibrosis was observed in 11 samples (3 moderate and 8 mild; median=1) and intimal fibroelastosis in 20 samples (severe in 3, moderate in 4, and mild in 13 cases; median=1). In 8 cases concurrent interstitial cellular rejection, and in 13 cases borderline changes were observed. Tubular HLA-DR positivity was noted in all biopsies with interstitial cellular rejection, and in 7 biopsies with

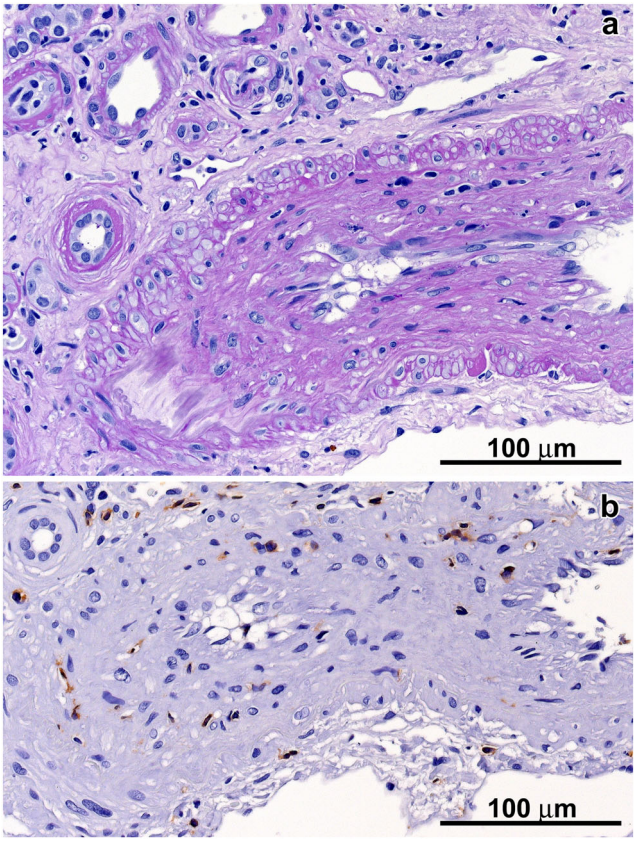

Fig. 1 Features of fibrosing intimal arteritis in adjacent sections. **a**: Scattered mononuclear cells are located in the fibrously thickened intima; the vessel lumen is markedly narrowed. PAS, magnification ×40; **b**: the majority of the mononuclear cells proved to be T-lymphocytes. Antibody to CD3, magnification ×40

borderline changes. The distributions of calcineurin inhibitor toxicity, glomerulonephritis and HCV positivity were not significantly different across the two study groups (Table 3).

The Spearman Correlation of the Morphological Variables

As expected, Spearman's rank order revealed robust correlations between the interstitial infiltrates in the nonscarred cortex and tubulitis and tubular HLA-DR expression, and between interstitial fibrosis and tubular atrophy. Tubulitis, interstitial inflammation, tubular atrophy, and interstitial fibrosis correlated with interstitial infiltrates in the scarred cortex and total interstitial inflammation. Weaker, but still significant correlations emerged between the scores of the active microcirculatory lesions (between glomerulitis and peritubular capillaritis and between peritubular capillaritis and C4d+). However, peritubular capillaritis also correlated with the interstitial infiltrates in the nonscarred cortex, tubulitis, interstitial infiltrates in the scarred cortex and total interstitial inflammation scores. A correlation was found between the chronic microcirculatory lesions (peritubular capillary basement membrane multilayering and cg). Peritubular capillary basement membrane multilayering also correlated with arterial intimal fibrosis and tubulointerstitial scarring. Fibrosing intimal arteritis correlated with interstitial infiltrates in the scarred cortex and total interstitial inflammation. Luminal narrowing related to intimal fibroelastosis did not correlate with other variables. The significant positive correlations between the morphological variables are to be seen in Fig. 2.

Hierarchical Cluster Analysis (HCA)

HCA confirmed the results of Spearman correlation: three groups of lesions were explored by clustering of the morphological variables (Fig. 3). The first group comprised inflammation in scarred areas, total cortical inflammation, inflammation in nonscarred areas, tubulitis, tubular HLA-DR expression, peritubular capillaritis, interstitial fibrosis, tubular atrophy, fibrosing intimal arteritis, C4d+, and glomerulitis. The second group comprised peritubular capillary basement membrane multilayering, arterial intimal fibrosis, cg and arteriolar hyalinosis. The third group comprised arterial intimal fibroelastosis.

Post-Biopsy Management and Graft Survival

The therapeutic interventions after the biopsy procedure are summarized in Table 4. All 13 patients with a histological diagnosis of acute TCMR Banff IA or IB received steroid pulse therapy. In 2 cases with arteritis and concurrent interstitial cellular rejection, anti-thymocyte globulin and plasmapheresis were also administered. For the patients who had concurrent borderline changes, the immunosuppression was intensified. Different therapeutic approaches were applied in the cases of isolated arteritis: 2 patients received steroid pulse therapy, 1 patient participated in intensified immunosuppression, and 2 patients continued the same therapeutic regimen as in the pre-biopsy period. In cases of coinciding chronic calcineurin inhibitor toxicity, the dosage was reduced or the drug was withdrawn. The median duration of the post-biopsy follow-up was 20 months (IQR=25.50). The means of estimated GFR in the cg with arteritis group and cg without arteritis group were not significantly different at the time of biopsy, but the results of estimated GFR measurements at 3, 6 and 12 months post-biopsy differed significantly (Fig. 4). Forty-two graft failures were attributed to rejection. From among the 17 censored cases, 9 patients died with a functioning allograft and 3 functioning grafts had been removed, in 2 patients due to sepsis, and in 1 patient because of massive bleeding after the biopsy puncture.

The median graft survival in the patients with arteritis was significantly lower than that in those without arteritis (7.5 (SE=2.554) vs. 29 months (SE=6.006), respectively (p= 0.001), Breslow method). After the cases with

Table 2 Immunophenotyping of arteritis and concurrent lesions

Case No.[a]	CD3+ cell density (cell/inflamed intima mm^2)	CD68+ cell density (cell/inflamed intima mm^2)	CD3+ cells / CD3+ plus CD68+ cells	CD8+/TIA-1 cell density (cell/inflamed intima mm^2)	Inflamed artery/ all artery	cg[b] score	ptcbmml[c] score	C4d[d] score	g[e] score	Con-current lesions	Tubular HLA-DR positivity
1	163.84	0	1	61.44	2/3	3	3	3	2	ICR[f]	yes
2	768.00	640.00	0.55	384.00	2/2	1	1	3	3	ICR	yes
3	465.45	1210.18	0.27	93.09	3/5	1	3	1	3	ICR	yes
4	76.80	0	1	76.80	2/2	1 (EM[h])	0	3	2	ICR	yes
5	460.80	0	1	51.20	2/2	3	0	3	3	ICR	yes
6	59.73	34.13	0.64	34.13	2/4	1	2	3	3	B.c.[i], CNI-tox[j]	yes
7	94.52	0	1	c.n.b.e.[k]	1/2	3	3	0	3	B.c.	yes
8	102.40	0	1	c.n.b.e.	1/4	1	2	0	1	B.c., GN[l]	yes
9	34.13	0	1	c.n.b.e.	1/3	2	2	3	1	B.c.; GN, CNI-tox	no
10	0	91.02	0.2	22.76	1/3	3	1	3	3	B.c.	yes
11	204.80	0	1	25.60	1/2	2	2	1	2	B.c., CNI-tox	yes
12	195.05	0	1	121.90	1/2	3	2	0	2	CNI-tox	no
13	74.47	0	1	c.n.b.e.	2/2	3	0	2	2	GN	no
14	312.89	0	1	c.n.b.e.	2/5	3	2	2	3	GN, CNI-tox	yes
15	204.80	0	1	c.n.b.e.	2/5	1	0	0	0	CNI-tox	no
16	199.80	12.49	0.94	99.90	4/4	3	3	0	3	none	no

[a] number

[b] transplant glomerulopathy

[c] peritubular capillary basement membrane multilayering

[d] C4d positivity of the peritubular capillaries

[e] glomerulitis

[f] interstitial cellular rejection

[g] light microscopy

[h] electron microscopy

[i] borderline changes

[j] calcineurin-inhibitor toxicity

[k] could not be evaluated

[l] glomerulonephritis

Table 3 Median values of Banff scores and other morphological variables, and distributions of coinciding lesions in the study groups

	cg[a] without arteritis	cg with arteritis	p
Banff scores and other studied morphological variables			
C4d[b]	1	2	0.276
g[c]	2	2.5	0.161
i[d]	1	1.5	0.038
t[e]	1	1	0.084
ptc[f]	2	2	0.044
ci[g]	2	2.5	0.409
ct[h]	2	2	0.492
i-IFTA[i]	1	1.5	0.027
ti[j]	1	2	0.018
Luminal narrowing[k]	1	3	<0.001
cg	3	2.5	0.895
ptcbmml[l]	2	2	0.986
ah[m]	3	3	0.89
Coinciding lesions (n)			
Interstitial cellular rejection	8	5	0.311
Borderline changes	13	6	0.755
Tubular HLA-DR positivity	18	11	0.242
CNI tox[n]	16	6	1
GN[o]	4	4	0.194
HCV[p] positivity	3	2	0.606

Banff v lesion occurred in 1 case only, and statistical analysis could therefore not be performed

[a] Transplant glomerulopathy

[b] Complement 4 d

[c] Glomerulitis

[d] Interstitial infiltrate in the nonscarred cortex

[e] Tubulitis

[f] Peritubular capillaritis

[g] Interstitial fibrosis

[h] Tubular atrophy

[i] Interstitial infiltrate in the scarred cortex

[j] Interstitial infiltrate in the entire cortex

[k] Chronic arterial changes were read as arterial intimal fibrosis, fibrosing intimal arteritis and arterial intimal fibroelastosis; the degree of luminal narrowing was compared between the study groups

[l] Peritubular capillary basement membrane multilayering

[m] Arteriolar hyalinosis

[n] Calcineurin inhibitor

[o] Glomerulonephritis

[p] Hepatitis C virus.

glomerulonephritis had been excluded, the median graft survival in the cg with arteritis group remained significantly lower (6 [SE=2.031] vs. 32 months [SE=4.988], respectively ($p<0.001$) (Fig. 5a, b). The median graft survival was significantly worse in the patients with arteritis (6 months [SE=2.031]) than in those non-arteritis patients who had interstitial cellular rejection (35 months [SE=17.570], $p=0.010$) (Fig. 5c). Further, grafts with fibrosing intimal arteritis with mononuclears had a worse median survival than grafts with intimal fibrosis or fibroelastosis (7.5 (SE=2.286) vs. 21 months (SE=4.954) vs. 35 (SE=7.099), respectively ($p<0.001$) (Fig. 5d).

Discussion

In this study, 59 consecutive indication biopsies displaying cg were analyzed with regard to the clinicopathological features of

Fig. 2 Spearman correlation coefficients (r_S) between the studied morphological variables. p-value for each r_S is shown below the corresponding correlation coefficient. Abbreviations: *C4d* C4d positivity of the peritubular capillaries, *g* glomerulitis, *ptc* peritubular capillaritis, *t* tubulitis, *i* interstitial infiltrates in the nonscarred cortex, *HLA-DR* HLA-DR expression of the tubular epithelium, cv_{mo} fibrosing intimal arteritis, *i-IF/TA* interstitial infiltrates in the scarred cortex, *ti* interstitial infiltrates in the entire cortex, *ct* tubular atrophy, *ci*-interstitial fibrosis, *cg* transplant glomerulopathy, *ptcbmml* peritubular capillary membrane multilayering, cv_{if} arterial intimal fibrosis, *ah* arteriolar hyalinosis and cv_{ife} arterial intimal fibroelastosis. Intimal arteritis (v) was recorded in only one patient thus it was not included in the correlation tests

concurrent arteritis. Although DSA data were available only in 1 patient, the biopsy findings with cg were consistent with chronic ABMR supported by additional histological and clinical data. Various combinations of peritubular capillary C4d positivity, peritubular capillary basement membrane multilayering, capillaritis, glomerulitis, and/or fibrous arterial intimal thickening accompanied all cases with cg. Furthermore, histological, immunofluorescent, ultrastructural and clinical evidence of hepatitis C virus-related membranoproliferative glomerulonephritis or thrombotic microangiopathy, that should be considered in the differential diagnosis of cg [24, 25], were not observed.

By the 2013 Banff classification (1), 93 % of the cases fulfilled the histological criteria of chronic active ABMR (evidence of chronic tissue injury and C4d+and/or glomerulitis and /or peritubular capillaritis≥2), while 7 % of the cases did not show activity, that may indicate the cessation of alloantibody production prior to the biopsy procedure. The prevalence of arteritis was 27 % (16/59).

To address the clinicopathological significance of arteritis concurrent to cg, the 'Banff' chronic vascular lesion was subdivided into three morphologically distinct entities: intimal fibrosis, fibrosing intimal arteritis with mononuclears, and intimal fibroelastosis. Our statistical tests (Spearman

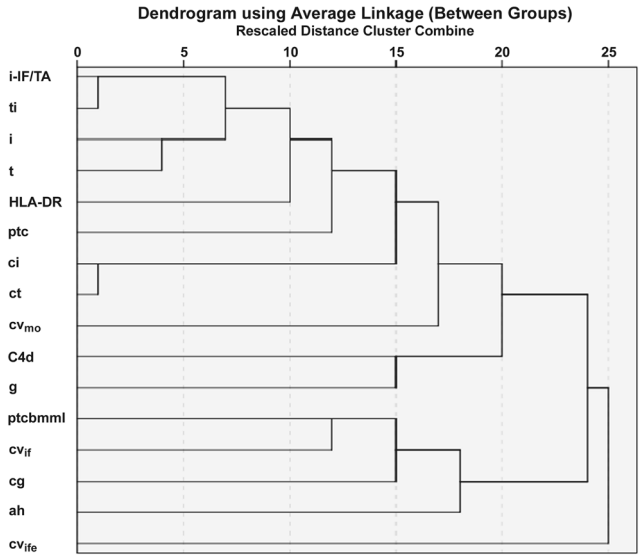

Fig. 3 Hierarchical cluster analysis diagram (dendrogramm) of the studied morphological variables. Clustering of the variables is based on Pearson correlation. Horizontal axis indicates the level of similarity at which two variables –based on their correlation coefficient- are fused into a cluster. Variables that are linked together at lower values (0–10) are more correlated to each other, than to those, that are linked to them at higher values (15–20). According to our model, three major clusters can be defined. The first cluster comprised of inflammation in scarred areas (i-IF/TA), total cortical inflammation (ti), inflammation in nonscarred areas (i), tubulitis (t), tubular HLA-DR expression (HLA-DR), peritubular capillaritis (ptc), interstitial fibrosis (ci), tubular atrophy (ct), fibrosing intimal arteritis (cv_{mo}), C4d positivity (C4d), and glomerulitis (g). The second cluster encompassed peritubular capillary basement membrane multilayering (ptcbmml), arterial intimal fibrosis (cv_{if}), transplant glomerulopathy (cg) and arteriolar hyalinosis (ah). A single variable showed complete separation from the two other clusters: arterial intimal fibroelastosis (cv_{ife}) characteristic of ageing/hypertension

correlation, HCA) connected these vascular lesions with different groups of morphological variables. These results confirmed the notion that intimal fibrosis with mononuclears is an active process (because it correlated/was clustered mainly with inflammatory lesions), while intimal fibrosis, along with transplant glomerulopathy and peritubular capillary multilayering is the result of a prolonged chronic tissue injury. As opposed to the former two, intimal fibroelastosis does not have statistical association with other morphological lesions characteristic of the alloimune response that reflects the fact that this lesion is due to ageing and/or hypertension.

We also characterized the histological features of fibrosing intimal arteritis. The median luminal narrowing was the worst in this group compared to the other two with chronic vascular lesion. In 10 biopsies, cytotoxic T-lymphocytes were identified in the fibrotic arterial intima. Further, in more than 2/3 of all cases, morphological evidence of concurrent T-cell-mediated alloimmune response (interstitial cellular rejection or borderline changes) was found. Cases with arteritis (irrespective of the severity of concurring intimal fibrosis) showed significantly higher grades of interstitial inflammation, interstitial inflammation in scarred cortex, and total inflammation (see Table 3). These findings collectively suggest that cellular rejection mechanisms may play a role in the evolution of arteritis in, at least some cases with chronic ABMR. However, the cellular composition of arteritis in some cases may also argue for antibody-mediated injury. There were 5 cases with CD68+ cells in the fibrotic arterial intima, four of them showed T-cell-mediated alloimmune response. The presence of CD68 positive cells (most likely macrophages) in these cases may be an indication of antibody-dependent cellular cytotoxicity, a potential pathogenetic mechanism of chronic ABMR [19]. We therefore conclude that both cellular and antibody-mediated mechanisms can contribute to the development of these lesions (although probably to a different extent from case to case), thus the precise separation of the two alloimmune pathway is impossible in this context.

Regarding the clinical relevance of the arteritis in cg, the mean estimated GFR measurements were significantly lower in the arteritis group at 3, 6, and 12 months after the biopsy. This finding is parallel to the much shorter median graft survival of these patients (7.5 vs. 29 months). Exclusion of the cases with coinciding glomerulonephritis did not lead to considerable changes in graft survival (6 vs. 32 months), which shows that the shorter survival seen in the arteritis group cannot

Table 4 Post-biopsy management of study groups

Chosen therapy	cg[a] without arteritis			cg with arteritis		
	no ICR[b] (n=22)	borderline changes (n=13)	TIS (n=8)	no ICR (n=5)	borderline changes (n=6)	ICR (n=5)
Steroid pulse	0	0	8	2	0	5
Intensification of maintenance immunsuppression	0	13	0	1	6	1
Anti-thymocyte globulin	0	0	0	0	0	2
Plasmapheresis	0	0	0	0	0	2
No therapeutic intervention	22	0	0	2	0	0

[a] Transplant glomerulopathy, [b] Interstitial cellular rejection

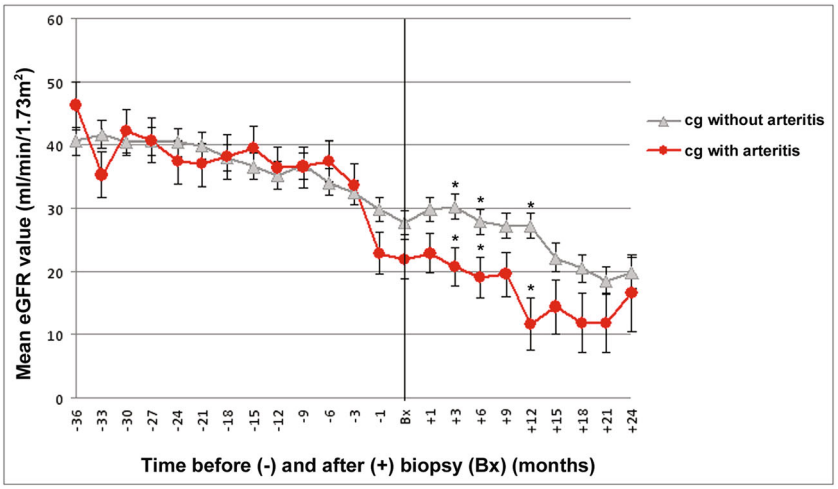

Fig. 4 Mean eGFR values in the cg group without arteritis and in the cg group with arteritis. The renal function in the cg group with arteritis was significantly poorer than that in the cg group without arteritis 3, 6 and 12 months post-biopsy (value pairs indicated by *)

be attributed to the confounding effect of glomerulonephritides. The graft survival in patients with arteritis was also shorter (7.5 months) than in patients with interstitial cellular rejection (35 months), which indicates that late-onset interstitial cellular rejection can be controlled more effectively than arteritis. We also found that grafts with fibrosing intimal arteritis had a worse survival than grafts with intimal fibroelastosis or intimal fibrosis, indicating that fibrosing intimal arteritis is a more aggressive lesion than intimal fibrosis without inflammatory cells, therefore the morphological distinction between the two has clinical relevance. The shorter survival in this group can probably be traced back to the more severe luminal narrowing than that observed in intimal fibrosis. Another factor that might explain the poorer survival of the patients with this lesion is the

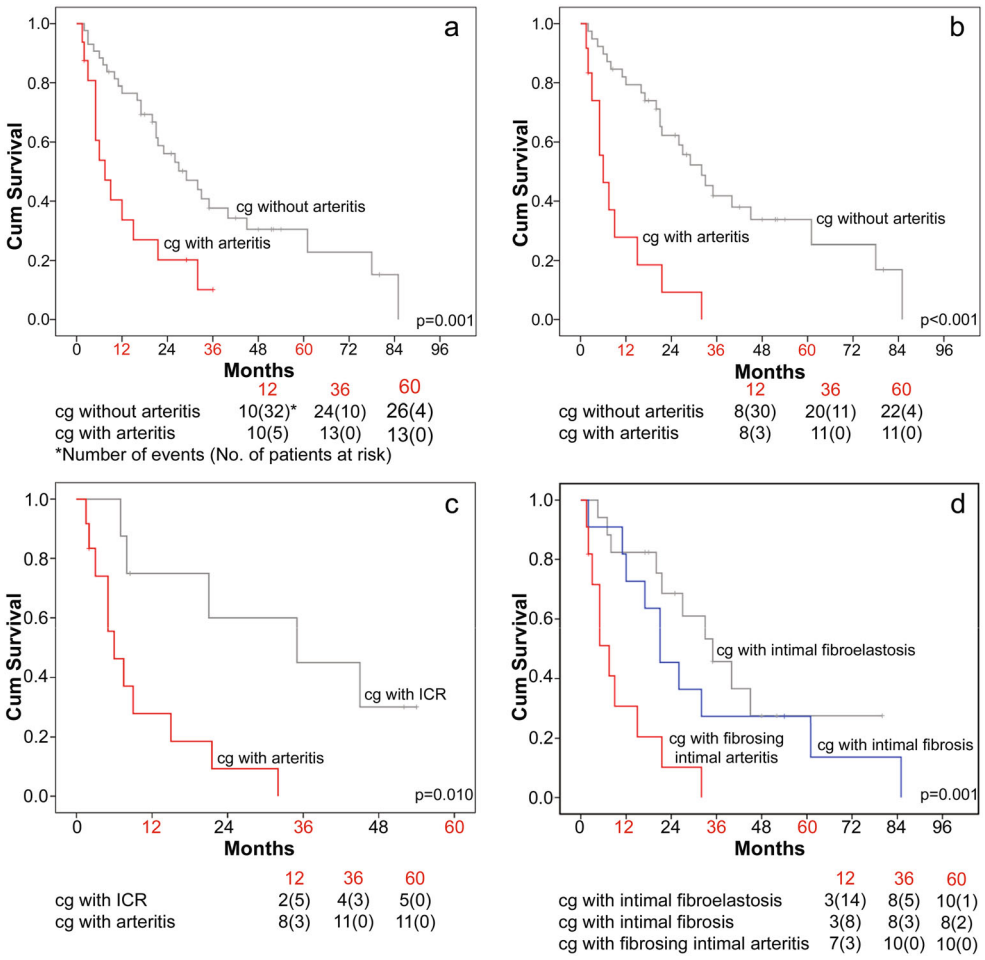

Fig. 5 Graft survival estimates: **a**, cg without arteritis vs. cg with arteritis (all patients $n=59$) **b**, same groups after patients with glomerulonephritis were excluded **c**, patients with arteritis vs. non-arteritis patients with interstitial cellular rejection (ICR) **d**, in cg groups with or without arteritis and in cases with arteritis with or without interstitial cellular rejection (ICR) vs.. cases with acute ICR. Cg with arteritis had a poorer prognosis than cg without arteritis in the first two settings: **a** all patients ($n=59$), **b** patients without concurrent glomerulonephritis ($n=51$), and cases with arteritis with or without ICR had a poorer prognosis than cases with acute ICR (**c**). Fibrosing intimal arteritis has the worst prognosis compared to intimal fibrosis and intimal fibroelastosis (**d**)

significantly higher inflammatory load, a known negative prognostic factor [16] in kidney biopsies.

In summary, the re-evaluation of a consecutive series of 59 cases with cg disclosed the relatively frequent coincidence of intimal arteritis. Arteritis was accompanied by severe intimal fibrosis in 11 of the 16 cases. The cellular infiltrate was dominated by T-cells, some of which were cytotoxic, and this, together with the frequent coincidence of interstitial cellular rejection of varying severity, raises the possibility that T-cell-mediated mechanisms may play a role in the evolution of fibrosing intimal arteritis. However, these findings do not exclude the possibility of ABMR also being an actor in the development of this lesion. There may be a link between antibody-mediated injury and fibrosing intimal arteritis, but evaluation of this hypothesis will require larger studies. The lesion exerted a strong negative effect on graft survival, likely via severe luminal narrowing.

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