BANTAO Journal 3 (2): p 123; 2005

ANCA-Associated Glomerulonephritis and Systemic Vasculitis in Kidney – A Pathologist's Perspective

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Introduction

Terminology (the names of diseases) and the definition of vasculitides (abnormalities that warrant assignment of the diagnostic terms) were proposed and agreed at the International Consensus Conference in Chapell Hill, USA, in 1993 but diagnostic criteria have yet to be established (1). In line with the Chapell Hill proposal, clinical-pathological syndromes representing various forms of systemic and organ limited vasculitides have been classified into three main groups according to the caliber of the involved blood vessels (1):

1. Vasculitides of the large blood vessels (aorta and its branches);
2. Vasculitides of the medium-sized blood vessels;
3. Small blood vessel vasculitides (involving distal arterial branches such as the intrarenal arcuate and interlobular arteries, arterioles, venules and capillaries, such as glomerular and pulmonary alveolar capillaries. This is the largest, most important group.

Already in 1988 Falk and Jennette (2) demonstrated that most patients with pauci-immune (without well defined immunoglobulin deposits) crescentic glomerulonephritis, including patients with or without small vessel systemic vasculitis, have antineutrophil cytoplasmic antibodies (ANCA). It has been found that when glomerular necrosis and consequent crescent formation are identified in the absence of well-defined immunohistologic evidence of immune complex or anti-GBM disease, the major differential diagnoses are pauci-immune kidney limited crescentic glomerulonephritis, Wegener's granulomatosis, microscopic polyangiitis and Churg-Strauss syndrome (1). Furthermore, it has become evident that ANCA are incriminated in the pathogenesis of pauci-immune crescentic glomerulonephritis, because ANCA are present in most patients with disease activity (3).

Falk et al (4) obtained a 75% overall renal survival rate at 24 months using high dose corticosteroids or corticosteroids combined with cyclophosphamide. It has been demonstrated that patients treated with corticosteroids alone had a lower remission rate and three times greater risk of relapse compared with patients treated with corticosteroids combined with cyclophosphamide (5). ANCA crescentic glomerulonephritis is an aggressive disease that warrants aggressive immunosupression and tends to respond better to treatment than in the case of patients with anti-GBM glomerulonephritis (6). Assessment of ANCA in serum and kidney biopsy enables rapid and precise diagnosis, so that the treatment can begin promptly. Furthermore, the monitoring of ANCA titer is a useful indicator of the disease activity, the effect of therapy and recurrences during the course of the disease.

Definition of ANCA systemic vasculitis and glomerulonephritis

ANCA positive pauci-immune necrotizing crescentic glomerulonephritis can be a kidney limited disease or may represent only one of the major target organ involvement in systemic vasculitides, including Wegener's granulomatosis, microscopic polyangiitis and Churg-Strauss syndrome (1, 7). The incidence of pauci-immune small vessel vasculitis in different organs varies, but pulmonary-renal syndrome is a common clinical manifestation of all forms of ANCA systemic vasculitides. Small blood vessels never show uniform diffuse, but multifocal irregular involvement, limited occasionally to only a few vascular segments, a small number of glomeruli or the involvement can be widespread, affecting the majority of glomeruli and numerous segments of extraglomerular intrarenal small blood vessels.

Microscopic polyangiitis is characterized by pauci-immune (2+ or less staining for immunoglobulins semiquantitatively scaled from 0 to 4+) necrotizing inflammation of small blood vessels, predominantly capillaries, arterioles and venules involving, with varying incidence, the digestive tract, skeletal muscles, heart, spleen, liver, skin, lungs and kidneys. Fibrinoid necrosis of the glomerular capillary loops is occasionally accompanied by mild leukocyte exudation. Leukocytoclastic small vessel vasculitis can be seen in the skin and alveolar capillaritis with a consequent intra-alveolar hemorrhage in the lungs, occasionally massive. In the kidneys, necrotizing crescentic glomerulonephritis is characterized by more or less widespread necrosis of the glomerular capillary loops, accompanied by an unfavorable extracapillary capsular crescentic reaction as a consequence. There is a frequent coincidence of active (acute) necrotizing exudative and proliferative inflammation, as well as chronic sclerosing irreversible scar lesions, which is in line with clinical observation of the natural or therapy modified course of the disease, showing more or less frequent recurrences and remissions (1).

According to the literature, in the acute stage of the disease ANCA are found in the serum of 82-90% of patients, in more than 80% P-ANCA with antigen specificity for myeloperoxidase (MPO) and in 12% for proteinase 3 (PR3), and even less frequently for other antigens (8).

Isolated pauci-immune crescentic glomerulonephritis (renal limited vasculitis) can represent only a variant of microscopic polyangiitis limited to the kidneys, with a

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similar profile of ANCA, usually P-ANCA with MPO antigen specificity (8, 9).

Wegener's granulomatosis shares similarities with microscopic polyangiitis with regard to pauci-immune small vessel vasculitis of various organs, including alveolar and glomerular capillaritis. However, the distinguishing features are irregular necrotizing granulomas of the upper and lower respiratory tract, including the nasopharynx, paranasal sinuses, trachea and lungs. In about 90% of patients with fully developed Wegener's granulomatosis, ANCA are found in the serum, in a great majority C-ANCA with antigen specificity for proteinase 3 (PR3), in about 10% P-ANCA with antigen specificity for MPO and only exceptionally specificity for other antigens (1, 8). Wegener's granulomatosis limited to the respiratory tract has been suggested to be a special variant of disease (1). It seems possible that Wegener's granulomatosis starts as a granulomatous inflammation in the respiratory tract with negative ANCA, which proceeds over years to ANCA positive systemic vasculitis (9, 10).

Churg-Strauss syndrome is by far the least common ANCA positive systemic pauci-immune necrotizing small vessel vasculitis, associated with granulomatous inflammation, usually with a less severe involvement of the kidneys. There are discriminatory features that enable differentiation from Wegener's granulomatosis: lower incidence of ANCA in the serum, usually with antigen specificity for MPO and, particularly, eosinophylia in the blood and tissues as well as bronchial asthma (1).

Our studies of ANCA vasculitis and significance of kidney biopsy

In patients with clinically suspected systemic vasculitis or rapidly progressive glomerulonephritis a differential diagnosis of extracapillary crescentic glomerulonephritis has to be solved by a nephropathologist exploring a kidney biopsy. Classification into one of the three main groups of crescentic glomerulonephritis has to be the first stage in the diagnostic procedure, which can usually be already achieved by traditional light microscopy (10). Various forms of immune complex glomerulonephritis, diagnosed in our biopsy files in 34.4% of 285 patients with crescentic glomerulonephritis, have in common a significant glomerular hypercellularity, in addition to extracapillary crescents. Anti-GBM (40-14.0%) and ANCA extracapillary glomerulonephritis (147-51.5%) show in addition to extracapillary crescents, fibrinoid necrosis, destruction and collapse of the glomerular capillary loops, usually widespread diffuse in anti-GBM glomerulonephritis and focal or irregular diffuse, showing lesions in various stages, in ANCA positive glomerulonephritis. Confirmation, further diagnostic separation and definite diagnosis are based on supplementary immunofluorescence and electron microscopical studies, as well as on clinical-pathological correlation (Table 1).

Table 1. Clinico-pathologic diagnosis in 135 patients with ANCA-associated glomerulonephritis

Clinico-pathologic diagnosis	No. of patients	ANCA antigen specificity		
		PR3 (n=55)	MPO (n=74)	Other (n=6)
Wegener's granulomatosis	56	47	8	1
Microscopic polyangiitis	50	6	42	2
Kidney-limited pauci-immune crescentic GN	28	2	23	3
Churg-Strauss syndrome	1	0	1	0

Kidney biopsy is usually of limited value in the differential diagnosis of various ANCA positive pauci-immune vasculitides, because true diagnostic interstitial granulomas in the kidney are very rare (Table 2) and serial sections frequently demonstrate only a periglomerular and perivascular granulomatous reaction, which is not diagnostic. According to our results, the finding of extraglomerular renal vasculitis suggests systemic vasculitis (Table 2).

Table 2. Comparison of histologic changes in 135 ANCA positive patients with renal involvement grouped in relation to ANCA antigen specificity

Histologic changes	ANCA antigen specificity			P value (PR3 vs. MPO)
	PR3 (n=55)	MPO (n=74)	Other (n=6)	
Glomerulonephritis - focal[a]	30 (54.5%)	18 (24.3%)	3/6	<0.001
diffuse[a]	24 (43.6%)	55 (74.3%)	2/6	
mesangial proliferation[b]	1.3±0.8	1.2±0.8	1.3±0.5	0.332
necrosis[b]	1.8±1.3	1.3±1.2	0.8±0.9	0.013
exudative reaction[b]	1.2±1.2	0.8±0.9	0.5±0.8	0.137
thrombosis[a]	13 (23.6%)	9 (12.2%)	1/6	0.093
extracapillary proliferation[c]	38.5±30.1	43.6±28.0	37.5±43.3	0.334
global sclerosis[c]	11.5±11.8	24.0±23.2	17.7±17.4	<0.001
segmental sclerosis[c]	7.0±11.0	13.9±14.2	4.2±10.2	0.002
Extraglomerular vasculitis - active[a]	12 (21.8%)	17 (23.0%)	1/6	0.877
chronic[a]	5 (9.1%)	10 (12.2%)	1/6	0.438
Interstitial cell infiltration[b]	2.5±1.1	2.9±0.7	2.7±1.0	0.013
Interstitial fibrosis[b]	1.3±1.1	2.2±1.2	2.2±1.0	<0.001
Interstitial granuloma[a]	3 (5.5%)	2 (2.7%)	1/6	0.433
Periglomerular or perivascular granuloma[a]	1(1.8%)	13(17.6%)	1/6	0.007

The variables are expressed as [a]proportions (%), [b]mean semiquantitative values ±SD, [c]mean percentage of involved glomeruli ±SD

It must be stressed that a kidney biopsy report must include the results of quantitative and semiquantitative assessment of active (acute), potentially reversible glomerular and extraglomerular lesions (expressed as a percentage of glomeruli involved by necrosis, exudation, proliferation, cellular and fibrocellular crescents), as well as chronic sclerosing irreversible changes. The histopathologic scoring system used in our studies (9) shares many similarities with that introduced by the European Study Group (11, 12, 13). Qualitative and precise quantitative data included in the kidney biopsy report by an experienced nephropathologist are the best indicators for clinical decisions about the most appropriate therapy planning, evaluation of the therapeutic results in rebiopsies and prognostic assessment.

Our study of 135 patients with renal involvement confirmed that a great majority of patients with a clinical-pathological diagnosis of Wegener's granulomatosis are PR3-ANCA-positive (47/56=83.9%) whereas the majority of patients with microscopic polyangiitis (42/50=84.0%) and renal limited vasculitis / isolated pauci-immune crescentic glomerulonephritis (23/28 = 82.1%) are MPO-ANCA positive (Table 1).

Furthermore, our biopsy study of 135 ANCA-positive patients (9), in agreement with the results of the European Vasculitis Study Group (12) shows that differences between PR3-ANCA and MPO-ANCA subgroups of patients are not qualitative but quantitative (Table 2). Focal glomerulonephritis was found to be more frequent in the PR3-ANCA subgroup, and diffuse glomerulonephritis in the MPO-ANCA subgroup. At the time of presentation, active glomerular inflammatory changes, e.g., fibrinoid necrosis, exudative reaction, capillary thrombosis and cellular crescents, were observed more frequently and extensively in the PR3-ANCA subgroup, whereas fibrous crescents, glomerulosclerosis and interstitial fibrosis were found to be significantly more prominent in the MPO-ANCA subgroup. Suggestive interstitial granulomas were more frequently associated with PR3-ANCA, a periglomerular and perivascular granulomatous reaction with MPO-ANCA. According to our findings, the presence of glomerular renal vasculitis suggests systemic vasculitis. Hauer et al (12) conclude that the pathogenesis of renal disease in these two ANCA subsets could be different. We believe that the quantitative differences in histopathology between the two subgroups can be explained by the observation that the kidney biopsy in PR3-ANCA patients with Wegener's granulomatosis, which primarily affects the respiratory tract, was performed soon after the first symptoms of renal involvement appeared, whereas in MPO-ANCA patients, kidney biopsy was done late in the course of the disease (9).

Focal segmental and focal global thrombotic microangiopathy-like glomerular changes were not infrequently observed in our study of ANCA-positive pauci-immune glomerulonephritis and systemic vasculitides, which has not been mentioned in other similar biopsy studies. We assume that endothelial cell swelling and subendothelial edema represent an early stage in the pathogenesis of ANCA induced fibrinoid necrosis.

Nevertheless, there are a few cases reports of a coincidence of clinical/laboratory and histologic lesions of hemolytic uremic syndrome and ANCA rapidly progressive glomerulonephritis (14).

There are only a limited number of kidney biopsy long-term follow-up studies in ANCA-associated vasculitis so far (12). An as yet unpublished study of 38 ANCA vasculitis patients with 46 rebiopsies, performed mostly due to a clinical suspicion of a recurrence, demonstrates significantly lower incidence and extension of active inflammatory changes and, on the other hand, an increase in the extent of glomerulosclerosis and interstitial fibrosis in the rebiopsy (Table 3). It appears that focal extracapillary crescentic glomerulonephritis in the first biopsy fairly frequently proceeds into diffuse, although the appropriate therapy, with a high dose of corticosteroids in combination with cyclophosphamide, not only significantly improves renal and overall patient survival but also allows part of the active lesions to revert to a normal phenotype, as demonstrated by the study by Hauer et al (12).

Table 3. Comparison of histologic changes in the first renal biopsy and rebiopsies of 38 patients with ANCA vasculitis

Histologic changes	First renal biopsy (n=38)	Rebiopsies (n=46)	P value
Normal histology	0	1 (2.2%)	
Glomerulonephritis - focal[a]	14 (36.8%)	11 (23.9%)	0.220
diffuse[a]	24 (63.2%)	34 (73.9%)	
- active[a]	11 (28.9%)	0	
active and chronic[a]	24 (63.2%)	16 (34.8%)	<0.005
chronic[a]	3 (7.9%)	29 (63.0%)	
Necrosis[b]	1.7±1.1	0.3±0.6	<0.005
Extracapillary crescents[c]	47.2±24.13	18.6±23.7	<0.005
Global sclerosis[c]	15.7±15.4	39.2±23.9	<0.005
Segmental sclerosis[c]	9.2±10.2	15.2±12.3	0.016
Extraglomerular vasculitis - active[a]	9 (23.6%)	2 (4.4%)	0.009
- chronic[a]	4 (10.5%)	6 (13.9%)	0.723
Interstitial fibrosis[b]	1.8±1.3	2.6±1.1	0.004

The variables are expressed as [a]proportions (%), [b]mean semiquantitative values ±SD, [c]mean percentage of involved glomeruli ±SD

Finally, one should be aware that different immune pathogenic mechanisms can coexist in patients with extracapillary crescentic glomerulonephritis. Of 11 of our patients with various forms of immune complex glomerulonephritis, anti-GBM antibodies in the serum and linear immunofluorescence in the kidney biopsy were demonstrated additionally in 5, and ANCA were positive in the serum in the other 6 (Table 4). Development of extracapillary crescents in these 11 patients appears to be related to the presence of anti-GBM and ANCA, respectively. Furthermore, of the total number of 44 patients with anti-GBM associated glomerulonephritis in our files during the period of 1977-2003, 6 (13.6%) patients also had positive P-MPO-ANCA in serum (Table 4) (15). All 6 patients showed a severe diffuse pure extracapillary crescentic glomerulonephritis, in 3 with active necrotizing lesions and crescents and the other 3 with already more advanced sclerosing crescentic glomerulonephritis. Despite aggressive immunosuppressive therapy, the outcome was poor in both recovery of renal function and mortality. Only one patient is still alive and in remission after 64 months of follow-up.

Table 4. Coexistence of pathogenic mechanisms in patients with crescentic glomerulonephritis

Type of glomerulonephritis with crescents	No. of patients	Immune complexes (Granular IF)	Anti-GBM (Linear IF)	ANCA (Pauci-immune)
Membranous GN	2	2	2	
Focal lupus GN	2	2		2
IgA nephropathy	5	5	2	3
Henoch-Schönlein GN	1	1	1	
Proliferative GN	1	1		1
Pure crescentic GN	6		6	6

Abbreviations: GN – glomerulonephritis, IF - immunofluorescence

Conclusions

ANCA monitoring and the assessment of ANCA antigen specificity, as well as kidney biopsy qualitative and quantitative evaluation play an important role in diagnosis and classification of small vessel pauci-immune vasculitides, as well in the most appropriate therapy planning and follow-up of the disease course. Our study confirms that a great majority of PR3-ANCA positive patients present with Wegener's granulomatosis and most MPO-ANCA positive patients have microscopic polyangiitis and kidney-limited pauci-immune crescentic glomerulonephritis, respectively.

There are quantitative and not qualitative histopathologic differences between PR3-ANCA and MPO-ANCA subgroups of patients, established by kidney biopsies at the time of clinical presentation, which suggests a common pathogenesis of ANCA-associated diseases but a different clinical course of Wegener's granulomatosis, microscopic polyangiitis and renal limited pauci-immune crescentic glomerulonephritis.

Although a combination of corticosteroid and cyclophosphamide therapy has been suggested, to allow part of the active histologic lesions to revert to normal phenotype, it appears that the majority of glomeruli presenting extensive necrosis and extracapillary crescents proceed into glomerulosclerosis. Our study shows that focal active crescentic glomerulonephritis in the first kidney biopsy of ANCA-positive patients fairly often turns into diffuse sclerosing, with or without active inflammatory changes, usually of a limited extent.

In patients with various biopsy proven forms of immune-complex glomerulonephritis with a prominent crescentic reaction, as well as in patients with pure extracapillary crescentic glomerulonephritis, screening for anti-GBM and ANCA should be undertaken due to the possibility of coexisting pathogenic mechanisms.

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