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Original Article

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ANCA-negative pauci-immune renal vasculitis: histology and outcome

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Abstract

Background. Pauci-immune renal vasculitis with focal glomerular necrosis and crescent formation is usually associated with anti-neutrophil cytoplasmic antibodies (ANCAs). However, ANCA's are absent in up to 10% of cases, which constitutes a rarely studied variant of renal vasculitis.

Methods. This retrospective multicentre cohort study analyzed the presenting features, renal histology and outcome in 20 patients with pauci-immune crescentic necrotizing renal vasculitis in whom indirect immunofluorescence did not detect ANCA.

Results. Renal histology revealed a high percentage of active glomerular lesions (50%), mainly cellular crescents, 28% of them with glomerular necrosis. Chronic tissue damage with glomerulosclerosis (21%) and diffuse interstitial fibrosis (40%) was already present at diagnosis, more prominent than in historical PR3-positive patients. Infiltrates of polymorphonuclear neutrophils in glomerular capillary loops were observed in 40% of all biopsies, mainly in necrotic lesions. The subsets of interstitially infiltrating leukocytes similar to ANCA-associated disease. Microscopic polyangiitis was diagnosed in 17 patients, Wegener's granulomatosis in two and renal-limited vasculitis in one. The patients median disease extent index (DEI) of 5 (range 4–11) reflected a systemic vasculitis. ANCA-negative vasculitis was not associated with infection or malignancy. Renal outcome was correlated to DEI ($P = 0.032$) and serum creatinine at diagnosis ($P = 0.04$). The mortality rate was high (35%) and closely related to age above 65 years at diagnosis ($P = 0.014$).

Conclusions. The histological findings and prognosis in ANCA-negative renal vasculitis are comparable with those of ANCA-positive disease. Our data underline the importance of the exact diagnosis in an active vasculitic disease process even in the absence of ANCAs.

Keywords: ANCA-negative; microscopic polyangiitis; neutrophils; pauci-immune renal vasculitis; renal biopsy; renal outcome; Wegener's granulomatosis

Introduction

Pauci-immune crescentic necrotizing glomerulonephritis (GN) is defined histologically by the presence of focal glomerular necrosis and extracapillary proliferation in the absence of significant glomerular immune deposits. This type of GN, which represents a frequent cause of acute renal failure, is due to small vessel vasculitis. It occurs during systemic diseases such as microscopic polyangiitis (MPA), Wegener's granulomatosis (WG) and more rarely Churg–Strauss syndrome or as a renal-limited vasculitis (RLV) [1].

The association of anti-neutrophil cytoplasmic antibodies (ANCAs) with WG, described in 1985 [2], was a major step in distinguishing pauci-immune crescentic necrotizing GN from other types of rapidly progressive GN, e.g. post-infectious, cryglobulinaemic, anti-glomerular basement membrane, IgA nephropathy and lupus nephritis.

Indirect immunofluorescence (IIF) [p-ANCA for myeloperoxidase (MPO); c-ANCA for proteinase 3 (PR3)] is highly sensitive to identify ANCA, in active untreated WG or MPA, with a positive test in >75–90% of patients [3–5]. Because of the strong association between disease and ANCA's, numerous studies have proposed a pathogenic role for ANCAs in the development of vasculitis involving small vessels [6].

However, in a significant number of cases of pauci-immune crescentic GN, ANCAs are absent. Earlier studies, comparing those with ANCA-positive cases

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suffer, because ANCA-negative groups are small or heterogeneously defined [3,7]. Therefore, we describe the clinical course, histological features and outcome in 20 patients with pauci-immune crescentic GN and a consistently negative test for ANCA.

These results will be compared to existing data of an ANCA-positive cohort.

Patients and methods

Patients and selection criteria

This retrospective study identified 20 patients who presented between 1990 and 2001 with a pauci-immune crescentic necrotizing GN and in whom no evidence for circulating ANCAs was found by immunofluorescence (IF) testing. The patient recruitment was performed in collaboration with pathologists and clinicians in three French hospitals from a cohort of ~625 patients with ANCA-associated vasculitis (17% anti-PR3/79% anti-MPO). In each patient, a renal biopsy had been performed at the time of diagnosis, and it was analysed for the purpose of this study. All patients' records were reviewed and relevant data were extracted.

Immunological data

Testing for ANCA was performed by IIF in ethanol-fixed neutrophils at initial clinical presentation and was obtained regularly during follow-up, particularly during relapses of vasculitis. Before 1997, ANCA tests were performed using an in-house method. After 1997, we used a commercial kit (Euroimmun, Germany, distributed by Bioadvance in France).

Tests for anti-MPO and anti-PR3 antibodies were performed in all sera available by enzyme-linked immunosorbent assay (ELISA; Euroimmun, Germany). In addition, in four cases (two WG, two MPA), a capture-ELISA technique (performed in the laboratory of A. Wiik) was used to confirm ANCA negativity. Other immunological markers are reported if performed.

Clinical data

Acute renal insufficiency was defined as an increase of at least 30% of baseline serum creatinine in <3 months. The glomerular filtration rate (GFR) was calculated using the Cockcroft–Gault formula, considering the highest initial creatinine at diagnosis and the latest available creatinine value during follow-up. Nephrotic syndrome was defined as proteinuria >3.5 g/day and a serum albumin <30 g/l. Arterial hypertension was defined as a blood pressure >140/90 mmHg or if antihypertensive medication was needed. MPA, WG and RLV were diagnosed according to the Chapel-Hill consensus conference criteria [8]. For classification purposes, chest and or sinus X-ray results were evaluated from all patients. Additionally, the results of any biopsy of extra-renal organs were considered.

Disease activity at initial clinical presentation was evaluated by using the Birmingham Vasculitis Assessment Score (BVAS) [9]. Briefly, the BVAS consists of a list of items that are predominantly based on clinical history and examination, supported by certain laboratory data such as serum creatinine and the presence and absence of blood or protein in the urine. Vasculitis-related organ involvement was assessed using the disease extent index (DEI) [10].

Renal biopsies

Paraffin-embedded renal sections were stained with trichrome, silver, periodic acid–Schiff and haematoxylin/eosin. IF microscopy results were available for all patients included in the study. The IF studies were performed by a classic direct IF technique using antibodies against IgA, IgG, IgM, C3, C1q, fibrin, albumin, and κ and λ light chain. The tested antisera either did not stain or the staining was scattered and thus were insufficient to suggest any main diagnosis other than pauci-immune crescentic GN.

All biopsies were reviewed by one experienced pathologist (L.H.N.) and renal lesions representative of disease activity and chronicity were scored using a standardized protocol for ANCA-associated systemic vasculitis as reported elsewhere [11]. These results were compared with existing historical data on ANCA-positive pauci-immune renal vasculitis [7] which were evaluated by the same histological scoring system.

In short, each glomerulus was scored separately for the presence or absence of fibrinoid necrosis, crescents (cellular-segmental, cellular-circumferential, fibrous) and global sclerosis. The number of glomeruli revealing these lesions was expressed as the percentage of the total number of glomeruli (100%) in the biopsy. The percentage of normal glomeruli was scored as well.

Other lesions were scored semi-quantitatively as mesangial proliferation, capillary loop infiltrates (0 = <5, 1 = >5 inflammatory cells per glomerulus), interstitial infiltrates (0 = absent, 1 = <20%, 2 = 20–50%, 3 = >50%) and interstitial fibrosis (0 = absent, 1 = focal, 2 = diffuse).

To assess cellular tissue infiltration, T cells and macrophages were stained immunohistochemically with, respectively anti-CD3 (dilution 1/300) and anti-CD68 (dilution 1/1000) (Dako) and were compared. Neutrophil cell infiltration (PN) per glomerular section or per interstitial infiltrate was calculated by the characteristic nuclear morphology. Arteries and arterioles were evaluated for the presence of vasculitis, arterial hyalinosis or sclerosis.

Statistics

All statistical analyses were carried out using the SPSS package for windows. Descriptive statistics were performed with standard tests. Kaplan–Meier curves were used to evaluate the influence of histological and selected clinical parameters on survival; the significance level was tested by log-rank. To identify variables predictive of renal outcome at follow-up, partial correlation for GFR change during follow-up adjusted to age was tested for histological and clinical parameters (two-tailed, significance level $P \leq 0.05$).

Results

Twenty patients were identified (15 males and five females) with a median age of 65 years at presentation (range 33–82 years).

Renal involvement (Table 1)

The majority of patients presented with acute renal insufficiency (17 out of 20 patients). Six of them showed acute renal failure with a creatinine >500 μmol/l; four required short-term haemodialysis at diagnosis (1–2 weeks). Median serum creatinine at presentation was 267.5 μmol/l (range 62–1219). Microhaematuria was present in all cases. The median level of proteinuria was 2.7 g/day (range 0.44–11). Nephrotic syndrome was present in four cases. Of the 20 patients, 11 were hypertensive. The median time between the appearance of first symptoms and renal biopsy was 2 months (range 0.5–36).

Extra-renal involvement

Seventeen out of 20 patients showed constitutional symptoms of vasculitis including fatigue, night sweats, weight loss and fever. Myalgia or arthralgia or both, were present in eight patients, and biopsy-proven muscle vasculitis in one. Clinical signs of vasculitic skin involvement were observed in 10 cases, and biopsy-proven skin vasculitis in seven. Sinusitis or rhinitis or both, associated with epistaxis in one, was judged as ENT involvement in four patients, corresponding histologically to giant cell granuloma in one and to non-specific inflammation in two patients.

Pulmonary infiltrates revealed by X-ray were seen in three cases, with cavitated nodules in two. Pericardial effusion with histological signs of inflammation and haemodynamic depression, necessitating drainage, was present in two patients, and minor effusion was present in one other patient. Ischaemic bowel disease was present in two cases. Three cases had neurological involvement in the form of peripheral neuropathy. Intracerebral haematoma was considered to be an initial clinical sign of vasculitis in patient 7, without any angiographic evidence of aneurism formation.

Altogether, in 11 patients extrarenal biopsies showed vasculitis. The median BVAS score was 18.5 (range 14–29). The median DEI score was 5 (range 4–11), representing the involvement of at least two organs.

Diagnostic classification

MPA was diagnosed in 17 out of 20 patients. Two patients were classified as WG (patients 1 and 7) and one patient as RLV (patient 2).

Concomitant diseases

Monoclonal gammopathy of undetermined significance was diagnosed in patients 5 and 14. Patient 4 had an alcohol-induced cirrhosis without signs of portal hypertension at initial presentation. Two patients had pulmonary embolism (patient 1 after the diagnosis of vasculitis and patient 3 a few weeks before). No medication known to induce a vasculitis has been identified retrospectively in the cohort. Infection with hepatitis B or C, nor any other virus, nor a concomitant neoplasia was observed.

Immunology

IF testing for ANCAs was negative in all patients at diagnosis, before the initiation of immunosuppressive therapy, and remained negative during follow-up. ELISA tests for anti-MPO or anti-PR3 or both were negative in all 11 tested patients. In all WG patients, additionally performed capture-ELISAs were negative. Anti-nuclear antibodies were positive in three patients out of 19, whereas anti-DNA antibodies were not detected in any of the patients (see Table 1). Antiphospholipid antibodies were positive (low range titre) in three out of 11 patients, and lupus anticoagulant was absent in four tested patients. Anti-GBM antibodies were negative in five tested patients. Cryoglobulinaemia was detected in three out of 16 patients, associated with a monoclonal gammopathy in one. Serum complement component levels were normal in all patients (data not shown).

Renal histology

Fifty percent of glomeruli had (predominantly cellular) crescents, 21% were globally sclerotic and 29% were normal (Table 2). Fibrinoid necrosis of the glomerular tuft was present in 28% of glomeruli with crescents. In two biopsies, necrosis was present in glomeruli without crescents. Mild capillary loop infiltration was observed in 75% of all biopsies, partly with the presence of polymorphonuclear cells. In five patients, polymorphonuclear neutrophils were observed in necrotic lesions. In three other patients, they were also present in the capillary lumen (more than five neutrophils per glomerulus), though this infiltration was irregularly distributed amongst the glomeruli.

Interstitial fibrosis was present in 80% of biopsies and, in half of these cases, it was scored as diffuse. Interstitial infiltrates were seen in 95% of all biopsies, being moderate or intense in 45%. In the later case, cellular typing revealed a mixed infiltrate of lymphocytes (CD3-positive), macrophages (CD68-positive) and PNs. Hyalinosis and arteriolosclerosis were present in 45 and 40% of all biopsies, respectively.

IF studies showed the absence of significant immune deposits in all but two patients. In case 7, IF studies showed rare irregular epimembranous IgG deposits in glomerular focal necrosis and crescents. In case 20, mild irregular glomerular C3 deposits were present on the initial biopsy. However, these deposits were absent in two subsequent biopsies performed because of persistent clinical disease. In two biopsies, giant cells (patients 1 and 15) were found, in patient 1 in combination with vascular granuloma.

Three patients had follow-up biopsies (time interval 6–12 months after initial biopsy), which revealed

Table 1. Patient characteristics: clinical and renal presentation at diagnosis, treatment and outcome

Patient	Age at presentation	Sex	Diagnosis	Renal involvement[a]	Disease extent[b]	Initial BVAS	Initial DEI	Maximal creatinine at diagnosis (μmol/l)	24 h urinary protein excretion	BP at presentation (mmHg)	Other immunological markers[c]	Treatment[d]		Duration of follow-up	Current/last creatinine	Outcome
												Initial	Current			
1	72	M	WG	ARF	K, L, A, B	21	5	584	0.44	121/65*	ANA 1/100	PC	PC	12 months	330	Stable renal function
2	43	F	RLV	ARF, oliguric	K, B	14	5	1219	0.75	170/110	ANA 1/80	PC, dialysis (PA)	None	10 years	79	Recovery
3	82	F	MPA	ARI, non-oliguric	K, S,	14	4	404	3	110/60	–	PC	Died	2 months	265	Death
4	53	M	MPA	ARI	K, S, A, B	17	7	210	1.62	140/80*	Antiphospholipid 28 U of GPL/ml	PC	P	2 years	187	Stable renal function
5	64	M	MPA	ARI, nephrotic syndrome	K, P, B	20	5	268	11.1	170/90	–	PC	PC	14 months	136	Stable renal function
6	33	M	WG	Proteinuria	K, E, L, A, P, B	29	9	115	1.75	Normotensive	Cryo pos.	PC	None	10 years	119	Recovery
7	62	M	MPA	Proteinuria	K, A, S, H, B	23	10	62	3.35	140/80*	–	PEX, PC	None	3 years (+4)	74	Recovery
8	54	M	MPA	ARI	K, S, H, B	20	5	275	1.8	120/80	–	PC/PA	None	2 years	152	Stable renal function
9	73	F	MPA	ARI, oliguric	K, S, GI, B	25	6	245	7.85	150/80	–	PC	None	2 years	142	Stable renal function
10	55	M	MPA	ARI	K, E, A, P, B	27	11	123	0.82	Normotensive	Cryo pos.	PC	None	3 years	89	Recovery
11	67	F	MPA	Proteinuria, ARI	K, L, B	18	5	71	3	Normotensive	Antiphospholipid, 16 U of GPL/ml	PC	Died	6 months	62	Death (sepsis)
12	46	M	MPA	Nephritic syndrome, ARI	K, H, B	17	5	200	1.1	140/80	–	PC	None	6 years	106	Recovery
13	66	M	MPA	Proteinuria, ARI	K, H, A,	18	7	196	3.42	138/80	–	PC	P	1 year	162	Stable renal function
14	81	M	MPA	Proteinuria, ARI	K, S, B	14	4	196	3.24	127/54*	Antiphospholipid. 18 U of GPL/ml, ANA 1/160	None	Died	1 year	200	Death (cardiac)
15	77	M	MPA	Proteinuria, ARI	K, E, B	21	6	443	2	145/78*	–	PC/PA	Died (PC)	11 months	194	Death (cardiac)
16	76	F	MPA	ARF, oligoanuric	K, S, B	18	5	643	1.68	150/80*	ANA 1/640	PC	Died (P)	7 months	162	Death (sepsis)
17	77	M	MPA	ARF, nephrotic syndrome	K, S, B	14	5	692	4.6	160/120	–	P	Died	3 months	300	Death
18	76	M	MPA	ARF non-oliguric	K, GI, S, A, B	24	7	743	2.4	140/90*	Anti-TPO pos.	PC	Died	1 month	706 (dialysis)	Death (vasculitis)
19	50	M	MPA	ARF, oliguric	K, E, A, B	17	6	843	6	Normotensive	–	P/Bactrim	None	6 years	Dialysis	Dialysis
20	60	M	MPA	Nephrotic syndrome	K, S, A	19	5	267	5.87	122/59	–	P	None	2 years	162	Stable renal function

[a]MPA = microscopic polyangiitis; WG = Wegener's granulomatosis, RLV = renal-limited vasculitis; ARF = acute renal failure; ARI = acute renal insufficiency.
[b]K = kidney; S = skin; E = ENT; A = arthralgia/myalgia; GI = gastrointestinal; P = peripheral nervous system; C = central nervous system; H = heart; L = lung and lower airway, B = constitutional symptoms.
[c]ANA = antinuclear antibodies; antiphospholipid = antiphospholipid antibodies; cryo = cryoglobulin; anti-TPO = anti-thyroid peroxidase antibodies.
[d]P = prednisone/methylprednisolone; C = cyclophosphamide; A = azathioprine; PEX = plasma exchange.

Table 2. Glomerular and interstitial lesions at initial biopsy of 20 ANCA-negative patients

	Glomerular lesions		Interstitial lesions				
	$n \pm$ SD	%		Grading (% of all biopsies)			
				0	1	2	3
No. of glomeruli/biopsy	17 ± 13.1	100	Fibrosis	20	40	40	–
Normal glomeruli	5 ± 3.9	29.4	Cellular infiltration	5	50	30	15
Glomerular sclerosis	3.6 ± 3.7	20.9					
Crescents	8.4 ± 11.5	49.7	Cellular infiltration	0/1	2/3		
Cellular circumferential	4.1 ± 10.3	24.1	Subtype				
Cellular segmental	2.7 ± 2.2	15.9	PN <5	10/11	2/9		
Fibrotic	1.6 ± 3.4	9.1	PN >5	1/11	7/9		
Necrotic glomeruli	8.4 ± 10.3	28.5	Type[a]				
Capillary loop infiltration	–	80	CD68 = CD3	–	5/9		
PN infiltrates			CD68 < CD3	–	2/9		
<5/glomerulus	–	70	CD68 > CD3	–	1/9		
>5/glomerulus	–	30					

[a]Only cellular infiltrates grade 2–3 were assessed for CD68 and CD3 staining.

active lesions in two as signs of persistent disease or relapse and predominantly chronic lesions in one patient.

Treatment

The majority of patients (16 out of 20) received a primary immunosuppressive protocol with oral and with or without intravenous steroids, and cyclophosphamide pulse therapy. Patient 8 had plasma exchange. Patient 19 received high doses oral prednisone and oral trimethoprim/sulfamethoxazole.

Three patients were treated initially with steroids only: patients 14 and 18 because of their age and patient 20 as it was post-infectious GN that was suspected initially. After a period of 6–12 months, cyclophosphamide was substituted by azathioprine in two patients, and by trimethoprim/sulfamethoxazole in one. Ten patients received low-dose steroid maintenance therapy only (median duration: 18 months, range 4–24).

Outcome

Survival was significantly determined by age at diagnosis (see Figure 1). Seven patients (35%) died, all in the first year following the diagnosis of vasculitis (median age 76.6 years *vs* 56.2 years in long-term surviving patients). Causes of death were myocardial infarction in two, septic shock in two and intestinal haemorrhage due to uncontrolled vasculitis in one patient. In two patients, the cause of death remained undetermined. In three cases, renal function improved prior to death.

Renal outcome was defined as the change of GFR during follow-up. Median GFR at the latest visit was 26 ml/min (range 4–49.5). Only one patient progressed to end-stage renal failure.

Initial creatinine and the DEI were significantly correlated with renal function change, adjusted for age

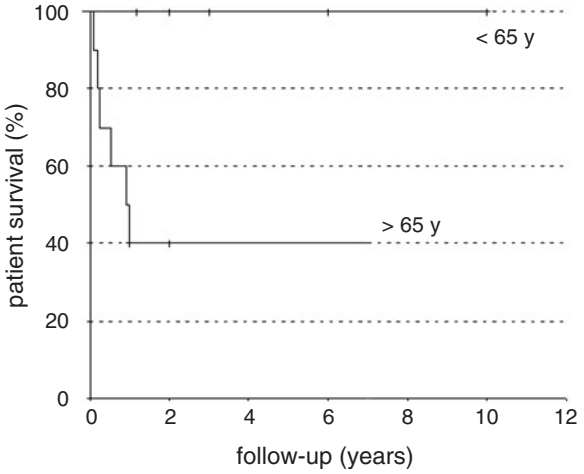

Fig. 1. Patient survival was dependent on age at diagnosis in ANCA-negative vasculitis. Median patient age of 65 years dichotomized the two groups ($P < 0.014$; $n = 10$ for each group).

($r = -0.49$, $P = 0.042$; $r = 0.47$, $P = 0.032$). Histological scores, like the percentage of normal glomeruli ($r = 0.07$, $P = 0.77$), crescents ($r = 0.10$, $P = 0.67$) or glomerular necrosis ($r = 0.41$, $P = 0.085$), did not correlate with renal outcome.

Relapse of disease requiring the reintroduction or an increase of immunosuppressive therapy occurred in 20% of all patients, with renal involvement in four out of five cases. The median time interval between diagnosis and relapse was 8.5 months (range 4–12).

Discussion

Pauci-immune necrotizing small vessel vasculitis is usually associated with the presence of ANCAs directed to proteinase 3 (PR3-ANCA) or myeloperoxidase (MPO-ANCA) [1,2].

More rarely ANCA's are absent in this type of vasculitis [12,13]. In WG, ANCAs may remain negative in the limited ENT form (granulomata) of the disease. However, ANCAs are usually detected when the disease progresses to a systemic vasculitic stage [14].

Published data regarding kidney involvement and outcome in ANCA-negative pauci-immune vasculitis are scarce and often lack a clear-cut proof of ANCA negativity [3,7]. One of the reasons is the difficulty of establishing the absence of ANCAs. We analyzed only patients with consistently negative ANCAs on IIF.

As recommended by the international consensus conference, we used IIF as first line diagnostic tool, because of its high sensitivity for detecting ANCAs [5,15,16]. Although the false-negative rate of IIF is relatively low [16], we have confirmed negative IIF by specific ELISAs for anti-PR3 and anti-MPO in the majority of patients.

In our ANCA-negative patients, there is a clear predominance of patients with MPA (85%). This finding could be related to the high incidence rate of MPA among pauci-immune vasculitis in Southern Europe [17], whereas WG is much more common in Northern Europe [18]. On the other hand, a Swedish vasculitis case-finding study [19], which considered only patients from a nephrology department, found more cases of MPA (79%) than WG (21%) in their cohort. Therefore, we cannot rule out that the predominance of MPA in our study group reflects selection bias.

ANCA-negative pauci-immune GN is part of a systemic vasculitic disease process. The latter usually involves additionally at least one extra-renal organ system as shown by the high DEI score of 5 for our comparable with cohort, a score comparable with that of ANCA-positive vasculitis [10]. Nearly all our patients presented with constitutional symptoms. Interestingly, we observed a high prevalence of extra-renal signs of skin (50%), joint and muscle (40%) involvement. Only a minor percentage presented with ENT and pulmonary symptoms. In an earlier study of 98 patients with ANCA-associated vasculitis [4], ENT and lung involvement was predominant, with a high prevalence of pulmonary haemorrhage (40%) in MPO-positive patients.

Renal vasculitic involvement presented with typical clinical signs in our ANCA-negative cohort. The absence of deposits in IF studies of renal biopsies excluded a post-infectious or immune complex origins of the disease. Scant deposits were observed in two patients. Patient 20 presented a C3 positivity for IF, which disappeared on two subsequent biopsies taken during persistent clinical disease activity, and patient 7 had segmental epimembranous deposits with a nephrotic syndrome at diagnosis. The coincidence of crescentic GN and membranous nephropathy is rarely observed. Tse *et al*. described 10 cases of membranous nephropathy complicated by vasculitic glomerulonephropathy with negative ANCA tests in five cases [20]. They observed a more aggressive clinical course than in membranous nephropathy alone.

Active renal disease was evident in all kidney biopsies in the present series.

We compared our findings to those of 58 MPO-ANCA-positive and 63 PR3-ANCA-positive patients of a recent multicentre study from the EUVAS (European vasculitis) network, using identical histopathological scoring criteria [7]. Active renal lesions, as assessed by the presence of crescents and fibrinoid necrosis and interstitial infiltrates, were similar in all serological subgroups (Figure 2a).

Severe interstitial fibrosis and glomerulosclerosis were more prominent in ANCA-negative and MPO-positive patients compared with PR3-positive patients (see Figure 2a and b).

A possible explanation is that MPO-positive and ANCA-negative disease might be entities that lead to rapid progression of irreversible renal lesions. It is more likely, that in patients with MPO-positive or ANCA-negative vasculitis, a delay in diagnosis exposes the kidney to a chronic smouldering disease activity. Ultimately, a large amount of interstitial fibrosis representing chronic renal damage may be the result.

ANCA-negative renal vasculitis is also characterized by glomerular infiltrating cells in up to 80% of our patients. Minor infiltrates of PN in glomerular capillary loops were observed in almost 40% of all biopsies,

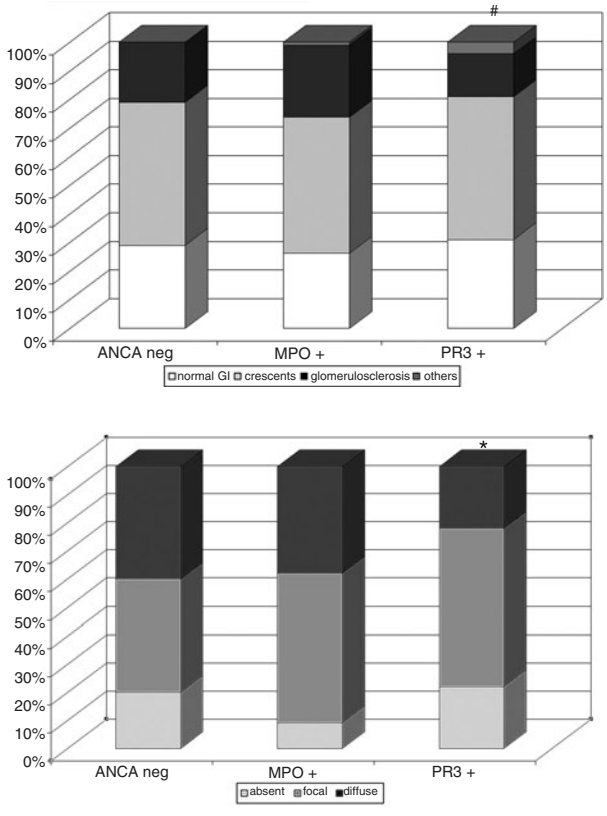

Fig. 2. Renal histology of ANCA-negative compared with MPO- and PR3-ANCA-positive biopsies. (**a**) Glomerular lesions. (**b**) Interstitial fibrosis. ($^{+}$MPO- and PR3-ANCA-positive groups with permission from Hauer *et al*. [7]. $^{\#}$PR3-positive *vs* MPO-positive or *vs* ANCA-negative $P < 0.05$ for glomerulosclerosis. *PR3-positive *vs* MPO-positive or *vs* ANCA-negative $P < 0.05$).

mainly in necrotic lesions. Additionally, we observed moderate to severe interstitial infiltrates in 45% of the biopsies, predominantly a mixed infiltrate of lymphocytes and macrophages with significant presence of PN. Weidner *et al.* [21] identified a comparable composition of cellular infiltrates in a careful analysis of 65 biopsies from ANCA-associated renal vasculitis.

The mechanisms leading to the occurrence of ANCA-negative vasculitis are unclear. In ANCA-positive vasculitis, these auto-antibodies activate neutrophils by different mechanisms (e.g. direct Fab′2 binding to ANCA antigens on leukocyte surfaces, or Fc receptor engagement by ANCA immune complexes) leading to neutrophil and endothelial cell apoptosis and necrosis [6]. However, in ANCA-negative disease, the presence of PN in pathological lesions occurs independently of circulating ANCAs, and may involve other unidentified autoantibodies or T-cell-dependent mechanisms [22].

The mortality rate of our ANCA-negative cohort is 35% over 5 years. In ANCA-positive vasculitis, mortality rates vary from 24 to 41% [3,23,24]. Patient survival was strongly correlated with age in our cohort, which has been already shown for ANCA-associated vasculitis [23,24]. Apparently, co-morbidity from cardiovascular disease and infectious complications due to immunosuppressive therapy accounts for the high mortality rate in older patients independently of the ANCA status.

Renal outcome was related to initial serum creatinine and to DEI, but not to any of the evaluated histological parameters. However, the amount of glomerular fibrinoid necrosis had a tendency to correlate positively with the improvement of renal function in our study. A recent European multicentre study in 160 patients with ANCA-associated vasculitis identified serum creatinine at presentation as the best predictor of renal function after 1 year [25]. Histological parameters, like the amount of normal glomeruli and fibrinoid necrosis, are correlated with renal outcome in ANCA-associated vasculitis [26]. Our failure to show any firm relationship between histological markers and renal outcome in ANCA-negative disease may be due to the limited size of our cohort.

In conclusion, pauci-immune crescentic necrotizing GN can in the absence of ANCAs but with clinical and histological findings indistinguishable from ANCA-associated vasculitis. Renal histology shows extensive chronic renal damage as found in MPO-positive vasculitis.

The initial creatinine, DEI and age are useful parameters for predicting renal or patient outcome in ANCA-negative vasculitis. Our finding of a high mortality rate, especially in older patients, underlines the importance of an exact diagnosis in the absence of ANCAs. Neutrophils may play a pathogenic role even in the absence of ANCAs, although the mechanism of neutrophil activation remains to be elucidated.

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Conflict of interest statement. None declared.

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