ANCA-associated pauci-immune glomerulonephritis: always pauci-immune?

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ABSTRACT

Objective. *Anti-neutrophil cytoplasmic antibody (ANCA)-associated glomerulonephritis (GN) is considered "pauci-immune" with absent or mild glomerular tuft staining for immunoglobulin (Ig) and/or complement. However, it is not unusual to see some immune deposits (ID) within glomeruli on immunofluorescence (IF). We determined to evaluate the prevalence and clinical significance of immune deposits in ANCA-associated GN.*

Methods. *We included all patients with ANCA associated vasculitis with renal biopsies between January 2002 and May 2014: granulomatosis with polyangiitis, eosinophilic granulomatosis with polyangiitis, microscopic polyangiitis and renal limited vasculitis. Patients were divided into Group A: biopsy without ID (≤2+ intensity of immunostaining) and Group B: biopsy with ID (>2+ intensity of immunostaining). Serum creatinine, estimated glomerular filtration rate (eGFR) at time of the biopsy, amount of proteinuria and haematuria, requirement of dialysis and extra renal involvement were recorded.*

Results. *Fifty-three patients (75.4% females) were included. Mean age at biopsy was 66.3 years. Typical pauci-immune GN was found in 39 patients (73.5%, group A). In 14 patients (26.4%, group B) examination revealed substantial deposition of Ig or complement in the mesangium and/or along the glomerular capillary wall. The only difference comparing both groups was significantly higher proteinuria in group B (mean 1.6/24 h (SD: 10.7) vs. 0.8/24 h (SD: 7.6), p=0.0036).*

Conclusion. *In ANCA GN at least a quarter of patients were not "pauci-immune" (26.4%). In this subgroup, immune deposits were only associated with a significantly higher proteinuria. Further basic and clinical research is needed to elucidate the significance of immune deposition in ANCA GN.*

Introduction

In kidney glomerular diseases, based on immunofluorescence studies of renal biopsies, three patterns are found: a linear fluorescence pattern due to a direct antibody-mediated attack such as in anti-GBM disease (Type I). An immune complex-mediated pattern with granular deposits of Ig, such as in Henoch-Schönlein purpura, cryoglobulinaemic vasculitis and systemic lupus erythematosus nephritis (Type II), and the so-called 'pauci-immune' pattern that is strongly associated with the presence of ANCA (Type III) (1).

ANCA-associated vasculitis consist of four separate syndromes: granulomatosis with polyangiitis (GPA), eosinophilic granulomatosis with polyangiitis (EGPA), microscopic polyangiitis (MPA) and renal limited vasculitis (RLV) (1). Pauci-immune indicates the relative lack of immunoglobulin and complement deposition within the kidney as demonstrated by indirect immunofluorescence techniques. Because of this relative paucity of complement in vessels, complement was not initially envisioned as an important participant in the pathogenesis of ANCA vasculitis and ANCA GN. However, alternative complement pathway activation is now recognised as an important mediator in the induction of ANCA-induced acute vascular inflammation because of evidence from experimental animal models, *in vitro* experiments, and clinical observations (2). A theoretical model about pathogenic mechanisms in ANCA-associated disease should be able to explain the development of the observed pathologic changes in the tissue. Evidence from animal models of the involvement of alternative complement pathway activation in the pathogenesis

of ANCA-associated disease prompted studies to identify supportive evidence in human disease. Examination of glomerular and vascular inflammatory lesions in biopsy specimens from patients with ANCA-associated disease revealed markers of alternative complement pathway activation including factor B and properdin, as well as factors shared by the classical and alternative pathways including C3d and membrane attack complex, but absence of components of the classical and lectin pathways such as C4d and mannose-binding lectin (3).

The anaphylatoxin C5a has a strong proinflammatory activity. In relation to ANCA-induced neutrophil activation, C5a is able to prime neutrophils, resulting, in the expression of proteinase 3 (PR3) on the neutrophil membrane (4). The interaction between C5a and its receptor on neutrophils has, indeed, been shown to cause a significant amplification loop for ANCA-induced neutrophil activation. C5a receptor appeared to be essential for development of MPO-ANCA crescentic glomerulonephritis in an animal model (5, 6). In ANCA glomerulonephritis, complement components and immunoglobulin are markedly accentuated at sites of inflammation and necrosis and less apparent or absent in segments and glomeruli with no histopathologic changes. This absence or paucity of complement and immunoglobulin in vessels without inflammation, and the accentuation of staining at sites of inflammation and necrosis, are consistent with a more interdependent involvement of complement activation with induction of inflammation primarily in the microenvironment at localised sites of inflammation and necrosis in ANCA disease (3, 4).

In recent years, attention has been drawn in the clinical setting, to patients who fulfill the criteria for ANCA associated vasculitis but without the classical pauciimmunity in renal biopsy.

The aim of this study was to evaluate the prevalence and clinical significance of immune deposits in ANCA-associated pauci-immune GN.

Methods

Patients

We retrospectively included all patients diagnosed with GPA, EGPA, MPA and RLV according with the 2012 Chapel Hill Consensus (7) who underwent renal biopsy between January 2002 and May 2014. Patients with anti-glomerular basement membrane (anti-GBM) disease, post infectious glomerulonephritis, Henoch-Schonlein purpura or other underlying disease causing secondary vasculitis (such as systemic lupus erythematosus, rheumatoid arthritis or drug induced vasculitis) were excluded. Patients were divided into 2 groups: Group A: biopsy without ID (equal or less than 2+ intensity of immunostaining) and Group B: biopsy with ID (more than 2+ intensity of immunostaining). Immunofluorescence (IF) included Immunoglobulins (Ig) (IgG, IgA and IgM) and complement components (C3 and C1q). Serum creatinine, estimated glomerular filtration rate (eGFR) at time of the biopsy, amount of proteinuria and haematuria, dialysis requirement and extra renal involvement were recorded along with the treatment received. Response to induction therapy was evaluated at six months.

Renal biopsies

Renal biopsies were reviewed by light microscopy by one pathologist who was blinded to the clinical history. Indirect immunofluorescence evaluation was based on the original pathology report and was not reviewed.

When IF was positive for Ig (IgA, IgM, IgG) or complement components (C1q or C3), the localisation within the glomerulus (mesangium or capillary wall) was determined. The intensity of immunostaining was scored as negative (0), weak (+1), weak-moderate (+2), moderate (+3) or strong (+4). Pauciimmunity was defined by negative staining (0) or up to +2.

Serology

All serum samples were tested for ANCA at the time of diagnosis before renal biopsy. 79% (42 out of 53 patients) were tested for anti-GBM antibodies. Indirect immunofluorescence was used for ANCA C and P screening. Only patients diagnosed after 2009 were tested for proteinase-3 (PR3) or myeloperoxidase (MPO) antibodies using enzyme-linked immunosorbent assay (ELISA).

Statistical analysis

Statistical analyses were performed using STATA v. 10. Continuous variables are presented as mean ± SD, or median and IQRs where appropriate. Categorical variables are presented as percentages. Chi-square or Fisher's exact test were used to determine significant differences between categorical data, and *t*-test when normally distributed, and Mann-Whitney test when not, for continuous variables. Two-sided *p*-values <0.05 were considered statistically significant.

Ethical approval

We received confirmation from our ethical committee that ethics approval was not required for this retrospective study.

Results

Fifty-three patients (75.4% females) were included. The mean age at the time of the biopsy was 66.3 (SD: 14.3). In terms of clinical diagnosis, 43% of patients were RLV, 28% GPA, 19% MPA and 10% EGPA. Regarding ANCA, 48 out of 53 patients were positive by IIF (90.56%). 64.1% were P ANCA, 26.4% C ANCA and 9.4% ANCA negative. Of the 48 patients with positive ANCA by IIF, 34 of them (70.8%) were tested by ELISA, 24 (70.6%) were MPO ANCA and 10 (29.4%) were PR3 ANCA.

Typical pauci-immune GN was found in 39 patients (73.5%, group A). In 14 patients (26.4%, group B) histopathological examination revealed substantial deposition of Ig or complement in the mesangium and/or along the glomerular capillary wall. ID deposition was more frequent in GPA and EGPA compared with MPA and RLV (Table I). C3 deposition on the capillary wall was the most frequent finding (64.2%), followed by C3 + IgG (21.4%) and IgG alone (14.2%), (Fig. 1). No patient presented positive staining for C1q. IgM and IgA were found only in two patients but along with C3. Over the 14 patients in group B, six were C ANCA, six were P ANCA and two were ANCA negative, of these two both of them were female

Table I. Differences between the groups.

	GROUP A, n=39 (less than 2+ intensity of immunostaining)	GROUP B, n=14 (more than 2+ intensity of immunostaining)	*p*
Mean age (SD)	67.6 (15.4)	62.6 (10.1)	0.26
Females, n (%)	29 (72.5)	10 (76.2)	0.75
Diagnosis, n (%, CI)			
- GPA	9 (60, 16.3-67.7)	6 (40, 32.3–83.7)	
- GPAE	3 (60, 14.7-94.7)	2 (40, 5.3–85.5)	
- MPA	9 (90, 55.5-99.7)	1 (10, 02–44.5)	
- RLV	1 (78, 56.3-92.5)	5 (22, 7.5–43.7)	
Mean baseline creatinine mg/dl, (SD)	3 (2.8)	3.4 (2.3)	0.69
Extra-renal involvement; n, (%)	19 (48.7)	7 (50)	0.93
Mean proteinuria g/24 h, (SD)	0.8 (7.6)	1.6 (10.7)	0.0036
Remission after induction treatment; n (%)	26 (74.2)	11 (68.7)	0.68

SD: standard deviation; CI: confidence interval .

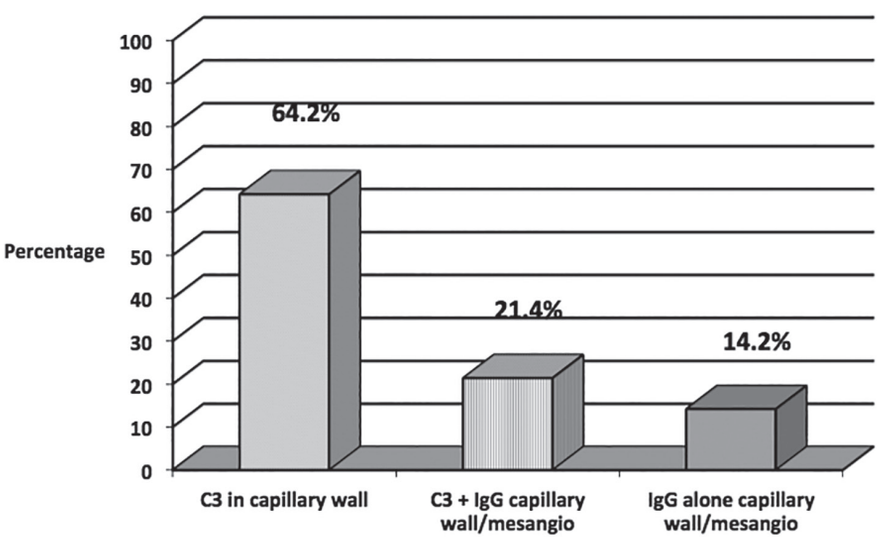

Fig. 1. Distribution by type of deposits.

and had diagnosis of EGPA. Compared with patients in group A, those in group B demonstrated significantly more 24 h proteinuria (mean 1.6/24 h (SD: 10.7) *vs*. 0.8/24 h (SD: 7.6), *p*=0.0036). No differences between groups were found related to age, gender, renal function, extra renal organ involvement at the time of biopsy and response to the induction therapy (Table I).

Regarding induction therapy, 34 (64.1%) patients received IV cyclophosphamide (CYC), 11 (20.7%) oral CYC, 2 (3.7%) rituximab (RTX), 1 (1.8%) mofetil mycophenolate (MMF), 1 (1.8%) methotrexate (MTX) and 4 (7.5%) received only steroids. Choices of treatment regimens were lived to the individual physician according to patient's symptoms and comorbidities. Six patients underwent plasma exchange and eight patients required dialysis.

Normal serum complement C3 and C4 levels were observed in 50 patients (94.33%), only 3 (5.6%) were found to have slightly low levels.

Discussion

Over the past decade, research trials in vasculitis led to a 2012 revision of the 1994 Chapel Hill Consensus Conference (CHCC) nomenclature, focusing on aetiology, pathogenesis, pathology and clinical characteristics as the basis for categorisation. The 2012 CHCC nomenclature of vasculitis syndromes only provides definitions and the ACR criteria are classification criteria. Diagnostic criteria are defining features of a particular disease that can be used to predict the emergence of disease in a particular patient. 'Gold-standard' diagnostic criteria are generally based on expert opinion (7). None of these nomenclatures or classification criteria help to predict the histopathologic feature on renal involvement.

In the pathophysiology of renal glomerulonephritis, both the innate and the acquired immune system are involved. The initial acute vascular lesions of ANCA disease, including glomerulonephritis, begin with the influx of neutrophils and their accumulation, followed by monocytes and macrophages with the induction of vascular necrosis. Despite of the lack of immunoglobulin and complement in the injured glomeruli and vessels, both experimental results and observations in patients support a pathogenic role for both immunoglobulin and complement in the mediation of this acute necrotising injury (8).

Xing *et al*. described that immunohistochemical examination of renal biopsy specimens from ANCA disease patients revealed Bb, C3d, and C5b-9 in glomeruli and in small arteries. This confirmed earlier findings reported by the same group in seven patients with MPO-ANCA GN that showed deposits of C5b-9, C3d, factor B and factor P in glomeruli and small blood vessels with active vasculitis. Mannose binding lectin (MBL) and C4d were not detected. These observations support a role for alternative complement pathway activation but not classic or lectin pathways in the pathogenesis of ANCA induced inflammation (3).

Haas *et al*. demonstrated the presence of electron-dense deposits in 68 cases (54%) among 126 patients of GN associated with ANCA and/or necrotising angiitis (9).

In a study of Clinical and pathological characteristics and outcomes of Chinese patients with ANCA GN, renal biopsy was performed in 74 patients with primary ANCA-associated systemic vasculitis, 23 cases (31%) possess immune complex deposition, as assessed by immunofluorescence and/or electron microscopy (10).

Neumann *et al*. studied 45 patients with ANCA-associated crescentic glomeru-

lonephritis and heavy proteinuria and reported that eight patients (17.7%) showed renal Ig deposits on histological examinations (11).

It has been reported that various forms of IC-related glomerulonephritis in ANCA-positive patients are more likely to be associated with necrotising or crescentic lesions than in similar ANCA-negative patients with IC-related glomerulonephritis (12), and that patients with ANCA associated GN and IC deposits have heavier proteinuria than patients with classical pauci-immune ANCA-associated GN (11).

In our study we found that immune deposition can be detected in 26% of patients, representing a considerable subset arguing against pauci-immune GN as the only pathogenetically relevant feature in patients with ANCA-associated GN. Our data are also in agreement with other reports, describing immune deposits in renal biopsies of patients with heavier proteinuria (11). We did not find correlation between C3 deposits in renal tissue and levels of C3 in peripheral blood. One possible explanation could be that there is not immune complex deposition, but rather expression of activation of the alternative complement pathway, without real consumption of C3.

There have been several studies examining patients with ANCA GN to determine the association between histology and clinical outcomes (1, 3, 5, 8-11). It is evident from these studies that there is a wide degree of variability in the histologic features found to be of prognostic significance in ANCA GN. There is an urgent need to develop a classification for ANCA glomerulonephritis with predictive value of the renal histopathological parameters and to assess the active and chronic lesions that can be universally used to unified criteria for treatment options either in induction therapy and maintenance (13).

Conclusions

Our results confirm that in ANCA GN a substantial percentage of patients have evidence of C3 or Ig deposition in renal biopsies (26.4%). The prevalence of C3 deposition could be related with the alternative pathway activation, described as an important mechanism in the pathogenesis of ANCA vasculitis. In this subgroup, ID was associated with a significantly greater degree of proteinuria. This article provides the first report about the presence of ID in ANCA GN in Latin American patients. Further research is needed to elucidate the significance of IC deposition in ANCA GN.

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