

Original Article

Histopathological features predictive of a clinical diagnosis of ophthalmic granulomatosis with polyangiitis (GPA)

Hazlita Isa^{1,2,3}, Sue Lightman^{1,2}, Philip J Luthert², Geoffrey E Rose⁴, David H Verity⁴, Simon RJ Taylor^{1,5}

¹Multidisciplinary Vasculitis Clinic, Hammersmith Hospital, Du Cane Road, London W12 0NN, UK; ²UCL Institute of Ophthalmology, 11-43 Bath Street, London EC1V 9E, UK; ³Department of Ophthalmology, Medical Faculty, University Kebangsaan Malaysia, 56000 Kuala Lumpur, Malaysia; ⁴Orbital Clinic, Moorfields Eye Hospital, City Road, London EC1V 2PD; ⁵Division of Immunology & Inflammation, Faculty of Medicine, Imperial College London, London, UK

Received July 14, 2012; Accepted August 2, 2012; Epub September 5, 2012; Published September 15, 2012

Abstract: Background: The limited form of Granulomatosis with Polyangiitis (GPA), formerly known as Wegener's Granulomatosis (WG) primarily involves the head and neck region, including the orbit, but is often a diagnostic challenge, particularly as it commonly lacks positive anti-neutrophil cytoplasm antibody (ANCA) titres or classical features on diagnostic orbital biopsies. The purpose of this study was to relate biopsy findings with clinical outcome and to determine which histopathological features are predictive of a clinical diagnosis of GPA. Methods: Retrospective case series of 234 patients identified from the database of the UCL Institute of Ophthalmology Department of Eye Pathology as having had orbital biopsies of orbital inflammatory disorders performed between 1988 and 2009. Clinical records were obtained for the patients and analysed to see whether patients had GPA or not, according to a standard set of diagnostic criteria (excluding any histopathological findings). Biopsy features were then correlated with the clinical diagnosis in univariate and multivariate analyses to determine factors predictive of GPA. Results: Of the 234 patients, 36 were diagnosed with GPA and 198 with other orbital pathologies. The majority of biopsies were from orbital masses (47%). Histology showed a range of acute and chronic inflammatory pictures in all biopsies, but the presence of neutrophils ($P<0.001$), vasculitis ($P<0.001$), necrosis ($P<0.001$), eosinophils ($P<0.02$) and macrophages ($P=0.05$) were significantly associated with a later clinical diagnosis of GPA. In a multivariate analysis, only tissue neutrophils ($OR=3.6$, $P=0.01$) and vasculitis ($OR=2.6$, $P=0.02$) were independently associated with GPA, in contrast to previous reports associating eosinophils and necrosis with the diagnosis. Conclusions: Neutrophil, eosinophil and macrophage infiltration of orbital tissues, together with vasculitis and necrosis, are all associated with a clinical diagnosis of GPA, but only neutrophil infiltration and vasculitis are independently associated with this diagnosis. These features may assist in the establishing the diagnosis of limited GPA among patients with early orbital disease, particularly in the absence of positive serum ANCA titres.

Keywords: Granulomatosis with polyangiitis, histopathology, eosinophils, nuclear dust

Introduction

Granulomatosis with Polyangiitis (GPA), formerly known as Wegener's granulomatosis (WG), is a systemic small-vessel vasculitis that is characterised by necrotising granulomatous inflammation affecting the renal, pulmonary, upper airways and ocular systems, and is associated with circulating anti-neutrophil cytoplasm antibodies (ANCA) [1]. The initial symptoms of GPA are observed in the head and neck region in

some 95% of patients, but making the diagnosis at this stage is often extremely challenging. GPA remains limited to ENT and ocular involvement in some patients, but can involve other organs, such as the lungs and kidneys [2], with severe consequences for the patient. Important issues in the management of GPA include both prediction of disease progression and the side-effects of the drugs required to treat active disease. Early diagnosis of limited GPA is useful, as it enables earlier introduction of the appro-

Histopathology of GPA

priate immunosuppressive therapy, presumably reducing the risk of local progression of the disease and of involvement of other organs, including the kidney, after which the prognosis dramatically worsens [3].

Ocular involvement occurs in 50-60% of patients and is the presenting feature in 8-16%, with severe ocular morbidity occurring as a complication of both localised and systemic GPA [4]. Orbital involvement occurs in approximately half of all patients with GPA, and may lead to orbital bone destruction and loss of vision from contraction and fibrosis around the optic nerve, despite treatment and resolution of inflammation [5]. Ocular features of GPA include scleritis, which can be necrotising and bilateral, and which can pose a major threat to the integrity of the globe. Nevertheless, the diagnosis of ocular GPA is particularly difficult as its clinical manifestations often overlap with other inflammatory conditions such as sarcoidosis and idiopathic inflammatory orbital disorders. In addition, in the limited form of GPA, anti-neutrophil cytoplasm antibody (ANCA) titres are positive in only 50-65% of patients [6, 7].

Although the histopathological findings are often diagnostic for GPA in renal disease [8, 9], this is not the case for orbital and upper airways disease, with classic findings being present in less than a third of patients, and fewer than this in recent-onset disease [10, 11]. Indeed, the histological features of GPA can mimic other forms of idiopathic orbital inflammation such as sarcoidosis, idiopathic inflammatory orbital disorders and lymphoid hyperplasia.

The histological features of orbital GPA are diverse and can include any of the following: granulomatous foci, collagen deposition, necrosis, nuclear dust, plasma cells and an infiltrating eosinophilic response [12]. The latter has been suggested to predict disease progression [13], analogous to the suggestion that, in renal biopsies in GPA, an increase CD8⁺ cells might also predict and increase in disease activity [14]. These descriptions are drawn from relatively small numbers of patients, and, as yet, there has not been a study which has systematically analysed a large number of orbital biopsies. We therefore performed such a retrospec-

tive investigation for a consecutive case series in which we examined histopathology reports of all patients who had undergone orbital biopsies for orbital inflammatory disorders over a period of 21 years, and related this to their clinical outcomes. To our knowledge, this is the first study of its kind.

Methods

The study was approved by the Moorfields & Whittington Research Ethic Committee and was a retrospective study of all patients who had undergone orbital or adnexal biopsy for orbital inflammatory disease at Moorfields Eye Hospital over 21 years. All patients who had undergone an orbital or adnexal biopsy between 1988 and 2009 were identified from the UCL Institute of Ophthalmology Department of Eye Pathology Database. The biopsy reports were reviewed, and the cell types and tissue responses documented qualitatively as present or absent. Cell types noted included neutrophils, eosinophils, plasma cells, multinucleated giant cells, macrophages and mast cells; tissue responses assessed included necrosis, sclerosis, lymphoid follicles, nuclear debris and granulomas.

A comprehensive review of the clinical notes was next performed to obtain information on patient demographics and clinical data including the final clinical diagnosis, orbital signs and symptoms, ocular signs and symptoms, other organ involvement, ANCA status, treatment and duration of follow up. Patients were classified as having clinical features consistent with a diagnosis of GPA if they met any two of the following clinical criteria: (1) Characteristic ocular features of GPA; (2) Characteristic ENT, pulmonary, renal or cardiac involvement; (3) Positive immunofluorescence for ANCA; (4) Positive ELISA for anti-PR3 antibodies. Crucially, histological findings and diagnosis were excluded from the list of diagnostic criteria to avoid confounding the study and patients were included in the "non-GPA" group if they did not meet the above criteria. Systemic and limited GPA were defined by the presence or absence of renal or lower airway involvement, respectively. Patients were also classified as 'newly-diagnosed' or 'known' GPA depending on whether their clinical diagnosis had been made prior to the biopsy procedure.

Histopathology of GPA

Table 1. Patient characteristics

	GPA (n=36)	Non GPA (n=198)	P
Age	50.7 ± 3.2 years	50.4 ± 1.2 years	0.92
Gender (M:F)	21:15 (57%:43%)	127:71 (64%:36%)	0.57
Biopsy structures			
- Orbital mass	20	91	0.29 ^a
- Lacrimal gland	8	79	
- Nasolacrimal/sinonasal	12	31	
- Extraocular muscle	2	28	
- Other	3	11	

^aP value represents χ^2 test of orbital mass vs. other biopsy site for each group

Table 2. Comparison of cellular profiles

Cell type	GPA (%) (n=36)	Not GPA (%) (n=198)	Unadjusted		Adjusted	
			OR	P	OR	P
PMN	19 (53%)	30 9 (15%)	5.9 (2.7 - 12.6)	<0.0001*	3.9 (1.4 - 11.2)	0.01*
Eosinophil	19 (53%)	62 (31%)	2.5 (1.2 - 5.0)	0.02*	0.7 (0.24 - 1.8)	0.42
Vasculitis	19 (53%)	11 (6%)	19.0 (7.8 - 46.4)	<0.0001*	4.8 (1.6 - 14.7)	0.006*
Necrosis	22 (39%)	21 (11%)	5.4 (2.4 - 12.0)	<0.0001*	2.4 (0.8 - 6.6)	0.10
Lymphocyte	19 (53%)	113 (57%)	0.8 (0.4 - 1.7)	0.86		
Fibrosis	22 (61%)	111 (56%)	1.2 (0.6 - 2.6)	0.72		
Macrophage	16 (44%)	55 (28%)	2.1 (1.0 - 4.3)	0.05*	2.0 (0.9 - 4.5)	0.11
Plasma cells	18 (50%)	85 (43%)	1.3 (0.7 - 2.7)	0.47		
Giant cells	2 (6%)	23 (12%)	0.4 (0.1 - 2.0)	0.39		
Follicles	4 (11%)	34 (17%)	0.6 (0.2 - 1.8)	0.47		
Nuclear debris	2 (6%)	6 (3%)	1.9 (0.4 - 9.7)	0.36		
Granuloma	12 (33%)	45 (23%)	1.7 (0.8 - 3.7)	0.21		

+ve = cells reported present in biopsy; -ve = cells not reported in biopsy; OR = odds ratio; CI = confidence interval; * = statistically significant difference (P<0.05); % = percentage of patients with positive occurrence of cell type or tissue response reported in biopsy within each group. Adjusted odds ratios are adjusted for all statistically significant variables (P<0.05) from the univariate analyses.

The unpaired student's T-test and Fisher's exact test were performed to examine differences between the two groups. Univariate analysis was performed, including the calculation of 95% confidence intervals. Multivariate analysis was performed to look for any independent association of a cellular profile or tissue response with the clinical diagnosis of GPA. For the multivariate logistic regression analyses, all statistically significant variables (P<0.05) from the univariate analyses were included. SPSS 17 and GraphPad Prism 5.01 were used to perform the statistical analysis.

Results

Two hundred and thirty-four patients were identified from the UCL Institute of Ophthalmology pathology database as having undergone orbit-

al biopsy for an orbital inflammatory condition between 1988 and 2009, and for whom full clinical details were available. Of these, 36 patients fulfilled our criteria for the diagnosis of GPA. Diseases in the non-GPA group included chronic idiopathic inflammation of the orbit (CIIO) in 74/198 patients (37%), lymphoid hyperplasia in 16 (8%), sarcoidosis in 13 (7%), myositis in 3 (2%), dacryoadenitis in 33 (17%), and thyroid eye disease in 12 (6%). Patient characteristics are included in **Table 1**, and indicate no significant differences between the GPA and non-GPA groups.

Clinical characteristics of orbital GPA

Of the 36 patients in the GPA group, two patients (6%) had systemic GPA (that is, with renal or lower airways involvement) and 34

(94%) had limited disease. Seventeen (47%) had been diagnosed with GPA before their ocular symptoms commenced and had received treatment in the form of corticosteroids, with or without second-line immunosuppression; the remaining 19 (53%) had a diagnosis of GPA based on their ocular disease. The most common ocular presentations in the GPA group were proptosis (55%), lid swelling (44%) and scleritis (32%). Other ocular presentations included diplopia, epiphora (nasolacrimal block), ocular pain and decreased vision. Interestingly, and in agreement with recent studies, no patients with limited GPA were observed to progress to the systemic form of GPA during the duration of this study [15, 16]. The duration of follow-up in this study was a median 36 months (range 24 to 190 months; 708 patient-years in total).

Biopsy features predictive of clinical features consistent with a diagnosis of GPA

In the univariate analysis, neutrophils, eosinophils, vasculitis, macrophages and necrosis were present significantly more often in the GPA group than in the non-GPA group (**Table 2**). A multivariate analysis, controlling for confounding factors, showed that neutrophils and vasculitis are independently associated with the clinical diagnosis of GPA - with odd ratios of 3.9 and 4.8 respectively.

Twelve patients were ANCA-positive, 11 were ANCA-negative and the remaining 13 patients did not have any ANCA levels on record - being patients with an established diagnosis of GPA prior to their ophthalmic presentation. There was a trend for eosinophils ($P=0.08$) and lymphocytes ($P=0.09$) to be seen more frequently in the ANCA-positive group than in the ANCA-negative group.

Just over a half (19/36) of the patients were newly diagnosed with GPA and 17 had a known diagnosis of GPA prior to ophthalmic presentation, including 2 patients with systemic GPA. All patients with established GPA had previously received, or were still on, immunosuppressive treatment. Presumably due to a lack of prior treatment, cellular infiltration was greater in patients without a prior diagnosis of GPA and the changes including the presence of nuclear debris and giant cells, both of which were seen only in patients without prior therapy.

Discussion

The diagnosis of ophthalmic GPA is difficult, as its clinical manifestations often overlap with other inflammatory disorders. Furthermore, ANCA titres are positive in only 50-65% of such patients, and classic histological features are also frequently absent from biopsy material; this being in contrast to disease affecting other organs, such as the kidney [5, 7, 11, 13]. Nevertheless, this investigation indicates that there are significant differences in the cellular and tissue profile in biopsies taken from patients who will later be assigned a diagnosis of GPA, as compared to other diseases, the presence of infiltrating neutrophils and vasculitis being independently associated with a clinical diagnosis of GPA. Biopsies from patients with GPA also show many more inflammatory cells as compared to other types of lymphocytic orbital inflammatory disorders, which reflects the tendency to a more fulminant disease with GPA.

Previous studies have suggested that eosinophils play a part in the pathogenesis of GPA and, in one study, the presence of eosinophils in biopsies of patients with limited GPA was said to be predictor of disease progression [7]. In our study, a univariate analysis did indicate significantly more eosinophils in patients with GPA, but this was lost when other confounding factors were controlled for, suggesting that the number of tissue eosinophils might be associated with disease severity, rather than being associated with GPA in particular.

Nuclear debris is an entity that has recently become a subject of interest in GPA and is thought to originate from the rupture of nuclei of neutrophils in the tissue of patients with GPA, possibly as a result of ANCA activity [9, 12]. In our study, nuclear debris was seen more frequently in the GPA group, but this might be underrepresented as pathologists may not have specifically reported this phenomenon in the past; further quantitative evaluation of this entity in orbital biopsies would be interesting and beneficial. In addition, the efficacy of rituximab in certain patients with GPA implicates a role for B cells in the aetiology and perpetuation of disease, and further characterisation of these cells at different stages of disease would be of interest [5, 17, 18].

Interestingly, despite the limitation that all of the cellular profiles in this study were based on histopathological reports, we were still able to detect significant differences in cellular activity between the GPA and non-GPA group. Developing a grading system for the cellular and tissue profiles should provide a clearer picture of cellular activity in these orbital biopsies, and thereby enable a more accurate comparison to be made between orbital biopsies.

Ethical approval

The study is approved by the Moorfields & Whittington Research Ethic Committee (REC ref. no. 09/H0721/75, LIGS 1023).

Declaration of competing/conflicts of interest

We declare that all authors have no competing/conflicting of interests that might be perceived to influence the results and/or discussion reported in this article.

Declaration of funding

SRJT was funded by the UK National Institute of Health Research and the work of the Multidisciplinary Vasculitis Clinic was supported by the Imperial NIHR Comprehensive Biomedical Research Centre. The sponsor or funding organization had no role in the design or conduct of this research. We declare that all authors have no financial support or relationships that may pose conflict of interest that might be perceived to influence the results and/or discussion reported in this article.

Address correspondence to: Dr. Simon RJ Taylor, Imperial College London Faculty of Medicine, 5th Floor Commonwealth Building, Hammersmith Hospital, London W12 0NN. Tel: +44 (0) 20 8383 2306; Fax: +44 (0) 20 7566 2266; E-mail: s.r.taylor@imperial.ac.uk

References

- [1] Knight A, Ekblom A, Brandt L and Askling J. Increasing incidence of Wegener's granulomatosis in Sweden, 1975-2001. *J Rheumatol* 2006; 33: 2060-2063.
- [2] Bacon PA. The spectrum of Wegener's granulomatosis and disease relapse. *N Engl J Med* 2005; 352: 330-332.
- [3] Harper SL, Letko E, Samson CM, Zafirakis P, Sangwan V, Nguyen Q, Uy H, Baltatzis S and Foster CS. Wegener's granulomatosis: the relationship between ocular and systemic disease. *J Rheumatol* 2001; 28: 1025-1032.
- [4] Pakrou N, Selva D and Leibovitch I. Wegener's granulomatosis: ophthalmic manifestations and management. *Semin Arthritis Rheum* 2006; 35: 284-292.
- [5] Taylor SR, Salama AD, Joshi L, Pusey CD and Lightman SL. Rituximab is effective in the treatment of refractory ophthalmic Wegener's granulomatosis. *Arthritis Rheum* 2009; 60: 1540-1547.
- [6] Tarabishy AB, Khan M, Bunyard M and Lowder CY. Retinal vasculitis associated with the anti-synthetase syndrome. *Ocul Immunol Inflamm* 2010; 18: 16-18.
- [7] Lamprecht P and Gross WL. Current knowledge on cellular interactions in the WG-granuloma. *Clin Exp Rheumatol* 2007; 25: S49-51.
- [8] Little MA and Pusey CD. Glomerulonephritis due to antineutrophil cytoplasm antibody-associated vasculitis: an update on approaches to management. *Nephrology (Carlton)* 2005; 10: 368-376.
- [9] Seo P and Stone JH. The antineutrophil cytoplasmic antibody-associated vasculitides. *Am J Med* 2004; 117: 39-50.
- [10] Kalina PH, Lie JT, Campbell RJ and Garrity JA. Diagnostic value and limitations of orbital biopsy in Wegener's granulomatosis. *Ophthalmology* 1992; 99: 120-124.
- [11] Raynaud P, Garrel R, Rigau V, Poizat F, Vic P, Cartier C, Riviere S, Baldet P and Costes V. [How can the diagnostic value of head and neck biopsies be increased in Wegener's granulomatosis: a clinicopathologic study of 49 biopsies in 21 patients]. *Ann Pathol* 2005; 25: 87-93.
- [12] Ahmed M, Niffenegger JH, Jakobiec FA, Ben-Arie-Weintrob Y, Gion N, Androudi S, Folberg R, Raizman MB, Margo CE, Smith ME, McLean IW, Caya JG and Foster CS. Diagnosis of limited ophthalmic Wegener granulomatosis: distinctive pathologic features with ANCA test confirmation. *Int Ophthalmol* 2008; 28: 35-46.
- [13] Choopong P, Khan N, Sangwan VS, Zafirakis P, Rallatos CL, Rojas B, Baltatzis S and Foster CS. Eosinophil activation in Wegener's granulomatosis: a harbinger of disease progression? *Ocul Immunol Inflamm* 2005; 13: 439-445.
- [14] Aasarod K, Bostad L, Hammerstrom J, Jorstad S and Iversen BM. Wegener's granulomatosis: inflammatory cells and markers of repair and fibrosis in renal biopsies—a clinicopathological study. *Scand J Urol Nephrol* 2001; 35: 401-410.

Histopathology of GPA

- [15] Holle JU, Gross WL, Holl-Ulrich K, Ambrosch P, Noelle B, Both M, Csernok E, Moosig F, Schinke S and Reinhold-Keller E. Prospective long-term follow-up of patients with localised Wegener's granulomatosis: does it occur as persistent disease stage? *Ann Rheum Dis* 2010; 69: 1934-1939.
- [16] Fechner FP, Faquin WC and Pilch BZ. Wegener's granulomatosis of the orbit: a clinicopathological study of 15 patients. *Laryngoscope* 2002; 112: 1945-1950.
- [17] Fervenza FC. Rituximab in ANCA-associated vasculitis: fad or fact? *Nephron Clin Pract* 2011; 118: c182-188; discussion c188.
- [18] Joshi L, Lightman SL, Salama AD, Shirodkar AL, Pusey CD and Taylor SR. Rituximab in refractory ophthalmic Wegener's granulomatosis: PR3 titers may predict relapse, but repeat treatment can be effective. *Ophthalmology* 2011; 118: 2498-2503.