

The antineutrophil antibody in uveitis

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Abstract

Ninety eight patients with uveitis of various types were tested for the presence of the antineutrophil antibody or ANCA by an indirect immunofluorescence method. This antibody is found in patients with diseases associated with small vessel vasculitis, including Wegener's granulomatosis and microscopic polyarteritis. Eleven true positive cases were found. A positive test was not associated with the anatomical site of the uveitis but was related to the time course of the disease. In particular single/repeated rather than non-recurrent uveitis was associated with a positive test. Three groups of patients seem more likely to have positive tests: those with bilateral chronic posterior uveitis; a group with an anterior uveitis which is single/repeated who were younger than 46 at disease onset and had isolated eye disease; and an older group, aged 60 years or more at onset, with single/repeated uveitis and systemic features.

Over the years many antibodies have been discovered. Some, such as the antinuclear antibody for disseminated lupus erythematosus or rheumatoid factor for rheumatoid arthritis, are specific for a limited range of conditions, though neither are absolute markers for these conditions. Others, such as smooth muscle antibodies or parietal cell antibodies, are non-specific and frequently found in immune mediated diseases. They do not identify particular diseases.

In the early 1980s an antibody specific for neutrophil cytoplasm was recognised in diseases associated with systemic vasculitis.¹ Later this antibody was found to be a marker for a narrow range of conditions all associated with small vessel vasculitis.

Small vessel vasculitis is found in several diseases, including Wegener's granulomatosis,² microscopic polyarteritis,³ and segmental focal glomerulonephritis.¹ These conditions may all have the antineutrophil antibody (ANCA) as a common antibody. The antibody reacts against components of the neutrophil cytoplasm giving rise to a characteristic appearance on indirect immunofluorescence.⁴

The antineutrophil cytoplasmic antibody reaction can give one of three appearances, two of which, cytoplasmic and perinuclear, are regarded as positive. The third is a diffuse pattern, which is a non-specific reaction due to an excess of miscellaneous antibodies.

The cytoplasmic appearance is called C-ANCA. The 'C' is for classical, and it is most often found in Wegener's granulomatosis. The P-ANCA or perinuclear pattern occurs if neutrophil myeloperoxidase is exposed and combines with nucleic acid during the alcohol fixing stage. This reaction is more commonly found in

microscopic polyarteritis, though neither of these patterns are absolute for a particular disease.

Initially it seemed that the antibody might occur in only a limited number of conditions, but the list is expanding and now includes Kawasaki's disease,⁵ antglomerulobasement membrane disease,⁶ and the renal-pulmonary syndrome due to an IgM antibody.⁶ All have a small vessel vasculitis.

A survey of uveitis⁷ showed that in 44% of patients with anterior uveitis and in 9.6% of those with posterior uveitis no cause for their disease was found. Immunologically mediated mechanisms are possible when no cause is found.⁸

The uveal tract, particularly the choroid, is very vascular, and a vasculitis involving arterioles and venules is a common feature of uveitis. Against this background it seemed reasonable to look for the ANCA in patients with apparent idiopathic uveitis in the hope of either defining a disease mechanism, or deciding if conditions such as Wegener's granulomatosis or microscopic polyarteritis were present. Therefore patients attending an uveitis assessment clinic were screened for the ANCA.

Material and methods

The ANCA test was done by indirect immunofluorescence as recommended by the 1st ANCA workshop in Copenhagen in 1988.⁹ After anticoagulation with edetic acid (EDTA) diluted 1 in 2 with tissue culture medium, dextran 110 (CP Pharmaceuticals Ltd) was added. The blood was incubated for 30 minutes at room temperature, the white cell rich supernatant was harvested, and the cells were washed once in the medium and then centrifuged on to microscope slides (Cytocentrifuge Shandon) and fixed in 95% ethanol at 4°C for 5 minutes.

Test and control sera were incubated with the cells on the slides in a moist chamber for 30 minutes at room temperature. They were then washed for 20 minutes in a bath of phosphate buffered saline, pH 7.2, and incubated with a dilution of fluorescein isothiocyanate (FITC)-labelled anti-human IgG (Binding Site Ltd) for 30 minutes at room temperature. After a further period of washing, a drop of DABCO diazobicyclo-octane (Aldrich) was added and the slides were examined under a fluorescence microscope (Leitz).

The patients were referred from a general ophthalmology clinic with the diagnosis of uveitis, cause unknown. The anatomical site, time course, and activity of the inflammation were ascertained from the referral request, the patients' symptoms, and the blood test results.

All patients had a full clinical examination and a set of investigations including erythrocyte

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sedimentation rate (ESR), full blood count, and measurement of urea, electrolytes, liver function (bilirubin, alkaline phosphatase, aspartate), immunoglobulins, C reactive protein, anti-nuclear factor, rheumatoid factor, immune complexes, and immunoglobulins (IgA, IgG, IgM), and chest x ray and urine testing for protein, glucose, and blood.

Other investigations were done if they seemed relevant. These included a test for herpes virus antibodies, Kveim test, Mantoux test, tissue biopsy (lacrimal, nasal mucosa), test for toxocara, toxoplasma titres, and syphilis serology.

The type of uveitis was defined according to the site of the predominant signs and classified after the recommendations of the International Uveitis study Group¹⁰ with the modification that 'long non-recurrent' is called chronic. If two sites were of equal prominence, this was noted. Those patients with a posterior uveitis whose major lesion was a retinal vasculitis were listed separately in some of the tables.

The time course of the disease was divided into single/repeated when the duration of each episode was less than six months and chronic if it was greater.

A questionnaire reflecting the features of microscopic polyarteritis, Wegener's granulomatosis, and glomerulonephritis was put to the ANCA-positive patients.

On the basis of all the clinical and laboratory information the patients were divided into those with isolated eye disease (IED) and those with systemic features and eye disease (SED). χ^2 or Fisher's exact tests were used to test for statis-

tical significance. The two-sample *t* test was used to compare the ages of the ANCA-positive and negative group. Percentages in the tables were calculated across the rows.

Abbreviations used are AU anterior uveitis, IU intermediate uveitis, PU posterior uveitis, APMPE acute posterior multifocal placoid pigment epitheliopathy, RV retinal vasculitis.

Results

The total number of patients screened was 98, comprising 41 males and 57 females. Among these 19 positive tests were found; three had the cytoplasmic pattern, eight the perinuclear, and eight the diffuse. This last group are included in the negatives, giving 11 true positives and 87 negatives.

There were five males in the positive group and 36 in the negative as compared with six female positives and 49 negatives. The average age of the positive group was 42.7 years compared with 36.9 in the other group. None of these differences were statistically significant.

The distribution of the positives over the various anatomical sites for uveitis was broadly in proportion to the number of patients with that type of uveitis. No predilection for a particular site was revealed.

The difference in the proportion of positives with single/repeated over the chronic variety of uveitis gave a χ^2 of 4.11, significant at the 5% level. ANCA positives were found more often in single/repeated uveitis.

This analysis was repeated for the largest diagnostic group. AU which was single or repeated was found in 45 of the 98 patients. In this group there were six positive ANCA results. In the 19 patients with chronic AU no positives were found. Though this suggests an association, it was not statistically significant.

Every patient with a positive test had inflammatory changes in the anterior chamber at some stage of their disease. In a few the changes were minor compared to features in other parts of the eye, so the diagnosis of AU was not made. Patient 6 had 1+ of flare and a few cells in the anterior chamber for a short time, but there were overwhelming features typical of APMPE visible in the fundus.

Both types of ANCA responses were found, though the perinuclear pattern was more common. The sex ratio (five females and six males) was equal. The age of onset was wide. Five patients had other clinical features suggesting a systemic disease. These included Behçet's disease, mild colitis, polymyalgia rheumatica, interstitial lung disease, and non-specific ill health. Six patients appeared to have isolated eye disease.

In nine patients the ANCA test became negative as their eye disease settled.

Different groups were tested to see if the proportion of positives in those with only eye disease differed (IED) from those with eye disease and systemic features (SED). No differences were found between ANCA-positive and negative patients when tested over the whole group, those with single or repeated uveitis, or those with only anterior uveitis.

Table 1 Site of uveitis and ANCA test result

	AU (%)	PU (%)	AU+PU (%)	IP (%)	RV (%)
ANCA +ve	5 (45)	1 (9)	2 (18)	1 (9)	2 (18)
ANCA -ve	58	11	9	2	7
Total	64 (65)	12 (12)	10 (10)	3 (3)	9 (9)

Table 2 Time course of uveitis and ANCA result - all patients

	Single/repeated (%)	Chronic (%)
ANCA +ve	9 (82)	2 (18)
ANCA -ve	43	44
Total	52 (53)	46 (47)

Table 3 Summary of patients with ANCA positive

Patient	ANCA type	Sex	Onset	Uveitis type	IED/SED	Persisting result
1	C	F	19	RV	S	No
2	C	M	21	AU B/R	I	No
3	C	F	54	RV	S	No
4	PN	M	8	AU+PU B/R	I	Yes
5	PN	F	12	AU+PU B/R	I	No
6	PN	M	32	APMPPE	I	No
7	PN	M	37	IU B/S	I	No
8	PN	M	45	AU U/S	I	No
9	PN	F	60	AU B/R	S	No
10	PN	M	70	AU B/R	S	Yes
11	PN	F	82	AU B/R	S	No

C=Cytoplasmic ANCA type. PN=perinuclear ANCA type. B=bilateral. U=unilateral. S=repeated.

Table 4 ANCA positives: cytoplasmic versus perinuclear responses

	Number	Male	Mean age	AU	AU+PU	IU/IPU	IED	SED
Cytoplasmic	3	1	31.3	1	1	1	1	2
Perinuclear	8	5	44.8	4	2	2	5	3

The perinuclear group contained more males, was older, and had fewer systemic features than the cytoplasmic group. Two of the patients in the cytoplasmic group had posterior uveitis which was bilateral and chronic. This conjunction of C-ANCA-positive and chronic posterior uveitis seems to identify a separate subgroup of ANCA-positive patients with uveitis.

Discussion

Those tested do not form a random sample of patients with uveitis. Selection occurred at three levels: at referral from the community to the eye hospital by the general practitioner; by the ophthalmologist, who referred those patients who were causing concern because of their severity, their non-responsiveness to therapy, or the association of systemic features; and finally within the clinic no attempt was made to test systematically all patients. Those tested were the ones without an initial obvious cause for their condition such as sarcoid or tuberculosis.

Positivity of this test depends in some circumstances on the disease activity. It is used as a marker to monitor the progress of Wegener's granulomatosis.² The test was performed when the patients were seen in the assessment clinic, and this was usually one to four weeks after their initial presentation, often when the condition was settling. It is possible the number of positives was underestimated.

In nine patients the test changed from positive to negative as their condition improved. In two (4 and 10) the test was persistently positive irrespective of the eye disease activity.

Patient 4 developed anterior uveitis when aged 8. It has continued intermittently for 28 years. Apart from some joint swelling lasting a few days he has had no other symptoms. He may have a chronic vasculitis which is restricted to the eye. The other patient (10) has systemic features suggestive of a more generalised disorder.

Any clinical features outside the eye which might possibly be related either to the eye or to a disease affecting the eye labelled the patient as having a systemic condition. This was used to divide patients into those having isolated eye disease and those not. No evidence was found to link a positive test to systemic disease.

The proportion of positive tests, about 11%, was similar in patients with AU, PU, or AU plus PU. The site of the uveitis was not therefore associated with a positive test.

Analysis of the time course of uveitis (Table 2) shows that single/repeated uveitis was more frequently associated with positivity than chronic disease. This association was not found when AU alone was considered, though the results were suggestive. Possibly the size of the groups was not large enough to give statistical significance.

In the positive group there was no consistency in sex, age of onset, or association with other diseases, nor in type of ANCA response and any of these features. If it is assumed that all patients with a positive ANCA response have a small vessel vasculitis, then it follows this disease process does not cause a single type of uveitis.

Two explanations might account for the gross

variation in patients with positive results. Firstly, the test is totally non-specific and occurs in a wide variety of immune mediated diseases which produce an excess of antibodies of all types. Hence a wide variety of uveitides will occur. This view is unlikely. Many diseases can give rise to an occasional positive test, but consistent positives are found only in those conditions which have a small vessel vasculitis, suggesting a specific pathogenesis.

Alternatively the test does reflect a specific abnormality which gives rise to a single pathological process. However, there are many initiating factors for this process, and it may encompass patients of differing ages and associated diseases. This process can be manifested in different parts of the uveal tract, giving different anatomical sites for the uveitis. In a similar way other common pathogeneses may appear in different ways in the eye: consider the widespread features of ocular sarcoid, for example.

Wegener's granulomatosis and microscopic polyarteritis, which are most specific for the ANCA test, for unknown reasons cause different clinical responses in different patients, even though the pathological process is common. It is possible there is a common pathogenesis in ANCA-positive patients which gives rise to different clinical features.

At present it is not possible to make statements on the significance of a positive ANCA test, but certain pointers are evident. Perhaps the most interesting feature of this study was the association of a positive test and isolated uveitis tending to be single or repeated. Four patients (2, 4, 5, 8) fell into this group. However, the numbers are small, and statistical significance was not achieved for this association. This group may represent a small part of the high proportion of patients with AU in which no causative agent is found. A positive test does suggest there is a vasculitis caused by an antibody which cross reacts with elements of the neutrophil cytoplasm.

The two types of positive ANCA tests seem to identify three different groups of patients. The cytoplasmic response was associated with a chronic posterior uveitis manifested as a retinal vasculitis (patients 1, 3). The cytoplasmic response is normally associated with Wegener's granulomatosis. Both of these patients had systemic features; patient 1 had Behçet's disease, and patient 3 a mild colitis; neither had Wegener's granulomatosis.

The perinuclear positives fell into two groups. All had single/repeated disease, usually with an isolated AU, though some had intermediate or a posterior uveitis as well. The first group (4, 5, 7, 8) was younger (disease started before the age of 46 years) and without systemic features. The second group (9, 10, 11) was older, aged 60 years or more at onset, and associated with systemic features or diseases.

Eleven positive responders are rather a weak foundation for such suggestions, but they are offered as starting points for other investigators. Until the immune processes which cause uveitis are better defined, the role of this test will remain unclear. In a few it will help the diagnosis of microscopic polyarteritis nodosa, but in the

majority it will be an interesting though unexplained finding.

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FIFTY YEARS AGO

Mustard gas and its implications

DEAR SIRS,—In reply to Messrs. John Eyre and Frank W Law may I state that I have seen one drop of 4 per cent solution of hyd. perchlor. in glycerine accidentally fall on the cornea and in a few seconds which elapsed before washing out the conjunctival sac, severe and lengthy, but not permanent damage was inflicted on almost the whole of the corneal epithelium. Here there was no question of absorption. I quite agree with them that in the field of biology there is nothing more misleading than the argument from analogy, which indeed led eminent men to assume and publish results on the assumption that because man could accommodate the mammals could also.

But the argument from analogy hardly applies in this instance just quoted.

As regards the late effects of mustard gas on the cornea, the few cases I have seen showed deep seated infiltration rather than ulceration and there was very little clear cornea left anywhere. So that I doubt

whether either diathermy or contact glasses would have assisted. If such cases, which I am glad to say are rare, present themselves, the method suggested will be borne in mind. It is true that these conditions do recur without obvious cause. No one will be more grateful than myself if the writers have devised an effective treatment of mustard gas in the field, and will look forward to their publication of their experimental results. But the practical use of any remedy of the kind in advanced positions of any army is very difficult but not insuperable.

Some idea of the difficulty of acting efficiently in advanced stations may be learnt from the Palestine campaign, where towards the end men with high temperature were given quinine at once, no matter what seemed to be the cause of the temperature. If the medical officers had waited until they could be sure that malaria was the cause it would at times have been too late. To neutralise the effects of mustard on the eyes, special instruction for the forward units will be a necessity. — Letter from James W Barrett. *Br J Ophthalmol* 1941; 25: 95.