A study of oligoclonal band negative multiple sclerosis

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Abstract

Objectives—to determine whether oligo-clonal band (OCB) negative multiple sclerosis is a reliable diagnosis and, if so, whether it has a distinctive prognosis.

Methods—retrospective and matched prospective comparison of the clinical and laboratory features of patients with clinical definite multiple sclerosis with and without intrathecal synthesis of oligoclonal IgG.

Results—thirty four patients were identified with apparent OCB negative clinically definite multiple sclerosis. The results of oligoclonal banding proved to have been equivocal in 14 of 34; the clinical diagnosis of multiple sclerosis was questionable in 8 of 34. The remaining 12 patients with "true" OCB negative multiple sclerosis were significantly less disabled than matched OCB positive controls. Re-examination of CSF-serum pairs from six OCB negative patients showed that three remained OCB negative while three showed evidence of intrathecal synthesis of OCBs.

Conclusions—OCB negative clinically definite multiple sclerosis is rare and should be diagnosed with caution; in unequivocal cases it seems to have a relatively benign prognosis.

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"No test has been found to be consistently positive or abnormal in 100% of cases of clinically definite multiple sclerosis. This was not to be expected as there are few such diagnostic tests in medicine, but there is a significant gap in our knowledge concerning those patients in whom the tests are negative . . ."[1]

Modern immunochemical techniques have made it possible to show intrathecal synthesis of oligoclonal IgG in around 95% of patients with clinically definite multiple sclerosis,[2 3] providing key evidence that the disease process has an immunological component.[4] Although intrathecal synthesis of oligoclonal IgG is not specific for multiple sclerosis,[5] its diagnostic significance in an appropriate clinical setting is acknowledged in the most widely used criteria for the diagnosis of this condition.[6]

The presence of oligoclonal bands (OCBs) also provides some prognostic information. In patients with isolated syndromes which might represent a first attack of multiple sclerosis, such as optic neuritis or transverse myelitis, evidence of intrathecal synthesis of oligoclonal IgG or IgM indicates an increased likelihood of progression to clinically definite disease.[7–9] The contribution of intrathecal IgG synthesis to the pathogenesis of multiple sclerosis, and its bearing on the aetiology of the disease, remain uncertain.[3 10]

Occasional patients with clinically definite multiple sclerosis lack evidence for intrathecal IgG synthesis. Rudick *et al* identified the absence of such evidence as one of five "red flag" features which should cast doubt on the diagnosis.[11] However, the postmortem study of Farrell *et al* confirmed that OCBs may be absent in multiple sclerosis established by necropsy.[12] Their absence was associated with low numbers of "active plaques" and of plasma cells in affected and unaffected white matter and meninges. Stendahl-Brodin *et al* concluded from their clinical comparison of OCB negative and positive patients that the absence of OCBs predicts a relatively benign prognosis.[13]

This study was designed to re-examine the practical and theoretical questions raised by OCB negative critically definite multiple sclerosis with the aid of up to date techniques of immunochemistry and neuroimaging.

Methods

PATIENTS AND STUDY DESIGN

The case notes of patients admitted to The National Hospital for Neurology and Neurosurgery for investigation of suspected multiple sclerosis between 1985 and 1990 were reviewed. Patients with clinically definite or progressive probable disease[14] whose CSF and serum had been examined by isoelectric focusing were identified. Some of these patients were OCB negative. A few additional patients were identified after a request to clinicians at the National Hospital to report any cases of OCB negative definite multiple sclerosis encountered in their clinics.

The clinical details and stored laboratory results of patients with putative OCB negative multiple sclerosis were scrutinised to identify patients with plausible alternative diagnoses to multiple sclerosis (WIM), and those with questionable laboratory results (EJT).

We compared the OCB negative and positive patients in two ways:

(1) the clinical features of the two groups, ascertained retrospectively from the notes, were compared.

(2) We performed a prospective comparison of the OCB negative patients with matched OCB positive controls. Each patient in whom both

the clinical and the laboratory diagnoses seemed secure was matched with an OCB positive patient drawn from the group of patients investigated in 1989–90 for sex, age, duration of disease, Poser category, and disease type (matching of the relapsing and remitting to the secondary progressive type was permitted).[15]

Matching was performed in the following manner. For each OCB negative case we identified the OCB positive patients investigated in 1989–90 who were of the same sex, within five years in age and duration of disease, and who were of the same diagnostic category and type. If one of the potential controls was closer to the index case in both age and duration of disease than any other, that control was selected for further study. If not, random numbers were assigned to the potential controls, and the control with the lowest random number was selected. The matching constraints were relaxed in two cases: an OCB negative patient with an exceptionally long duration of disease (37 years) was matched with an OCB positive control with a disease duration of 22 years; a 58 year old OCB negative patient was matched with an OCP positive control aged 50.

Consenting matched OCB negative and OCB positive patients underwent: (a) a full history and neurological examination (AZJZ); (b) a blind disability assessment, using the Kurtzke expanded disability status scale (EDSS)[16] performed by a single assessor (DK); (c) MRI of brain and spinal cord before and after an infusion of gadolinium-DTPA; (d) venepuncture for HLA typing and immunochemistry (all patients); (e) lumbar puncture for CSF immunochemistry (oligoclonal band negative patients only). Patients who consented to (a) and (b) only were nevertheless included in the study.

IMMUNOCHEMISTRY
Both initial and follow up analysis of CSF-serum pairs involved: (a) isoelectric focusing of CSF and serum with protein transfer to nitrocellulose and visualisation of IgG using an enzyme labelled double antibody method as previously described[17]; (b) polyacrylamide gel electrophoresis (PAGE) of CSF[18]; (c) immunoblotting of the PAGE gel with visualisation of free and bound light chains.[18]

MAGNETIC RESONANCE IMAGING
Brain and spinal cord MRI was performed in nine consenting OCB negative-OCB positive patient pairs with a Signa 1·5 T superconducting system (GE Milwaukee), before and after enhancement with 0·01 mmol/kg gadolinium DTPA (two patients declined enhancement). Axial T2 and proton density weighted images of the brain were acquired using a fast spin echo (FSE) sequence (13 patients) or a standard dual spin echo sequence (five patients). Sagittal FSE images of the spinal cord were obtained in 13 patients, standard spin echo images in five. Axial images of the cord were acquired using a gradient echo sequence.

Scans were reported by a neuroradiologist (BEK) who was blinded to clinical details.

Lesions in brain and spinal cord were quantified as previously described.[19 20]

HLA TYPING
Genomic DNA was isolated from 30 ml venous blood by a phenol-chloroform extraction procedure. DR, DQA1, and DQB1 alleles were determined by standard methods.

STATISTICS
Statistical analysis was performed using the Arcus statistical package. The Wilcoxon signed rank test for non-parametric data was used to compare the characteristics of the matched OCB negative and OCB positive patient pairs. The Mann-Whitney test and the χ^2 squared test with Yates' correction were used to compare the characteristics of the OCB positive and negative patient groups.

Results
PREVALENCE OF OCB NEGATIVE CLINICALLY DEFINITE MULTIPLE SCLEROSIS
Two hundred and ninety patients with clinically definite or progressive probable multiple sclerosis had their CSF examined by isoelectric focusing at the National Hospital between 1985 and 1990. Thirty one were OCB negative, 25 of whom were clinically definite, six progressive probable.[14] Three further OCB negative patients, all clinically definite, were identified by clinicians.

On review, the original laboratory results proved to be equivocal in 13 patients and in error in one. The reasons for equivocal results were suboptimal loading of CSF IgG at isoelectric focusing in four cases and the presence of extremely faint bands which had not been reported in nine cases.

Review of the clinical records disclosed the possibility of an alternative diagnosis to multiple sclerosis in five cases, and two cases were so atypical of multiple sclerosis that it was considered unwise to include them. The alternative diagnoses were: inherited cerebellar degeneration in two cases, and systemic lupus erythematosus, idiopathic cortical degeneration, and hysteria in one case each.

Thirteen cases remained in which both clinical diagnosis and laboratory result were considered secure. All were clinically definite by the criteria of Poser[6] and either relapsing and remitting or secondary progressive in type. One of these "true" OCB negative patients proved to have cervical cord compression as an alternative, or additional, diagnosis to multiple sclerosis on the basis of investigations performed as a part of this study. Thus the final OCB negative group contained 12 patients (table 1). Nine derived from the group of patients studied between 1985 and 1990, indicating a frequency of true clinically definite OCB negative multiple sclerosis of 3·1% (nine of 290) in this population.

COMPARISON OF OCB NEGATIVE WITH OCB POSITIVE PATIENTS
Retrospective group comparison
Table 1 shows the comparison of the clinical and laboratory features of the OCB negative

Table 1 Comparison of clinical and laboratory features of OCB negative patients and OCB positive control patients 1989–90

	OCB positive	OCB negative	P value
Sex ratio (F:M)	94:50 (1·9:1)	6:6 (1:1)	NS
Poser (CDMSA 1:2)	105:39 (2·7:1)	8:4 (2:1)	NS
Type (RR:2°P)	94:50 (1·9:1)	7:5 (1·4:1)	NS
Mean age (y)	41 (41)	42 (43)	NS
Mean duration	11 (10)	14 (12)	NS
Mean duration to LP	8 (6)	11 (10)	NS
Disability	4 (4)	3 (3·5)	0·02
Polyacryl OCBs	82/128	0/12	0·0005
Total light chains	95/115	0/12	<0·0001
Free light chains	72/115	0/12	0·0001
Total	144	12	

Poser (CDMSA1:CDMSA2) = ratio of patients with CDMSA1 by the Poser classification[5]; patients with CDMSA2; type (RR:2°P) = ratio of patients with relapsing and remitting to patients with secondary progressive disease[14]; mean duration = mean duration of disease from onset of symptoms; mean duration to LP = mean interval between onset of symptoms and initial lumbar puncture; disability = mean Kurtzke EDSS,[15] assessed retrospectively from the clinical notes; polyacryl OCBs = numbers of patients with oligoclonal bands on polyacrylamide gel electrophoresis[17]; total light chains = numbers of patients in whom CSF light chains were detected by immunoblotting of polyacrylamide gels[17]; free light chains = numbers of patients in whom CSF free light chains were detected by immunoblotting of polyacrylamide gels.

group with the group of clinically definite OCB positive patients admitted in 1989–90.

Prospective case-control comparison
Eleven of 12 OCB negative patients consented to further study. Eleven OCB positive patients were matched for age, sex, duration of disease, Poser category, and disease type. Ten of these patients agreed to take part; one patient failed to respond to two approaches. Another suitable OCB positive patient was approached and agreed to participate.

There were no significant differences in age or duration of disease between patients in the OCB negative-OCB positive pairs by the Wilcoxon signed rank test. Sex and Poser group (clinically definite multiple sclerosis) were identical within each pair. Six OCB negative patients were relapsing and remitting, five secondary progressive; three OCB positive patients were relapsing and remitting, eight secondary progressive.

Disability was assessed in every patient. Table 2 summarises the scores.

Brain and spinal cord MRI was performed in all 11 OCB positive patients with gadolinium enhancement in 10, and in nine OCB negative patients with gadolinium enhancement in eight. The spinal cord images were technically unsatisfactory in one OCB negative patient.

Table 2 summarises the cranial and lesion loads. Although the cranial lesion score was higher in the OCB positive patients in seven of nine pairs these differences were not significant at P < 0·05 (Wilcoxon signed rank test one tailed P = 0·21). Gadolinium enhancing lesions on cranial MRI were infrequent in both

groups, occurring in two of 10 OCB positive patients and one of eight OCB negative patients. The ratio of periventricular to deep white matter lesions was similar in the two patient groups. Spinal cord lesions were identified in seven of eight OCB negative patients and nine of 11 OCB positive patients with a mean spinal lesion score of 3 in both groups.

There were no significant differences in the frequencies of HLA alleles between the two groups.

FOLLOW UP CSF FINDINGS IN OCB NEGATIVE PATIENTS
Consent to follow up lumbar puncture was obtained from six of the 11 OCB negative patients included in the matched comparison, at a mean interval of four years since the initial lumbar puncture, and from the OCB negative patient in whom the study disclosed cervical cord compression. Four cases remained OCB negative by isoelectric focusing, including the patient with cervical cord compression. Three cases had become OCB positive, one of the three displaying serum bands as well as bands restricted to the CSF. The less sensitive technique of PAGE with a non-specific protein stain (Coomassie blue) failed to detect OCBs in any of the follow up samples, indicating that the response shown by isoelectric focusing was subtle. Free CSF light chains were detected by immunoblotting in two of the three cases with oligoclonal CSF.

Discussion
This study suggests three main conclusions:
(1) It confirms that the absence of OCBs in a putative case of definite multiple sclerosis should lead to a review of the clinical diagnosis and of the relevant investigations. Repeated neuroimaging and re-examination of CSF may be worthwhile in difficult cases. Nevertheless, true OCB negative clinically definite multiple sclerosis occurs, and some patients with undoubted multiple sclerosis remain persistently OCB negative over many years.

(2) The study adds to the evidence that the small group of patients with true OCB negative clinically definite multiple sclerosis has a relatively benign prognosis. Patients in the OCB negative group were significantly less disabled than the matched OCB positive patients when reassessed in this study (table 2). Retrospective scoring of disability from the clinical notes had suggested a similar conclusion (table 1).

Although we cannot be certain of the diagnosis of multiple sclerosis in the "true" OCB negative cases, these patients conformed to the Poser criteria for CDMS[6] after careful review of the clinical evidence. The diagnosis was supported by the MRI appearances in the nine patients who consented to this investigation.

We matched OCB negative patients and positive controls for age, sex, disease duration, and Poser category. However, we allowed the matching of relapsing and remitting patients to secondary progressive controls and vice versa. It so happened that there were five patients

Table 2 Kurtzke disability scores and MRI lesion loads in OCB negative patients and OCB positive control patients

		OCB positive (n)	OCB negative (n)	P value (Wilcoxon)
EDSS	Mean	5·5 (11)	4·0 (11)	
	Median	6·0 (11)	3·5 (11)	0·008
MR lsn load	Mean	48 (11)	37 (9)	
	Median	51 (11)	31 (9)	0·21

EDSS = Kurtzke disability score[15]; MR lsn load = cranial MRI lesion load (see text).

with secondary progressive disease in the final OCB negative group and eight in the OCB positive group. A decision to avoid such pairings would have been liable to obscure any relation between OCB status and disability, the main variable under study, as the onset of significant disability is associated with the onset of the secondary progressive phase of disease.[21] In other words, the hypothesis that the presence of OCBs is associated with worse disability predicted that there would be a higher proportion of patients with secondary progressive disease in the OCB positive group than among the OCB negative patients.

Examination of the pattern of scores on the Kurtzke functional systems scales[16] in OCB positive and negative cases (results not shown) does not suggest that the difference in overall disability between the two groups is explained by different patterns of functional involvement. Similar numbers in the two groups, for example, showed evidence of involvement of sensory and visual functions.

(3) The study raises the possibility that the absence of OCBs is associated with a low plaque burden. The trend towards such an association is in keeping with other reports of positive, if weak, correlations between lesion load, quantitative measures of intrathecal synthesis, and measures of disability.[22 23]

In conclusion, in the sample studied only 3% of patients with undoubted multiple sclerosis lack evidence of intrathecal synthesis of oligoclonal IgG. Failure to show such synthesis in a patient with suspected clinically definite multiple sclerosis should prompt a review of the clinical data, laboratory results, and neuroimaging. It may be worthwhile to repeat some or all of the investigations, as an alternative diagnosis may come to light, and a proportion of patients with OCB negative multiple sclerosis will acquire intrathecal OCBs with time. Nevertheless, some patients with undoubted multiple sclerosis remain persistently OCB negative. The present study supports the conclusion of Stendahl-Brodin and Link[13] that the prognosis of true OCB negative multiple sclerosis is relatively benign. It is uncertain whether this relation between OCB status and prognosis is explained by an association with lesion load, with the intensity of inflammation, or with some other factor.

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