RESEARCH PAPER

Cerebrospinal fluid oligoclonal bands in multiple sclerosis and clinically isolated syndromes: a meta-analysis of prevalence, prognosis and effect of latitude

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ABSTRACT

Background Oligoclonal bands (OCBs) unique to the cerebrospinal fluid are used in the diagnosis of multiple sclerosis (MS). The precise prevalence of OCBs in MS and clinically isolated syndrome (CIS) is unknown. The influence of OCBs on clinical outcomes has not been quantified. OCB prevalence has been associated with latitude in a single study, if confirmed this would provide avenues for further study.

Methods Using a systematic review and meta-analysis approach, the proportion of OCB-positive MS and CIS and the influence of OCBs on clinical outcomes were calculated. The relationship between latitude and OCB prevalence was calculated using linear regression.

Results Seventy-one articles were included. Overall, 87.7% of 12 253 MS and 68.6% of 2685 CIS patients were OCB positive. OCB-positive MS patients had an OR of 1.96 of reaching disability outcomes, although a number of negative studies did not provide data. OCB-positive CIS patients had an OR of 9.88 of conversion to MS. Latitude predicted OCB status in MS patients (p=0.009) but not in CIS patients.

Conclusions This is the largest study of OCB prevalence in MS and CIS. OCB positivity strongly predicts conversion from CIS to MS. The relationship between latitude and OCBs is confirmed, and this finding warrants further investigation.

INTRODUCTION

IgG oligoclonal bands (OCBs) represent IgG unique to the cerebrospinal fluid (CSF), that is, without corresponding IgG in the serum. They are commonly used as part of the diagnostic workup for multiple sclerosis (MS) but are not essential to make the diagnosis.1 Many studies have been performed examining their frequency, sensitivity and specificity; others have attempted to determine their utility as a prognostic marker. OCBs are not unique to MS; rather they provide evidence of intrathecal IgG synthesis thought to reflect the compartmentalised central nervous system (CNS) humoral immune activation present in MS. OCBs are found in other inflammatory and infectious diseases affecting the CNS, although these can be differentiated from MS using additional CSF and/or clinical findings.

A number of techniques have been developed to detect CSF OCBs. The gold standard is isoelectric focusing (IEF) on agarose gel followed by immunoblotting or immunofixation for IgG2 with paired CSF and serum. The sensitivity for detection of OCBs is over 95% using this technique.3–5 Alternative techniques, including silver staining, demonstrate reduced sensitivity and specificity in MS.5 There are six classic patterns of CSF and serum staining:

- Type 1 (C−/S−): no bands in either CSF or serum;
- Type 2 (C+/S−): oligoclonal IgG bands in CSF but not in serum, indicating intrathecal IgG synthesis;
- Type 3 (C++/S+): oligoclonal IgG bands in CSF with additional identical IgG bands in CSF and serum, also indicating intrathecal IgG synthesis;
- Type 4 (C+/S+): identical IgG bands in CSF and serum, indicating systemic immune reaction;
- Type 5 (Cm/Sm): monoclonal IgG bands in CSF and serum, indicating the presence of a systemic paraprotein;
- Type 6 (Cm/S−): monoclonal IgG band in the CSF but not in the serum, indicating the presence of an intrathecal monoclonal; this can occur as part of the early evolution of an intrathecal oligoclonal response or represent an intrathecal paraprotein.6

The presence of a single OCB limited to the CSF is considered a negative study. There is some evidence that a second lumbar puncture (after an interval of at least 6 months) should be considered in those patients with a single OCB, because those patients who convert to a ‘full’ OCB pattern are more likely to be subsequently diagnosed with MS.6

The precise prevalence of OCB positivity in MS is not known. There are a large number of studies examining this as either a primary or a secondary research question. The largest of these enrolled around 3000 patients.7 Although the advent of large MS databases has enabled population-based studies, there are limitations to using these, as different centres often use different techniques for detecting OCBs.

There are a number of studies examining the prognostic significance of OCB positivity (and negativity) when assessing a patient with a clinically isolated syndrome (CIS). The presence or absence of OCBs can give important information regarding
the likelihood of progression to clinically definite MS, however, the magnitude of this prognostication has yet to be determined in a population size greater than 500.

The relationship between the presence or absence of OCBs and disease outcomes in those with clinically definite MS has also been an area of interest. To date, there is no definitive answer as to whether the presence of OCBs confers a better outcome in terms of disability progression.

It has recently been suggested that the latitude at which a patient resides is related to the probability of being OCB positive. The reasons behind this variability are unclear, and this finding requires replication.

Given the volume of literature that has previously been published surrounding OCBs and MS and the variation in techniques that have been used, there is a need to clarify the use of OCBs in the clinical arena. Through a systematic review of the literature and a meta-analysis, this study aims to clarify the prevalence of OCBs in clinically definite MS and CIS. The relationship between OCB positivity and MS, conversion from CIS to clinically definite MS and any potential relationship between OCB status and latitude in MS and CIS are also examined.

METHODS
Inclusion criteria

The inclusion criteria were prespecified. The search was limited to papers published after 1980 as IEF with immunofixation was not commonly used before this time. In order to include papers, studies had to include CSF data on a minimum of 10 patients with MS, suspected MS, or CIS. Those papers that specified the inclusion of patients with neuromyelitis optica (NMO; Devic’s disease) were excluded. Studies including patients with ‘Asian optico-spinal MS’ were not included from the initial analysis as this entity appears to overlap with MS and NMO. These papers were excluded from the conservative analyses. In those papers including patients with MS and CIS, the two groups had to be clearly separated. The number of patients found to be OCB positive and OCB negative had to be clearly stated. The technique used to determine OCBs was recorded. For those studies used to determine the prevalence of OCB in MS, the study had to specify that OCBs were determined by IEF with immunofixation; studies using alternative techniques were excluded. Those papers that used a small number (<20) of patients with the primary aim of comparing methods for detecting OCBs were rejected at this stage.

In order to be included in the prognostic data analysis, papers had to give data regarding the number of OCB-positive and OCB-negative patients who met a predefined clinical endpoint. Data regarding all endpoints were gathered. Data from studies using all techniques for OCB detection and from those not specifying methods were recorded. Those studies giving narrative information about clinical outcomes were also recorded, but they could not be included in the formal data analysis.

Search strategy

PubMed was searched using the terms ‘multiple sclerosis and oligoclonal bands’, ‘multiple sclerosis and OCBs’, ‘multiple sclerosis and OCB’, ‘clinically isolated syndrome and oligoclonal bands’, clinically isolated syndrome and OCB’ and ‘clinically isolated syndrome and OCBs’. The resulting abstracts were hand searched for publications meeting the inclusion criteria. The results from each search were cross-referenced as many duplicate results were identified.

Statistical analysis

Prevalence, sensitivity and specificity and positive and negative predictive values (PPVs and NPVs) were calculated using standard formulae. The OR of clinical outcomes according to OCB status was calculated using the generic inverse variance model in RevMan 5.1. A random-effects model was applied unless I² was ≤25%, in which case a fixed-effects model was used.

Between-study heterogeneity was assessed for each calculation using Cochran’s Q, χ² test and I². Bias was assessed using visual inspection of funnel plots and quantified using an Egger p value.

Where clinical outcomes were studied, those studies using IEF and immunofixation were initially studied in isolation. The studies using other techniques were then added to the cohort in order to increase the number of patients included in the analysis. Separate analyses were performed for MS and CIS. When examining outcomes in CIS, two analyses were performed—one with all patients with CIS and a further subgroup analysis of patients presenting with optic neuritis (ON). Fisher’s exact test was used to compare the proportion of OCB-positive and OCB-negative patients reaching the clinical outcomes. Linear regression (modelled in PASW V18 (SPSS)) was used to examine whether any relationship existed between conversion rates and duration of follow-up in OCB-positive and OCB-negative groups.

Effect of latitude on OCB status

The location of each study was determined using the data provided in the manuscript, and the latitude was determined using Google maps (http://www.maps.google.com). Where samples had been taken from a regional or national cohort, the latitude of the midpoint of that country or area was used for the analysis. Papers describing samples taken from international collaborations were excluded from this analysis. MS and CIS were analysed separately.

A linear regression model was used for this analysis (PASW V18 (SPSS)). The proportion of CSF samples found to be OCB positive were regressed on the population latitude. The dependent variable was the proportion of OCB-positive samples and the independent variable latitude, and the contribution of latitude to the equation O:E ratio=(latitude×X)+constant was assessed. An additional independent variable for sample size was then added to the model in order to assess whether this affected the results obtained.

RESULTS

Included papers

Following the initial screening, 350 unique papers were identified. The abstracts and full text of these papers were then hand searched for papers meeting the inclusion criteria. Seventy-one articles were selected for inclusion in the final analysis (see online supplementary appendix 1). The reasons for rejecting papers at this stage were varied, but most commonly included papers that selected small numbers of patients for methodological studies (n=58), papers that selected OCB-positive or OCB-negative patients only (n=15), review papers (n=18) and papers examining IgM OCB only (n=12). The selection process is summarised in figure 1.

Forty-eight studies were used to assess the prevalence of OCB in MS and CIS, with 32 giving information on OCB prevalence in MS and 21 in CIS (some papers covered both MS and CIS). 36 papers were used to calculate the relationship between OCB and clinical outcomes and 12 papers gave qualitative information regarding the relationship between clinical outcomes and OCB status (see online supplementary appendix 1). Of the 36 papers
used to calculate outcomes, 18 used IEF with immunofixation, in 12 the technique was not specified and the remaining 6 used a specified technique other than IEF with immunofixation, most commonly electrophoresis with silver staining. Ten studies used in the clinical outcomes data analysis studied outcomes in MS, and of the remaining 26 examining CIS, 9 selected patients with ON. Forty-four papers were used in the latitudinal analysis, with some papers providing information on both MS and CIS. 28 papers were used to determine the relationship between latitude and OCB status in MS, and 19 in CIS.

**OCB prevalence in MS and CIS**

There was OCB data meeting the inclusion criteria in a total of 12,253 MS patients, of whom 10,751 were OCB positive and 1,577 OCB negative; overall, 87.7% patients with MS were OCB positive. When the three Asian studies were excluded, 10,719 of 12,171 (88.1%) MS patients were found to be OCB positive. When all studies were included regardless of the population and the technique used to detect OCB, 16,678 of 19,773 (84.3%) MS patients were OCB positive. A conservative analysis, where only those papers using IEF with immunofixation were included, and all papers possibly using duplicate cases (ie, those originating from the same centre) and the Asian studies were excluded, showed 5,495 of 6,118 (89.8%) patients with MS were OCB positive. There was a significant difference in the OCB positivity rate between the ‘all studies’ and the conservative analysis (p<0.0001; χ² with Yates’ correction).

There was OCB data meeting the inclusion criteria in a total of 2,685 patients with CIS, of whom 1,841 were OCB positive and 844 OCB negative; overall, 68.6% patients with CIS were OCB positive. There were no studies examining OCBs in CIS in Asian patients. When all studies were included regardless of techniques, 3,580 of 5,154 (69.5%) patients were OCB positive. A conservative analysis showed 1,489 of 2,205 (67.5%) CIS patients to be OCB positive. There was no significant difference in the OCB positivity rate between the ‘all studies’ and the conservative analysis.

**Relationship between OCB status and clinical outcomes in MS**

Ten studies gave data about clinical outcomes in patients with MS. Of these, four used IEF with immunofixation. In all of the studies using IEF with immunofixation, Expanded Disability Status Scale (EDSS)-related outcome measures were used to define clinical outcomes; one used EDSS of 4 at 10 years disease duration, two used EDSS 6 during follow-up and one used an increase of ≥1 EDSS point in 5 years. When the results were combined, 667 of 1,764 (37.8%) OCB-positive patients reached the disability outcome measures specified in the study compared with 42 of 154 (27.2%) OCB-negative patients (p<0.0001, Fisher’s exact test). When the meta-analysis was performed, this gave an OR of reaching the disability outcome of 1.96 (95% CI 1.31 to 2.94; p=0.001) with no between-study heterogeneity (I²=0%; X²=2.95, df=3, p=0.40) (figure 2). There was no significant publication bias (Egger p value=0.12). A subgroup analysis of the two studies using EDSS 6 as an endpoint gave an OR of reaching EDSS 6 of 2.03 (95% CI 1.24 to 3.33; p=0.005) with no heterogeneity (I²=0%; X²=0.87, df=1, p=0.35).

When the six studies using other techniques for measuring OCBs were included (see online supplementary appendix 1), the range of outcome measures used increased. EDSS outcome was used by a number of studies, including EDSS 6, 7.5 or 8 between 5 and 10 years disease duration. One study used worsening of EDSS by 1 point over 2 years, and another study used ‘poor recovery from relapses’. Seven hundred and seventy of 2,202 (35.0%)
OCB-positive patients reached the disability outcome measure associated with the study compared with 66 of 333 (19.8%) OCB-negative patients (p<0.0001, Fisher’s exact test). Inclusion of the studies using alternative techniques and EDSS-defined outcome measurements gave an OR of meeting the study endpoint of 1.65 (95% CI 1.27 to 2.13; p=0.0002) with moderate heterogeneity (I²=48%; X²=22.97, df=13, p=0.03) (data not shown). This was not significantly different from when only IEF with immunofixation was used.

Thirteen studies gave narrative results without absolute numbers. One study found a significantly lower relapse rate in OCB-negative patients (relapse rate 1.45±0.69 in OCB positive and 0.58±0.64 in OCB negative, p=0.001). None of the other studies demonstrated any relationship between the presence of OCBs and the disability outcomes collected, including relapse rate, EDSS and MS severity score.

Relationship between OCB status and outcomes in CIS

Fourteen studies examined the relationship between OCB detected by IEF with immunofixation and outcomes in CIS. Two of these studies specified ON, and one study specified a brainstem syndrome as the CIS. Twelve studies used conversion to clinically definitive multiple sclerosis (CDMS) as the outcome, one used radiological conversion to MS and one used the number of patients reaching EDSS 6 at 5 years. The study using EDSS 6 as the outcome measure was excluded given the very different outcome measure, leaving 13 studies in the analysis (see online supplementary appendix). Seven hundred and thirty-three of 1143 (64.1%) OCB-positive patients converted to MS compared with 139 of 616 (22.6%) OCB-negative patients (p<0.0001, Fisher’s exact test). This gave a sensitivity of 0.84 and a specificity of 0.54 when using OCB to predict conversion to CDMS. The positive predictive value was 0.64 and the NPV was 0.77.

When the meta-analysis was performed, there was an OR of conversion to MS of 9.88 (95% CI 5.44 to 17.94; p<0.00001) in the OCB-positive patients (figure 3). However, there was significant between-study heterogeneity (I²=71%; X²=40.79, df=12, p<0.0001). There was no evidence of publication bias (Egger p value=0.20). Excluding the study using radiological conversion did not significantly alter this result. Other attempts to explore the underlying causes of the heterogeneity observed were similarly unsuccessful.

When all of the studies examining the relationship between OCB and conversion to MS (regardless of the technique used to detect OCB) were considered (an additional 12 studies; see online supplementary appendix 1), 973 of 1584 (61.4%) OCB-positive CIS patients converted to MS compared with 173 of 927 (18.7%) OCB-negative CIS patients (p<0.0001, Fisher’s exact test). This gave an OR of conversion to MS of 9.99 (95% CI 6.54 to 15.27; p<0.00001) in the OCB-positive patients (data not shown). There was significant between-study heterogeneity (I²=57%; X²=56.27, df=24, p=0.0002), which proved impossible to eliminate. This result was not significantly different from that when only those studies using IEF with immunofixation were used.

When all studies were included, there appeared to be a relationship between the proportion of OCB-positive patients converting to CDMS and the duration of follow-up (using linear regression, p=0.042, R²=0.1833) (figure 4). However, when only those studies using IEF with immunofixation were included, this relationship was no longer significant. There was no relationship between the proportion of OCB-negative patients converting to CDMS and the duration of follow-up. Given the low conversion rate in the OCB-negative group together with the lack of any relationship between conversion rate and duration of follow-up in the OCB-negative group, it was not possible to determine whether conversion occurs sooner in those who are OCB positive.

Given the large number of studies examining outcomes in ON, these were studied separately. The majority (7 of 9) did not specify that IEF with immunofixation was used. The results were similar to those obtained with all CIS, with 474 of 743 (63.8%) OCB-positive patients developing MS compared with 98 of 429 (22.8%) OCB-negative patients (p<0.0001, Fisher’s exact test). The meta-analysis gave an OR of conversion to MS of 10.13 (95% CI 7.11 to 14.44; p<0.00001) in the OCB-positive patients with no heterogeneity (I²=0%; X²=6.96, df=8, p=0.54).

Relationship between OCB and latitude

Twenty-eight studies giving data on OCB in MS were used to determine the effect of latitude on the proportion of MS samples positive for OCBs. Only studies using IEF with immunofixation were included in this section of the analysis. Linear regression revealed a significant relationship between OCB positivity and latitude (p=0.002, figure 5) with a correlation coefficient (R²) of 0.31. This relationship was maintained when an
additional variable for sample size was included in the model (for effect of latitude, p=0.009; for effect of sample size, p=0.833). When the Asian studies were excluded, a significant relationship remained (p=0.005 in the linear regression model; R²=0.169).

Nineteen studies were included in the latitudinal regression model for CIS. There was no significant relationship between the proportion of OCB-positive samples and the latitude (p=0.099; data not shown); this was not altered by the inclusion of sample size in the model (for effect of latitude, p=0.119; for effect of sample size, p=0.856).

CONCLUSIONS
By pooling a large number of studies with information regarding OCB status in MS and CIS, this study is able to inform clinicians regarding the clinical significance of OCBs in patients with suspected and definite MS. Just under 90% patients with MS and around 68% patients with CIS are OCB positive. In patients with CIS, the presence of OCBs is associated with a markedly increased risk of conversion to MS. The magnitude of this risk equates to an OR of 9.9, seemingly regardless of the anatomical location of the CIS.

The proportion of the approximately 10% patients who have been diagnosed with ‘OCB-negative MS’, who actually have MS, remains unclear. It is likely that at least some of these patients will not actually have pathologically definite MS; 95% surveyed neurologists felt that they had evaluated a misdiagnosed patient in the last year. The most common suspected alternative diagnoses included non-specific white matter abnormalities on MRI, small vessel ischaemic disease and migraine. All of these conditions may cause MRI abnormalities that may be mistaken for MS; however, none are associated with OCB. Solomon et al argue that the inappropriate use of imaging criteria for diagnosis may play an important role in misdiagnosis. Given the high rate of OCB positivity in our meta-analysis, we argue that OCB positivity plays an important role in MS diagnosis, and that those patients who are OCB negative should have their diagnosis closely considered.

The relationship between the presence of OCB and clinical outcomes in those people who have developed MS is less clear, with the heterogeneity between outcome measures used, length of follow-up and publication/reporting bias clouding the analysis. While the initial analysis would suggest that those patients with MS who are OCB positive have an OR of 1.96 of reaching specified disability outcomes at follow-up compared with those who are OCB negative, this must be qualified by examining the studies giving narrative negative results. While the negative studies, in general, did not provide raw data regarding the number of patients reaching disability milestones, they must be taken into account.

The confirmation of the finding that latitude does appear to be associated with OCB status in MS (but not in CIS) is interesting. In theory, the relationship between OCB positivity rate and latitude in people with MS should be independent of latitude if all people diagnosed with MS actually have MS. However, if one assumes that an incorrect diagnosis of MS, resulting in a diagnosis of ‘OCB-negative MS’, is one of the major causes of OCB-negative MS, and that this misdiagnosis rate is independent of the prevalence of MS, then this would have a greater effect on the perceived OCB prevalence in those countries with lower rates of MS. Thus, a potential factor underlying the relationship between OCB positivity and latitude would be a higher rate of MS misdiagnosis relative to absolute MS prevalence in those countries where MS is rarer.

It has previously been shown that in the Northern Hemisphere, the prevalence, but not the incidence, of MS varies in a latitudinal manner. The reasons for this difference are unclear but may reflect changing cultural habits, including attitudes to sunbathing and sunscreen, which in turn affect population vitamin D levels. The latitudinal variation in OCBs may therefore reflect a shared underlying aetiology with the variation in prevalence rates, and it will be interesting to see whether this gradient changes or disappears altogether.

Further studies are therefore required in order to determine why latitude appears to affect OCB status in this way. The relationship between OCB status and clinical outcome in MS remains unclear, and large-scale prospective studies are required in order to overcome the significant selection and publication bias that may underlie many of the results in the literature to date.
Multiple sclerosis

Contributors RD and GG conceived the idea of this paper. RD performed the literature search, RD and SR performed the statistical analysis. RD wrote the original draft of the manuscript, with important contributions from SR and AD. All authors provided input into the final manuscript.

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