Oligoclonal bands and the IgG index in multiple sclerosis: uses and limitations

P D GILES*, J P HEATH† and S J WROE†
From the Departments of *Clinical Chemistry and †Neurology, The Royal Infirmary, Edinburgh EH3 9YW, UK

SUMMARY. The relationship between two tests commonly used in the investigation of multiple sclerosis (MS), the IgG index and oligoclonal bands, has been assessed. Using an immunoblotting technique specific for IgG, analysis of cerebrospinal fluid for oligoclonal bands was found to provide greater diagnostic sensitivity than the IgG index without any loss of specificity. In patients without oligoclonal bands the IgG index had no diagnostic value for MS and in the presence of bands the magnitude of the index was unrelated to the clinical certainty of the diagnosis. High values of the IgG index were invariably associated with the presence of oligoclonal bands and the IgG index appeared to have no clinical significance independent of this relationship. Even as a screening test the IgG index has serious limitations.

No completely reliable test for multiple sclerosis (MS) has yet been devised and clinical diagnostic criteria remain extremely important. However, in recent years several techniques have been developed which are of value in this context, including electrophysiological measurements (evoked potentials), nuclear magnetic resonance imaging and cerebrospinal fluid (CSF) immunoglobulin analysis.

According to recent diagnostic criteria,1 evoked potential and tissue imaging methods can be considered as extensions of the clinical examination in that they demonstrate subclinical dissemination of disease, although such abnormalities are not specific for MS. The information provided by immunoglobulin studies, however, is of a different type and complements that available from electrophysiological and imaging techniques. Although the pathogenetic significance of intrathecal immunoglobulin synthesis is unclear, a diagnosis of MS can be considered to be laboratory-supported or not according to the presence or absence of evidence of intrathecal immunoglobulin production.1 It is important to note, however, that intrathecal immunoglobulin synthesis also occurs in several other diseases, including neurosyphilis, subacute sclerosing panencephalitis and systemic lupus erythematosus (SLE).2 Tests of antibody production within the central nervous system must therefore be combined with other types of evidence before a diagnosis of MS can be made.

Indicators of intrathecal immunoglobulin synthesis are either quantitative or qualitative. Quantitative tests include the IgG index3–5 Tourtellotte’s formula6–8 the gamma globulin index9 and the CSF IgG/albumin ratio.8 Oligoclonal band analysis is a qualitative test.

There has been a debate on the relative merits of quantitative and qualitative tests of intrathecal immunoglobulin production. In a comparison of the IgG index and oligoclonal bands in 44 patients with borderline indices, Thompson et al.10 found that the proportion of samples giving a normal index but showing oligoclonal bands varied between 34% and 43% according to the cut-off chosen for the index. However, these authors considered the effect of varying the decision level for the index on the ability to predict the outcome of oligoclonal band analysis and did not evaluate the implications for diagnostic accuracy in MS as assessed by independent clinical criteria. Bloomer and Bray6 compared oligoclonal bands with both Tourtellotte’s formula and the CSF IgG/albumin ratio in the diagnosis of MS. Although the detection of oligoclonal bands was found to be the best single test, a combination of this analysis with Tourtell-
lotte's formula was found to give a greater sensitivity in definite and probable MS, although the effect of using this combination on diagnostic specificity was not discussed in detail. French et al.9 however, in a study of the gamma protein index and oligoclonal bands in MS, concluded that the combination of their two tests offered greater specificity than the use of the index on its own.

There is therefore a commonly held view that, while the detection of oligoclonal bands may be the best individual test of local immunoglobulin synthesis in MS, nonetheless there may be an advantage in combining this analysis with a quantitative test to enhance either diagnostic sensitivity or specificity.

However, the potential gain in performing both types of analysis is limited by the extent to which the two tests are independent of each other. In this paper the interdependence of the IgG index (one of the most commonly used quantitative tests) and analysis for oligoclonal bands is evaluated, and the degree to which the clinical value of each test depends on the outcome of the other is assessed.

### Materials and methods

Over an approximately 12-month period, 250 requests for the IgG index were received on patients undergoing diagnostic lumbar puncture for a variety of neurological disorders. Of these requests, 216 were included in the present study. Thirty-one patients were excluded either because there was insufficient CSF to permit all the analyses to be performed or because the CSF was blood-stained. Three repeat requests on patients already included in the series were also excluded.

The patients were classified according to diagnosis without reference to laboratory data (Table 1). Multiple sclerosis was definitely excluded in 117 cases. A clinical diagnosis of MS was made in 97 patients and these were divided into definite, probable and possible cases according to the purely clinical criteria of McAlpine.11 In addition, two requests were received from patients with optic neuritis. This lesion presenting in isolation is not considered in the three basic McAlpine classes. However, in the present study, these cases have been added to the 'possible MS' group on the grounds that the commonest cause of optic neuritis in adults is demyelination, between 50% and 70% of patients being found to have MS on long-term follow-up.12

CSF and serum IgG and albumin concentrations were measured by kinetic immunoturbidimetry using a Cobas Bio centrifugal analyser. The between-batch coefficients of variation for the two proteins in both fluids were between 4% and 6%. The antiserum for the IgG assay was obtained from the Scottish Antibody Production Unit, Carluke, Lanarkshire, UK, and that for the albumin assay from Cambridge Life Sciences, Milton Road, Cambridge, UK. The IgG assays were standardised with SPS-O1 (Protein Reference Unit, Royal Hallamshire Hospital, Sheffield, UK). The albumin assays were standardised with human serum albumin (Hoechst U.K. Ltd, Salisbury Road, Hounslow, UK) in phosphate-buffered saline pH 7·4.

The IgG index was calculated from the ratios of the IgG concentration, [IgG], in CSF and serum and of albumin concentration, [alb], in CSF and serum:

\[
\text{IgG index} = \frac{[\text{IgG}]_{\text{CSF}}}{[\text{IgG}]_{\text{serum}}} \cdot \frac{[\text{alb}]_{\text{serum}}}{[\text{alb}]_{\text{CSF}}}
\]

Oligoclonal band analysis was performed on paired CSF and serum samples according to the method of Walker et al.13 In brief, unconcentrated CSF and diluted serum (1:100 in 0·9% saline) were separated by isoelectric focusing on 0·5 mm agarose (agarose Z and ampholine pH 3·5–10 from LKB-Pharmacia, Pharmacia House, Milton Keynes, UK) and blotted on to nitrocellulose (Schleicher and Schuell membranes supplied by
Anderman & Co Ltd, Kingston-upon-Thames, UK). After blocking in 2% gelatin (Bio-Rad, Caxton Way, Watford Business Park, Watford, UK) the membrane was stained by a double-antibody immunoperoxidase technique (first antibody rabbit anti-human IgG; Dako, High Wycombe, UK, diluted 1:1000 and second antibody goat anti-rabbit IgG horseradish peroxidase conjugate; Bio-Rad 170-6515, diluted 1:3000). 4-Chloro-1-naphthol (Bio-Rad 170-6534) was used for colour development. The results were interpreted without knowledge of the IgG index, and were regarded as positive if two or more discrete bands were detected in the CSF in the absence of matching bands in the corresponding serum specimen.

Unpaired numerical data were compared by the Mann-Whitney U-test.

Results

(1) The Relationship between the IgG Index and Oligoclonal Banding

The distribution of the IgG index according to the presence or absence of oligoclonal bands (irrespective of clinical diagnosis) for the 216 sets of analyses is shown in Fig. 1. Seventy-five of these patients were positive for oligoclonal bands. In the oligoclonal band negative patients the IgG index has an approximately Gaussian distribution with a mean of 0.46 and standard deviation 0.098. Oligoclonal bands were not found in any case in which the index was below 0.46 but were present in all cases with an index greater than 0.73. Outside these limits, therefore, the outcome of oligoclonal band analysis could be confidently predicted from the IgG index.

Ninety-four (44%) of the 216 specimens had IgG indices between 0.46 and 0.73. Oligoclonal bands were found in 23 (24%) of these, representing 31% of all the oligoclonal band positive results.

(2) The Clinical Significance of the IgG Index in the Absence of Oligoclonal Bands

Of the total of 141 CSF specimens in which no oligoclonal bands were found, 112 came from patients in whom MS had definitely been excluded ('non-MS'). In the remaining 29 patients, MS was the final clinical diagnosis and these were classified as 18 possible, nine probable and two definite cases. Figure 1 (c and d) shows the distribution of values of the IgG index on the oligoclonal band-negative patients according to whether a clinical diagnosis of MS had been made or not. There is complete overlap between these two groups of patients. The mean IgG index in the MS patients (0.45) is not significantly different ($P = 0.67$) from that in the non-MS patients (0.46).

The outcome is not changed if patients with possible MS are excluded and a comparison is made between definite and probable cases on the one hand and non-MS on the other. Figure 1 (c and e) shows that, in patients without CSF oligoclonal bands, the distribution of values of the IgG index in definite and probable MS lies entirely within that found in non-MS patients, no statistically significant difference being demonstrable between the MS and non-MS patients ($P = 0.8$). In these patients without CSF oligoclo-
nal bands, therefore, the IgG index had no diagnostic value for MS.

(3) THE CLINICAL SIGNIFICANCE OF OLGOCOLONAL BANDS IN THE ABSENCE OF AN ELEVATED IGG INDEX
The highest value of the IgG index found in oligoclonal band-negative patients was 0.73. This occurred in a non-MS patient with a lateral popliteal nerve palsy. For the purpose of this discussion, 0.73 is taken as the decision level for the IgG index, values greater than this being regarded as 'raised'. The effect of varying the decision level is considered in section 5.

In patients with an IgG index below 0.73, oligoclonal bands were found in the CSF in 22 cases with a clinical diagnosis of MS, comprising eight definite, four probable and 10 possible cases. These patients represented 31% of the cases thought to have MS on clinical grounds and in whom oligoclonal bands were found.

In general, sensitivity is defined as the incidence of true-positive results obtained when a test is applied to patients known to have a particular disease. Specificity is calculated as the incidence of true-negative results obtained when the test is applied to subjects known to be free of the disease in question. In the present context, the calculation of the diagnostic sensitivities and specificities of oligoclonal bands and the IgG index is complicated by the fact that, in probable and possible MS, there is uncertainty about the true diagnosis. However, estimates of sensitivity and specificity can be obtained by considering respectively the clinically definite cases and the patients from whom MS has definitely been excluded.

Of the 33 clinically definite MS cases in the present study, 31 (94%) had CSF oligoclonal bands whereas only 23 (70%) had IgG indices greater than 0.73 (all these cases also having oligoclonal bands). This difference represents the increase in sensitivity due to the detection of oligoclonal bands in the absence of an elevated IgG index.

Of the 117 non-MS cases, 112 (96%) were oligoclonal band-negative as compared with 113 (97%) with an IgG index below 0.73. The specificities of the two tests were therefore very similar.

(4) THE CLINICAL SIGNIFICANCE OF THE IGG INDEX IN THE PRESENCE OF CSF OLGOCOLONAL BANDS
Figure 2 shows the values of the IgG index found in patients with oligoclonal bands according to their clinical diagnoses. The overlap in the ranges found in the different diagnostic categories is very substantial. Some of the lowest IgG indices in oligoclonal band-positive patients occurred in cases of definite MS. By contrast, IgG indices of 1.0 or greater were found in 10 of the 22 patients in whom the diagnosis of MS was only 'possible', and in four of the five patients with oligoclonal bands due to other diseases.

There was no statistically significant difference between the values of the IgG index in patients with oligoclonal bands due to clinically definite MS and those with oligoclonal bands in the other diagnostic classes, including diseases other than MS. In the presence of oligoclonal bands, therefore, the magnitude of the IgG index was unre-
Diagnostic tests in multiple sclerosis

Fig. 3. ROC curves for the IgG index. (A) used as a diagnostic test for MS; (B) used to predict the outcome of oligoclonal band analysis.

related to the clinical certainty of the diagnosis of MS, and did not discriminate between patients with MS and those with oligoclonal bands due to other diseases.

(5) THE EFFECT OF DECISION LEVEL ON THE PERFORMANCE OF THE IgG INDEX

Figure 3 shows two receiver-operating characteristic (ROC) curves for the IgG index.

Curve A represents the IgG index as a diagnostic test for MS and is based on the data from patients with (a) definite MS and (b) disease other than MS. It shows the loss of specificity (increasing false-positive rate) that results as the sensitivity (true-positive rate) of the index for MS is increased by reducing the decision level chosen to divide ‘positive’ from ‘negative’ IgG index results. Using a cut-off of 0.55, the sensitivity of the index for MS matches that of oligoclonal bands (94%). However, at this level the index has a specificity of only 79%, as compared with 96% obtained with oligoclonal bands (see section 3).

Curve B represents the index as a test for the presence or absence of oligoclonal bands and is based on the data from all the patients irrespective of diagnosis. It is apparent that regardless of the choice of decision level, the ability of the index to discriminate between MS and non-MS patients is no better than its capacity to separate oligoclonal band positive and negative groups.

The upper right-hand portions of curves A and B (representing the true-positive and false-positive rates at low cut-off values for the index) are almost perfectly super-imposable. By contrast, the lower left-hand portions of the curves (corresponding to high cut-off values for the index) diverge, curve A lying below curve B. The explanation for this is that, when a low decision level is chosen and the IgG index is used as a test for MS, most of the true-positive results are oligoclonal band-positive, whereas nearly all of the false-positive results are oligoclonal band-negative. For example, with a cut-off of 0.45, 31 of the 33 true-positive IgG index results (94%) were from patients with CSF oligoclonal bands while 59 of the 64 patients with false-positive indices (92%) were oligoclonal band-negative. With low decision levels, therefore, the discriminatory power of the IgG index for MS closely reflects its ability to separate patients with and without oligoclonal bands. However, as the cut-off value is raised, an increasing proportion of the false-positive results obtained when the IgG index is used to diagnose MS are also oligoclonal band-positive. Above 0.73, all IgG index results are associated with the presence of oligoclonal bands irrespective of the diagnosis. It follows, therefore, that a decision level greater than 0.73 will result in no false-positives when the index is used to predict the outcome of oligoclonal band analysis, but when it is used as a test for MS the index is limited by the false-positive rate of oligoclonal bands for this diagnosis.

(6) INTRATHECAL IMMUNOGLOBULIN SYNTHESIS IN DISEASE OTHER THAN MS

Table 2 shows the diagnoses of the five patients with diseases other than MS in whom CSF oligoclonal bands were found. The IgG index was raised in all but one of these. Neurosyphilis, subacute sclerosing panencephalitis (SSPE) and encephalitis are recognised causes of intrathecal immunoglobulin synthesis but, to our know-

Table 2. Five non-MS patients with oligoclonal bands

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>IgG index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurosyphilis</td>
<td>2.83</td>
</tr>
<tr>
<td>Subacute sclerosing panencephalitis (SSPE)</td>
<td>1.33</td>
</tr>
<tr>
<td>Encephalitis</td>
<td>0.50</td>
</tr>
<tr>
<td>Congenital torsion dystonia</td>
<td>1.78</td>
</tr>
<tr>
<td>Cryptogenic juvenile chorea</td>
<td>2.50</td>
</tr>
</tbody>
</table>
ledge, oligoclonal bands have not previously been described in the other two disorders.

Although neither test was completely specific for MS, in none of these cases were the CSF findings a cause of confusion, the true diagnosis being readily established by other criteria.

Discussion

This study has examined the extent to which two tests commonly used in the investigation of MS, the IgG index and CSF oligoclonal bands, have independent clinical value.

There was a relationship between the magnitude of the IgG index and the outcome of oligoclonal band analysis such that bands were invariably present when the index exceeded 0.73 and were never detected in patients with an index below 0.46.

In oligoclonal band-negative cases the index did not discriminate between non-MS and MS patients (including clinically definite and probable cases). By contrast, even when the index was not elevated, oligoclonal band analysis increased diagnostic sensitivity for MS without impairing specificity. There was no evidence that the information available from oligoclonal band analysis was enhanced by the addition of the IgG index and in oligoclonal band-positive patients the magnitude of the index was unrelated to the clinical certainty of the diagnosis.

These results suggest that the diagnostic value of the IgG index for MS is entirely secondary to the association between elevated levels and the presence of CSF oligoclonal bands. By contrast, the clinical significance of CSF oligoclonal bands is independent of the value of the IgG index.

These conclusions may not apply to methods using non-specific protein stains, such as Coomassie Brilliant Blue or silver, rather than a specific immunochemical procedure for the detection of oligoclonal bands, as was used here. In a direct comparison, George et al. found that with CSF specimens subjected to electrophoresis on agarose, the interpretation of 9% of specimens with apparent oligoclonal banding detected by Coomassie Brilliant Blue was altered by the immuno fixation. The risk of false positive results when isoelectric focusing is used with non-specific stains has also been emphasized. The conclusions from the present study may also not apply to less sensitive methods for the detection of oligoclonal bands. Such methods may fail to detect banding even when quantitative tests of intrathecal IgG production are abnormal. These considerations may explain the contrast between the conclusions of French et al. and those of the present study. Using a non-specific stain to detect oligoclonal bands and densitometry to determine the concentrations of albumin and gamma globulin in CSF and serum, these authors found that the combination of an abnormal gamma protein index and the presence of oligoclonal bands provided a higher diagnostic specificity for MS than an abnormality in either test on its own, while in a minority of MS cases the index was raised in the absence of oligoclonal bands.

The specificity of both the IgG index and oligoclonal bands for MS depends on the composition of the population being studied. The patients in the present report were investigated in a specialist neurological unit and include a high proportion of patients with diseases other than MS. The situation may be different in a district general hospital, where requests for these analyses may be largely confined to patients with suspected MS. However, although the absolute specificities of the two tests for MS may vary between different patient populations, the relationship between the two tests would be expected to be the same since this appears to be independent of the clinical diagnosis.

The advantage of the IgG index is that it is cheap and easy to determine, especially if the component analyses can be performed on automated equipment. By contrast, oligoclonal band analysis is technically more demanding and time consuming, and requires skill for its interpretation. However, because the detection of oligoclonal bands improves diagnostic sensitivity by about a third without any compromise in specificity, the ease of performing and interpreting the IgG index cannot be considered as justification for its use as an alternative to oligoclonal band analysis.

Because in this study IgG indices outside defined limits reliably predicted the presence or absence of oligoclonal bands, it might be thought that the index could be used as a screening test, direct analysis for oligoclonal bands being unnecessary except when the index fell between about 0.50 and 0.75. However, even as a screening test, the IgG index has limited efficiency since nearly half of the requests in the present study fell within this range. Furthermore, reference ranges for the IgG index are very method-dependent and vary between laboratories. It would therefore be unsafe for a laboratory providing only the IgG index to select samples to refer elsewhere for oligoclonal band analysis on the basis of the limits quoted here.

Downloaded from acb.sagepub.com by guest on September 28, 2016
Acknowledgements

Thanks are due to Professor L G Whitby and Dr A F Smith for helpful criticism of the manuscript, and to Mrs S Milner for technical assistance. We are grateful to Professor C P Warlow, Dr B Ashworth, Dr R E Cull, Dr P A G Sandercock, Dr B Pentland and Dr R Will for permission to study their patients.

References


Accepted for publication 21 March 1989