Chapter 13
The Myelin-*Acinetobacter*-Neurofilament Index in an Attempt to Diagnose Multiple Sclerosis

13.1 Introduction: The Need for a Laboratory Test of Multiple Sclerosis

The diagnosis of multiple sclerosis is difficult and depends on clinical criteria because the pathological process may affect many different nerves which lead to various and protean neurological signs and symptoms.

Some mimicking diseases can be excluded such as Lyme disease, but there is no simple blood test for multiple sclerosis.

However over 80 % of multiple sclerosis patients have detectable abnormalities in their cerebro-spinal fluid (CSF), such as oligoclonal bands (Link and Huang 2006).

The presence of two or more oligoclonal bands suggests the presence of disease activity in multiple sclerosis patients (Davies et al. 2003).

Other groups have suggested that antibodies to myelin or "myelin oligodendrocyte glycoprotein" (MOG) could be used to detect patients who will go on to develop multiple sclerosis following an initial neurological event (Klawiter et al. 2010).

Antibodies to MOG have been reported to be present in 38 % of multiple sclerosis patients compared to antibodies to myelin basic protein being present in only 28 % of such patients (Reindl et al. 1999).

Neurodegeneration with leakage of axonal components such as neurofilaments would appear to be a component of pathological damage in multiple sclerosis (Teunissen and Khalil 2012).

Antibodies to neurofilaments may also be used in the assessment of disease activity (Gahan et al. 1985) and release of axonal proteins would appear to be associated with progressive disease in multiple sclerosis patients as well as with clinical disability (Semra et al. 2002).

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Previous studies have shown that animals affected by bovine spongiform encephalopathy could be detected in many cases by using a composite parameter, the "Myelin-*Acinetobacter*-Neurofilament" antibody index.

Since antibodies to these three components have been investigated, it is proposed to assess whether a similar composite index the "myelin-*Acinetobacter*-neurofilament" assay could be calculated for multiple sclerosis patients.

13.2 Materials and Methods: Serum Samples, Bacteria and ELISA

Serum Samples

Sera from 26 multiple sclerosis patients were obtained from the Institute of Neurology at the Hospital for Nervous Diseases, Queen Square, London, as previously described.

Diagnosis was made according to the Poser criteria and the following abbreviations have been used to describe the patients:

CVA: Serum samples were obtained from 20 patients in the Department of Geriatric Medicine at University College Hospital, London who had suffered from a unilateral hemiplegia due to a "cerebro-vascular accident" or stroke.

B: "Benign multiple sclerosis" patients are characterised by infrequent exacerbations but leading to complete recovery.

RR: "Relapsing remitting" course are patients who have relapses followed by remissions.

ARR: "Acute relapsing remitting multiple sclerosis" patients are those who have more frequent exacerbations followed by partial or complete remission.

Secondary P: "Secondary progressive multiple sclerosis" patients are defined as those who continue to deteriorate without remission following an initial "relapsing remitting" course of disease.

PP: "Primary progressive multiple sclerosis" are patients who are characterised by a continuous deterioration of symptoms without remission from the onset of disease.

Trans: "Transitional multiple sclerosis" patients are those who are deemed to be between "relapsing remitting" and "secondary progressive" stages.

Sera from 25 subjects attending the London Blood Donor services were used as healthy controls.

Bacterial Cultures and ELISA

Bacterial cultures and ELISA studies were carried out as previously described. The coefficient of variation was less than 10 % for all ELISA tests carried out.

Statistical Analysis

Statistical calculations were carried out as previously described.

13.3 Calculation of the Myelin.*Acinetobacter.*Neurofilament Index in Multiple Sclerosis Groups

The MAN (Myelin-*Acinetobacter*-Neurofilament) index was calculated in both multiple sclerosis patients and healthy controls, as previously described for "bovine spongiform encephalopathy":

$$\text{M.A.N. Index} = (\text{Ig MBP} \quad 10) \times (\text{Ig } \textit{Acinetobacter} \times 10) \times (\text{Ig Neurofilament} \times 10)$$

The 99.9 % confidence limits (CL) of the controls were calculated as follows: = Mean ± 3 SD (standard deviations)

The MAN index was expressed as $\log_{10}$ in sera and controls.

13.4 Results of the "Myelin.*Acinetobacter.*Neurofilament" Index Calculations in Multiple Sclerosis Patients

The "myelin.*Acinetobacter.*neurofilament" index was calculated for five strains of *Acinetobacter* species and once with *Pseudomonas* bacteria.

Since only IgG antibodies are likely to cross the blood-brain barrier, the MAN index was calculated using this immunoglobulin isotype.

All multiple sclerosis patients had values above the 99.9 % confidence limits of the controls, when the MAN index was calculated with *Acinetobacter lwoffii 5866* (Fig. 13.1), *Acinetobacter sp, strain 11171* (Fig. 13.2), *Acinetobacter radioresistens* (Fig. 13.3), *Acinetobacter sp. strain 19004* (Fig. 13.4) and *Acinetobacter junii 17908* (Fig. 13.5).

Only 88.5 % of multiple sclerosis were shown to have a MAN index above the 99.9 % confidence limit, when calculating with *Pseudomonas aeruginosa* antibodies (Fig. 13.6).

In all cases only one control was shown to lie above the 99.9 % confidence limit.

Seven CVA patients were shown to have values above the 99.9 % confidence limits when the MAN index was calculated using the *Acinetobacter sp. strain 11171* (Fig. 13.2).

For all other strains of *Acinetobacter* and *Pseudomonas aeruginosa* more than 12 CVA patients had MAN indices above the 99.9 % confidence limits.

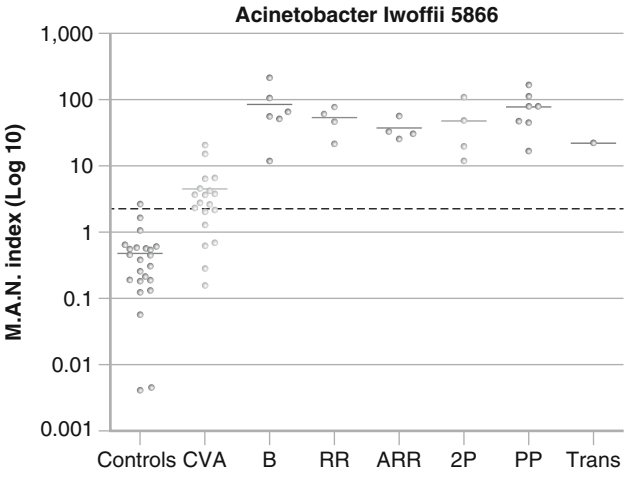

Fig. 13.1 MAN index calculated for *Acinetobacter lwoffii 5866*, expressed as $\log_{10}$ in sera from 26 multiple sclerosis patients (*B* benign, *RR* relapsing remitting, *ARR* acute relapsing remitting, *2P* secondary progressive, *PP* primary progressive, *Trans* transitional), 20 CVA patients and 25 healthy blood donors. *Dashed line* represents 99.9 % confidence limit of controls (Copyright © American Society for Microbiology, Hughes et al. (2001))

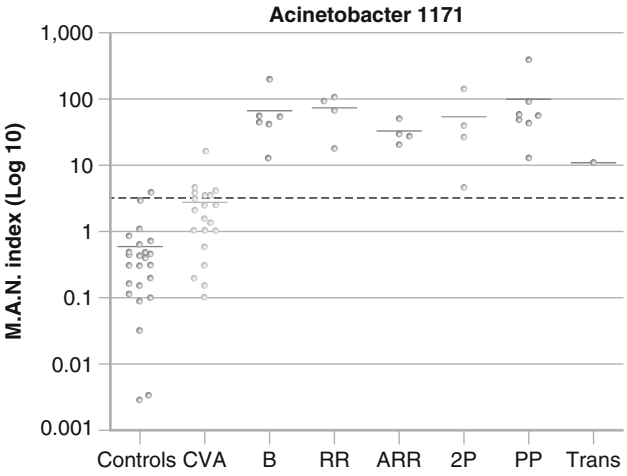

Fig. 13.2 MAN index calculated for *Acinetobacter 11171*, expressed as $\log_{10}$ in sera from 26 multiple sclerosis patients (*B* benign, *RR* relapsing remitting, *ARR* acute relapsing remitting, *2P* secondary progressive, *PP* primary progressive, *Trans* transitional), 20 CVA patients and 25 healthy blood donors. *Dashed line* represents 99.9 % confidence limit of controls (Copyright © American Society for Microbiology, Hughes et al. (2001))

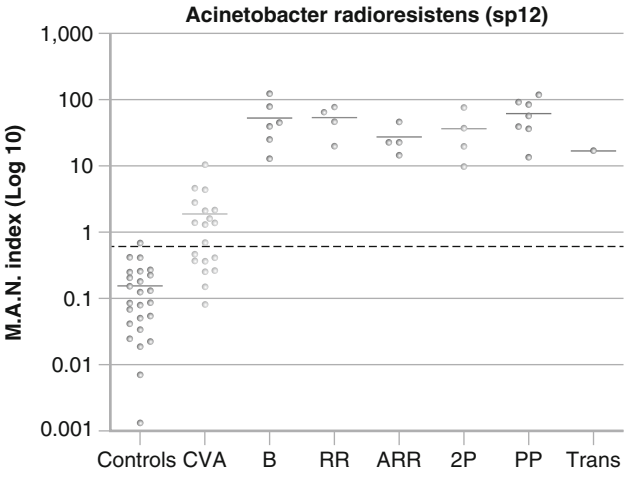

Fig. 13.3 MAN index calculated for *Acinetobacter radioresistens (sp 12)*, expressed as $\log_{10}$ in sera from 26 multiple sclerosis patients (*B* benign, *RR* relapsing remitting, *ARR* acute relapsing remitting, *2P* secondary progressive, *PP* primary progressive, *Trans* transitional), 20 CVA patients and 25 healthy blood donors. *Dashed line* represents 99.9 % confidence limit of controls (Copyright © American Society for Microbiology, Hughes et al. (2001))

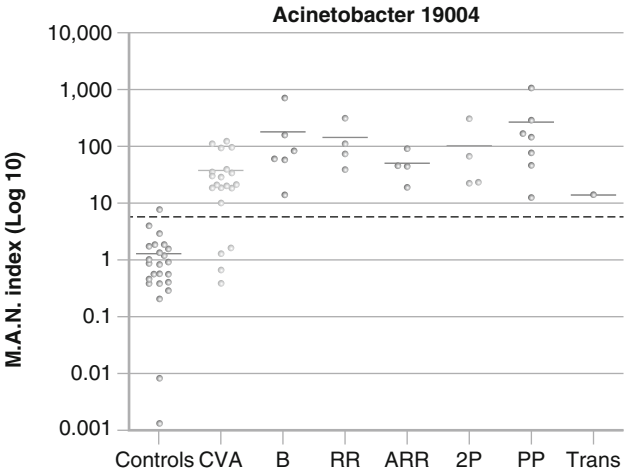

Fig. 13.4 MAN index calculated for *Acinetobacter 19004*, expressed as $\log_{10}$ in sera from 26 multiple sclerosis patients (*B* benign, *RR* relapsing remitting, *ARR* acute relapsing remitting, *2P* secondary progressive, *PP* primary progressive, *Trans* transitional), 20 CVA patients and 25 healthy blood donors. *Dashed line* represents 99.9 % confidence limit of controls (Copyright © American Society for Microbiology, Hughes et al. (2001))

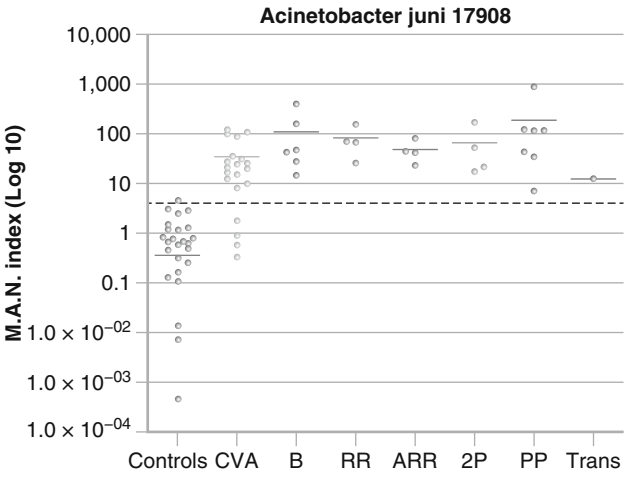

Fig. 13.5 MAN index calculated for *Acinetobacter junii 17908*, expressed as $\log_{10}$ in sera from 26 multiple sclerosis patients (*B* benign, *RR* relapsing remitting, *ARR* acute relapsing remitting, *2P* secondary progressive, *PP* primary progressive, *Trans* transitional), 20 CVA patients and 25 healthy blood donors. *Dashed line* represents 99.9 % confidence limit of controls (Copyright © American Society for Microbiology, Hughes et al. (2001))

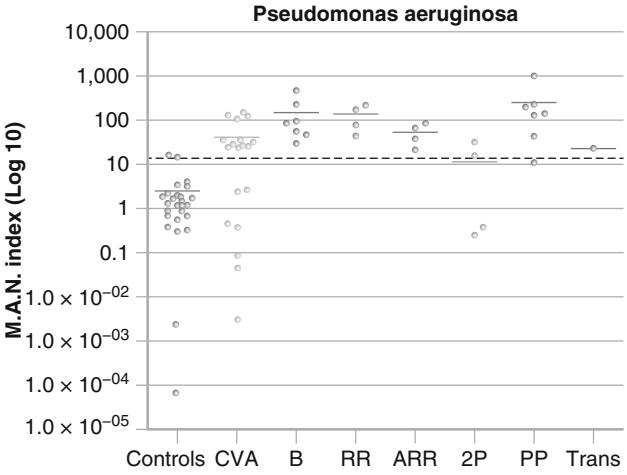

Fig. 13.6 MAN index calculated for *Pseudomonas aeruginosa*, expressed as $\log_{10}$ in sera from 26 multiple sclerosis patients (*B* benign, *RR* relapsing remitting, *ARR* acute relapsing remitting, *2P* secondary progressive, *PP* primary progressive, *Trans* transitional), 20 CVA patients and 25 healthy blood donors. *Dashed line* represents 99.9 % confidence limit of controls (Copyright © American Society for Microbiology, Hughes et al. (2001))

No significant differences were seen in the MAN indices between the different multiple sclerosis groups.

However when calculating the MAN index using *Acinetobacter radioresistens* all multiple sclerosis patients could be distinguished from the two control groups, the healthy individuals and the CVA patients acting as a "disease control" group (Fig. 13.3).

When removing the *Acinetobacter* parameter, the MAN index could distinguish multiple sclerosis patients from healthy controls but not from the CVA group.

When the MAN index was calculated with IgA and IgM antibodies, immunoglobulin isotypes that do not cross readily the blood-brain barrier, the differences between the multiple sclerosis patients and control groups were not as significant, when compared to the calculations carried out with the IgG isotype.

Whether the MAN index can be useful in the diagnosis, progression and follow-up of multiple sclerosis patients requires further studies involving many neurological centres.

13.5 Intrathecal Production of Antibodies and *Acinetobacter*

The origin of antibodies to environmental agents such as viruses or bacteria in causing a neurological disease poses the question as to where is the site of production of such immunological markers.

For instance anti-*streptococcal* antibodies in Sydenham's chorea are produced in the tonsils and related lymph nodes. Then the IgG isotype antibodies cross the blood-brain barrier and affect the basal ganglia with consequent clinical and neurological complications.

Auto-antibodies specific for myelin and myelin oligodendrocyte glycoprotein (MOG) were bound to disintegrating myelin around axons in lesions of acute multiple sclerosis and also in the marmoset model of experimental allergic encephalomyelitis (Genain et al. 1999).

Intrathecal anti-MOG antibody production is elevated in patients with multiple sclerosis. The recombinant myelin oligodendrocyte glycoprotein (rMOG) consists of the 120 amino acid of the extracellular domain of MOG. The rMOG index was elevated in multiple sclerosis patients compared to controls.

Patients with progressive multiple sclerosis had higher indices than patients with relapsing-remitting multiple sclerosis (Klawiter et al. 2010).

Previous studies have shown that over 90 % of patients with clinically definite or probable multiple sclerosis showed abnormal polyacrylamide gel electrophersis patterns in the forms of oligoclonal bands together with cytological presence in the cerebro spinal fluid (CSF) of atypical large lymphocytes and plasma cells (Thompson et al. 1979).

The origin of these antibodies was ascribed to intrathecal production by immune cells which had been stimulated by agents present within the nervous tissues or by autoimmune activity (Newcombe et al. 1985).

Studies on intrathecal synthesis of antibodies to a number of viruses, such as measles, rubella, para-influenza type 2, respiratory syncytial virus, mumps, influenza A, influenza B, adeno and herpes simplex virus showed fluctuations which did not correlate with the clinical course of the disease (Arnadottir et al. 1982). The authors concluded that the viral antibodies studied were not relevant to the aetiology and pathogenesis of multiple sclerosis.

Multiple sclerosis is a disease with extensive heterogeneity in the clinical course as well as in pathological lesions. Some post-mortem studies have shown that the patterns of demyelination are heterogeneous between patients (Lucchinetti et al. 2000).

13.6 Pathological Implications: *Acinetobacter* as an Extra-thecal Aetiological Agent in Multiple Sclerosis

The question arises where were the anti-*Acinetobacter* antibodies produced which had been observed in patients with multiple sclerosis. Paired serum and cerebrospinal fluid samples from multiple sclerosis were compared to other neurological diseases. There was no greater incidence of high affinity antibodies in the CSF compared to serum in multiple sclerosis patients compared to patients suffering from other neurological diseases. This suggests that there is no intrathecal production of antibodies to *Acinetobacter* bacteria (Chapman et al. 2005).

Clearly it would appear that antibodies to *Acinetobacter* behave in the same way as anti-*streptococcal* antibodies in rheumatic fever and Sydenham's chorea.

The antibodies are produced in an extra-thecal space, in the case of Sydenham's chorea in the tonsils and related lymph nodes and in the case of multiple sclerosis probably in the lymph nodes associated with the upper respiratory tract and nasal sinuses.

The antibodies then cross from the serum across the blood-brain barrier to the cerebro-spinal fluid and if present in sufficiently high concentrations, then IgG1 and IgG3 antibodies will activate the complement cascade and cause myelin and neurofilament pathology.

The *Acinetobacter* microbe is frequently isolated from nasal sinuses (Casiano et al. 2001) and sinusitis has been described to be present in many multiple sclerosis patients (Gay et al. 1986).

These observations would appear to be consistent with the previous reports that respiratory infections are somehow associated with the onset or progression of multiple sclerosis.

Autoantibodies occur following tissue damage, such as those observed after myocardial infarctions or burns. A similar situation would appear to occur in CVA patients following damage to brain tissues with production of autoantibodies binding to myelin basic protein or neurofilaments.

Further studies are clearly required to assess whether particular sequences of *Acinetobacter* would provide better antigenic epitopes to study and follow the progression of patients affected by multiple sclerosis.

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