Intrathecal oligoclonal IgG synthesis in multiple sclerosis

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Abstract

Evidence of intrathecal oligoclonal immunoglobulin G (IgG) synthesis has been used as a diagnostic test in patients with suspected multiple sclerosis (MS) for about 50 years. Over the last decade the test allowed to substitute for dissemination in space. Here a case is presented to illustrate some of the practical consequences of removing this test from 2010 revision of the McDonald criteria. Next, the pathophysiology of intrathecal IgG synthesis and methodological aspects of the test are reviewed. Reassuringly the introduction of new commercially available kits improved the inter-laboratory agreement of the test substantially (kappa > 0.8) with an high analytical sensitivity (92-98%) of 114 laboratories participating in the UK external national quality assessment service between 2008-2011. Presence of intrathecal oligoclonal IgG is a sign of pathology in a number of diseases other then MS and some these pose a differential diagnostic challenge in daily clinical practice. This argument is further supported by a meta–analysis (13,467 patients) which illustrates that the diagnostic specificity drops from 94% to 61% if such cases are considered. Taken together there is no evidence to suggest that the test may be a substitute for radiological dissemination in space. There is however data to suggest that the test may be of prognostic value. Based on the prognostic value and the high analytical sensitivity, a conceptional change may be possible by prospectively testing if the test may be considered as a potential substitute for dissemination in time.

Keywords:

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Introduction

Evidence of intrathecally–produced immunoglobulin G (IgG) was used from 1950– 2011 as an additional diagnostic test for multiple sclerosis. Stringent brain imaging criteria can demonstrate dissemination in space (DIS) with such accuracy that an additional CSF examination is not necessary.[1] The vigour of the public debate following the publication of this paper on the future diagnostic value of this test[2–4] published in this Journal stimulated the present point of view and review. The practical implications for day-to-day neurological practice are illustrated by a own patient.

Case #1 In February 2011, a 41–year old, right–handed man experienced an episode of vertigo. His general practitioner (GP) noticed a nystagmus and referred him to the Ear, Nose and Throat (ENT) specialist. The vertigo was thought to be central in origin and a MRI was requested. This MRI demonstrated multiple paraventricular T2–lesions (Figure 1 A). By May 2011 the patient had made a full recovery.

In July 2011 he developed pain on eye–movements in his right eye. About one week later his vision started to deteriorate. He was referred to a Neurologist, who diagnosed optic neuritis. Visual evoked potentials (VEP) of the right eye were severely prolonged (P100, 125 ms). A repeat MRI did not show any new lesions (Figure 1 A). A subsequent lumbar puncture revealed intrathecally–synthesised oligoclonal bands.

Taken together there were two attacks, one of which was clinically confirmed by a neurologist. Radiologically this patient did not fulfil DIS or DIT.[1] A diagnosis of MS could not be made in 2011.[1] A year earlier, however the patient would been diagnosed with MS because evidence of intrathecally–produced IgG would have been a substitute for radiological DIS.[5,6]

Methods

Search strategy and selection criteria A systematic review of the literature was conducted on all CSF studies in MS since publication of the first consensus report recommending the use of IEF for qualitative analysis of intrathecally–produced IgG in MS[7] between 1994 and October 2011, including manuscripts published ahead of print and conference abstracts irrespective of language using Pubmed, EMBASE, Medline, Web of Science and the Cochrane Register of Diagnostic Test Accuracy Studies using the search terms: multiple sclerosis, MS, cerebrospinal fluid and CSF. From 2164 studies identified, 2115 were excluded either because they were reviews, did not include a control group, were not performed in adult humans, did not perform analyses of oligoclonal bands or IEF as recommended in the original consensus guidelines,[7] did not specify how a diagnosis of MS was made or because missing data could not be obtained from the authors by email contact. A total of 49 studies were included.[8–56]

Statistical analysis The data analysis used the Cochrane Collaboration's Review Manager software package (RevMan5) following the guidance of the Diagnostic Test Accuracy (DTA) Working Group. The meta–analysis of the diagnostic accuracy was performed using a hierarchical summary receiver–operating characteristic (HSROC) model in SAS (version 9.1.3).[57]

What is intrathecal oligoclonal IgG synthesis?

The immune system requires B–cells to produce IgG. In the central nervous system (CNS) B-cells reside in the meninges and parenchyma.[58] Importantly, only a small number of B–cell clones are present in the CNS. Therefore any intrathecally–produced IgG can only ever be oligoclonal. Clonally–expanded B–cells from the CSF were shown to be the source of matching CSF IgG.[59] Readily distinguishable IgG bands seen on IEF are called "oligoclonal bands" (OCB).[60] The practical points to remember about OCB are summarised in Synopsis 1.

SYNOPSIS 1 — Five keys to intrathecally–produced IgG

- In normal CSF all IgG comes from the blood by passive diffusion
- In normal CSF and serum IgG is polyclonal
- Oligoclonal bands in blood give a mirror pattern in CSF
- Intrathecal (local) IgG synthesis is present when there are bands in the CSF that are not visible in the serum
- Oligoclonal bands are (generally) a sign of pathology

What are the target antigens for intrathecally–produced IgG?

Intrathecally–produced IgG has been used in an attempt to identify aetiologically relevant antigens, but to date this has not been successful in MS.

Of the many candidate antigens studied, myelin–associated lipids have been found to be present most consistently[61] (and references therein). Analysis of recombinant IgG1 antibodies from single CSF plasma blast clones suggests that about 27% of the antibodies are directed against lipid complexes which frequently contain sulfatide.[62] The pathological significance of this finding remains speculative.

Is the pattern of intrathecally–produced oligoclonal IgG preserved in MS?

Most studies report that the OCB pattern in MS, once established, remains stable over time[63,64] (and references therein). Only a minority of studies reported sequential changes of the OCB pattern such as more bands, less bands or change of band intensity during the course of MS.[65–68]

How specific is intrathecal oligoclonal IgG for MS?

Any process triggering a B–cell response may lead to the presence of IgG in the CSF. Diseases known to produce an intrathecal oligoclonal IgG response are summarised in Table [1].

Poor analytical quality triggers the development of international guidelines

It was suggested that one problem arising from the worldwide introduction of intrathecal IgG analysis for MS diagnostics was a loss of analytical quality.[69] The reported frequencies of CSF OCB in MS ranged from 45% to 77%. A diagnostic sensitivity of 45% is clearly not acceptable, therefore panel recommendations for CSF analysis were developed.[70] These consensus criteria also spell out the relevance of a standardised general CSF analysis as a basis for the interpretation of the IEF findings (Synopsis 2). Good clinical selection and a standardised CSF analysis help to minimise *pre–analytical* pitfalls leading to false–positive or false–negative CSF OCB results.

SYNOPSIS 2 — General CSF examination

- CSF cytology:
  - A high red blood cell count (5×10^9/L to 7×10^9/L) in the absence of bilirubin (assessed by spectophotometry[71]) suggests a traumatic tap. This may render other quantitative tests non interpretable
  - A slightly raised white cell count (> 5×10^6/L) may be found in up to 34% of patients with MS[72]
  - A high white cell count (> 50×10^6/L) is unusual in MS
- CSF total protein: a very high CSF total protein content (> 1 g/L) suggests an infectious or neoplastic process.
- CSF/serum albumin quotient: allows assessment of the integrity of the blood–CSF barrier and is the basis for quantitative models of intrathecal immunoglobulins
- CSF glucose: the CSF/serum ratio should be >0.4; a lower ratio suggests an infectious process[70]
- CSF lactate: an increase in CSF lactate (> 2.4 nmol/L) is unusual in MS and may suggest mitochondrial pathology, ischaemia or infections

Analytical quality reappraised

A Spanish study on the reporting of OCBs found the inter–laboratory agreement between the 19 participating laboratories to be almost perfect (kappa >0.8).[73] In the United Kingdom an external national quality assessment service (UK NEQAS) documented the analytical accuracy of reporting of OCBs since 1996. At time of writing there are 114 participating laboratories and electronic records were available from 2008. The analytical sensitivity ranged from 92–98% and the analytical specificity from 95–98% (Figure 2). In conclusion, an almost perfect inter–laboratory agreement can be expected.

What are the OCB patterns?

For a qualitative technique such as IEF, pattern recognition is crucial. An example of typical IEF OCB patterns on agarose gels with immunoblotting is shown in Figure 3. It was suggested that the observed patterns be designated as “Type 1” to “Type 5”.[70] For didactic reasons, *mnemonics* are used in Synopsis 3 to summarise these patterns.

SYNOPSIS 3 — Classification of CSF OCB patterns.

- **Normal**: no bands in CSF and serum (type 1[70])
- **Local**: oligoclonal bands in CSF but not in the serum, indicative of isolated intrathecal oligoclonal IgG synthesis (type 2[70])
- **Mirror**: identical oligoclonal bands in CSF and serum, indicating a systemic rather than an intrathecal immune reaction where oligoclonal bands are passively transferred into the CSF (type 4[70])
- **Mirror plus**: oligoclonal bands in the CSF and additional identical oligoclonal bands in CSF and serum samples, the space between bands is irregular (type 3[70])
- **Mirror steps**: monoclonal bands in the CSF and serum sample seen in the presence of a paraprotein (monoclonal IgG component), spaced in symmetric steps (type 5[70])

- **Artifact**: bands caused by pre–analytical or analytical problems

Interpretation of the OCB pattern

A **normal** test result (Type 1) does not always exclude pathology and may be found very early in the disease course, as illustrated in Figure 4. At the first lumbar puncture this patient fulfilled the diagnostic criteria for a CIS and at the second lumbar puncture for clinically definite MS.

Local synthesis: oligoclonal bands are present in the CSF but not in the serum (Type 2). This pattern is observed in patients with MS. As mentioned above, OCBs are also seen in a number of other diseases, with Table 1 likely to be incomplete.

The interpretation of the **mirror** patterns (Types 3, 4 and 5) is more complex and relies on additional information from the general CSF examination (Synopsis 2). One needs to consider systemic inflammation with or without additional local IgG synthesis.70 **Mirror steps** (Type 5) indicates the presence of a monoclonal gammopathy.

What happens to CSF monoclonal bands?

Monoclonal CSF bands are rare. The differential diagnosis includes clinically–definite MS, probable MS, CIS, SLE, paraneoplastic syndrome, vascular disease, encephalitis, peripheral neuropathies, superficial siderosis, torsion dystonia, lymphoma and lymphomatoid granulomatosis within or adjacent to the nervous system.74–76 In one study, a repeat lumbar puncture demonstrated that all patients who developed clinically–definite MS also showed evidence of intrathecal IgG synthesis in the second CSF sample.76

CSF bands: to count or not to count?

The hypothesis behind counting bands is that a higher number of bands may be of *prognostic* or *diagnostic* value. Some investigators found more than 10 bands in the CSF to be of high *diagnostic specificity* for MS.10 Others found that the absence of OCBs in the CSF of patient

with MS was a *good prognostic* sign[77,78] (and references therein). In contrast, two studies did not find any relationship between the presence and number (or absence) of CSF OCB bands and either disease progression or MS subgroups (RR, SP, PP disease).[79,80]

There are conceptional and methodological problems to be considered in counting bands. Firstly, the number of bands may not be a true reflection of the number of relevant B–cell clones. In order to address the biological relevance of OCBs, the number of clones producing the bands may turn out to be more relevant than the number of bands present. Secondly, clonally–expanded intrathecal B cells can appear before OCBs. This may explain why some patients only develop OCBs during the course of their disease (see reference[77] and Figure 4).

What information can CSF light chains add?

A single B–cell clone can only express either kappa or lambda light chains. Because kappa is rearranged first, it is quantitatively the dominant light chain in the human body. Therefore the kappa light chain (free and bound) is found more frequently in the CSF than lambda. In practice, immunoblotting for kappa/lambda light chains is helpful in the following situations:

- to decide whether IgG is monoclonal when “mirror steps” are seen. Monoclonal IgG only stains for one light chain.
- if it is uncertain whether or not very faint bands are present.
- in cases of “negative staining” (looking very white) at the beginning of the blot (towards the cathode). This may be due to IgM which is not picked up by the IgG staining, and kappa/lambda can be of help.

What information can CSF IgM add?

As in any immune–response, IgM levels increase in the serum and CSF before IgG develops. Detection of CSF oligoclonal IgM bands is possible using IEF.[81] An analytical drawback is that the pentameric IgM antibodies need to be dissociated for IEF and the association to single–cell

clones is therefore lost. As with IgG, IgM is not specific for MS but is also found in other inflammatory CNS diseases.[82] It has been suggested that oligoclonal CSF IgM is of prognostic relevance in MS.[83]

What is the diagnostic value of intrathecally–produced IgG in MS?

Meta–analysis — part I

The diagnostic sensitivity of CSF OCB using state–of–the–art methods is reported by pioneering experts in the field to be above 95%.[70,84] This estimate is consistent with the present meta–analysis of 49 studies[8–56] (11,136 patients) which calculates a pooled diagnostic sensitivity for MS of 93% with a specificity of 94% (Figure 7A). The forest plot (Figure 5) illustrates that the sensitivity of individual studies ranges from 1.0 (95%CI 0.88–1.00)[53] to 0.53 (95%CI 0.44–0.63).[56]

Of note, the majority of studies included healthy controls, patients without neurological diseases or patients with non–inflammatory neurological conditions. In reality, MS is frequently in the clinical differential diagnosis of those conditions listed in Table 1.

Meta–analysis — part II

What is the influence of other inflammatory conditions on the diagnostic value of CSF OCB? A repeat meta–analysis only considering those patients with MS or other inflammatory conditions[8–10,12,15–19,23,30,37,40–42,45,50–53] (2,331 patients) shows a reduced diagnostic specificity of 61% (Figure 6). The change of the specificity level is best appreciated by the rightward shift of the red dot in the HSROC plots (Figure 7 A & B).

What is the influence of ethnicity on the diagnostic value of CSF OCB?

Most studies reporting a diagnostic sensitivity above 95% were performed on patients with a predominantly Caucasian background. A much lower diagnostic sensitivity (7%–63%) was

reported for Asian patients from China, Japan, and Taiwan[30,85,86] and Brazilian patients.[19] In addition, there was an association between latitude (thought to be related to ethnic distribution) and the proportion of MS patients with evidence of intrathecally–produced IgG in a large (n=4481) multicenter study.[78] Together, this data suggests that the diagnostic sensitivity of CSF OCB may be less in non–Caucasian patients.

What is the prognostic value of intrathecally–produced IgG in predicting conversion from CIS to MS?

At first presentation, patients fulfilling radiological DIS but not DIT are classified as clinically–isolated syndrome (CIS), and some will go on to develop MS. The question is whether presence of intrathecally–produced IgG at this time gives any added prognostic information? The following case illustrates this point.

Case #2 A 39–year old right–handed woman woke up with numbness and a feeling of pins and needles in both legs about four months ago. In the following days she noticed increasing dizziness, nausea, and fatigue and found it more difficult to concentrate at work. She started to avoid neck flexion because this gave rise to short lasting, sudden, unpleasant sensations down her spine. There was no past–medical history of note, but one paternal aunt suffered from MS.

The only findings on examination were absent abdominal reflexes and a positive Lhermitte's sign.

The MRI scan of the brain and whole spine revealed five non–contrast enhancing lesions: one juxtacortical, one infratentorial, and three in the spinal cord.

The CSF IgG index was increased at 0.99 (normal value at the referring lab <0.63). Isoelectic focusing (IEF) was not performed at the referring hospital and CSF was not stored for further analyses. The remaining extensive laboratory investigations were normal.

Radiologically this patient fulfilled the 2010 criteria for *dissemination in space* (DIS).[1] There was

no radiological evidence for DIT1 and importantly she did not fulfil the clinical criteria for DIT. She therefore has a diagnosis of CIS.1 In this case some of us may consider that the risk of this patient developing MS is reasonably high. The prognostic evidence of intrathecally–produced IgG in such patients will be reviewed in the following two sections.

Optic neuritis

A meta–analysis on the prognostic value of intrathecally–produced IgG in patients presenting with monocular optic neuritis identified 10 studies including 646 patients.87 Within a mean follow–up time of 5.4 years (range 10 days to 20 years), 36% had converted to MS based on different diagnostic criteria. CSF was taken from 601 of these patients and tested using either agarose gel electrophoresis, IEF, agarose IEF combined with immunoblotting and avidin–biotin amplified double-antibody peroxidase staining, IEF and immunodetection with anti–human IgG labelled with alkaline phosphatase, or high–resolution immunofixation electrophoresis.87

Not surprisingly, given the variation of follow-up time, diagnostic criteria and laboratory methods employed, the odds–ratio for predicting conversion to MS ranged from 2.75 to 171.87

Other CIS

A prospective study by Tintoré *et al* pooled CIS patients with brainstem symptoms, spinal cord syndrome, optic neuritis, hemispheric, polyregional, or undetermined topographic presentation.88 In the pooled analyses the odds–ratio for developing clinically–definite MS according to the Poser criteria89 was 1.7 (95%CI 1.1–2.7).88 Of the 113 CIS patients with normal MRI, 30 evidence of intrathecal oligoclonal IgG and 7 of these developed CDMS within an average of 53 months.88 In a prospective Brazilian cohort the odds–ratio for developing clinically definite MS according to the Poser criteria89 was 5.3 (95%CI 1.6–9.5).88 In another prospective, longitudinal cohort 53% of CSF OCB–positive CIS patients with MRI not showing DIS (45% of 118 patients) were shown to develop clinically–definite MS within an average of 3.8 years.56 In contrast, a French study did not find intrathecally–produced IgG to be of statistical

significance if used in isolation (odds–ratio 1.15, 95%CI 0.58–1.97, p=0.5).[90] Furthermore, the prognostic value of CSF OCB positivity was statistically annihilated by MRI evidence of DIS.[90–92] In two studies the combined results of CSF OCB and MRI were a better and highly significant predictor for conversion to clinically–definite MS than either test alone.[88,90]

Conclusion

Over the past 50 years multiple sclerosis has been considered to be a disease in which DIS and DIT needed to be demonstrated in order to make a diagnosis.[1,89,93,94] Brain imaging is an ideal tool to show DIS and DIT and consequently became the cornerstone of MS diagnosis with the introduction of the McDonald criteria in 1998.[6] In the face of clinical assessment and brain imaging it seems rather challenging to demonstrate DIS and DIT based on evidence for intrathecally–produced oligoclonal IgG. Having said this, in the past CSF OCBs were regarded as a diagnostic test which could substitute for radiological *DIS*.[5,6]

Importantly, the present meta–analysis illustrates that CSF OCB can only be of low diagnostic specificity when other inflammatory conditions come into the differential diagnosis. This may be regarded as an additional argument for no longer considering CSF OCB as a substitute for non–specific MRI lesions, which fail the radiological criteria for DIS. In summary, one non–specific test result should not be used to substitute for another non–specific test result.

However, the interpretation of the results of laboratory testing for CSF OCBs (Synopsis 3) is truly an extension of a robust CSF examination (Synopsis 2) and clinical reasoning[95] (Table 1). Importantly, the recent appearance on the market of reliable kits for determination of CSF OCBs opens up opportunities for well–designed prospective, longitudinal, multi–ethnic, multi-center studies investigating the potential added prognostic value of CSF OCBs.

In conclusion, one can test that intrathecally–produced oligoclonal IgG and IgM are of additional prognostic value in patients were *DIT* cannot be demonstrated. This approach represents a conceptional change, focused rather on the prognostic than the diagnostic value of CSF OCB.

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Disclosures

The author has nothing to disclose.

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Tables

Table 1: *Diseases in which intrathecal oligoclonal IgG has been reported.*[8–10,12,15–19,23,30,37,40–42,4 *remitting relapsing MS, SPMS = secondary progressive MS, CIS = clinically isolated syndrome, C system, NMO = neuromyelitis optica, ADEM = acute demyelinating encephalomyelitis, LETM = lo transverse myelitis, SLE = systemic lupus erythematosus, BIH = benign intracranial hypertension, Syndrome.*

MS type	Autoimmune	Inflammation	Other
RRMS	SLE	Neurosyphilis	Paraneoplastic disorders
SPMS	Behcet's disease	Neuroborreliosis	Aseptic meningitis
PPMS	Neurosarcoidosis	HIV infection	Cerebral tumors
CIS	Sjögren's syndrome	Herpes viridae	Cerebral lymphoma
NMO	Morvan syndrome	Chlamydia	Vertigo
ADEM	Anti–NMDA encephalitis	Neurotuberculosis	Alzheimer's
LETM	Anticardiolipin syndrome	HTLV myelopathy	Prion disease
	Autoimmune encephalopathy	Schistosomiasis	Migraine
	Stiff–man syndrome	Cerebral cysticercosis	Syncope
	GBS	CNS vasculitis	BIH

Figure Legends

Figure 1: *MRI brain of a 41 year–old man demonstrating non–contrast enhancing T–2 lesions exclusively located in the paraventricular regions in (A) April 2011 and (B) July 2011.*

Figure 2: *Forest plot of the analytical accuracy of reporting CSF OCB from 114 laboratories participating in an external quality control scheme (data kindly provided by UK NEQAS, 12.10.2011).*

Figure 3: *The OCB patterns shown are **(A)** normal (no evidence for intrathecally–produced oligoclonal IgG, Type 1), **(B)** local synthesis (Type 2), **(C)** a mirror plus pattern (more bands in the CSF compared to the serum, Type 4), **(D)** a mirror pattern (equal number of matched bands in CSF and serum, Type 3), **(E)** mirror steps (monoclonal bands, Type 5), **(F)** an artifact* Shown is the original photograph to the left and an illustrative sketch to the right of the image.*

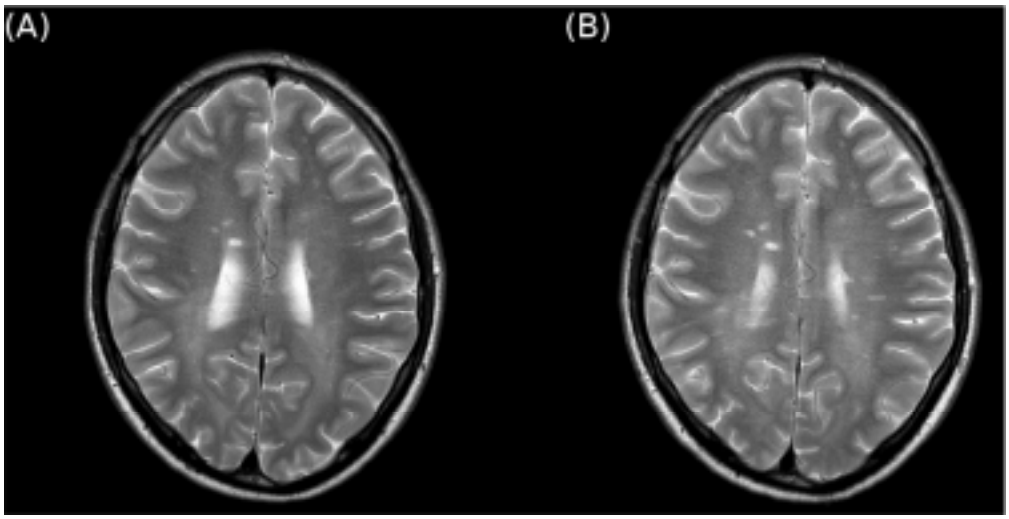
Figure 4: *The CSF in a patient presenting with CIS who showed no evidence of intrathecal IgG in 2004 but developed oligoclonal IgG bands in 2005.*

Figure 5: *Forest plot of the sensitivity and specificity of CSF OCB in patients diagnosed with MS according to consensus criteria.5,6,89 The controls comprise healthy patients, patients with non–inflammatory and inflammatory CNS disorders and patients with non–neurological conditions.*

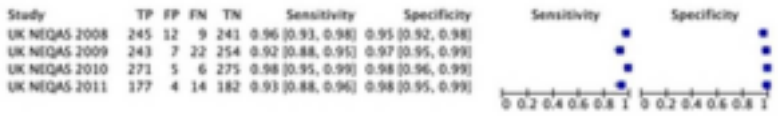
Figure 6: *The specificity of CSF OCB is 61% if patients diagnosed with MS according to consensus criteria5,6,89 are compared to patients with inflammatory neurological conditions.*

Figure 7: *The HSROC plots illustrate the bias introduced through selection of the control group. The high diagnostic specificity of CSF OCB for MS (93%)* ***(A)*** *is clearly reduced by comparing patients with MS to* ***(B)*** *those with other inflammatory neurological diseases (61%).*

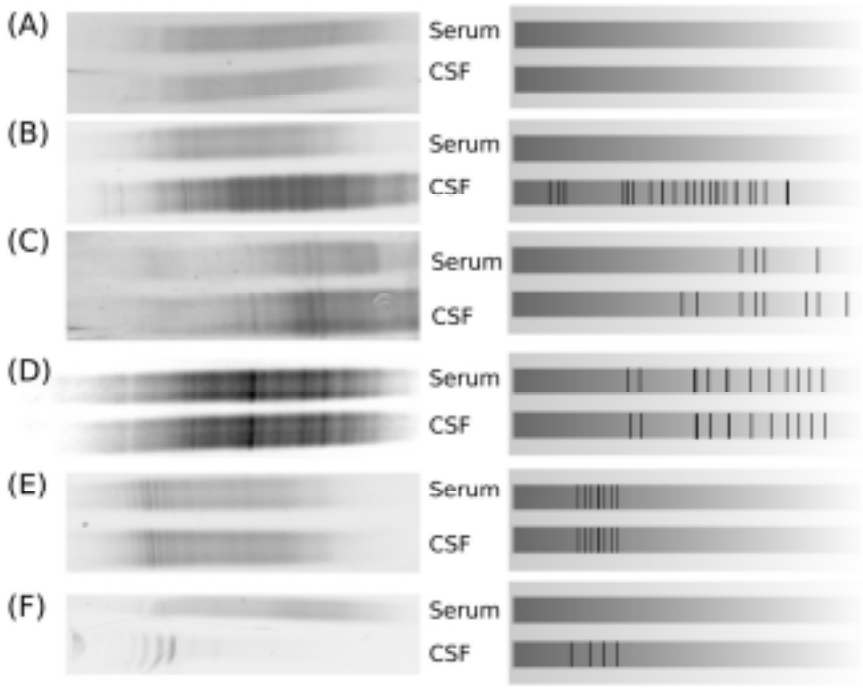
Figure 8: *MRI brain and spinal cord of a 29 year old woman demonstrating non–contrast enhancing lesions indicated by red arrows: 1 infratentorial, 1 juxtacortical, 3 spinal cord.*



MRI brain of a 41 year–old man demonstrating non–contrast enhancing T–2 lesions exclusively located in the paraventricular regions in (A) April 2011 and (B) July 2011.
166x84mm (300 x 300 DPI)

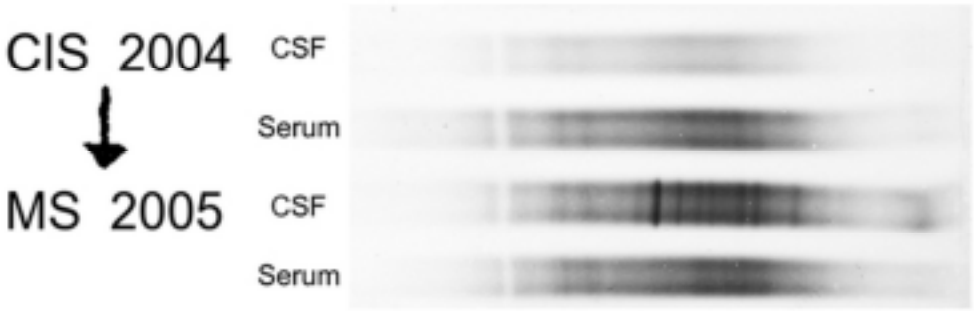


Forest plot of the analytical accuracy of reporting CSF OCB from 114 laboratories participating in an external quality control scheme (data kindly provided by UK NEQAS, 12.10.2011).
33x5mm (300 x 300 DPI)

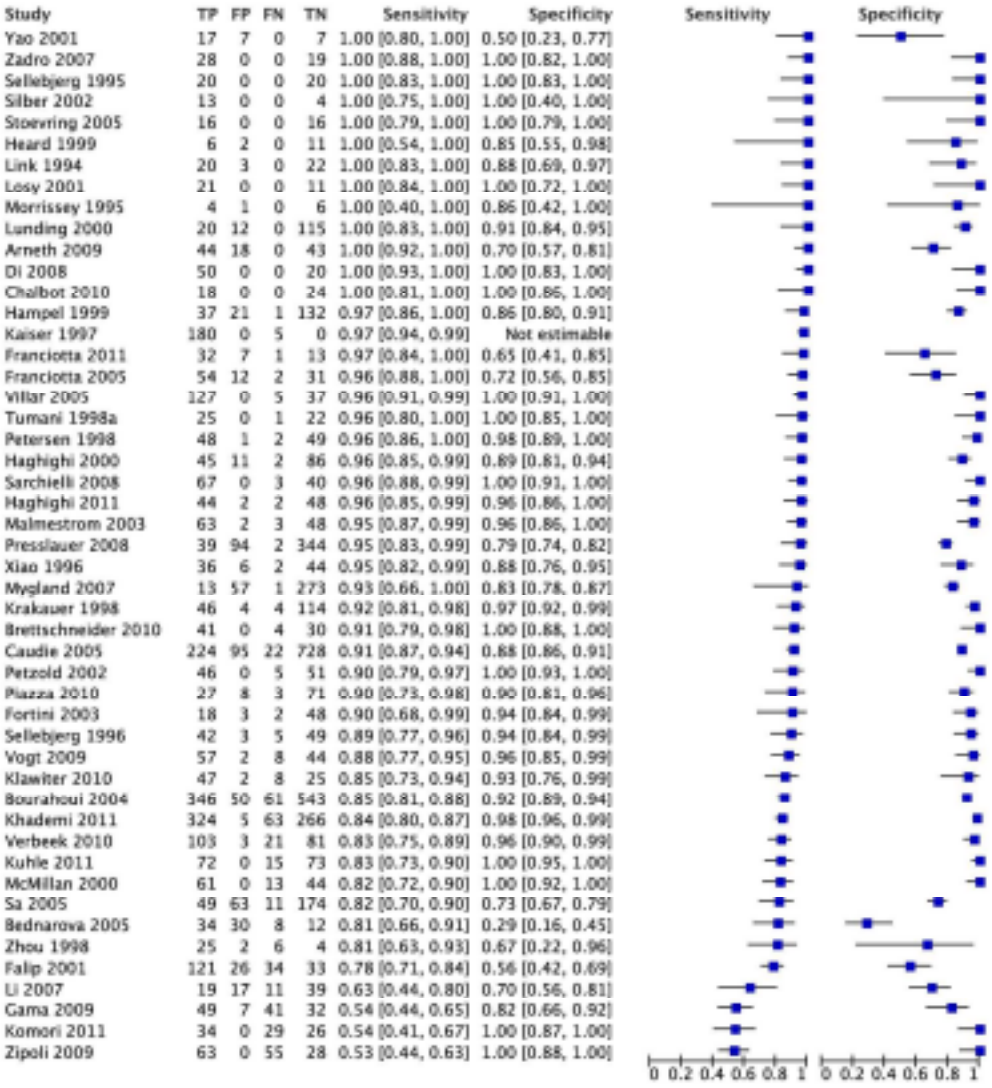


The OCB patterns shown are (A) normal (no evidence for intrathecally–produced oligoclonal IgG, Type 1), (B) local synthesis (Type 2), (C) a mirror plus pattern (more bands in the CSF compared to the serum, Type 4), (D) a mirror pattern (equal number of matched bands in CSF and serum, Type 3), (E) mirror steps (monoclonal bands, Type 5), (F) an artifact * Shown is the original photograph to the left and an illustrative sketch to the right of the image.

(*) The bands seen are all located very close to the negatively charged cathode. In fact these bands did almost not migrate at all toward the positively charged anode which is very unusual for IgG which is made up of a number of negatively charged amino-acids. The serum shows a polyclonal response visible as diffuse staining across the entire figure. In contrast, there is no visible staining to the right (towards the anode) in the CSF. So whilst the bands seen formally fit the definition of a "type 2" pattern , they represent an artifact. What happened here was that the CSF sample was contaminated. Contamination with either a fungus or bacteria leads to breakup of CSF proteins into smaller peptides and digestion of the sugar residues such that only small peptipes remain which are stripped of most of their original negative charge. These appear to the very left close to the cathode. There are almost no larger, stronger charged substances left which could migrate towards the anode. In conclusion this represents an artifact which can easily be misinterpreted.
160x110mm (300 x 300 DPI)

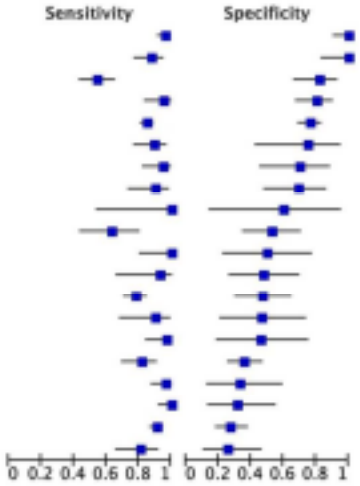


The CSF in a patient presenting with CIS who showed no evidence of intrathecal IgG in 2004 but developed oligoclonal IgG bands in 2005.
53x17mm (300 x 300 DPI)

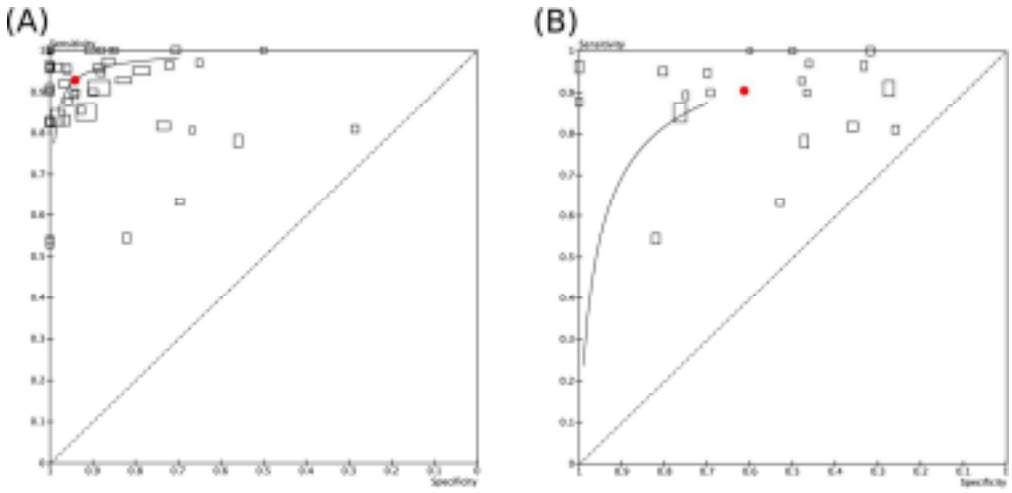


Forest plot of the sensitivity and specificity of CSF OCB in patients diagnosed with MS according to consensus criteria [5,6,89]. The controls comprise healthy patients, patients with non–inflammatory and inflammatory CNS disorders and patients with non–neurological conditions.
236x261mm (300 x 300 DPI)

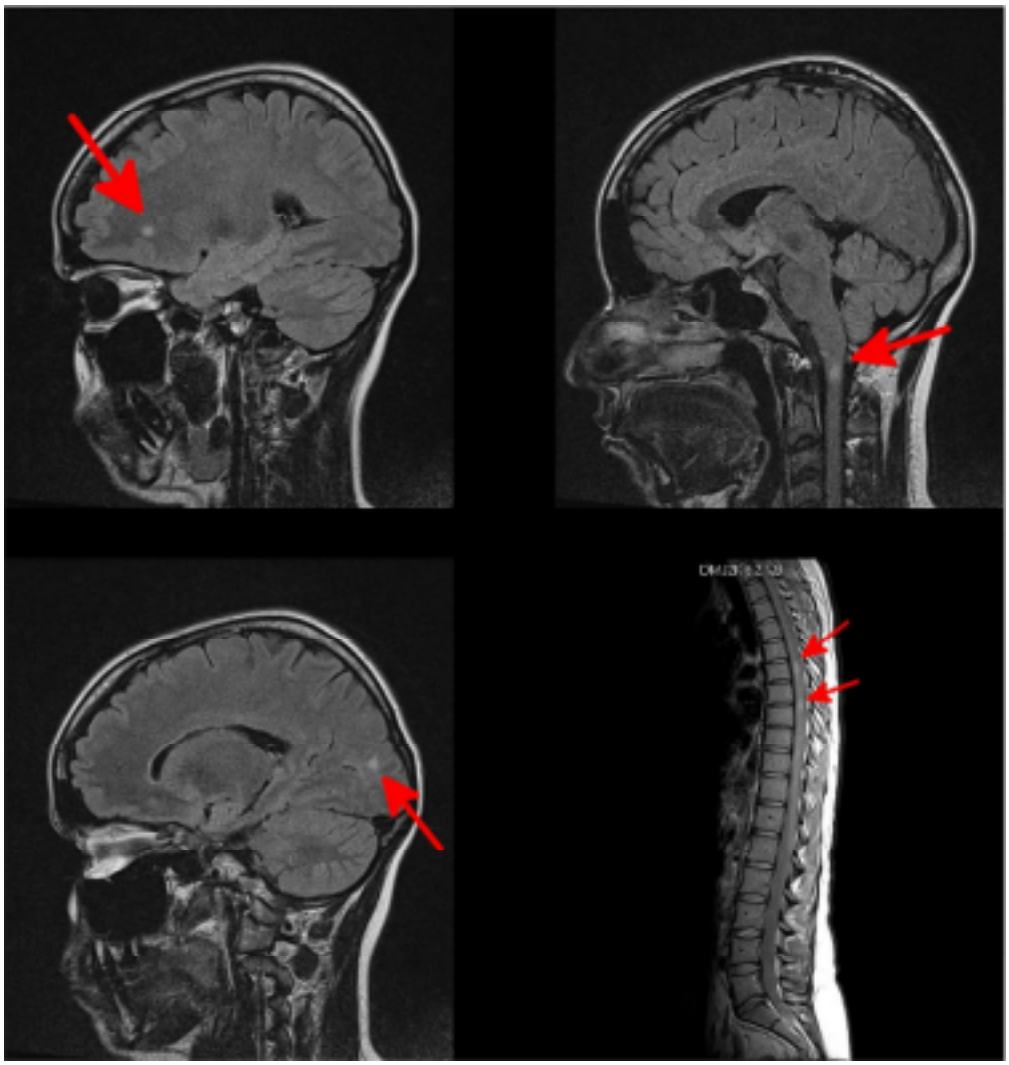
Study	TP	FP	FN	TN	Sensitivity	Specificity
Villar 2005	127	0	5	37	0.96 [0.91, 0.99]	1.00 [0.91, 1.00]
Vogt 2009	57	0	8	20	0.88 [0.77, 0.95]	1.00 [0.83, 1.00]
Gama 2009	49	7	41	32	0.54 [0.44, 0.65]	0.82 [0.66, 0.92]
Presslauer 2008	39	11	2	45	0.95 [0.83, 0.99]	0.80 [0.68, 0.90]
Bourahoui 2004	346	38	61	123	0.85 [0.81, 0.88]	0.76 [0.69, 0.83]
Sellebjerg 1996	42	3	5	9	0.89 [0.77, 0.96]	0.75 [0.43, 0.95]
Xiao 1996	36	6	2	14	0.95 [0.82, 0.99]	0.70 [0.46, 0.88]
Piazza 2010	27	8	3	18	0.90 [0.73, 0.98]	0.69 [0.48, 0.86]
Heard 1999	6	2	0	3	1.00 [0.54, 1.00]	0.60 [0.15, 0.95]
Li 2007	19	16	11	18	0.63 [0.44, 0.80]	0.53 [0.35, 0.70]
Yao 2001	17	7	0	7	1.00 [0.80, 1.00]	0.50 [0.23, 0.77]
Mygland 2007	13	12	1	11	0.93 [0.66, 1.00]	0.48 [0.27, 0.69]
Falip 2001	121	19	34	17	0.78 [0.71, 0.84]	0.47 [0.30, 0.65]
Fortini 2003	18	8	2	7	0.90 [0.68, 0.99]	0.47 [0.21, 0.73]
Franciotta 2011	32	7	1	6	0.97 [0.84, 1.00]	0.46 [0.19, 0.75]
Sa 2005	49	57	11	32	0.82 [0.70, 0.90]	0.36 [0.26, 0.47]
Franciotta 2005	54	12	2	6	0.96 [0.88, 1.00]	0.33 [0.13, 0.59]
Ameth 2009	44	15	0	7	1.00 [0.92, 1.00]	0.32 [0.14, 0.55]
Caudie 2005	224	66	22	25	0.91 [0.87, 0.94]	0.27 [0.19, 0.38]
Bednarova 2005	34	20	8	7	0.81 [0.66, 0.91]	0.26 [0.11, 0.46]



The specificity of CSF OCB is 61% if patients diagnosed with MS according to consensus criteria [5,6,89] are compared to patients with inflammatory neurological conditions.
105x53mm (300 x 300 DPI)



The HSROC plots illustrate the bias introduced through selection of the control group. The high diagnostic specificity of CSF OCB for MS (93%) (A) is clearly reduced by comparing patients with MS to (B) those with other inflammatory neurological diseases (61%).
175x84mm (300 x 300 DPI)



MRI brain and spinal cord of a 29 year old woman demonstrating non–contrast enhancing lesions indicated by red arrows: 1 infratentorial, 1 juxtacortical, 3 spinal cord.
259x273mm (300 x 300 DPI)