Intrathecal oligoclonal IgG synthesis in multiple sclerosis

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Abstract

The diagnosis of multiple sclerosis is based on dissemination in time and space. Before 2010 lack of evidence for dissemination in space could be substituted by a paraclinical test, cerebrospinal fluid (CSF) oligoclonal bands (OCB). The present meta-analysis (13,467 patients) shows that the diagnostic specificity of OCB drops from 94% to 61% if inflammatory etiologies are considered. Importantly, this was not caused by poor laboratory practice. This review on CSF OCB further illustrates the conceptional problem of substituting dissemination in space with a biomarker. The potential prognostic value of intrathecal OCB will need to be tested prospectively.

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1. Introduction

Evidence of intrathecally-produced immunoglobulin G (IgG) was used from around 1954 as an additional diagnostic test for multiple sclerosis (MS).[88] Stringent brain imaging criteria can demonstrate dissemination in space (DIS) with such accuracy that an additional CSF examination is not necessary.[74] A debate followed on the value of cerebrospinal fluid (CSF) analysis *in general*.[25,74,88,93] Two aspects of this debate have to be considered, one is focused on the potential utility of CSF analysis in general and the other on the specific value of OCB for a set of diagnostic criteria aimed to optimise early sensitivity and specificity. The relevance of a state-of-the-art general CSF analysis for the differential diagnosis of MS has been extensively reviewed.[88,93] In contrast, the present review and meta-analysis is *solely* focused on intrathecal oligoclonal IgG synthesis in MS. The review starts with a discussion of the basic biology and pathophysiology of intrathecal oligoclonal IgG synthesis. This short and pragmatic neuroimmunological review prepares the ground for a meta-analysis on the diagnostic value of CSF oligoclonal bands (OCB) in MS. The review closes with a revision of the potential prognostic value of the test.

2. 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[2] 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,[2] 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.[3,5,8,10–12,17,19–22,26,29–31,34,38,41,42,44–46,49,51,52,55,56,61,63,64,66,69,71,76,81,82,84

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 9.3).[80]

3. 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.[32,53] Importantly, only a small number of B-cell clones are present in the CNS.[77] 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.[62,65] Readily distinguishable IgG bands seen on IEF

are called "oligoclonal bands" (OCB).[54] 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

4. 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[47,98] (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.[9] The pathological significance of this finding remains speculative.

5. 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[14,15,50,103] (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.[4,27,57,90] A very recent report demonstrated that OCB disappeared in 12/73 (16%, $p<0.003$) of patients between a baseline lumbar puncture and a later lumbar puncture after treatment with natazilumab was started.[33]

6. 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.

7. 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.[40] 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.[23] These consensus criteria also spell out the relevance of standardised general

Table 1: *Diseases in which intrathecal oligoclonal IgG has been reported.*[3,5,8,11,19–22,26,34,49,64,71,76,81,84,99–102] *RRMS = remitting relapsing MS, SPMS = secondary progressive MS, CIS = clinically isolated syndrome, CNS = central nervous system, NMO = neuromyelitis optica, ADEM = acute demyelinating encephalomyelitis, LETM = longitudinal extensive transverse myelitis, SLE = systemic lupus erythematosus, BIH = benign intracranial hypertension, GBS = Guillain-Barré 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

CSF analyses 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 spectrophotometry[68]) suggests a traumatic tap. This may render other quantitative tests uninterpretable
  - A slightly raised white cell count ($> 5 \times 10^6$/L) may be found in up to 34% of patients with MS[tourtellotte1985'79]
  - A high white cell count ($> 50 \times 10^6$/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[23]
- CSF lactate: an increase in CSF lactate (> 2.4 nmol/L) is unusual in MS and may suggest mitochondrial pathology, ischaemia or infections

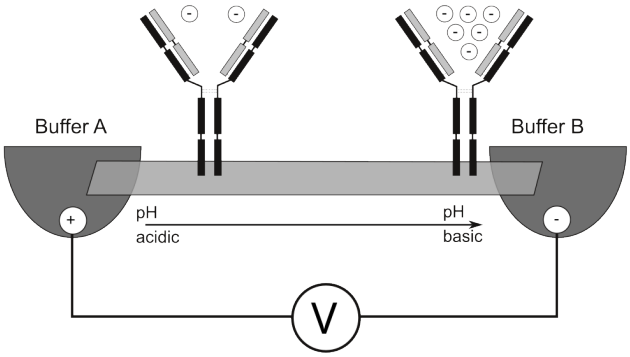

Figure 1: *The physics of IgG IEF. More negatively-charged IgG molecules (composed of more negatively-charged amino-acids) migrate further to the anode (⊕) and less negatively-charged IgG remains closer to the cathode (⊖). For high quality resolution a smooth pI gradient is essential.*

8. Analytical aspects

Analytically, electrophoresis was the first technique which permitted to accurately distinguish different proteins in the human CSF. An important limitation of the method was the poor sensitivity requiring large amounts (about 70 mL) of CSF.[37] Major advancements were made with the introduction of isoelectric focusing (IEF, see Figure 1) on agarose gels followed by immunoblotting.[39] At present only about 2-4 μL of CSF are required for detection of OCB.[23]

Until recently, many laboratories relied on in-house developed tests which carried a number of analyticaal challenges, ranging from the preparation of the gels and buffers to the immunoblotting. With the availability of commercial tests following consensus guidelines standardisation became easier. Some of the key pitfalls remaining are line-artifacts due to pI gradient which is not smooth, which can give the false impression of matched bands between CSF and serum. Typically these artifacts are seen across the entire gel. Likewise, a very high concentration of IgG in one sample may "leak" across the gel into the adjacent samples. As a general rule the use of positive and negative quality control samples and participation in a quality control program will help to identify some of these problems.

9. Quality control

For optimal results, standardised protocols are mandatory. These protocols should specify the key steps from sample acquisition, sample handling and analysis to sample storage.[78,88]

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

Study	TP	FP	FN	TN	Sensitivity	Specificity
UK NEQAS 2008	245	12	9	241	0.96 [0.93, 0.98]	0.95 [0.92, 0.98]
UK NEQAS 2009	243	7	22	254	0.92 [0.88, 0.95]	0.97 [0.95, 0.99]
UK NEQAS 2010	271	5	6	275	0.98 [0.95, 0.99]	0.98 [0.96, 0.99]
UK NEQAS 2011	177	4	14	182	0.93 [0.88, 0.96]	0.98 [0.95, 0.99]

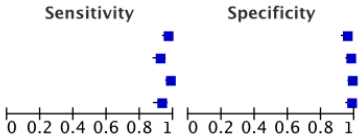

Figure 2: *Forest plot of the analytical accuracy of reporting CSF OCB from 114 laboratories participating in an external quality control scheme (data kindly provided by UK NEQAS, 12.10.2011).*

there are 114 participating laboratories and electronic records were available from 2008. Most frequently the Sebia assay was used, followed by the Helena assay and in-house methods,[39] with only a minority using the Phadia, Webb Scientific or Diasorin methods. Taken together, 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.

Likewise, 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).[1]

10. 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".[23] 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[23])
- **Local**: oligoclonal bands in CSF but not in the serum, indicative of isolated intrathecal oligoclonal IgG synthesis (type 2[23])
- **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[23])
- **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[23])
- **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[23])
- **Artifact**: bands caused by pre-analytical or analytical problems

10.1. 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.[23] **Mirror steps** (Type 5) indicates the presence of a monoclonal gammopathy.

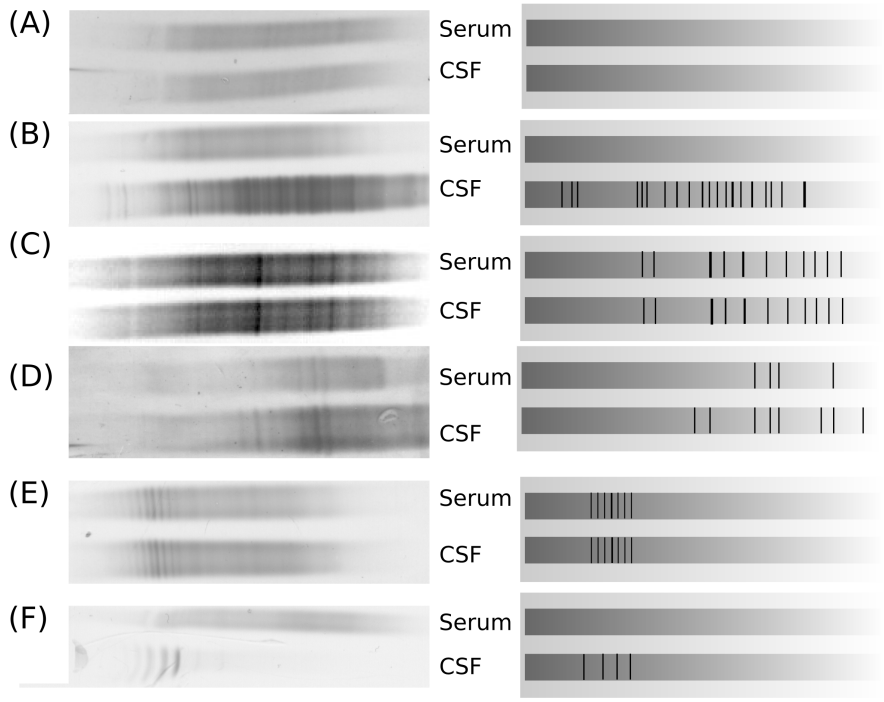

Figure 3: *The OCB patterns shown are* ***(A)*** *normal (no evidence for intrathecally-produced oligoclonal IgG, Type 1),* ***(B)*** *local synthesis (Type 2),* ***(C)*** *a mirror plus pattern (more bands in the CSF compared to the serum, Type 4),* ***(D)*** *a mirror pattern (equal number of matched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

11. 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.[6,16,59] 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.[16]

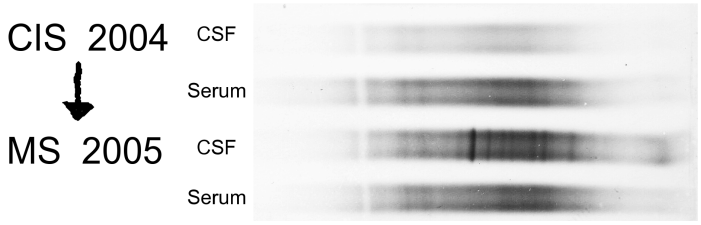

Figure 4: *The CSF in a patient presenting with CIS who showed no evidence of intrathecal IgG in 2004 but developed oligoclonal IgG bands in 2005.*

12. 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.[8] Others found that the absence of OCBs in the CSF of patient with MS was a *good prognostic* sign[48,105] (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).[35,43]

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[105] and Figure 4).

13. 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.

14. 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.[95] 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.[96] It has been suggested that oligoclonal CSF IgM is of prognostic relevance in MS.[97]

15. What is the diagnostic value of intrathecally-produced IgG in MS?

15.1. 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%.[23,50] This estimate is consistent with the present meta-analysis of 49 studies[3,5,8,10–12,17,19–22,26,29–31,34,38,41,42,44–46,49,51,52,55,56,61,63,64,66,69,71,76,81,82,84–86,89,
(11,136 patients) which calculates a pooled diagnostic sensitivity for MS of 93% with a specificity of 94% (Figure 7A).[67] The forest plot (Figure 5) illustrates that the sensitivity of individual studies ranges from 1.0 (95%CI 0.88-1.00)[102] to 0.53 (95%CI 0.44-0.63).[107]

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.

15.2. 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[3,5,8,11,19–22,26,34,49,64,71,76,81,84,99–102] (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).

15.3. Potential sources of bias

A number of potential biases need to be considered which will influence the accuracy of a test. An *index test bias* may be introduced if subjects were solely to be included depending on the result of an index (reference) test.[7] This is to be distinguished from a *double gold standard bias* were different reference standards are used based on the results of the index test. Next, there is the scenario of an *inclusion bias* if the reference standard and index test are dependent.

Finally, a *selection bias* may suggest impressive levels of the sensitivity and specificity of a particular test, but be based on the comparison of a hyper-normal control group with a clearly diseased group. This would for example be the comparison of CSF samples from patients with definite MS with CSF samples from a healthy, non-inflammatory control group. The results of the meta-analysis suggest that the high diagnostic sensitivity and specificity levels of CSF OCB for MS may, at least in part, be caused by a selection bias. Future studies in the field would need to be careful to consider potential sources of bias.

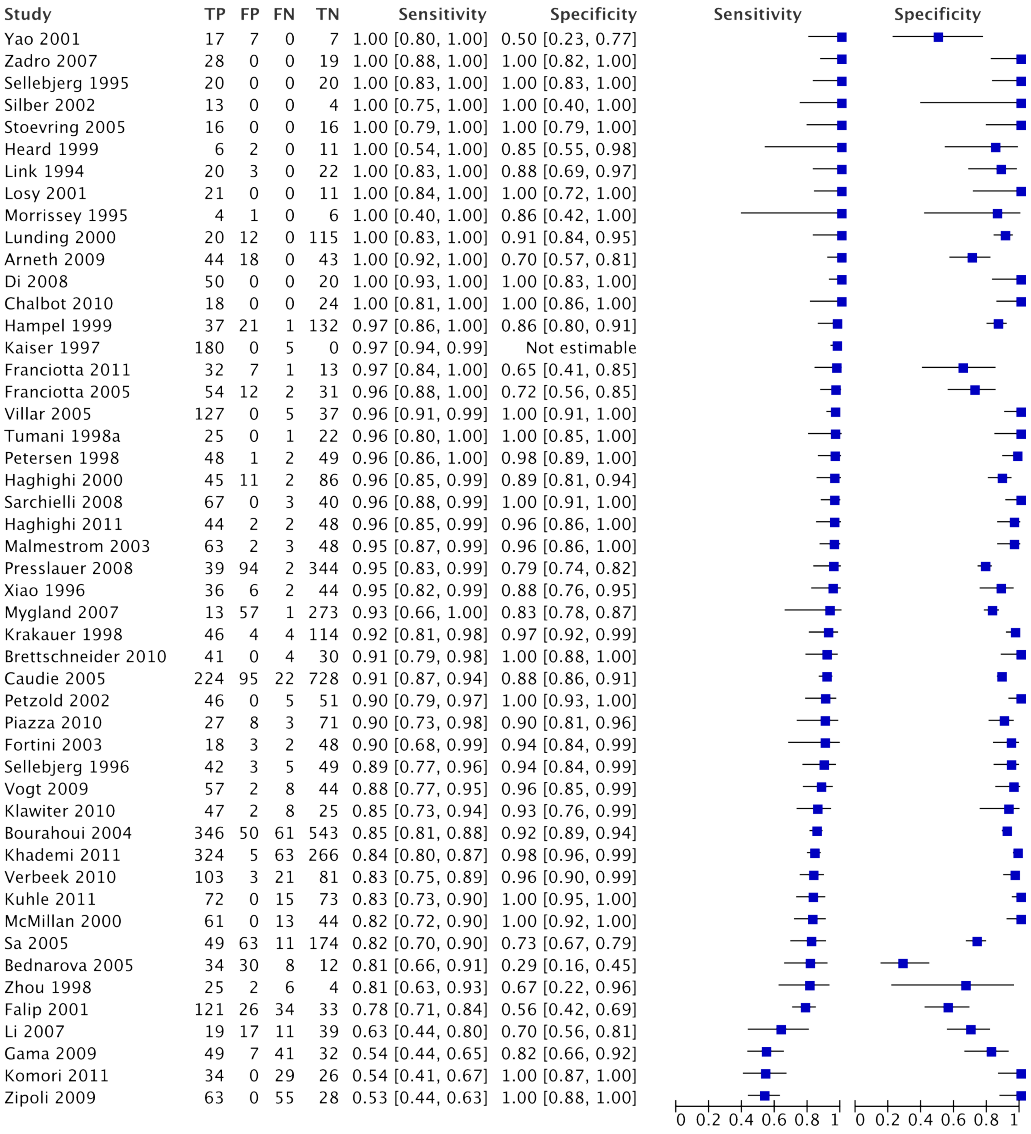

Figure 5: *Forest plot of the sensitivity and specificity of CSF OCB in patients diagnosed with MS according to consensus criteria.*[60,73,75] *The controls comprise healthy patients, patients with non-inflammatory and inflammatory CNS disorders and patients with non-neurological conditions.*

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]
Arneth 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]

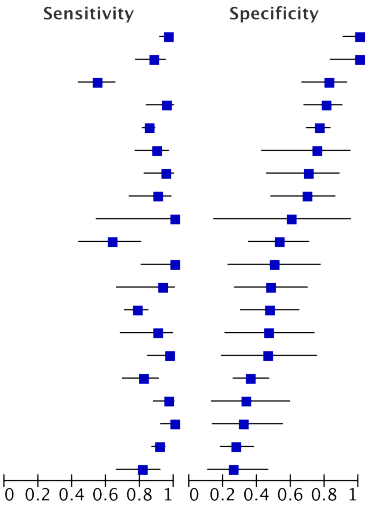

Figure 6: *The specificity of CSF OCB is 61% if patients diagnosed with MS according to consensus criteria*[60,73,75] *are compared to patients with inflammatory neurological conditions.*

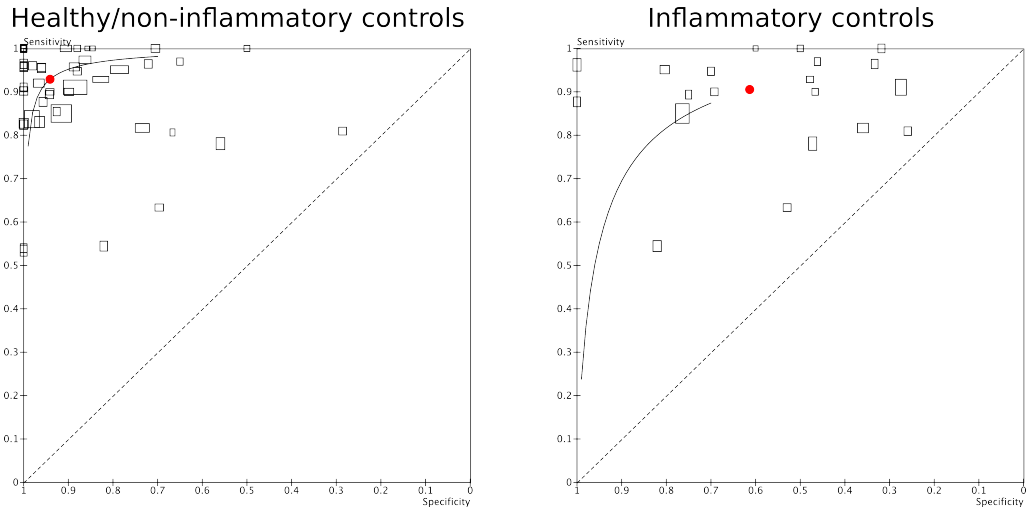

Figure 7: *The HSROC plots illustrate the bias introduced through selection of the control group. The high diagnostic specificity of CSF OCB for MS (93%)* ***(A)*** *is clearly reduced by comparing patients with MS to* ***(B)*** *those with other inflammatory neurological diseases (61%).*

15.4. 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[13,24,49] and Brazilian patients.[26] 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.[48] Together, this data suggests that the diagnostic sensitivity of CSF OCB may be less in non-Caucasian patients.

16. 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?

16.1. 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]

16.2. 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.[91] In the pooled analyses the odds-ratio for developing clinically-definite MS according to the Poser criteria[75] was 1.7 (95%CI 1.1-2.7).[91] 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.[91] In a prospective Brazilian cohort the odds-ratio for developing clinically definite MS according to the Poser criteria[75] was 5.3 (95%CI 1.6-9.5).[91] 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.[107] 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).[28] Furthermore, the prognostic value of CSF OCB positivity was statistically annihilated by MRI evidence of DIS.[28,36,79] 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.[28,91] A finding supported by a meta-analysis.[18]

There is a need for prospective, multi-center studies to test the potential prognostic value of CSF OCB and other biomarkers. Such studies will require to be clear about the definition of *prognosis*. First, prognosis as discussed in the review refers to conversion to MS of a patient who has a history very suggestive of MS, was CSF OCB positive, but did not show radiological DIS and DIT. Second, prognosis may also refer to clinical disability which implies the ability to recover from a disabling event, for which biomarkers other then OCB may be more appropriate.[70,88]

17. 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.[58,74,75,83] 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.[60] 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*.[60,73]

Overall a state-of-the-art CSF examination should be used as an extension of clinical reasoning to make optimal use of the added value of the tests requested.[72,88] An unselected request of CSF OCB on all patients will reduce the diagnostic specificity. Taken together, there are four potential scenarios. First, if the clinical picture and MRI are non-specific, likelihood is that CSF OCB may not be particularly helpful. Second, if the clinical picture is highly suggestive for MS, but there is no evidence for radiological DIS or DIT, CSF OCB may be of potential prognostic value. Third, if the clinical presentation is unspecific, but radiological DIS and DIT are present as are CSF OCB, one would be very suspicious about a diagnosis of MS. Finally, given the high sensitivity of CSF OCB in patients with MS, one might be careful about making a diagnosis of MS in a patient with a doubtful clinical history in whom CSF OCB are absent.

Importantly, the present meta-analysis provides class I evidence 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.

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References

[1] V. Abraira et al. "Utility of oligoclonal IgG band detection for MS diagnosis in daily clinical practice." *J Immunol Methods* 371 (2011), pp. 170–173. DOI: 10.1016/j.jim.2011.06.009.

[2] M Andersson, J Alvarez-Cermeno, G Bernardi, et al. "Cerebrospinal fluid in the diagnosis of multiple sclerosis: a consensus report." *J Neurol Neurosurg Psychiatry* 57 (1994), pp. 897–902. DOI: 10.1136/jnnp.57.8.897.

[3] B. Arneth and F. Birklein. "High sensitivity of free lambda and free kappa light chains for detection of intrathecal immunoglobulin synthesis in cerebrospinal fluid." *Acta Neurol Scand* 119 (2009), pp. 39–44. DOI: 10.1111/j.1600-0404.2008.01058.x.

[4] M Axelsson et al., eds. *Change in number and pattern of CSF oligoclonal bands in MS patients over time*. Vol. 17. Multiple Sclerosis Journal, 2011, S281.

[5] J. Bednárová, P. Stourac, and P. Adam. "Relevance of immunological variables in neuroborreliosis and multiple sclerosis." *Acta Neurol Scand* 112 (2005), pp. 97–102. DOI: 10.1111/j.1600-0404.2005.00418.x.

[6] T. Ben-Hur, O. Abramsky, and Y. River. "The clinical significance of a single abnormal immunoglobulin band in cerebrospinal fluid electrophoresis." *J Neurol Sci* 136 (1996), pp. 159–61.

[7] Patrick M Bossuyt et al. "Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative." *BMJ* 326 (2003), pp. 41–44.

[8] A. Bourahoui et al. "CSF isoelectrofocusing in a large cohort of MS and other neurological diseases." *Eur J Neurol* 11 (2004), pp. 525–529. DOI: 10.1111/j.1468-1331.2004.00822.x.

[9] Kathryn M. Brennan et al. "Lipid arrays identify myelin-derived lipids and lipid complexes as prominent targets for oligoclonal band antibodies in multiple sclerosis." *J Neuroimmunol* 238 (2011), pp. 87–95. DOI: 10.1016/j.jneuroim.2011.08.002.

[10] Johannes Brettschneider et al. "The chemokine CXCL13 is a prognostic marker in clinically isolated syndrome (CIS)." *PLoS One* 5 (2010), e11986. DOI: 10.1371/journal.pone.0011986.

[11] Christiane Caudie et al. "Cytoimmunological profile of cerebrospinal fluid in diagnosis of multiple sclerosis." *Pathol Biol (Paris)* 53 (2005), pp. 68–74. DOI: 10.1016/j.patbio.2004.04.001.

[12] Sonia Chalbot et al. "Cerebrospinal fluid secretory Ca2+-dependent phospholipase A2 activity: a biomarker of blood-cerebrospinal fluid barrier permeability." *Neurosci Lett* 478 (2010), pp. 179–183. DOI: 10.1016/j.neulet.2010.05.012.

[13] K. H. Chang et al. "Clinical characteristics of multiple sclerosis in Taiwan: a cross-sectional study." *Mult Scler* 12 (2006), pp. 501–506.

[14] C. Confavreux et al. "Oligoclonal "fingerprint" of CSF IgG in multiple sclerosis patients is not modified following intrathecal administration of natural beta-interferon." *J Neurol Neurosurg Psychiatry* 49 (1986), pp. 1308–1312.

[15] Jorge Correale and MarÃa de los Milagros Bassani Molinas. "Oligoclonal bands and antibody responses in multiple sclerosis." *J Neurol* 249 (2002), pp. 375–389. DOI: 10.1007/s004150200026.

[16] G Davies et al. "The clinical significance of an intrathecal monoclonal immunoglobulin band: a follow-up study." *Neurology* 60 (2003), pp. 1163–1106.

[17] M. Di Filippo et al. "Abnormalities in the cerebrospinal fluid levels of endocannabinoids in multiple sclerosis." *J Neurol Neurosurg Psychiatry* 79 (2008), pp. 1224–1229. DOI: 10.1136/jnnp.2007.139071.

[18] Ruth Dobson et al. "Assessing fracture risk in people with MS: a service development study comparing three fracture risk scoring systems." *BMJ Open* 3 (2013). DOI: 10.1136/bmjopen-2012-002508.

[19] M. Falip et al. "[Clinical usefulness of oligoclonal bands]." *Rev Neurol* 32 (2001), pp. 1120–1124.

[20] Alexandre S. Fortini et al. "Cerebrospinal fluid oligoclonal bands in the diagnosis of multiple sclerosis. Isoelectric focusing with IgG immunoblotting compared with high-resolution agarose gel electrophoresis and cerebrospinal fluid IgG index." *Am J Clin Pathol* 120 (2003), pp. 672–675. DOI: 10.1309/EM7K-CQR4-GLMH-RCX4.

[21] D. Franciotta et al. "Analysis of Chlamydia pneumoniae-specific oligoclonal bands in multiple sclerosis and other neurologic diseases." *Acta Neurol Scand* 112 (2005), pp. 238–241. DOI: 10.1111/j.1600-0404.2005.00407.x.

[22] Diego Franciotta et al. "Cerebrospinal BAFF and Epstein-Barr virus-specific oligoclonal bands in multiple sclerosis and other inflammatory demyelinating neurological diseases." *J Neuroimmunol* 230 (2011), pp. 160–163. DOI: 10.1016/j.jneuroim.2010.10.027.

[23] M.S. Freedman et al. "Recommended standard of cerebrospinal fluid analysis in the diagnosis of multiple sclerosis: a consensus statement." *Arch Neurol* 62 (2005), pp. 865–870. DOI: 10.1001/archneur.62.6.865.

[24] T. Fukazawa et al. "The significance of oligoclonal bands in multiple sclerosis in Japan: relevance of immunogenetic backgrounds." *J Neurol Sci* 158 (1998), pp. 209–214.

[25] Ian Galea, Mark S. Freedman, and Edward J. Thompson. "Cerebrospinal fluid analysis in the 2010 revised McDonaldś multiple sclerosis diagnostic criteria." *Ann Neurol* 70 (2011), 183, author reply 183–183, author reply 184. DOI: 10.1002/ana.22466.

[26] Paulo Diniz da Gama et al. "Study of oligoclonal bands restricted to the cerebrospinal fluid in multiple sclerosis patients in the city of São Paulo." *Arq Neuropsiquiatr* 67 (2009), pp. 1017–1022. DOI: 10.1590/s0004-282x2009000600011.

[27] Felipe von Glehn et al. "Disappearance of cerebrospinal fluid oligoclonal bands after natalizumab treatment of multiple sclerosis patients." *Mult Scler* (2011). DOI: 10.1177/1352458511428465.

[28] Olivier Gout et al. "Prognostic value of cerebrospinal fluid analysis at the time of a first demyelinating event." *Mult Scler* 17 (2011), pp. 164–172. DOI: 10.1177/1352458510385506.

[29] S. Haghighi et al. "Incidence of CSF abnormalities in siblings of multiple sclerosis patients and unrelated controls." *J Neurol* 247 (2000), pp. 616–622.

[30] S. Haghighi et al. "Myelin glycosphingolipid immunoreactivity and CSF levels in multiple sclerosis." *Acta Neurol Scand* (2011). DOI: 10.1111/j.1600-0404.2011.01554.x.

[31] H. Hampel et al. "Oligoclonal bands and blood–cerebrospinal-fluid barrier dysfunction in a subset of patients with Alzheimer disease: comparison with vascular dementia, major depression, and multiple sclerosis." *Alzheimer Dis Assoc Disord* 13 (1999), pp. 9–19.

[32] Christopher Harp et al. "Cerebrospinal fluid B cells from multiple sclerosis patients are subject to normal germinal center selection." *J Neuroimmunol* 183 (2007), pp. 189–199. DOI: 10.1016/j.jneuroim.2006.10.020.

[33] A. Harrer et al. "Cerebrospinal fluid parameters of B cell-related activity in patients with active disease during natalizumab therapy." *Mult Scler* (2012). DOI: 10.1177/1352458512463483.

[34] R. N. Heard et al. "Lack of restriction of T cell receptor beta variable gene usage in cerebrospinal fluid lymphocytes in acute optic neuritis." *J Neurol Neurosurg Psychiatry* 67 (1999), pp. 585–590.

[35] Kerstin Imrell et al. "Multiple sclerosis with and without CSF bands: clinically indistinguishable but immunogenetically distinct." *Neurology* 67 (2006), pp. 1062–1064. DOI: 10.1212/01.wnl.0000237343.93389.35.

[36] L. D. Jacobs et al. "Correlation of clinical, magnetic resonance imaging, and cerebrospinal fluid findings in optic neuritis." *Ann Neurol* 41 (1997), pp. 392–398. DOI: 10.1002/ana.410410315.

[37] E. A. Kabat, D. H. Moore, and H. Landow. "An electrophoretic study of the protein components in cerebrospinal fluid and their relationship to the serum proteins." *J Clin Invest* 21 (1942), pp. 571–577. DOI: 10.1172/JCI101335.

[38] R. Kaiser et al. "IgG-antibodies to CNS proteins in patients with multiple sclerosis." *Eur J Med Res* 2 (1997), pp. 169–172.

[39] G Keir, RW Luxton, and EJ Thompson. "Isoelectric focusing of cerebrospinal fluid immunoglobulin G: an annotated update." *Ann Clin Biochem* 27 (1990), pp. 436–443.

[40] D. F. Keren. "Optimizing detection of oligoclonal bands in cerebrospinal fluid by use of isoelectric focusing with IgG immunoblotting." *Am J Clin Pathol* 120 (2003), pp. 649–51.

[41] Mohsen Khademi et al. "Cerebrospinal fluid CXCL13 in multiple sclerosis: a suggestive prognostic marker for the disease course." *Mult Scler* 17 (2011), pp. 335–343. DOI: 10.1177/1352458510389102.

[42] Eric C. Klawiter et al. "Elevated intrathecal myelin oligodendrocyte glycoprotein antibodies in multiple sclerosis." *Arch Neurol* 67 (2010), pp. 1102–1108. DOI: 10.1001/archneurol.2010.197.

[43] M. Koch et al. "Cerebrospinal fluid oligoclonal bands and progression of disability in multiple sclerosis." *Eur J Neurol* 14 (2007), pp. 797–800.

[44] M. et al Komori. "Proteomic pattern analysis discriminates among multiple sclerosis-related disorders." *Ann Neurol* (2011).

[45] M. Krakauer et al. "Intrathecal synthesis of free immunoglobulin light chains in multiple sclerosis." *Acta Neurol Scand* 98 (1998), pp. 161–165.

[46] J. Kuhle et al. "Neurofilament heavy chain in CSF correlates with relapses and disability in multiple sclerosis." *Neurology* 76 (14 2011), pp. 1206–1213. ISSN: 1526-632X. DOI: 10.1212/WNL.0b013e31821432ff.

[47] Doris Lambracht-Washington et al. "Antigen specificity of clonally expanded and receptor edited cerebrospinal fluid B cells from patients with relapsing remitting MS." *J Neuroimmunol* 186 (2007), pp. 164–176. DOI: 10.1016/j.jneuroim.2007.03.002.

[48] J. Lechner-Scott et al. "The frequency of CSF oligoclonal banding in multiple sclerosis increases with latitude." *Mult Scler* (2011). DOI: 10.1177/1352458511431729.

[49] B. Li et al. "Cerebrospinal fluid IgG profiles and oligoclonal bands in Chinese patients with multiple sclerosis." *Acta Neurol Scand* 115 (2007), pp. 319–324. DOI: 10.1111/j.1600-0404.2006.00753.x.

[50] H. Link and Y. M. Huang. "Oligoclonal bands in multiple sclerosis cerebrospinal fluid: an update on methodology and clinical usefulness." *J Neuroimmunol* 180 (2006), pp. 17–28.

[51] J. Link et al. "Increased transforming growth factor-beta, interleukin-4, and interferon-gamma in multiple sclerosis." *Ann Neurol* 36 (1994), pp. 379–386. DOI: 10.1002/ana.410360309.

[52] J. Losy and A. Niezgoda. "IL-18 in patients with multiple sclerosis." *Acta Neurol Scand* 104 (2001), pp. 171–173.

[53] Laura Lovato et al. "Related B cell clones populate the meninges and parenchyma of patients with multiple sclerosis." *Brain* 134 (2011), pp. 534–541. DOI: 10.1093/brain/awq350.

[54] A Lowenthal, D Karcher, and M Van Sande. "The differential diagnosis of neurological diseases by fractionating electrophoretically the CSF gamma-globulins." *J Neurochem* 6 (1960), pp. 51–56.

[55] J. Lunding, R. Midgard, and C. A. Vedeler. "Oligoclonal bands in cerebrospinal fluid: a comparative study of isoelectric focusing, agarose gel electrophoresis and IgG index." *Acta Neurol Scand* 102 (2000), pp. 322–325.

[56] C. Malmeström et al. "Neurofilament light protein and glial fibrillary acidic protein as biological markers in MS." *Neurology* 61 (2003), pp. 1720–1725.

[57] D. H. Mattson, R. P. Roos, and B. G. Arnason. "Isoelectric focusing of IgG eluted from multiple sclerosis and subacute sclerosing panencephalitis brains." *Nature* 287 (1980), pp. 335–337.

[58] D. McAlpine. "Multiple Sckrosis. A Reappraisal." *Churchill Livingstone* (1972), pp. 225–226.

[59] P. A. McCombe et al. "Monoclonal immunoglobulin bands in the cerebrospinal fluid." *Aust N Z J Med* 21 (1991), pp. 227–9.

[60] W. Ian McDonald et al. "Recommended diagnostic criteria for multiple sclerosis: Guidelines from the international panel on the diagnosis of multiple sclerosis." *Annals of Neurology* 50 (2001), pp. 121–127. DOI: 10.1002/ana.1032.

[61] S. A. McMillan et al. "Evaluation of the clinical utility of cerebrospinal fluid (CSF) indices of inflammatory markers in multiple sclerosis." *Acta Neurol Scand* 101 (2000), pp. 239–243.

[62] Nancy L. Monson et al. "Receptor revision and atypical mutational characteristics in clonally expanded B cells from the cerebrospinal fluid of recently diagnosed multiple sclerosis patients." *J Neuroimmunol* 158 (2005), pp. 170–181. DOI: 10.1016/j.jneuroim.2004.04.022.

[63] S. P. Morrissey et al. "Bilateral simultaneous optic neuropathy in adults: clinical, imaging, serological, and genetic studies." *J Neurol Neurosurg Psychiatry* 58 (1995), pp. 70–74.

[64] A. Mygland et al. "Isoelectric focusing is superior to immunofixation electrophoresis in diagnosing CNS inflammation." *Acta Neurol Scand* 115 (2007), pp. 122–125. DOI: 10.1111/j.1600-0404.2006.00763.x.

[65] Birgit Obermeier et al. "Matching of oligoclonal immunoglobulin transcriptomes and proteomes of cerebrospinal fluid in multiple sclerosis." *Nat Med* 14 (2008), pp. 688–693. DOI: 10.1038/nm1714.

[66] A. A. Petersen et al. "Soluble ICAM-1, demyelination, and inflammation in multiple sclerosis and acute optic neuritis." *J Neuroimmunol* 88 (1998), pp. 120–127.

[67] A Petzold. "Biological Markers." *Multiple Sclerosis Journal* 18 (2012), p. 121.

[68] A Petzold, G Keir, and LT Sharpe. "Spectrophotometry for xanthochromia." *N Engl J Med* 351 (2004), pp. 1695–1696.

[69] A Petzold et al. "Markers for different glial cell responses in multiple sclerosis: clinical and pathological correlations." *Brain* 125 (2002), pp. 1462–1473. DOI: 10.1093/brain/awf165.

[70] Axel Petzold. "Neurofilament phosphoforms: Surrogate markers for axonal injury, degeneration and loss." *J Neurol Sci* 233 (2005), pp. 183–198. DOI: 10.1016/j.jns.2005.03.015.

[71] F. Piazza et al. "Cerebrospinal fluid levels of BAFF and APRIL in untreated multiple sclerosis." *J Neuroimmunol* 220 (2010), pp. 104–107. DOI: 10.1016/j.jneuroim.2010.01.011.

[72] C. H. Polman, J. C. Koetsier, and E. C. Wolters. "Multiple sclerosis: incorporation of results of laboratory techniques in the diagnosis." *Clin Neurol Neurosurg* 87 (1985), pp. 187–192.

[73] C.H. Polman et al. "Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria"." *Ann Neurol* 58 (2005), pp. 840–6.

[74] Chris H. Polman et al. "Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria." *Ann Neurol* 69 (2011), pp. 292–302. DOI: 10.1002/ana.22366.

[75] C. M. Poser et al. "New diagnostic criteria for multiple sclerosis: guidelines for research protocols." *Ann Neurol* 13 (1983), pp. 227–231. DOI: 10.1002/ana.410130302.

[76] Stefan Presslauer et al. "Elevated levels of kappa free light chains in CSF support the diagnosis of multiple sclerosis." *J Neurol* 255 (2008), pp. 1508–1514. DOI: 10.1007/s00415-008-0954-z.

[77] Michael K. Racke. "The role of B cells in multiple sclerosis: rationale for B-cell-targeted therapies." *Curr Opin Neurol* 21 Suppl 1 (2008), S9–S18. DOI: 10.1097/01.wco.0000313359.61176.15.

[78] H. Reiber. "External quality assessment in clinical neurochemistry: survey of analysis for cerebrospinal fluid (CSF) proteins based on CSF/serum quotients." *Clin Chem* 41 (1995), pp. 256–263.

[79] L. A. Rolak et al. "Cerebrospinal fluid in acute optic neuritis: experience of the optic neuritis treatment trial." *Neurology* 46 (1996), pp. 368–372.

[80] C. M. Rutter and C. A. Gatsonis. "A hierarchical regression approach to meta-analysis of diagnostic test accuracy evaluations." *Stat Med* 20 (2001), pp. 2865–2884.

[81] Maria José Sá et al. "[Oligoclonal IgG bands in the cerebrospinal fluid of portuguese patients with multiple sclerosis: negative results indicate benign disease]." *Arq Neuropsiquiatr* 63 (2005), pp. 375–379.

[82] Paola Sarchielli et al. "Fibroblast growth factor-2 levels are elevated in the cerebrospinal fluid of multiple sclerosis patients." *Neurosci Lett* 435 (2008), pp. 223–228. DOI: 10.1016/j.neulet.2008.02.040.

[83] GA Schumacher, G Beebe, RF Kebler, et al. "Problems of experimental trials of therapy in multiple sclerosis." *Ann. NY Acad, Sci* 122 (1965), pp. 552–568.

[84] F. Sellebjerg and M. Christiansen. "Qualitative assessment of intrathecal IgG synthesis by isoelectric focusing and immunodetection: interlaboratory reproducibility and interobserver agreement." *Scand J Clin Lab Invest* 56 (1996), pp. 135–143.

[85] F. Sellebjerg et al. "Acute optic neuritis: myelin basic protein and proteolipid protein antibodies, affinity, and the HLA system." *Ann Neurol* 38 (1995), pp. 943–950. DOI: 10.1002/ana.410380616.

[86] E. Silber et al. "Patients with progressive multiple sclerosis have elevated antibodies to neurofilament subunit." *Neurology* 58 (2002), pp. 1372–1381.

[87] A. G. Skov, T. Skov, and J. L. Frederiksen. "Oligoclonal bands predict multiple sclerosis after optic neuritis: a literature survey." *Mult Scler* 17 (2011), pp. 404–410. DOI: 10.1177/1352458510391340.

[88] Martin Stangel et al. "The utility of cerebrospinal fluid analysis in patients with multiple sclerosis." *Nat Rev Neurol* 9 (2013), pp. 267–276. DOI: 10.1038/nrneurol.2013.41.

[89] Birgitte Stoevring, Ole Vang, and Michael Christiansen. "(alpha)B-crystallin in cerebrospinal fluid of patients with multiple sclerosis." *Clin Chim Acta* 356 (2005), pp. 95–101. DOI: 10.1016/j.cccn.2005.01.034.

[90] E. J. Thompson, P. Kaufmann, and P. Rudge. "Sequential changes in oligoclonal patterns during the course of multiple sclerosis." *J Neurol Neurosurg Psychiatry* 46 (1983), pp. 547–550.

[91] M. Tintoré et al. "Do oligoclonal bands add information to MRI in first attacks of multiple sclerosis?" *Neurology* 70 (2008), pp. 1079–1083. DOI: 10.1212/01.wnl.0000280576.73609.c6.

[92] H. Tumani et al. "Acute optic neuritis: combined immunological markers and magnetic resonance imaging predict subsequent development of multiple sclerosis. The Optic Neuritis Study Group." *J Neurol Sci* 155 (1998), pp. 44–49.

[93] Hayrettin Tumani et al. "Revised McDonald criteria: The persisting importance of cerebrospinal fluid analysis." *Ann Neurol* (2011). DOI: 10.1002/ana.22508.

[94] M. M. Verbeek et al. "Increased cerebrospinal fluid chitotriosidase index in patients with multiple sclerosis." *Acta Neurol Scand* 121 (2010), pp. 309–314. DOI: 10.1111/j.1600-0404.2009.01242.x.

[95] L. M. Villar et al. "A sensitive and reproducible method for the detection of oligoclonal IgM bands." *J Immunol Methods* 258 (2001), pp. 151–5.

[96] L. M. Villar et al. "Intrathecal IgM synthesis in neurologic diseases: relationship with disability in MS." *Neurology* 58 (2002), pp. 824–826.

[97] L. M. Villar et al. "Intrathecal IgM synthesis is a prognostic factor in multiple sclerosis." *Ann Neurol* 53 (2003), pp. 222–6.

[98] L.M. Villar et al. "Intrathecal synthesis of oligoclonal IgM against myelin lipids predicts an aggressive disease course in MS." *J Clin Invest* 115 (2005), pp. 187–194. DOI: 10.1172/JCI22833.

[99] Luisa M. Villar et al. "Early differential diagnosis of multiple sclerosis using a new oligoclonal band test." *Arch Neurol* 62 (2005), pp. 574–577. DOI: 10.1001/archneur.62.4.574.

[100] M H J. Vogt et al. "Cerebrospinal fluid anti-myelin antibodies are related to magnetic resonance measures of disease activity in multiple sclerosis." *J Neurol Neurosurg Psychiatry* 80 (2009), pp. 1110–1115. DOI: 10.1136/jnnp.2008.146357.

[101] B. G. Xiao et al. "The cerebrospinal fluid from patients with multiple sclerosis promotes neuronal and oligodendrocyte damage by delayed production of nitric oxide in vitro." *J Neurol Sci* 142 (1996), pp. 114–120.

[102] S. Y. Yao et al. "CSF oligoclonal bands in MS include antibodies against Chlamydophila antigens." *Neurology* 56 (2001), pp. 1168–1176.

[103] Xiaoli Yu et al. "Intrathecally synthesized IgG in multiple sclerosis cerebrospinal fluid recognizes identical epitopes over time." *J Neuroimmunol* 240-241 (2011), pp. 129–136. DOI: 10.1016/j.jneuroim.2011.10.009.

[104] Ivana Zadro et al. "Clinical relevance of antibodies against myelin oligodendrocyte glycoprotein in different clinical types of multiple sclerosis." *Clin Neurol Neurosurg* 109 (2007), pp. 23–26. DOI: 10.1016/j.clineuro.2006.04.006.

[105] A. Z. Zeman et al. “A study of oligoclonal band negative multiple sclerosis.” *J Neurol Neurosurg Psychiatry* 60 (1996), pp. 27–30.

[106] S. R. Zhou et al. “A cross-reactive anti-myelin basic protein idiotope in cerebrospinal fluid cells in multiple sclerosis.” *Neurology* 50 (1998), pp. 411–417.

[107] V. Zipoli et al. “The contribution of cerebrospinal fluid oligoclonal bands to the early diagnosis of multiple sclerosis.” *Mult Scler* 15 (2009), pp. 472–478. DOI: 10.1177/1352458508100502.