CSF in MS: Current Assays & Significance

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Disclosures

- None pertinent to this talk

Top 10 Reasons Neurologists do Not order CSF Analysis

10. Test always unreliable
9. Tests not reproducible
8. Don't need it if there is MRI
7. LP - too much pain, not enough gain
6. Does anybody do this anymore?
5. The lab always loses the samples
4. We don't get paid enough to do LP's
3. I forgot how to do LP's
2. Too many false positives
1. I know the patient has MS

What Can the CSF Tell Us?

- QUALITATIVE:
  - Integrity of the BBB (blood-CSF barrier)
  - Presence of oligoclonal IgG production
- QUANTITATIVE:
  - Lymphocyte counts
  - Immunoglobulin synthesis
  - Cytokine measurement (e.g. TNFα)
  - Soluble receptors (e.g. sIL2-R; sICAM-1)
  - Degradation products (e.g. neurofilament, myelin proteins)

INTEGRITY of THE BBB: The ALBUMIN INDEX

- The BBB gets leakier with age
- Albumin is not synthesized in the CNS so any albumin measured in the CSF is due to diffusion from the serum (CSF albumin ~1/200 that of serum)
- Ratio of CSF:Serum albumin increases proportionally with increased leakiness of the BBB (as well as age)

AGE (range)	Albumin Index*
<15	5
15-29	6
30-39	7
40-59	8
>60	9

$$\text{*Albumin Index} = \frac{\text{CSF Albumin}}{\text{Serum Albumin}} \times 10^3$$

Oligoclonal Bands (OCB) in MS

- Produced by clonally expanded, terminally differentiated B cells within the CNS compartment
- Mark a highly targeted immune response against a specific target antigen(s)
- OCBs are among the strongest indicators of an antigen-driven humoral immune process in MS

Bankoti J et al. 2014; Ann Neurol;75:266–276

OCB in MS

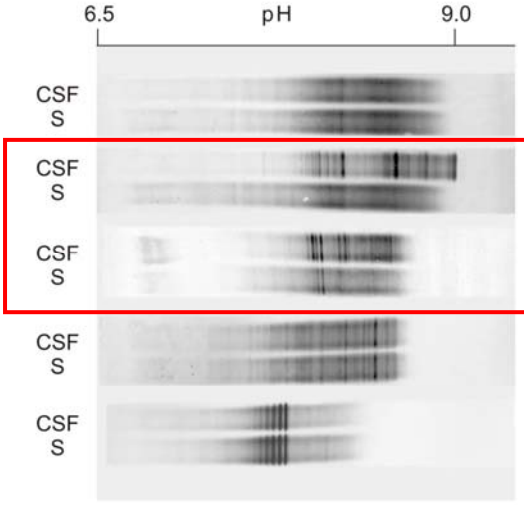
- Appear early in disease course
- Individual "fingerprint"
- Does not vary with either disease state (relapse/remission) or treatment (e.g. corticosteroids)
- Mostly due to IgG (though IgM OCB may offer additional information)
- Best detected by agarose immunoelectrophoresis (IEF) and immunoblotting or immunofixation (sensitivity >95% in proven MS cases)
- Criteria: ≥ 2 distinct bands in the CSF electrophoretic profile that are NOT present in the corresponding profile of serum

Accounting for False + CSF Results

- Infections (e.g. Borreliosis) and its complications
  - ~15% of OCB+*
- Inflammatory conditions
  - Localized synthesis indistinguishable from MS in conditions such as sarcoidosis, SLE, Behçet's or Sjögren's
- If only clinical "suspicion" (i.e. rule out other disease) then the cell (count, differential) and biochemistry profiles (glucose, protein, albumin index, IgG synthesis) can help, but not in ALL cases

*Based on 1007 suspected cases, McLean et al, 1990

OCB in MS (IEF-Immunoblotting)*



Type 1 No Bands

Type 2 SB

Type 3 SB and NSB

Type 4 NSB +CSF

Type 5 MCB

SB = specific banding
NSB = non-specific banding
MCB = monoclonal banding

*Courtesy of Prof. H. Reiber, Germany

QUANTITATION of CSF IgG: The IgG INDEX*

- Ideally the most simple and reliable estimate of localized synthesis of IgG
- Quantitatively valid even in the presence of obvious BBB damage (leakiness)
- A positive index is >70%

$$\text{*IgG Index} = \frac{\text{(IgG/albumin) CSF}}{\text{(IgG/albumin) serum}} \times 100\%$$

- New formulas for IgG account for the Ibumin Index using Q_{alb} as part of the equation
- Not a substitute for Qualitative analysis (OCB)
- Complimentary results to Qualitative measures

Revised 2010 MS Diagnostic Criteria

Historical Attacks	Clinically Evident Lesions	Additional Information Required
≥2	2	Nothing, but… caution if both MRI and CSF are negative
≥2	1	DIS criteria[a]: MRI+ OR Await 2nd clinical attack

[a]satisfy criteria of Table 1 for DIS (≥1 lesion in ≥2 of 4 areas: juxtacortical, periventricular, infratentorial or spinal cord)

Polman CH et al, Ann Neurol 2011; 69:292–302

Revised RRMS Diagnostic Criteria

Historical Attacks	Clinically Evident Lesions	Additional Information Required
1	≥2 **CIS**	DIT criteria[a]: ≥1 NEW T2 or Gd+ lesion at any time OR ≥1 Gd+ AND Gd- lesions OR Await 2nd clinical attack

[a]satisfy criteria of Table 2 for DIT (≥1 T2 or Gd+ lesion on follow-up MRI, with reference to a baseline scan, irrespective of the timing of the baseline MRI OR the simultaneous presence of asymptomatic Gd+ and Gd- lesions at any time)

Polman CH et al, Ann Neurol 2011; 69:292–302

Revised RRMS Diagnostic Criteria

Historical Attacks	Clinically Evident Lesions	Additional Information Required: DIS and DIT
1	1	DIS (Table 1) OR Await a 2nd clinical attack showing a different CNS region AND DIT (Table 2) OR Await a 2nd clinical attack

CIS

Polman CH et al, Ann Neurol 2011; 69:292–302;

Revised PPMS Diagnostic Criteria

Historical Attacks	Additional Information Required: 2 out of 3 of the following
≥1 year of disease progression (retro- or prospectively determined)	a. Evidence for DIS in the brain (≥1 T2 lesions in ≥1 area characteristic for MS (periventricular, juxtacortical or infratentorial) b. Evidence for DIS in the spinal cord based on ≥2 T2+ lesions c. + CSF (isoelectric focusing evidence of OCB or elevated IgG index)

If a subject has a brainstem or spinal cord syndrome, all symptomatic lesions are excluded from the Criteria

Polman CH et al, Ann Neurol 2011; 69:292–302;

Utility of CSF Analysis in Predicting CDMS in Optic Neuritis*

- n=147, followed up for 5 years (mean 2.1 years)
- OCB+ in 72%
- IgG Index increased in 41%
  - All these patients had +OCB
- N CSF in 22%
- 55% had ≥3 T2 lesions on MRI
  - only 128 patients underwent MRI studies, 12 whose scans were rejected due to tardiness (>6/12 after study start) = 116 total scans

*Söderström M et al, Neurology 50:708-714, 1998

Utility of CSF Analysis in Predicting CDMS in Optic Neuritis*

- 4 MRI+ patients were OCB-
  - 2/4 were OCB+ on subsequent CSF test
- 31% of +OCB had MRI ≤ 3 lesions
- N CSF in 22/116 MRI studied patients, and 17/22 (77%) had N MRI and N CSF
- N MRI in 41, but 20 with +CSF
- CDMS in 36% (53/147)

*only 128 patients underwent MRI studies, >6/12 after study start

Utility of CSF Analysis in Predicting CDMS in Optic Neuritis

- OCB:
  - Sensitivity- 96%
  - Specificity- 42%
  - PPV- 49%
  - NPV- 95%
- +OCB or +MRI:
  - Sensitivity- 100%
  - Specificity- 53%
  - PPV- 63%
  - NPV- 100%
- 25% (5/20) of MRI-patients with +CSF developed CDMS
  - 27% (3/11) in the NA ONTT
- 4% (2/53) of CDMS had N CSF at presentation but both had +CSF at a later time before CDMS
- N MRI and N CSF virtually ruled out MS

*Söderström M et al, Neurology 50:708-714, 1998

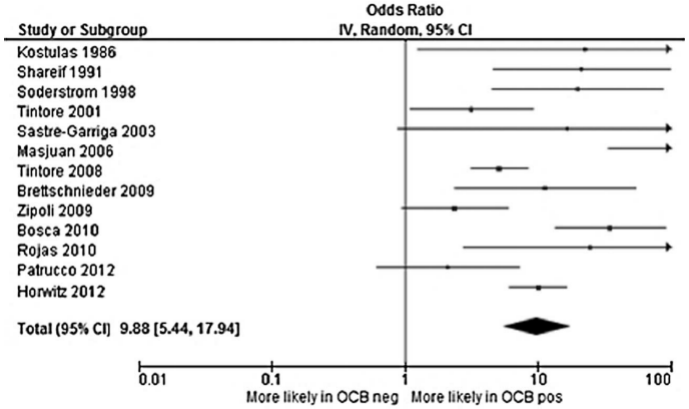
Utility of CSF Analysis in Predicting CDMS in ATM

- 85 patients with an acute partial TM presenting as CIS
- +OCBs associated with an odds ratio of 15.76 (95% CI, 2.95–84.24) of CDMS after a mean follow-up period of 104.8 (29.8) months

Bourre et al. 2012; Arch Neurol 69:357–362

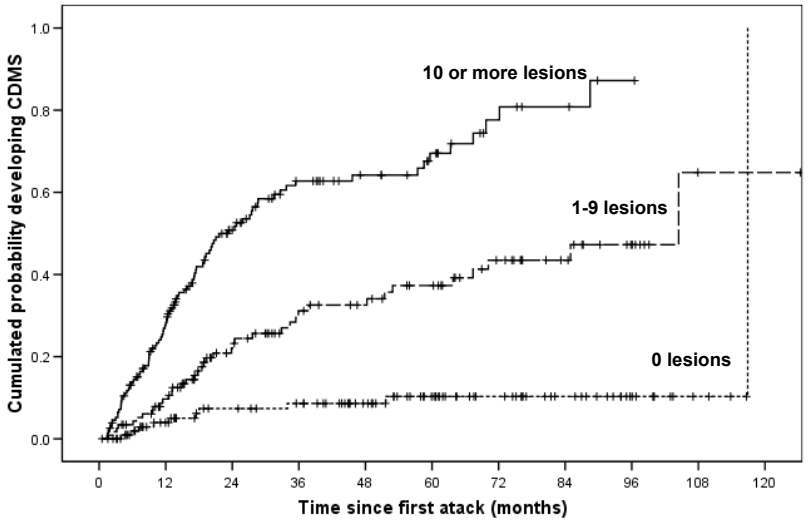
Utility of CSF Analysis in Predicting CDMS in CIS

- Meta-analysis
  - 68.6% of 2685 patients with CIS were OCB+
  - OR of 9.88 of conversion to CDMS



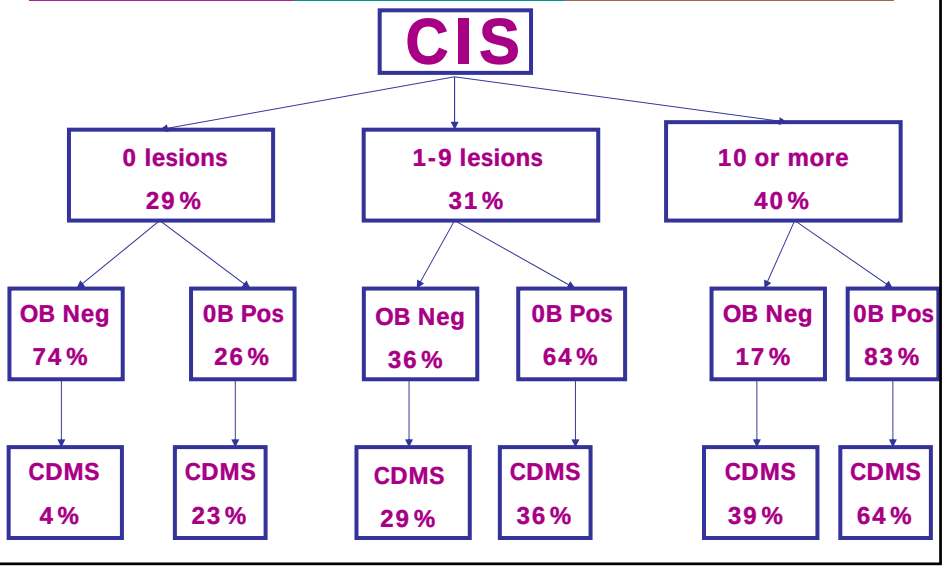
Dobson et al. 2013; JNNP 84: 909–914

Conversion to CDMS based on MRI: Baseline number of MRI lesions

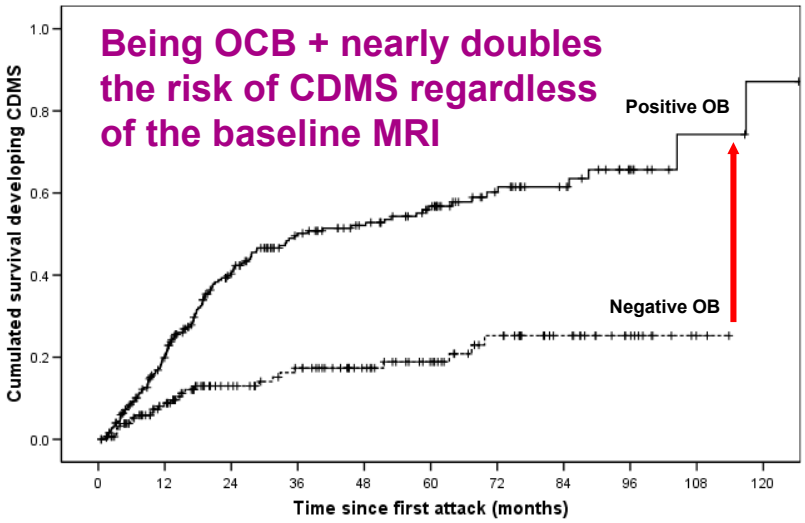


Tintore M et al, Neurology 2008;70;1079-1083

Conversion to CDMS based on CSF: Baseline OCB positive or negative



Conversion to CDMS based on CSF: Baseline OCB positive or negative



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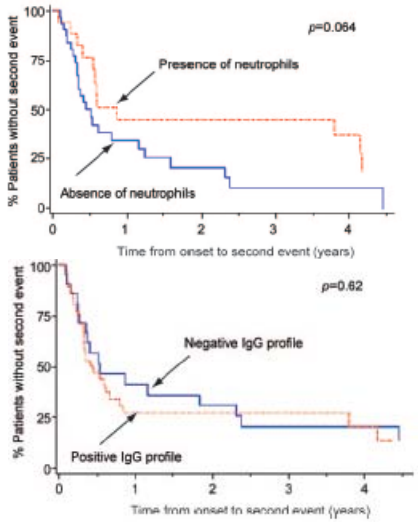
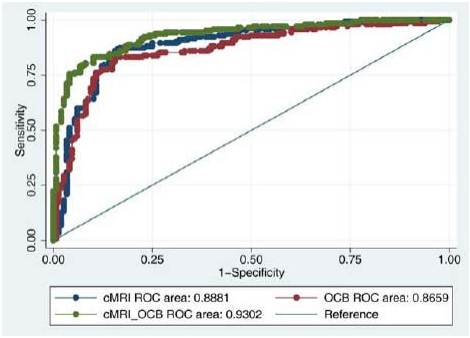
Paediatric vs. Adult CSF in MS



- Neutrophilia is not uncommon in younger patients presenting with early signs of MS
- There are fewer younger patients with a raised IgG Index (35 vs 68%, p=0.031), as well as fewer patients with positive OCB

Chabas D et al, Neurology 2010;74:399–405

OCB in Paediatric MS



- 357 children with isolated ON as a first demyelinating event with median follow-up of 4.0 years
- Combined cMRI & OCB positivity indicated a 26.84-fold higher HR for CDMS compared to double negativity (95% CI 12.26 - 58.74, p<0.001)

Heussinger, N et al 2015;Ann Neurol 77:1076–1082

Fate of Initial Single Band on IEF*

CSF Findings	# of Patients	Diagnosis
Conversion from MCB to OCB	9	3 MS 2 CIS ?MS 1 CNS Inflammation 2 Vascular disease 1 No diagnosis
Persisting MCB	13	1 CIS ?MS 2 Encephalitis 1 Cerebral Lymphoma 1 Axonal neuropathy 7 No evidence of inflammation
Initial MCB but N on follow-up	5	1 CIS ?MS 2 Encephalitis 1 Axonal neuropathy

**Davies et al, Neurology 60:1163, 2003*

CSF Light Chains

- Light chain (λ, κ) analysis can resolve equivocal electrophoretic patterns
  - Free light chains in the serum are excreted by the kidney, so if any are found in the CSF, this has to be due to localized synthesis
  - κ>λ, implies MS, whereas λ>κ is non-specific
  - Will be detectable in rare cases where oligoclonal banding is due to the presence of IgA or IgM (not detectable on IgG staining)

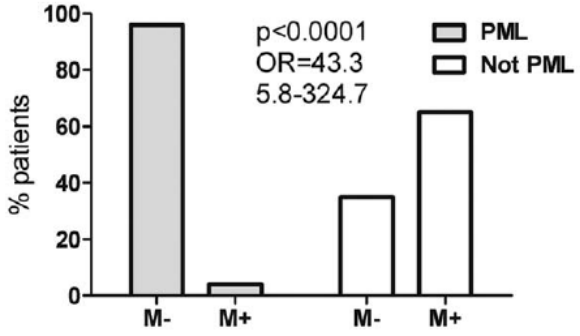
IgM Oligoclonal Banding

- Detected in 30-60% MS, especially early in the course, may indicate a worse prognosis
- Patients with +IgM OCB may have a more favourable response to disease modifying therapies
- PPMS patients with +IgM OCB were more likely to have Gd+ scans, possibly identifying them as potentially treatable

Masjuan J et al. 2006; Neurology, 66, 576–578
Villar LM et al. 2014; Ann Neurol 76:231–240

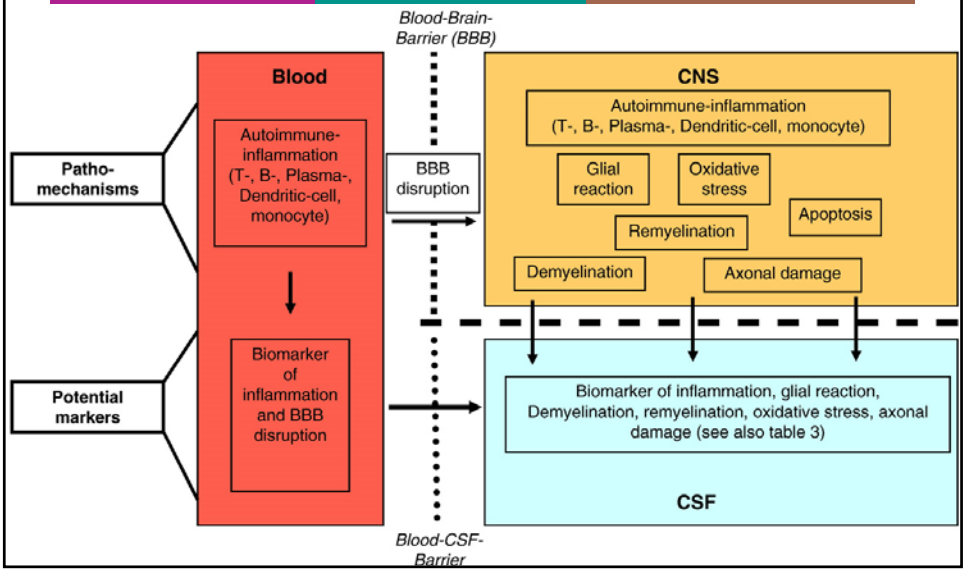
IgM Oligoclonal Banding

- In 24 patients on Natalizumab, lipid-specific IgM OCB were associated with a reduced chance of getting PML



Villar LM et al. 2015; Ann Neurol 77:447–457 66

CSF BioMarkers



Novel CSF Markers

- Soluble vascular cell adhesion molucle-1 (sVCAM-1)
- 24S-hydroxycholestrol
- Neurofilaments (NF)
- Soluble intercellular adhesion molecule-1 (sICAM-1)
- Soluble (s) E-selectin
- Soluble (s) CD30
- Platelet/endothelial cell adhesion molecule-1(PECAM-1)
- Neural cell adhesion molecule (NCAM)
- Glial fibrillary acidic protein (GFAP)
- Nitrous oxide (NO) metabolites
- Fetuin-A
- MBP
- Soluble human leukocyte antigen (HLA) class I and II antigens
- Tumor necrosis factor (TNF) alpha
- CXCL13
- Interleukin (IL) 6
- Interleukin (IL) 12
- Anti GM3 antibody
- Metalloproteinase-9 (MMP-9)
- Antibodies against heavy chain isoform
- Tau
- Actin
- Tubulin
- 14-3-3 protein

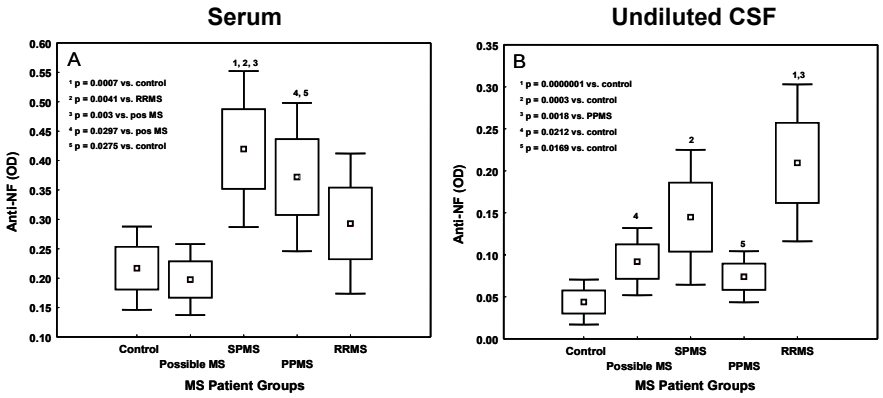
Novel CSF Biomarkers: CXCL-13

- Most potent B-cell chemoattractant and follicular B helper T cells via CXCR5
- Increase not specific for MS (also in viral/bacterial infections)
- Important prognostic marker in CIS, as it predict conversion to CDMS
- Associated with disease exacerbations and unfavourable prognosis in RRMS
- Levels correlated to the amount of CSF B-cells, plasmablasts, and intrathecal Ig production

Novel CSF Biomarkers: Fetuin-A

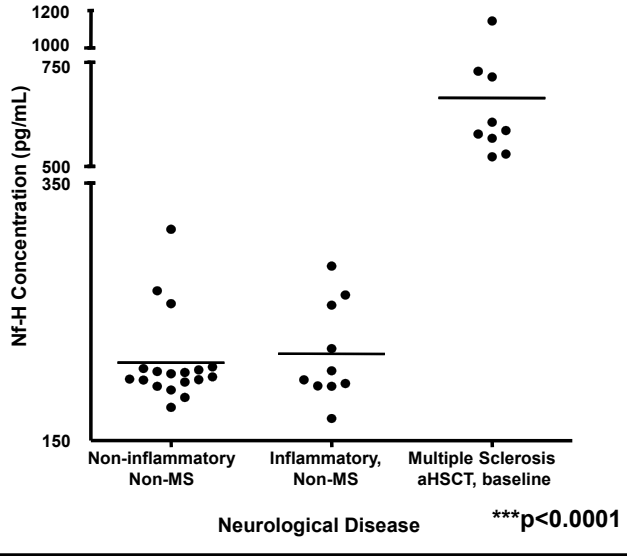
- (α-2-HS-glycoprotein, AHSG) a serum protein secreted primarily from the liver
- Altered levels of CSF fetuin-A in MS associated with early conversion to CDMS
- Elevated levels in SPMS but not PPMS
- Elevated levels correlate with disease activity
- In natalizumab-treated patients, levels reduced 1 year post treatment, correlating with therapeutic response
  - 69% of patients had decreased fetuin-A levels, similar to known clinical response to natalizumab

Detection of anti-Neurofascin Antibodies in MS Patient Sera and CSF (OD)

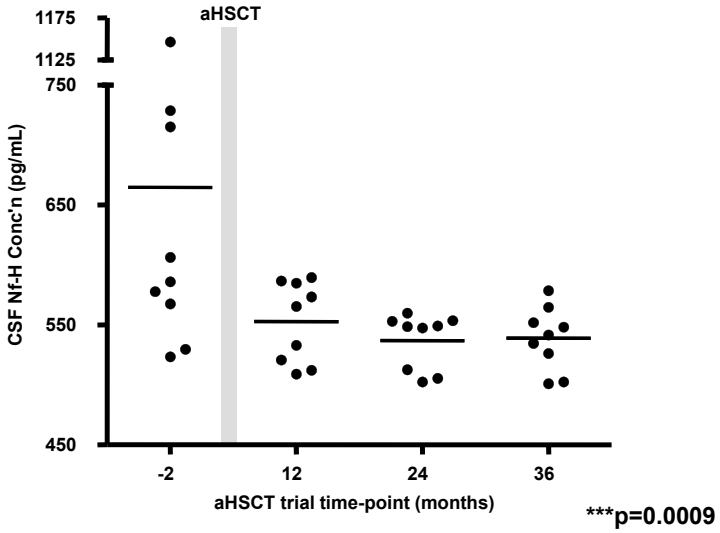


Detection of anti-neurofascin antibodies in MS patient sera and CSF. n = 178 [Control: 38; Possible MS: 27; SPMS: 25; PPMS: 39; RRMS: 49] and 121 [Control: 31; Possible MS: 17; SPMS: 17; PPMS: 21; RRMS: 35] for sera and CSF, respectively. Box-and-Whisker plots denote the mean ± 1.96SE. Statistics were calculated by applying the Kruskal-Wallis ANOVA, followed by the Mann-Whitney U Test.

Neurofilament Enriched in MS CSF



CSF Neurofilament Decreases Following Immunoablation & aHSCT



Utility of CSF Analysis in MS Diagnosis

- Especially useful when MRI is negative or fail to show typical (Barkhof) lesions
- Qualitative (OCB) vs. quantitative (IgG Index) offers greater sensitivity and specificity
- Increases the risk of MS in monosymptomatic disease (CIS) and probably in asymptomatic MS (RIS), though the natural history of this RIS group is currently unknown
- In juvenile MS, there is a lower sensitivity and the absence of OCB or raised IgG Index does not rule out MS