TAPPING INTO THE POTENTIAL OF CSF FOR THE INVESTIGATION OF MULTIPLE SCLEROSIS

HARMONIZED LABORATORY REPORTING AND EMERGING BIOMARKERS

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Declaration of Conflict of Interest

- I do not have any conflict of interest and I do not discuss any off-label use of drugs/devices.

LEARNING OBJECTIVES

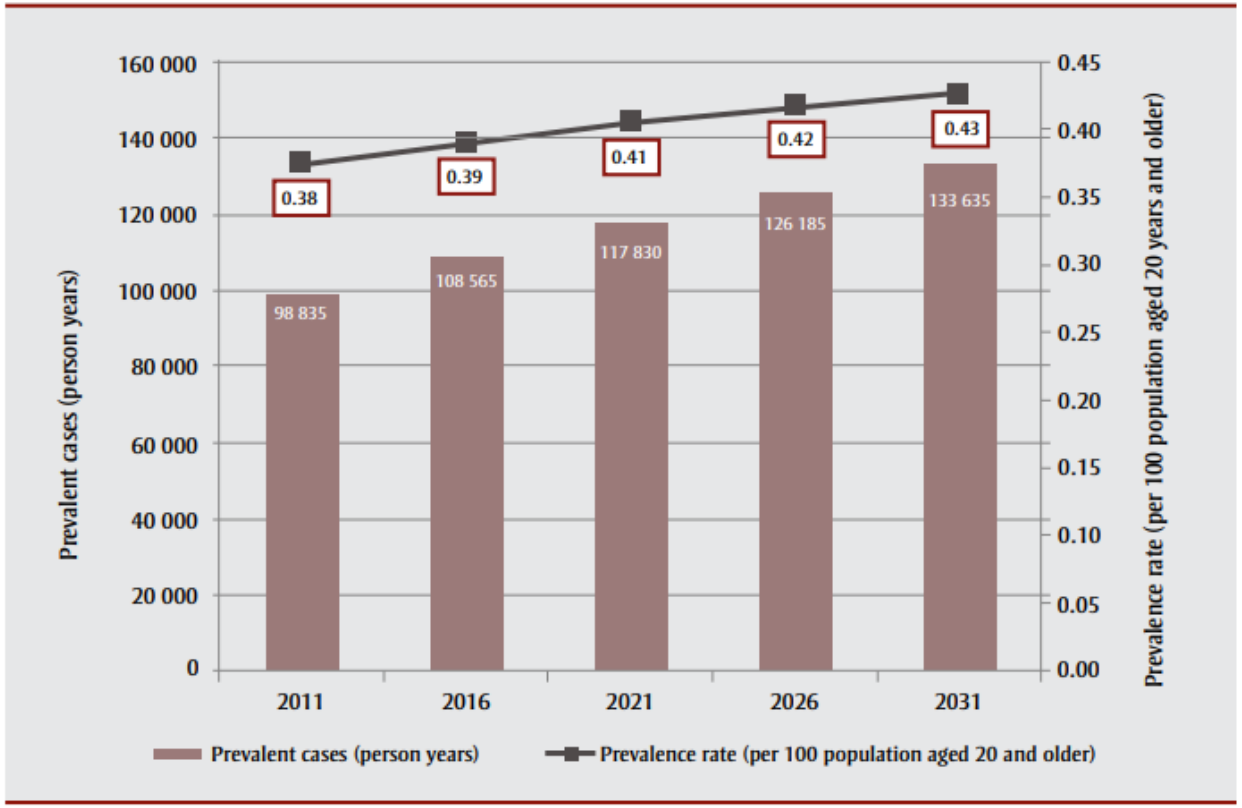
1. Review the draft harmonized reporting recommendations for CSF oligoclonal banding developed by the Harmonized CSF Analysis for MS Investigation (hCAMI) Committee of the Canadian Society of Clinical Chemists (CSCC)

2. Discuss the roles of kappa free light chain (kFLC) index and serum neurofilament light chain (sNfL) for diagnosing and monitoring multiple sclerosis

MULTIPLE SCLEROSIS (MS)

- Most common non-traumatic disabling neurological condition among young adults in Canada
- Typical onset between 20-40 years of age
- 3x more common in females than males
- People living with MS have reduced life expectancy and poorer health-related quality of life
  - Unemployment, long-term disability

Projected multiple sclerosis prevalence count (person years) and rate, population aged 20 years and older, both sexes, Canada, 2011–2031



Amankwah N, et al. Health Promotion and Chronic Disease Prevention in Canada. 2017; 37(2) 37-48

MULTIPLE SCLEROSIS (MS)

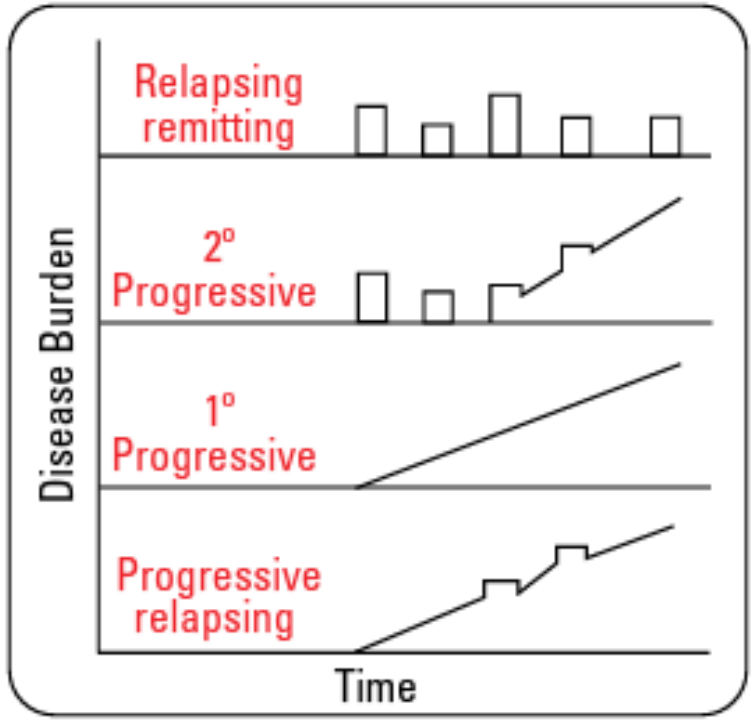
- Chronic, inflammatory, demyelinating disease of the CNS
- Immune-mediated disorder characterized by autoreactive lymphocytes

Clinical Features

- Numbness, visual disturbances (optic neuritis), fatigue, weakness, spasticity, impaired gait, vertigo, bladder dysfunction
- Uhthoff's phenomenon: worsening of symptoms in the heat

Clinical Patterns

- 85%: Relapsing Remitting MS (2-3%/y Secondary Progressive MS)
- 10% - Primary Progressive MS
- 5% - Progressive Relapsing MS
- Clinically Isolated Syndrome: monophasic clinical episode



Toronto Note 2020; Filippi M, et al. Nature Reviews 2018

2017 MCDONALD CRITERIA FOR MS DIAGNOSIS

Dissemination in Space Development of lesions in distinct anatomic locations within the CNS	**Dissemination in Time** Development or appearance of new CNS lesions over time
• ≥1 hyperintense T2 lesions on MRI in ≥2 MS-typical CNS regions (periventricular, juxtacortical, infratentorial, spinal cord) **OR** • Development of a second attack that implicates a different CNS site	• ≥2 attacks **OR** • Simultaneous presence of gadolinium-enhancing and non-enhancing lesions on MRI at any time or a new T2 and/or gadolinium-enhancing lesion(s) on follow-up MRI **OR** • Presence of ≥2 CSF-specific oligoclonal bands (OCB)

*Updated McDonald criteria expected to be published early next year!

Thompson AJ, et al. The Lancet: Neurology 2018

CSF OLIGOCLONAL BANDS IN MS

- Indicates chronic immune-activation in the CNS
- No definite association of OCB with a consistent antigen in MS patients
- OCB do not distinguish between clinical MS subtypes
- Clinically isolated syndrome: Presence of OCB predicts conversion to MS
  - Hazard ratio: 2.18 (CI: 1.71-2.77)

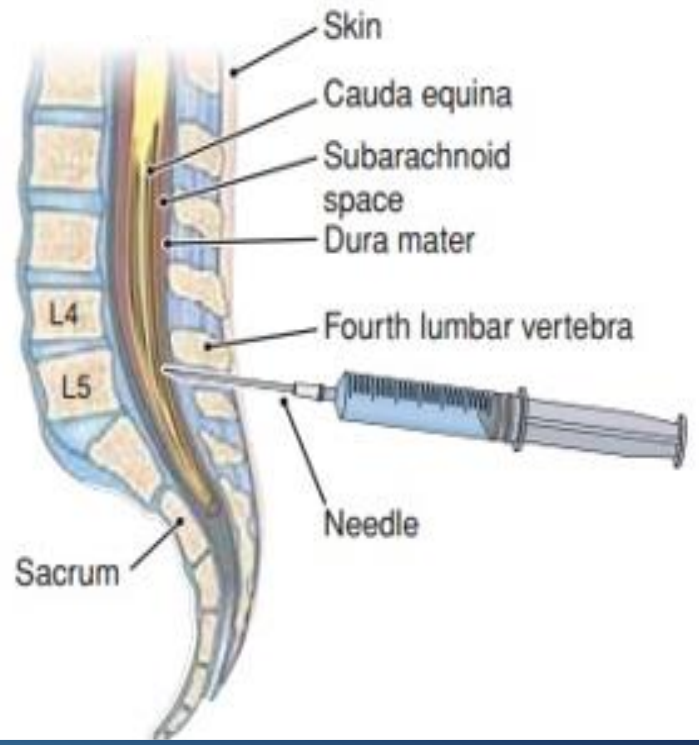
Clinical Disorder	Approximate Incidence of CSF OCB (%)
Subacute sclerosing panencephalitis	100
Multiple sclerosis	95
Neurosyphilis	95
Neuro-Lyme disease	80
Neuro-AIDS	80
Cysticercosis	80
Guillain-Barré syndrome	60
Neuro-SLE	50
Neurosarcoidosis	40

UpToDate 2021; Freedman MS, Arch Neurol 2005; Stangel M, et al. Nat Rev Neurol 2013; Tietz Textbook 6th Edition 2018

ROLE OF THE CLINICAL LABORATORY

$$IgG\ Index = \frac{\frac{CSF\ IgG}{Serum\ IgG}}{\frac{CSF\ albumin}{Serum\ albumin}}$$

- CSF & Serum Albumin
- CSF & Serum IgG
- Albumin Quotient
- IgG Index
- IgG Synthesis Rate



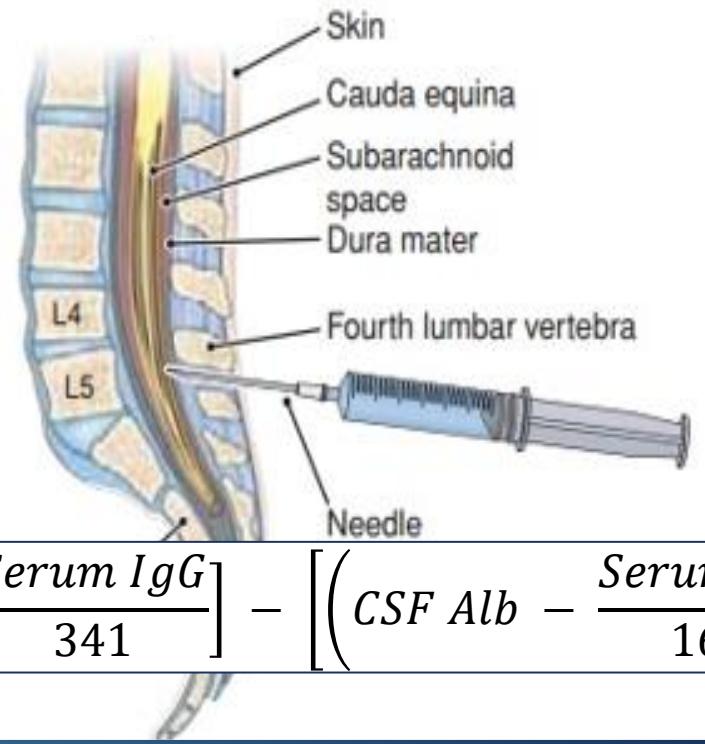
CSF Oligoclonal Banding

Automated

High Complexity

ROLE OF THE CLINICAL LABORATORY

- CSF & Serum Albumin
- CSF & Serum IgG
- Albumin Quotient
- IgG Index
- IgG Synthesis Rate



CSF Oligoclonal Banding

$$IgG\ Synthesis\ Rate = \left[CSF\ IgG - \frac{Serum\ IgG}{341}\right] - \left[\left(CSF\ Alb - \frac{Serum\ Alb}{169}\right)\left(\frac{Serum\ IgG}{Serum\ Alb}\right)(0.43)\right] x\ 5$$

Automated

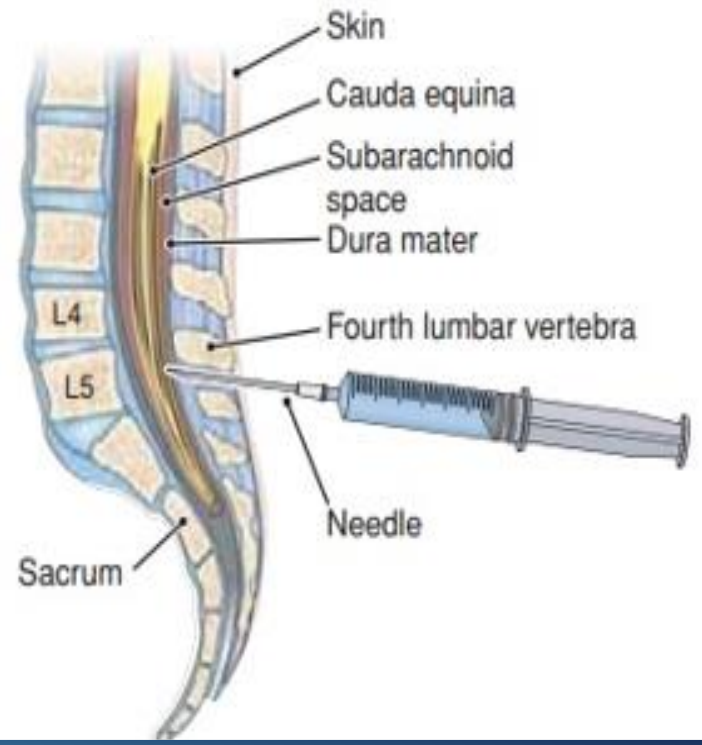
High Complexity

ROLE OF THE CLINICAL LABORATORY

- CSF & Serum Albumin
- CSF & Serum IgG
- Albumin Quotient
- IgG Index
- IgG Synthesis Rate

Emerging Biomarkers

- kFLC index
- sNfL

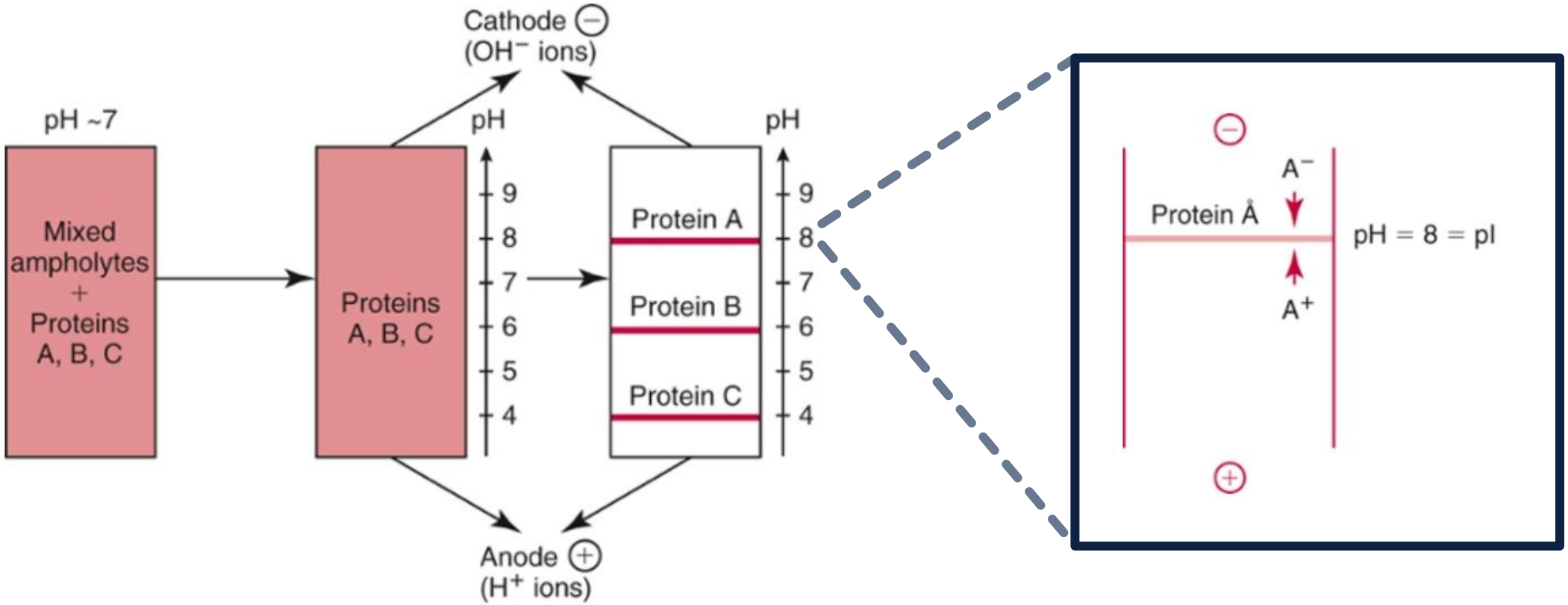


CSF Oligoclonal Banding

Automated

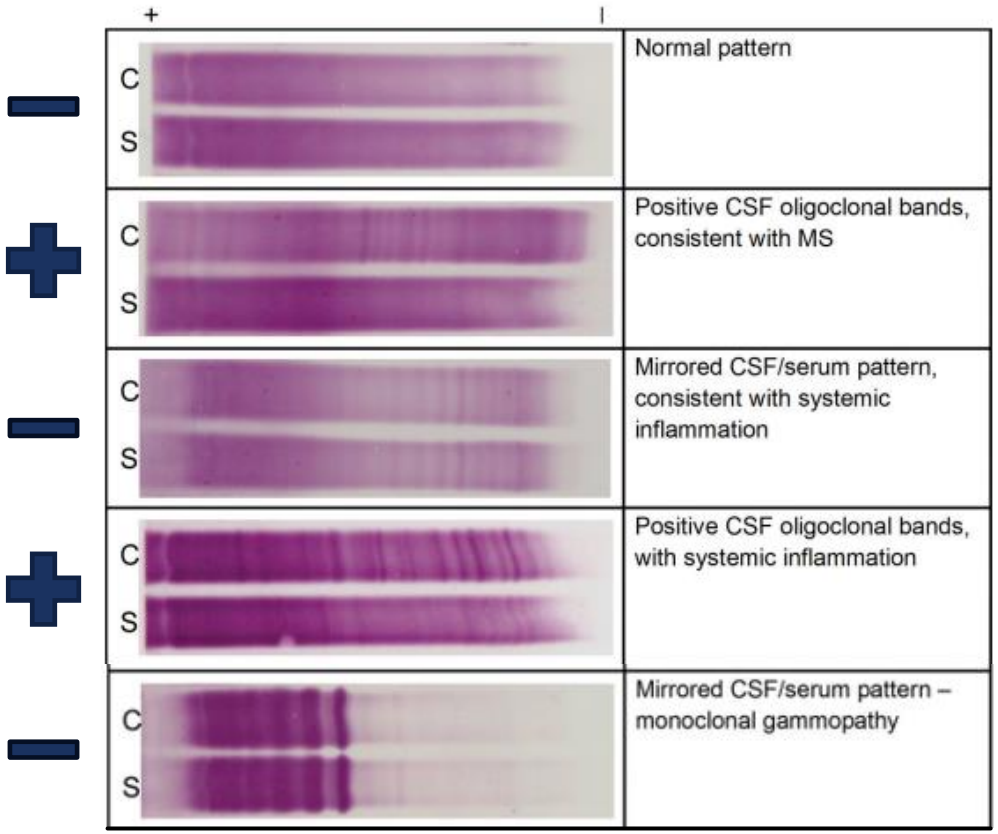
High Complexity

ISOELECTRIC FOCUSING ELECTROPHORESIS (IEF)



Tietz Textbook 6th Edition 2018

CSF OLIGOCLONAL BANDING



Limitations:

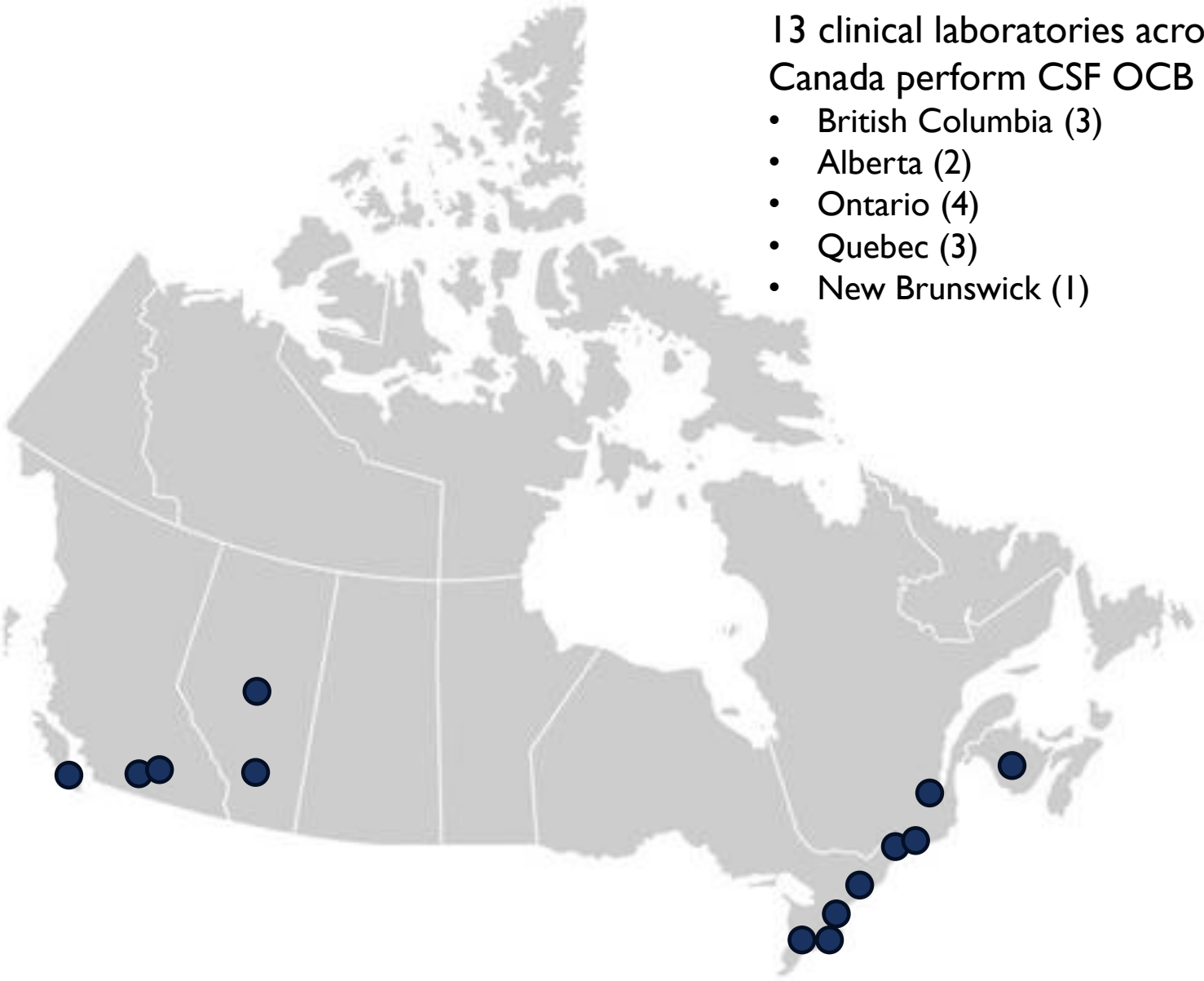
- Time-consuming
- Manual handling & technically demanding
- Requires expertise/resources
- Interpretation (variation)
- Analytical challenges (variation, artifacts)
- Limited prognostic utility

Chen Y, et al. JALM 2018; Tietz Textbook; St. Michael's Hospital SOP; Freedman MS, Arch Neurol 2005

CSF OCB TESTING LANDSCAPE IN CANADA

13 clinical laboratories across Canada perform CSF OCB testing:

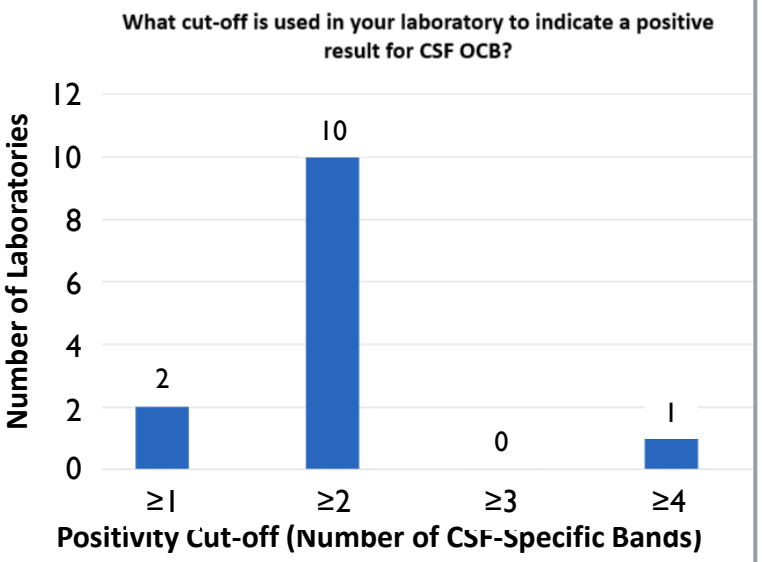
- British Columbia (3)
- Alberta (2)
- Ontario (4)
- Quebec (3)
- New Brunswick (1)

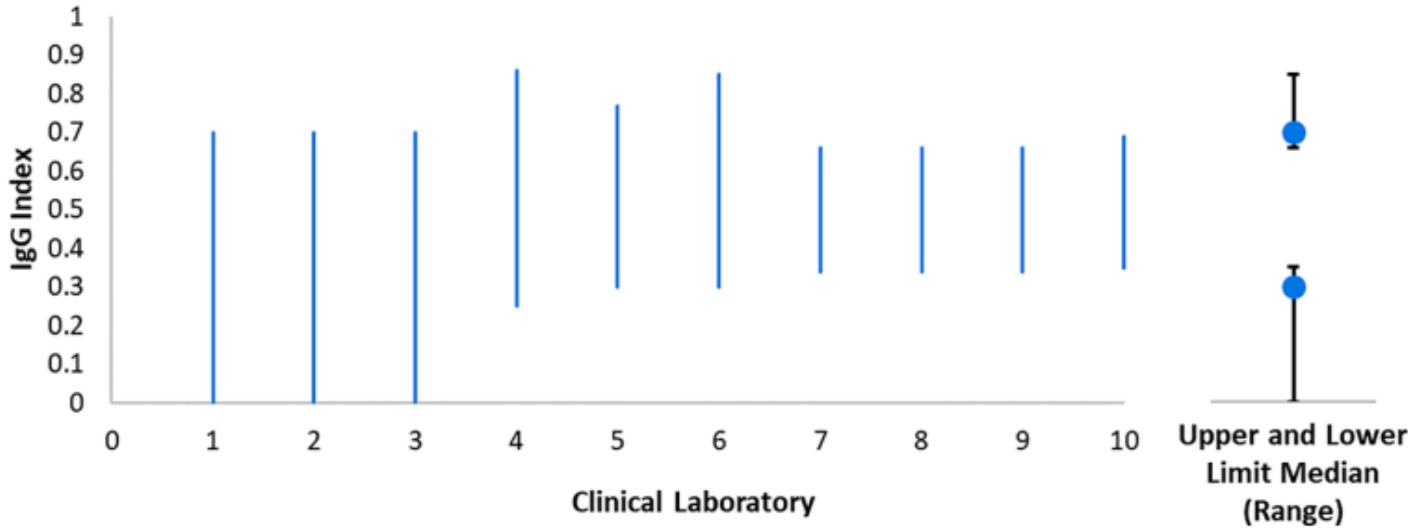


*All 13 clinical laboratories responded to our survey

CSCC CANADIAN SOCIETY OF CLINICAL CHEMISTS

Higgins V et al. Clinical Biochemistry. 2023 Jun 1;116:105-12





VARIABILITY IN CSF OCB REPORTING

Higgins V et al. Clinical Biochemistry. 2023 Jun 1;116:105-12

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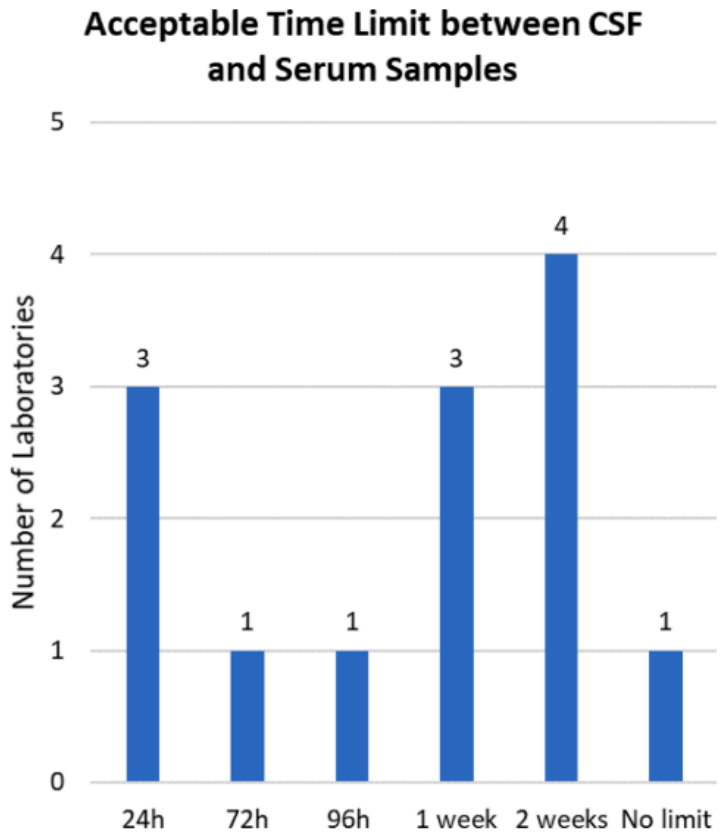
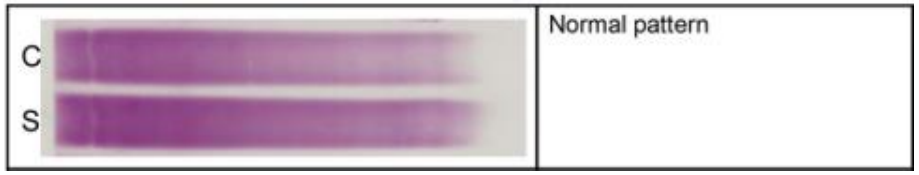


Associated CSF tests and calculated indices reported by 13 Canadian clinical laboratories

Biochemical Test/Index	# of Laboratories Reporting the Test/Index
CSF IgG	8
CSF Albumin	8
Serum IgG	2
Serum Albumin	2
Albumin Quotient	5
IgG Index	10
IgG Synthesis Rate	4

Higgins V et al. Clinical Biochemistry. 2023 Jun 1;116:105-12

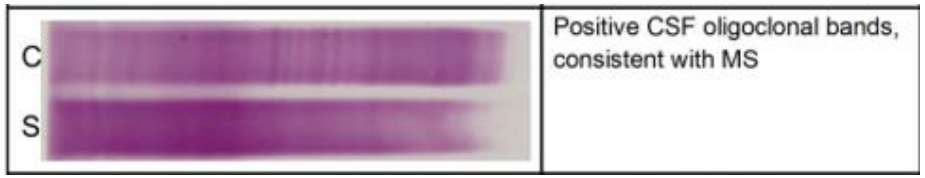
VARIABILITY IN CSF OCB REPORTING IN CANADA



Normal Pattern

1. No oligoclonal bands present in serum or CSF.
2. The oligoclonal band assay detected 0 or 1 CSF-specific band. This is a NEGATIVE result.
3. Absence of oligoclonal bands, with a normal CSF IgG index of XXX.
4. Isoelectric focusing performed on CSF and serum samples shows homogenous staining with no discordant bands between the two sample types. SUMMARY: NORMAL CSF PATTERN. NO OLIGOCLONAL PATTERN OBSERVED.

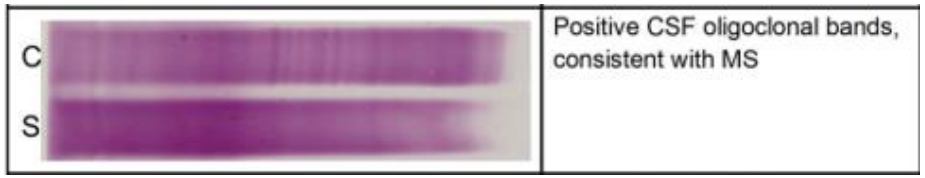
VARIABILITY IN CSF OCB REPORTING IN CANADA



Positive CSF OCB Pattern

1. The oligoclonal band assay detected 2 or more CSF-specific IgG bands. This is a POSITIVE result. CSF studies can be used for the diagnosis of multiple sclerosis (MS) by identifying intrathecal IgG synthesis (oligoclonal bands). The presence of two or more CSF-specific oligoclonal bands can support a diagnosis of MS according to the 2017 revised McDonald criteria. These findings, however, are not 100% specific for MS. CSF-specific IgG synthesis may also be found in patients with other neurologic diseases including infectious, inflammatory, cerebrovascular, and paraneoplastic disorders. Clinical correlation recommended.
2. Oligoclonal IgG bands present in CSF with no corresponding bands in serum. Indicative of intrathecal IgG synthesis. Typical pattern associated with multiple sclerosis.

VARIABILITY IN CSF OCB REPORTING IN CANADA



Positive CSF OCB Pattern

3. Isoelectric focusing performed on CSF and serum samples shows at least 4 bands in the CSF that are not observed in serum. This finding is consistent with increased IgG synthesis within the CNS and can be seen in demyelinating disorders such as multiple sclerosis. However, CSF oligoclonal banding is not specific to multiple sclerosis. There are many infectious and non-infectious inflammatory conditions that can generate CSF oligoclonal bands, such as systemic lupus erythematosus, neurosyphilis, neurological paraneoplastic disorders, aseptic meningitis, neurosarcoidosis, and infectious or autoimmune encephalitis. These conditions can usually be differentiated on clinical grounds assisted by other CSF findings in order to establish a definitive diagnosis. SUMMARY: OLIGOCLONAL BANDING NOTED ON ISOELECTRIC FOCUSING.

VARIABILITY IN CSF OCB REPORTING IN CANADA

Monoclonal Gammopathy Pattern

1. There is a monoclonal banding pattern seen in both the CSF and serum. The bands likely represent a systemic monoclonal gammopathy. Clinical correlation recommended.
2. CSF oligoclonal banding negative. Pattern consistent with the presence of a monoclonal IgG immunoglobulin. Suggest serum protein electrophoresis for confirmation if clinically indicated.
3. Isoelectric focusing performed on CSF and serum samples shows no discordant bands between the two sample types. However, multiple identical bands in both CSF and serum samples were observed in a pattern consistent with the presence of this patient's known IgG kappa monoclonal protein. SUMMARY: PRESENCE OF IDENTICAL BANDS IN CSF AND SERUM SAMPLES CONSISTENT WITH KNOWN MONOCLONAL PROTEIN. NO OLIGOCLONAL PATTERN OBSERVED.
   - Separate comments for possible vs. known monoclonal

THE HARMONIZED CSF ANALYSIS FOR MS INVESTIGATION (HCAMI) COMMITTEE

Aim:
Establish recommendations for laboratory processes and reporting of CSF OCB and associated tests supporting MS diagnosis

- Committee formed Spring 2023
- Monthly meetings

Clinical Chemists

- Daniel Beriault (Unity Health)
- Michelle Parker (Alberta Precision Labs)
- Basma Ahmed (McMaster University)
- Vipin Bhayana (London Health Sciences Centre)
- Ronald Booth (EORLA)
- Yu Chen (Dalhousie University)
- Christine Collier
- Myriam Gagne (CHU de Québec)
- Jessica Gifford (Alberta Precision Labs)
- Ola Ismail (London Health Sciences Centre)
- Joseph Macri (Hamilton Health Sciences)
- Ashley Newbigging (Fraser Health Authority)
- Lily Olayinka (Alberta Precision Labs)
- Karina Rodriguez-Capote (Interior Health Authority)
- Liju Yang (London Health Sciences Centre)

Neurologists

- Mark Freedman (The Ottawa Hospital)
- Fabrizio Giuliani (Alberta Health Services)
- Craig Moore (Memorial University)
- Ilia Poliakov (Saskatoon City Hospital)
- Raphael Schneider (Unity Health)
- Simon Thebault (The Ottawa Hospital)

CSCC CANADIAN SOCIETY OF CLINICAL CHEMISTS

HCAMI WORKING GROUP

1. Quality assurance

    1. What is the recommended QC material (patient samples vs. commercial material, CSF and/or serum samples, both positive and negative samples)
    2. What is the recommended frequency (how many lanes on the gel should be used for QC)?

HCAMI WORKING GROUP – SIX KEY AREAS

1. Quality assurance
2. Acceptable time limit between collection of matched CSF and serum samples

1. What is the stability of IgG in serum and CSF in vitro and in vivo?
2. Does this differ in patients with MS or acute inflammation?
3. How should CSF samples received without a paired serum be handled/reported?

HCAMI WORKING GROUP – SIX KEY AREAS

1. Quality assurance
2. Acceptable time limit between collection of matched CSF and serum samples
3. If and how to report the number of CSF-specific and/or CSF-serum matched bands

1. Is there utility in reporting the number of CSF-specific bands?
2. Does band count relate to prognosis? Likelihood or severity of disease?
3. Should bands be reported as an absolute count or a range of bands?
4. What is the intra- and inter-observer variability in reporting the number of CSF-specific bands?
5. What is the analytical reproducibility of band counts?

HCAMI WORKING GROUP – SIX KEY AREAS

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1. Quality assurance
2. Acceptable time limit between collection of matched CSF and serum samples
3. If and how to report the number of CSF-specific and/or CSF-serum matched bands
4. Mirrored pattern interpretation & follow-up (e.g., inflammatory response, monoclonal gammopathy)

1. What is the threshold of CSF-serum matched bands to identify an inflammatory response pattern?
2. What should be included in the interpretive comment for an inflammatory response pattern?
3. What action should be taken by the laboratory when a monoclonal gammopathy pattern is suspected (e.g., should they be confirmed by serum protein electrophoresis)?
4. What should be included in the interpretive comment for a monoclonal gammopathy pattern?

1. Quality assurance
2. Acceptable time limit between collection of matched CSF and serum samples
3. If and how to report the number of CSF-specific and/or CSF-serum matched bands
4. Mirrored pattern interpretation & follow-up (e.g., inflammatory response, monoclonal gammopathy)
5. Interpretation of matched bands with differing intensity between CSF and serum

    1. Should these bands be interpreted and reported as CSF-specific or CSF-serum matched bands?

HCAMI WORKING GROUP – SIX KEY AREAS

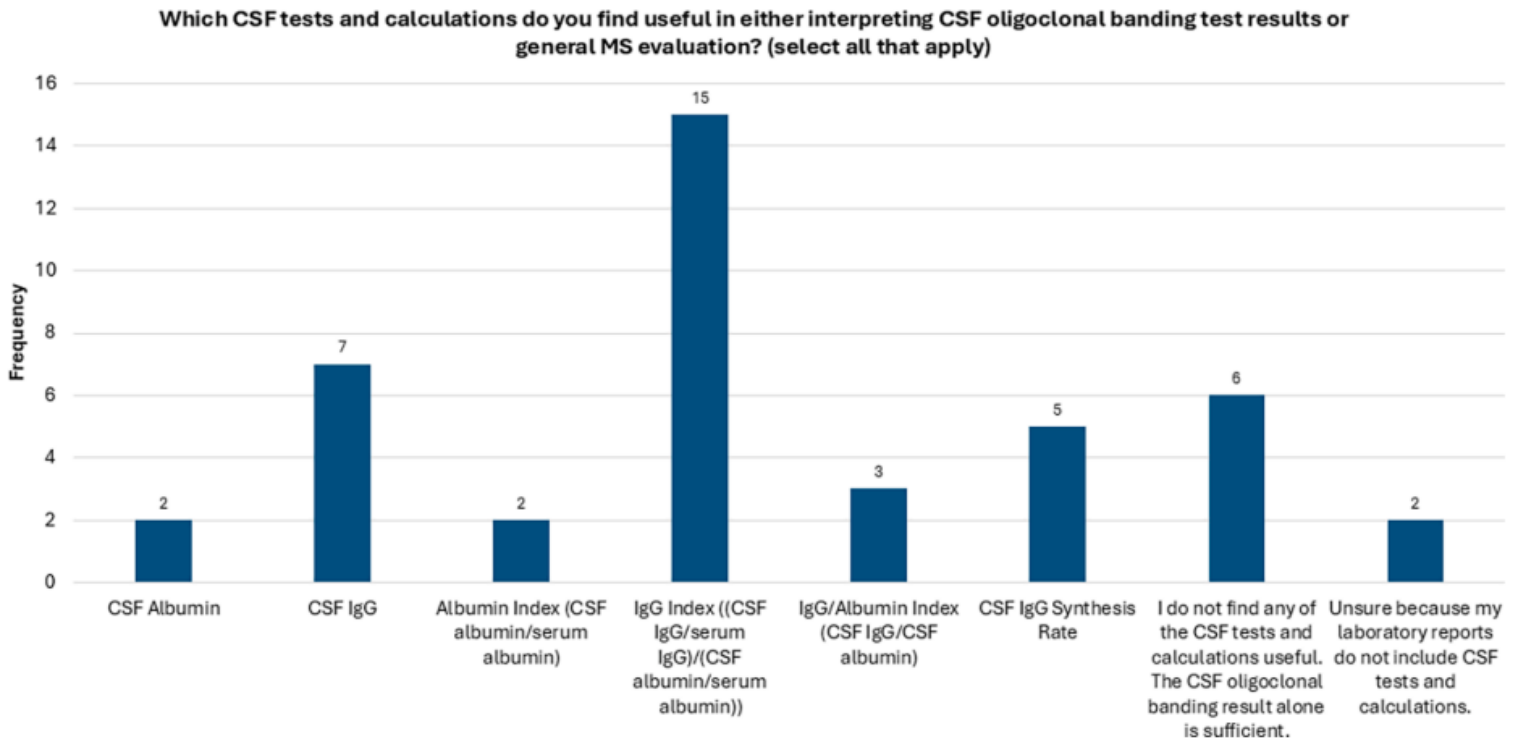
1. Quality assurance
2. Acceptable time limit between collection of matched CSF and serum samples
3. If and how to report the number of CSF-specific and/or CSF-serum matched bands
4. Mirrored pattern interpretation & follow-up (e.g., inflammatory response, monoclonal gammopathy)
5. Interpretation of matched bands with differing intensity between CSF and serum
6. Panel components and reference intervals/decision limits for number of CSF-specific bands (OCB) and associated tests and calculated indices

HCAI

1. Should the components of calculations/indices be reported (e.g., CSF IgG & albumin, Serum IgG & albumin)?
2. What associated tests and calculations should be included in a CSF OCB ordering panel?
3. What terminology, units, equations, reference intervals and/or decision limits should be used?

Neurologist Survey Results

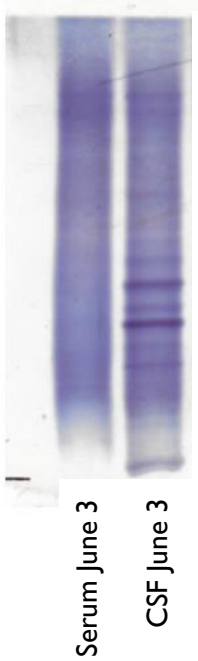
- 22 neurologists from 9 provinces participated, with a median practice length of 13 years
- Most (64 %) preferred a 24-hour limit for paired serum and CSF sample collection
- Most (73 %) favored a cutoff of ≥ 2 CSF-specific bands for positivity, aligning with the 2017 McDonald criteria



NEUROLOGISTS' PERSPECTIVE

Higgins et al. Clinical Biochemistry. 2024 22:135:110855

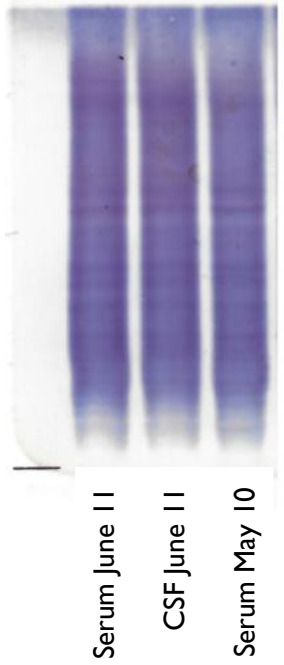
KEY AREA 2: Acceptable time limit between collection of matched CSF and serum samples



- 17 cases reviewed
- Time interval: 3-32 days

Study Results:

- Only one case changed interpretation
  - OCB present with mirrored pattern changed to without mirrored pattern (26 days)
- 9/17 exceeded serum albumin TEA (3-29 days)
- 4/17 exceeded serum IgG TEA (5-32 days)



Example 1

	June 3	June 19	Difference (abs or %)	TEA
Albumin, serum	42.6	47.3	11%	3 g/L or 8%
IgG, serum	8.98	9.54	6.2%	0.4 g/L or 20%
OCB pattern interpretation	OCB present	Same as original		
Time interval	16 days			

Example 2

	June 11	May 10	Difference (abs or %)	TEA
Albumin, serum	34.0	34.8	0.8	3 g/L or 8%
IgG, serum	11.2	8.12	27.5%	0.4 g/L or 20%
OCB pattern interpretation	Mirrored pattern	Same as original		
Time interval	32 days			

Thanks to Dr. Lily Olayinka

KEY AREA 2: Acceptable time limit between collection of matched CSF and serum samples

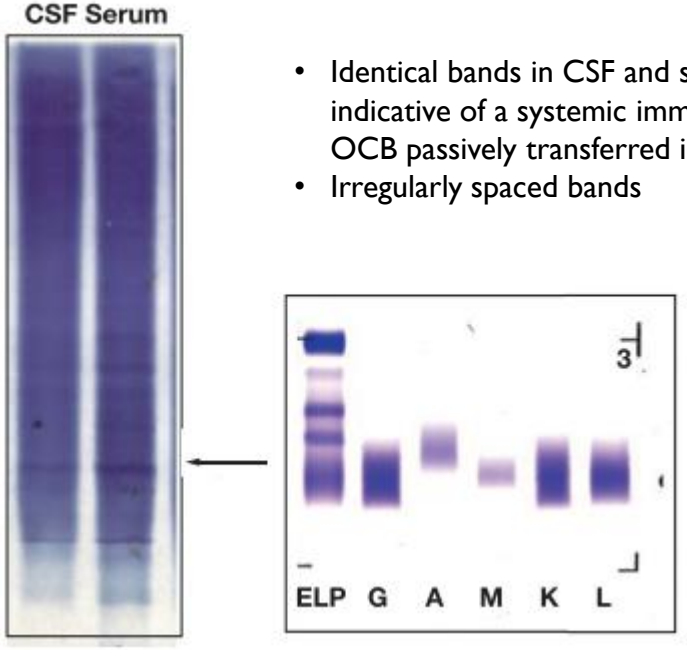
Draft Recommendation Statements

1. CSF and serum/plasma samples are ideally collected on the same day but can be considered paired for CSF oligoclonal banding analysis if collected within 3 weeks of each other.
2. Clinical laboratories should only report indices that include serum albumin and/or serum IgG when there is a serum/plasma sample collected on the same day as the CSF sample.
3. If a paired serum/plasma sample is unavailable, clinical laboratories should perform isoelectric focusing electrophoresis on the CSF sample.
    a. No band observed in the CSF sample: report as negative.
    b. 1 or more bands observed in the CSF sample: If feasible, the laboratory should attempt to obtain a paired serum/plasma sample. If a paired serum/plasma sample cannot be obtained and,
        i. the number of CSF bands is below the laboratory's cut-off for positivity, indicate the number of bands in the CSF and report as negative
        ii. the number of CSF bands is above the laboratory's cut-off for positivity, indicate the number of bands in the CSF and report as inconclusive

KEY AREA 4: Mirrored pattern interpretation & follow-up (e.g., inflammatory response, monoclonal gammopathy)

- 86 patients with mirrored patterns → serum immunofixation electrophoresis (sIFE) performed to confirm if monoclonal protein present or not

Typical Inflammatory Response Pattern

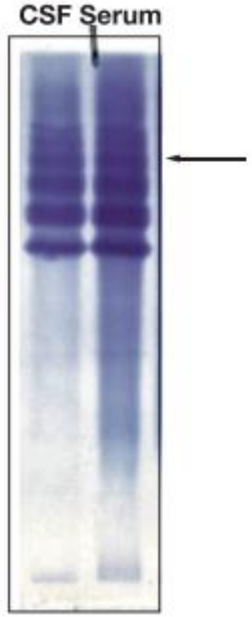


- Identical bands in CSF and serum samples, indicative of a systemic immune reaction, with OCB passively transferred into CSF
- Irregularly spaced bands

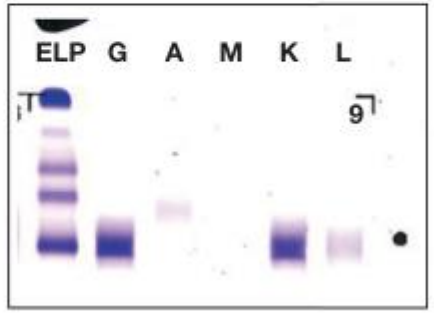
11/86 (12.8%)

Polyclonal immunoglobulins, with no monoclonal protein present

Typical Monoclonal Protein Pattern



- Monoclonal bands in CSF and serum samples, indicate the presence of a monoclonal IgG
- Characteristic symmetric spaces between bands, more prominent



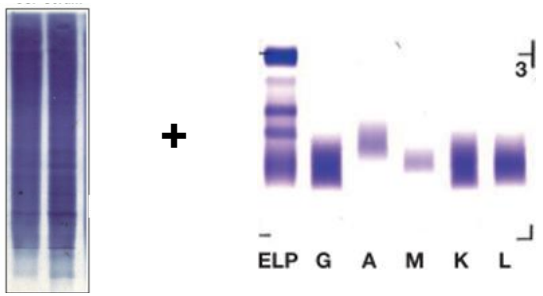
6/86 (6.9%)

Monoclonal IgG kappa present in the gamma region on sIFE

Wang et al. Laboratory Medicine. 2023;54:380-387

KEY AREA 4: Mirrored pattern interpretation & follow-up (e.g., inflammatory response, monoclonal gammopathy)

- 69/86 (80.2%) of sample were deemed 'atypical' (lower intensity of bands, smaller number of bands, or both)



		IEF + sIFE (Truth)		
		Inflammatory Pattern	Monoclonal Pattern	
IEF alone	Inflammatory Pattern	16	10	26
	Monoclonal Pattern	28	15	43
		44	25	69

- Of 44 inflammatory response patterns, only 16 (36%) were correctly called based on IEF alone

Wang et al. Laboratory Medicine. 2023;54:380-387

KEY AREA 4: Mirrored pattern interpretation & follow-up (e.g., inflammatory response, monoclonal gammopathy)

- 69/86 (80.2%) of sample were deemed 'atypical' (lower intensity of bands, smaller number of bands, or both)

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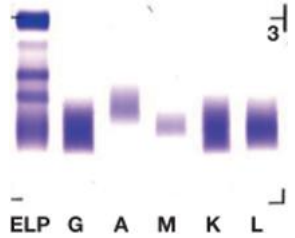


		IEF + sIFE (Truth)		
		Inflammatory Pattern	Monoclonal Pattern	
IEF alone	Inflammatory Pattern	16	10	26
	Monoclonal Pattern	28	15	43
		44	25	69

- Of 25 monoclonal protein patterns, only 15 (60%) were correctly called based on IEF alone.

Wang et al. Laboratory Medicine. 2023;54:380-387

KEY AREA 4: Other pattern interpretation & follow-up (e.g., inflammatory response, monoclonal gammopathy)

<u>Draft</u> Recommendation Statements

1. Clinical laboratories performing CSF OCB testing should report on the presence of a mirrored pattern (i.e., monoclonal gammopathy and/or inflammatory response), if observed. The mirrored pattern comment should be stated after indicating whether the result is positive or negative for CSF OCB.
2. If a mirrored pattern is observed suggestive of a monoclonal protein and a review of the patient chart indicates they have a known IgG monoclonal gammopathy, a comment should be added to the report indicating that a mirrored pattern is observed that is consistent with the presence of the patient's known IgG ** monoclonal protein (**specify if monoclonal protein is IgG kappa or IgG lambda).
3. For any mirrored pattern not associated with a known IgG monoclonal gammopathy or the interpreter does not have access to the patient chart, a comment should be added to the report indicating
    1. A mirrored pattern is observed that likely reflects a systemic inflammatory response, but the presence of a monoclonal protein cannot be excluded, and
    2. To interpret this result in combination with other appropriate laboratory and clinical findings and follow up with serum protein electrophoresis if clinically indicated.

DELPHI PROCESS TO DEVELOP HARMONIZED RECOMMENDATIONS

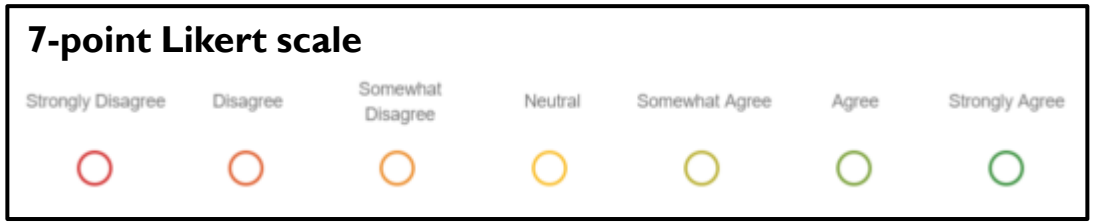
Steering Committee creates draft recommendation statements

Google Forms

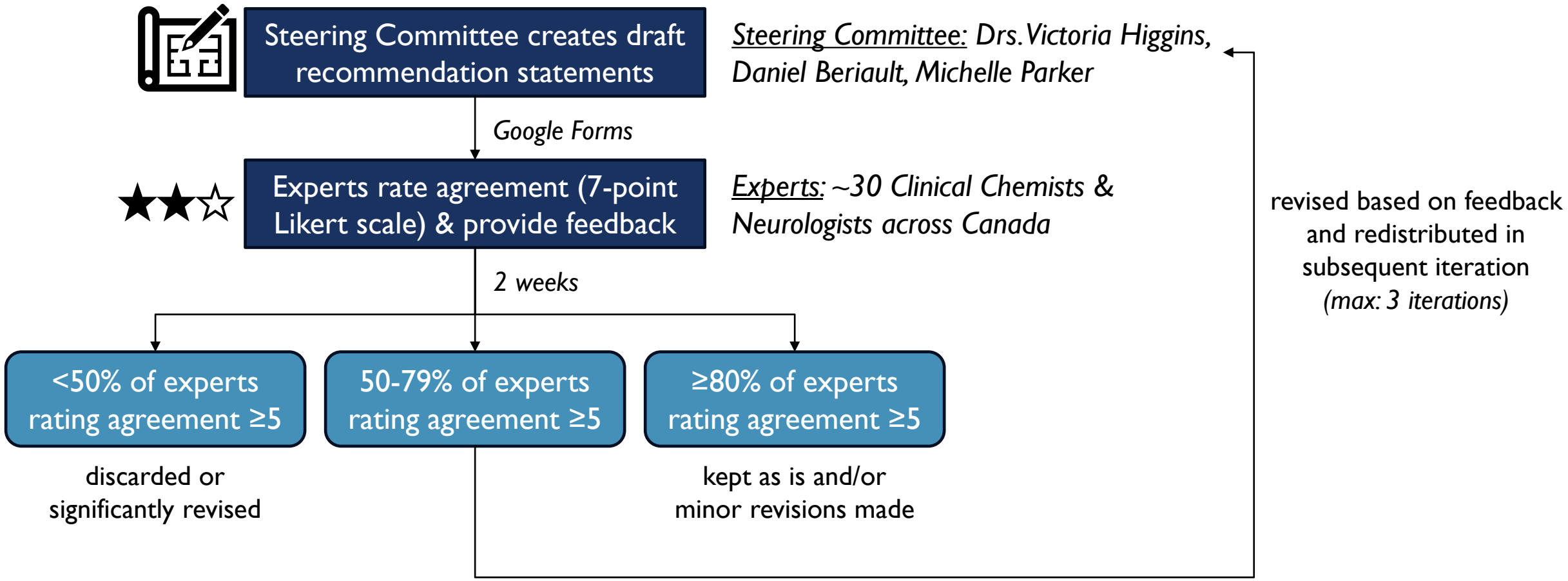
★★☆ Experts rate agreement (7-point Likert scale) & provide feedback

<u>Steering Committee:</u> Drs. Victoria Higgins, Daniel Beriault, Michelle Parker

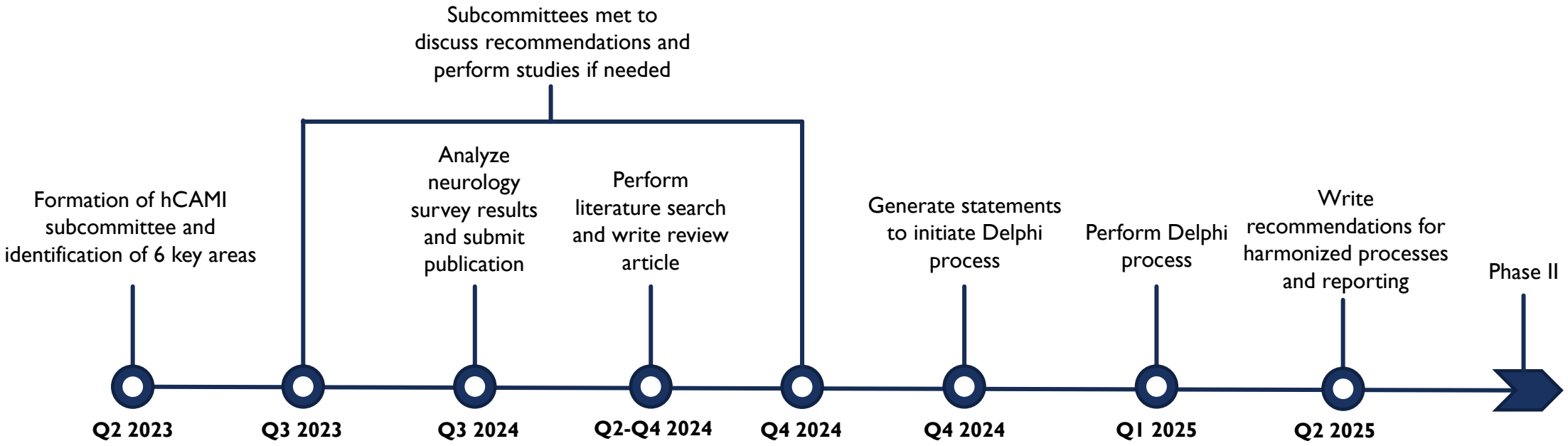
<u>Experts:</u> ~30 Clinical Chemists & Neurologists across Canada



DELPHI PROCESS TO DEVELOP HARMONIZED RECOMMENDATIONS



THE HARMONIZED CSF ANALYSIS FOR MS INVESTIGATION (HCAMI) COMMITTEE



CSCC CANADIAN SOCIETY OF CLINICAL CHEMISTS

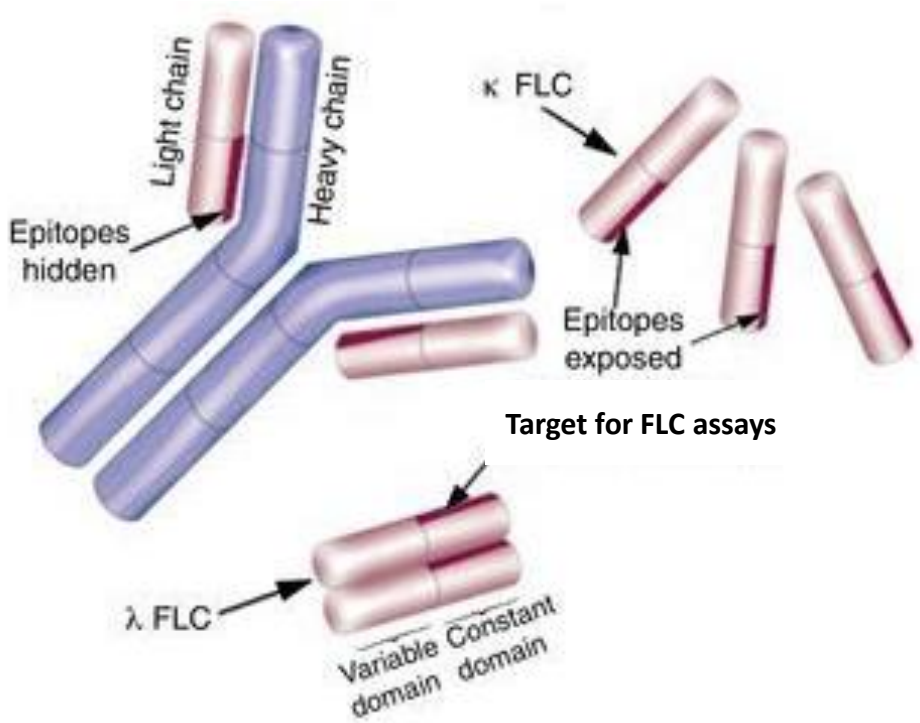
LEARNING OBJECTIVES

1. Review the draft harmonized reporting recommendations for CSF oligoclonal banding developed by the Harmonized CSF Analysis for MS Investigation (hCAMI) Committee of the Canadian Society of Clinical Chemists (CSCC)

2. Discuss the roles of kappa free light chain (kFLC) index and serum neurofilament light chain (sNfL) for diagnosing and monitoring multiple sclerosis

FREE LIGHT CHAINS

- Light chains are secreted by B cells along with intact immunoglobulins (10-40% excess over heavy chains)
- Free light chains have a short half-life in blood (2-4 hours) due to rapid renal clearance
  - FLCs produced intrathecally are not subject to clearance
- Free light chains accumulate in the CSF when there is chronic intrathecal inflammation
- κFLC have shown superior utility than λFLC



Tietsche de Moraes Hungria V, et al. Rev Bras Hematol Hemoter 2016

KAPPA FREE LIGHT CHAINS

What indicator of intrathecal κFLC should be used?

$$Qalb = \frac{CSF\ albumin}{Serum\ albumin}$$

1. CSF κFLC concentration *does not consider serum κFLC concentration or blood-brain barrier permeability (albumin quotient (Qalb))
2. κFLC quotient ($Q_{κFLC}$) *does not consider blood-brain barrier permeability (albumin quotient (Qalb))

$$Q_{\kappa-FLC} = \frac{CSF\ \kappa\text{-}FLC}{Serum\ \kappa\text{-}FLC}$$

3. κFLC index

$$\kappa - FLC\ index\ = \frac{\kappa-FLC_{CSF}\ /\ \kappa-FLC_{Serum}}{Q_{alb}}$$

**to be recommended in the new McDonald criteria*

4. Intrathecal κFLC fraction ($IF_{κFLC}$)

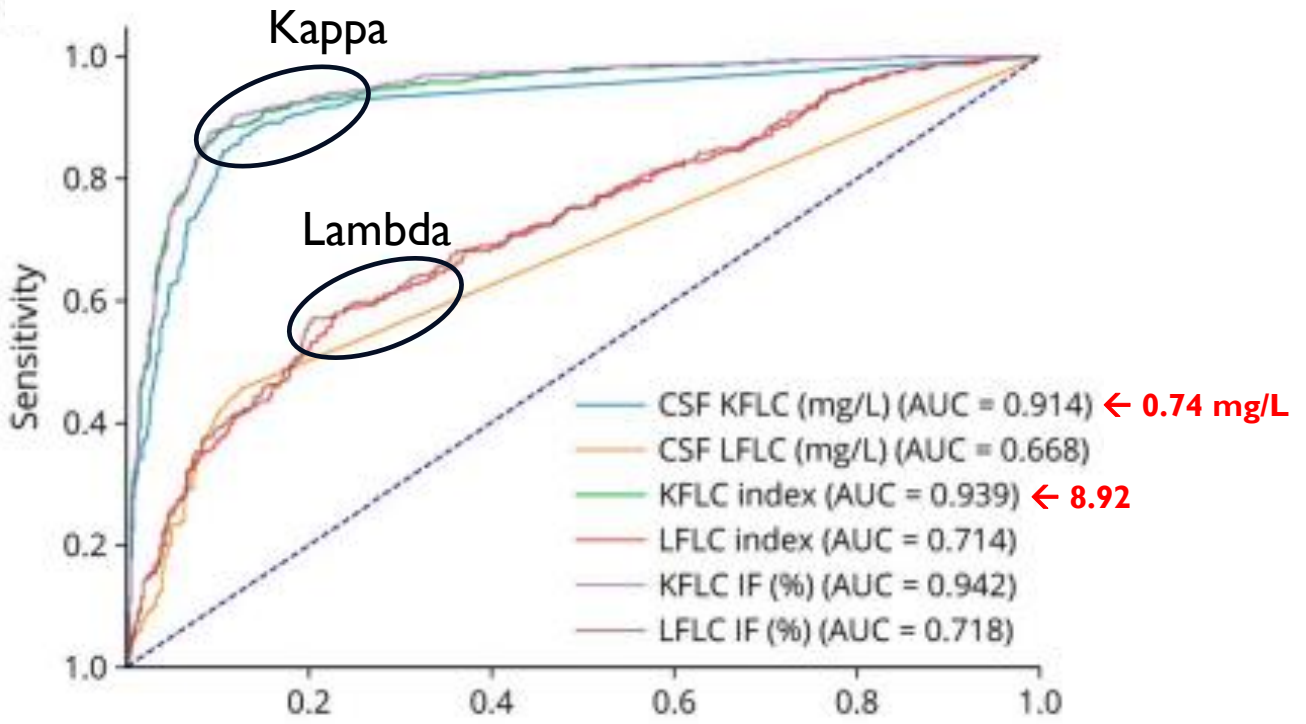
$$\kappa - FLC_{Loc} = (Q_{\kappa-FLC} - Q_{\lim\ \kappa-FLC}) \cdot \kappa - FLC_{Serum}$$

$$IF_{\kappa-FLC} = \frac{\kappa-FLC_{Loc}}{\kappa-FLC_{CSF}} \cdot 100$$

Presslauer 2014	$Q_{lim\ \kappa-FLC} = 0.9357 \cdot Qalb^{0.6687}$
Hegen 2019	$Q_{lim\ \kappa-FLC} = 3.1276 \cdot Qalb^{0.8001}$
Senel 2019	$Q_{lim\ \kappa-FLC} = 9.50 + 2.08 \cdot Q_{alb}$
Reiber 2019	$Q_{lim\ \kappa-FLC} = 3.27 \cdot \left(Q_{alb}{}^{2} + 33\right)^{0.5} - 8.2 \cdot 10^{-3}$

Gurtner KM, et al. Clin Chem Lab Med 2018

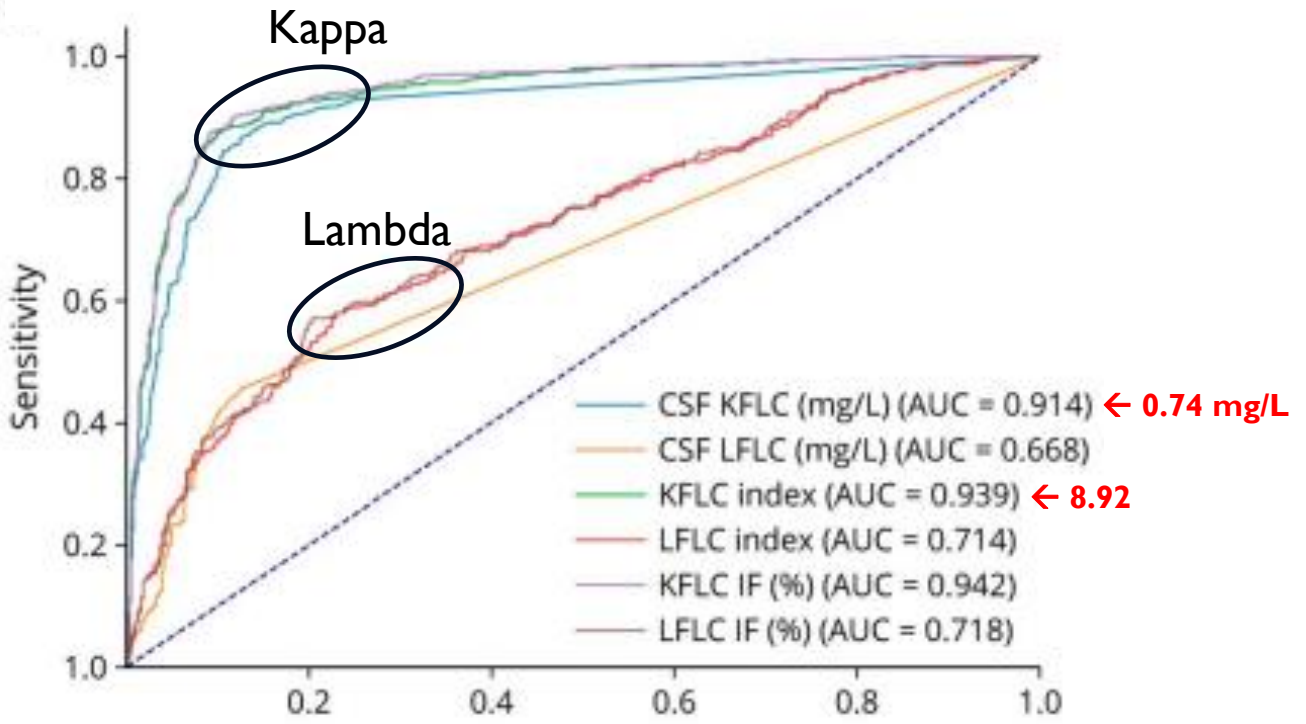
KFLC PERFORMANCE



	CSF OCB (≥2 CSF-specific bands)	CSF kFLC >0.74 mg/L	p-value OCB vs. CSF kFLC	kFLC index >8.92	p-value OCB vs. kFLC index
Sensitivity	81.99 (79.21, 84.77)	87.72 (85.35, 90.10)	<0.001	88.24 (85.95, 90.52)	<0.001
Specificity	90.16 (88.10, 92.22)	86.43 (84.06, 88.79)	<0.001	89.36 (87.29, 91.42)	0.339
PPV	88.38 (85.97, 90.79)	85.51 (82.99, 88.02)	0.008	88.12 (85.83, 90.41)	0.852
NPV	84.58 (82.16, 87.00)	88.52 (86.29, 90.75)	<0.001	89.46 (87.4, 91.52)	<0.001

Levraut M, et al. Neurol Neuroimmunol Neuroinflamm 2023

KFLC PERFORMANCE



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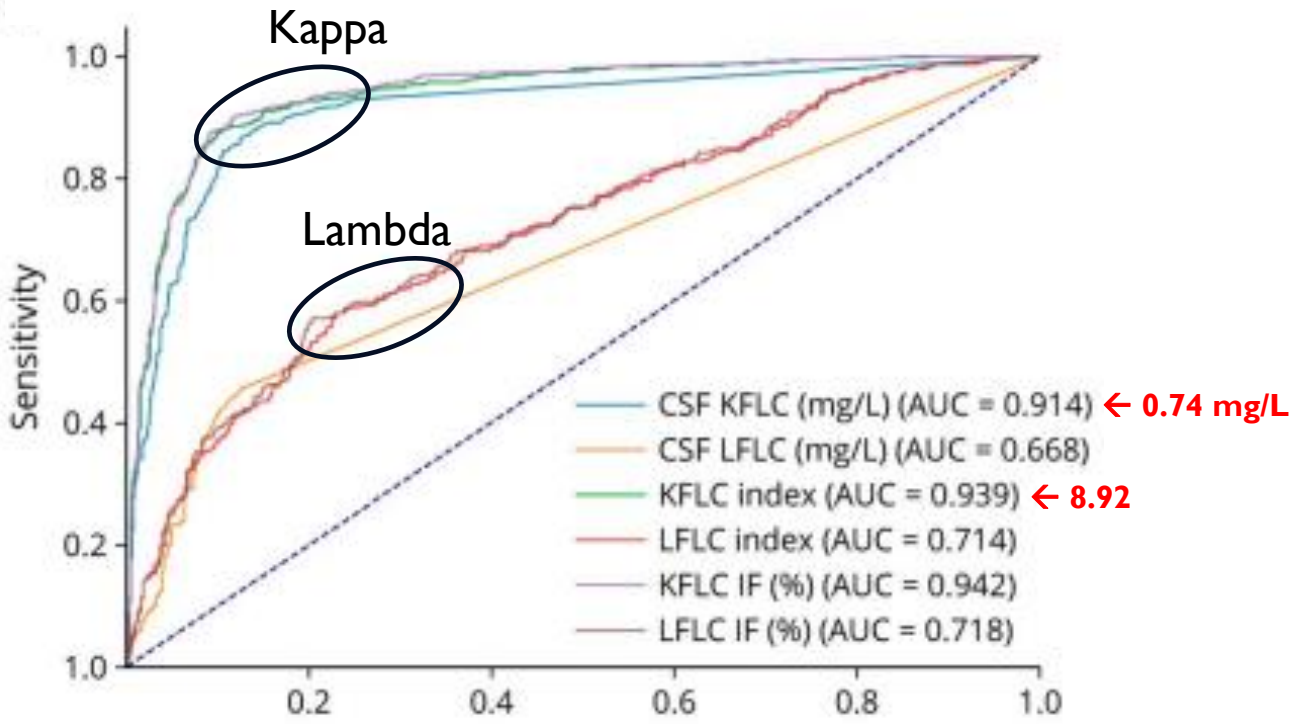

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No sig. difference!

Levraut M, et al. Neurol Neuroimmunol Neuroinflamm 2023

DIAGNOSTIC ACCURACY OF KFLC INDEX FOR MS

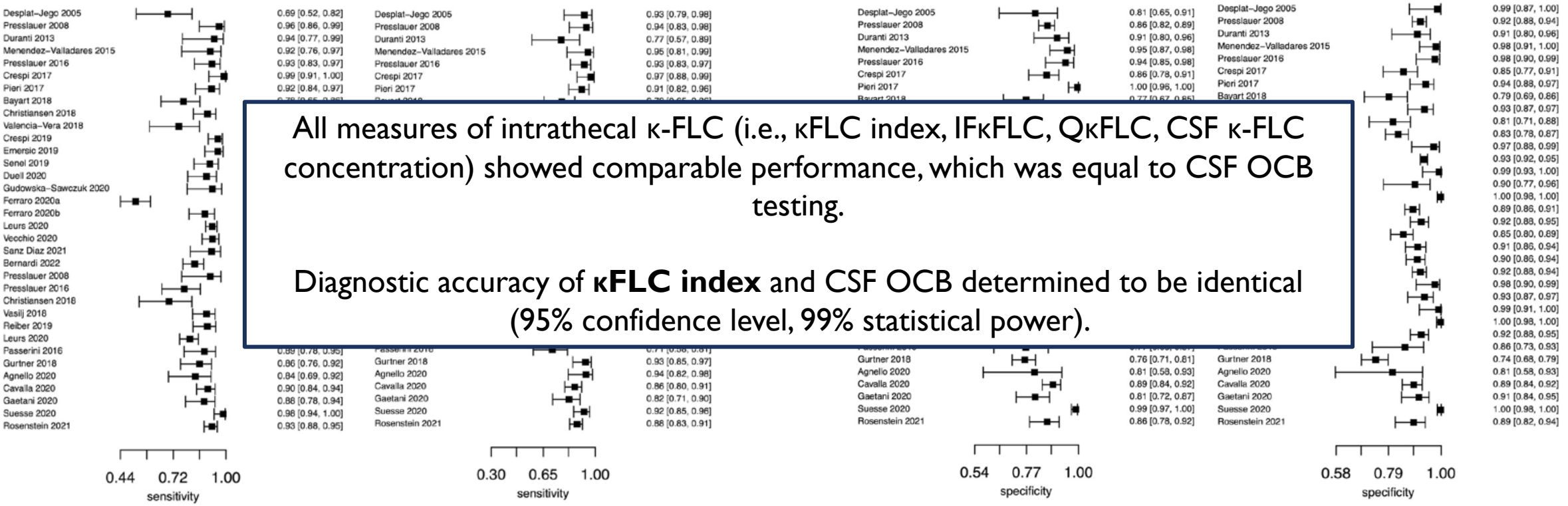
- Meta-analysis (32 studies) – 3322 patients with CIS/MS, 5849 controls

κFLC index sensitivity
(52-100%, weighted average: 88%)

CSF OCB sensitivity
(37-100%, weighted average: 85%)

κFLC index specificity
(69-100%, weighted average: 89%)

CSF OCB specificity
(74-100%, weighted average: 92%)

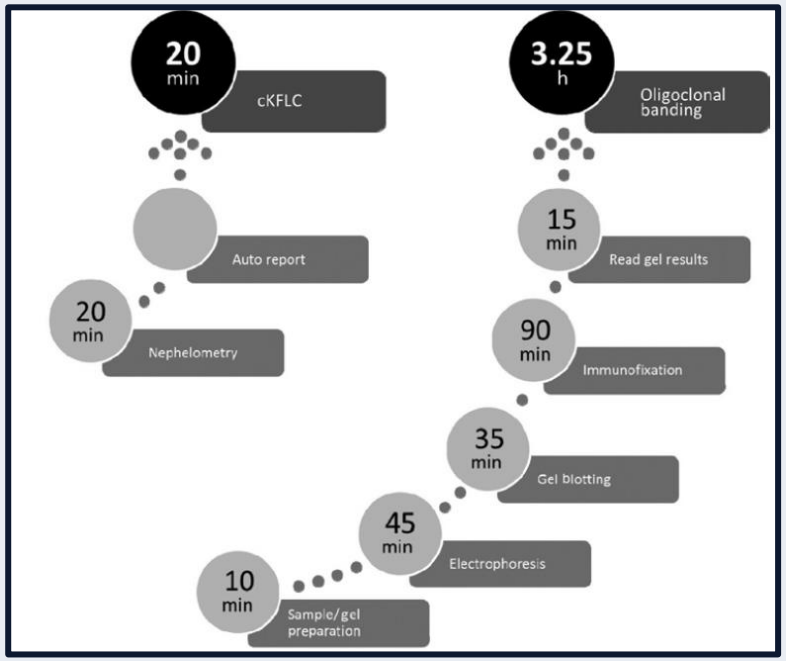


All measures of intrathecal κ-FLC (i.e., κFLC index, IFκFLC, QκFLC, CSF κ-FLC concentration) showed comparable performance, which was equal to CSF OCB testing.

Diagnostic accuracy of **κFLC index** and CSF OCB determined to be identical (95% confidence level, 99% statistical power).

Hegen H, et al. Multiple Sclerosis Journal 2022

KFLC INDEX



CSF κFLC advantages:

Improve TAT, simplify workflow, automated, objective interpretation, may be useful predictor of high disease activity (time to relapse, higher number of relapses)

Limitations/Challenges

- No agreed upon cut-off – range: 2.4 – 20
  - population differences, optimizing sensitivity or specificity, methodology differences
- CSF OCB infrastructure remains – reduced expertise?
- Batch testing – no improved TAT?

Available assays

- Freelite Mx (The Binding Site)
  - Turbidimetry (Optilite, SpaPlus, Roche cobas)
  - Nephelometry (Siemens BN, Beckman Immage)
- N Latex (Siemens)
  - Siemens BN (nephelometry)

Gurtner KM, et al. Clin Chem Lab Med 2018; Salavisa, M., et al., J Neuroimmunol, 2020; Berek, K., et al., Neurol Neuroimmunol Neuroinflamm, 2021

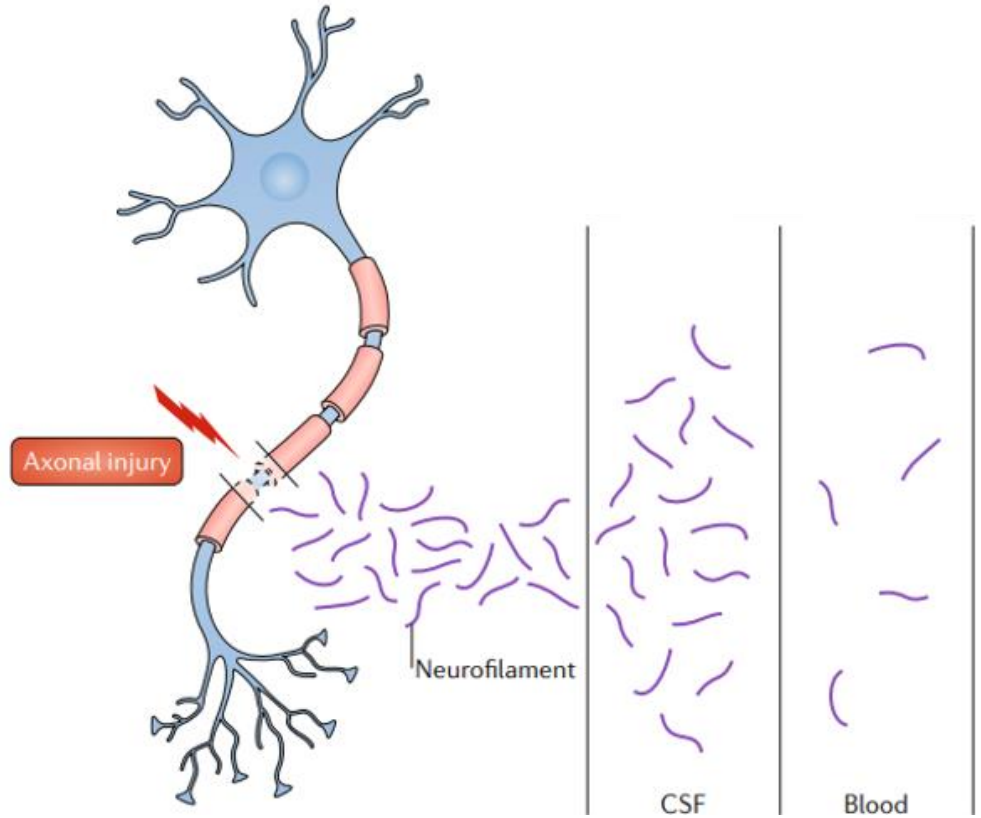
NEUROFILAMENT LIGHT CHAIN (NFL)

- Reasonably effective diagnostic biomarkers, but biomarkers of disease activity and outcomes are lacking
  - Important for timely intervention

- Current gold standard for disease activity monitoring: annual MRI
  - Limitations: miss clinically silent disease activity, high cost, inconvenient & labor intensive, frequent gadolinium exposure

- NfL is a neuroaxonal skeletal protein released into CSF, and eventually blood, from neuronal injury
  - Exclusively expressed in neurons
  - Neuronal injury can be due to neurodegenerative, inflammatory, vascular, and traumatic diseases

Neurofilament release after axonal damage



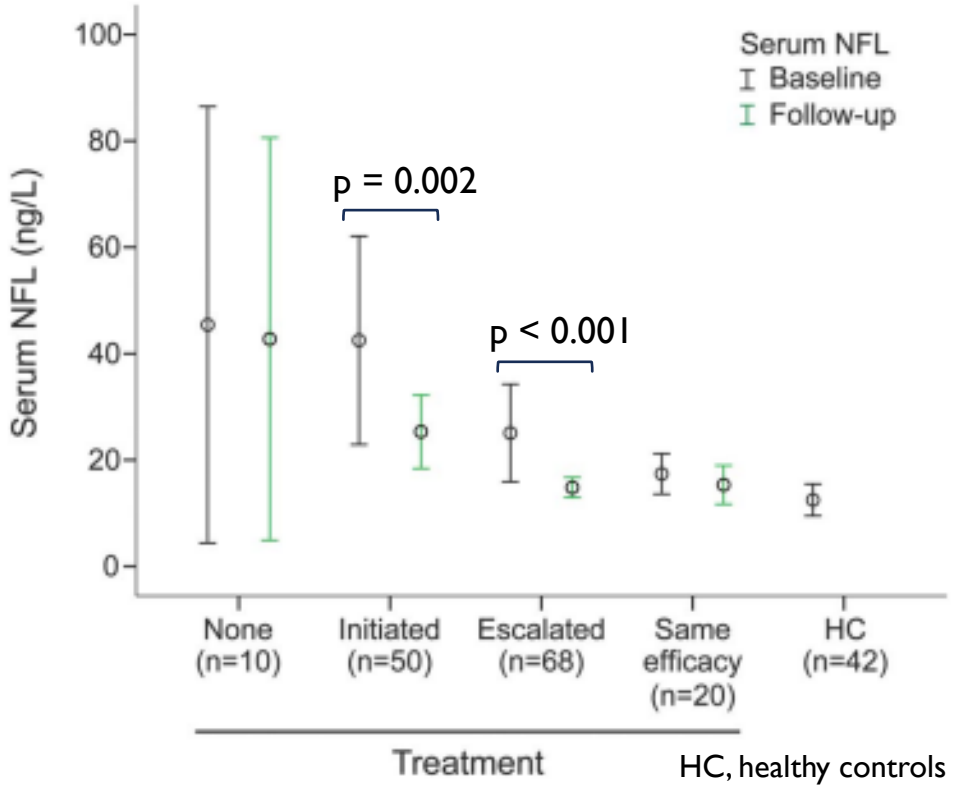
Thebault et al. Multiple Sclerosis Journal. 2022 Sept,28(10):1491-1497Khalil et al. Nature Reviews Neurology. 2018 Oct;14(10):577-89.

NEUROFILAMENT LIGHT CHAIN

Proposed clinical utility

1. **Inflammatory disease activity** – relapses, MRI lesions
2. **Disease progression** – disease severity scores (e.g., EDSS), cognition, visual acuity, progressive vs. relapsing phenotype)
3. **Treatment response** – monitor treatment efficacy, endpoint in clinical trials of novel agents
4. **Prediction/prognosis** – prodromal/preclinical phase, CIS conversion, short- and long-term prognosis

sNfL in patients with MS at baseline and follow-up and in healthy controls

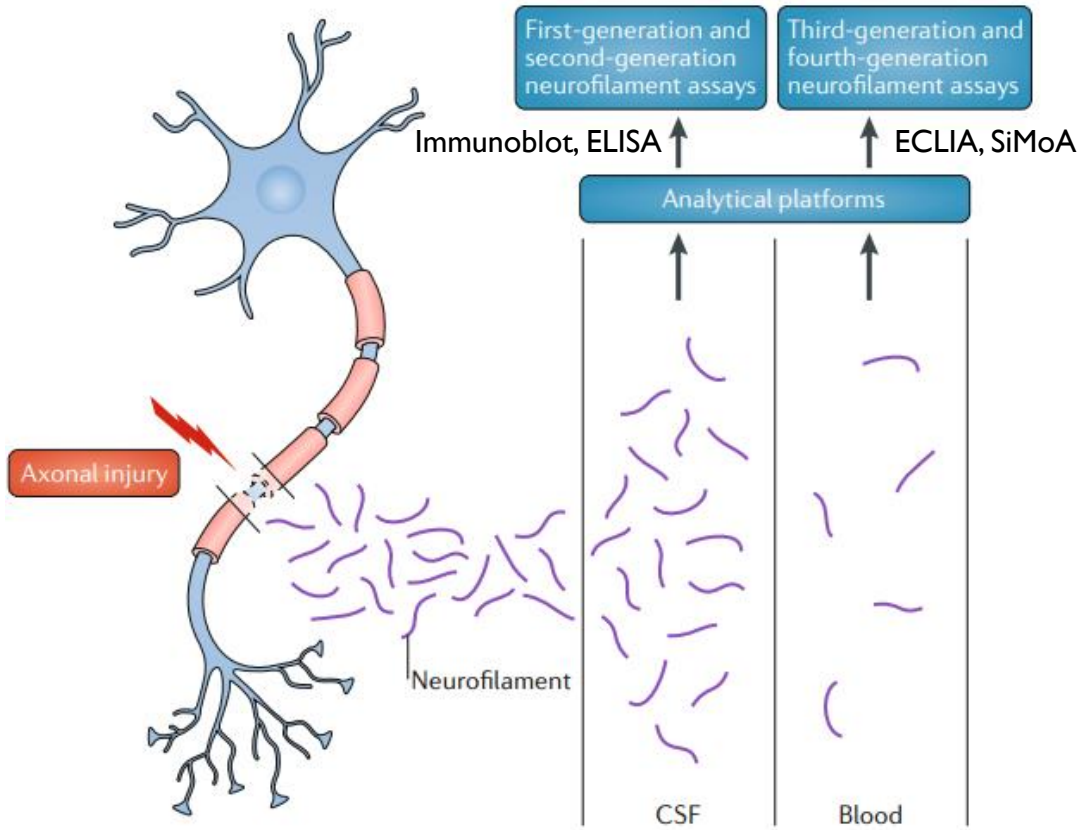


HC, healthy controls

Novakova L, Neurology. 2017. 89:2230–2237.

NEUROFILAMENT LIGHT CHAIN

Neurofilament release after axonal damage



Limitations/Challenges

- Non-specific for MS
- Confounding factors: increase with age, head injuries, vascular risk factors, renal function, decrease with high BMI
- Assay standardization

Available assays

- SiMoA (EORLA)
- Roche (St. Michael's Hospital)
- Siemens (Health Canada approved)

ECLIA, electrochemiluminescent immunoassay, ELISA, enzyme-linked immunosorbent assay; EORLA, Eastern Ontario Regional Laboratory Association; SiMoA, single-molecule array

Khalil et al. Nature Reviews Neurology. 2018 Oct;14(10):577-89.

SUMMARY

- Variation in reporting practices for CSF OCB across Canada
- The hCAMI Committee of the CSCC is developing harmonized reporting recommendations via the Delphi Process through collaboration between clinical chemists and neurologists
- kFLC index is anticipated to be included in the updated McDonald criteria as an equivalent test to CSF OCB
- sNfL shows promising clinical utility as a marker of inflammatory disease activity, disease progression, treatment response, and prognosis

THANK YOU! QUESTIONS?

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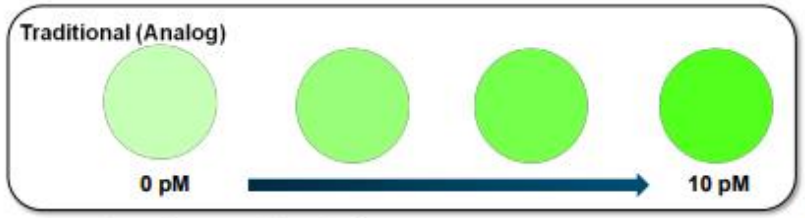
ADDITIONAL SLIDES

SNFL AVAILABILITY

First offered by EORLA in Ottawa (January 2021)

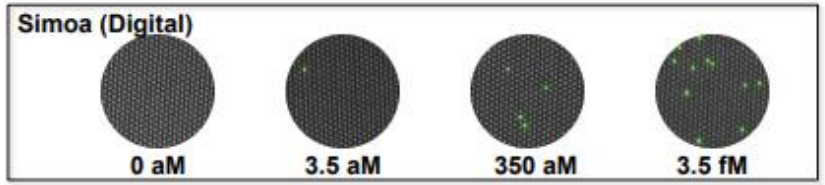
LABORATORY MEASUREMENT of NfL using SIMOA TECHNOLOGY

- SIMOA is a high sensitivity digital ELISA, allowing measurement of protein biomarkers in the fg/mL range with precision acceptable for clinical use.



- Reaction volume = 100 $\times 10^{-6}$ L
- Diffusion = dilution = low sensitivity
- Millions of molecules needed to reach detection limit

Microliters (µL)



- Reaction volume = 50 $\times 10^{-15}$ L **(2 billion times smaller)**
- Diffusion defeated = single molecule resolution = ultimate sensitivity
- One molecule needed to reach detection limit

Femtoliters (fL)

https://www.eorla.ca/wp-content/uploads/2021/01/Neurofilament-light-chain-Clinical-Information.pdf

SINGLE MOLECULAR ARRAY (SIMOA)