

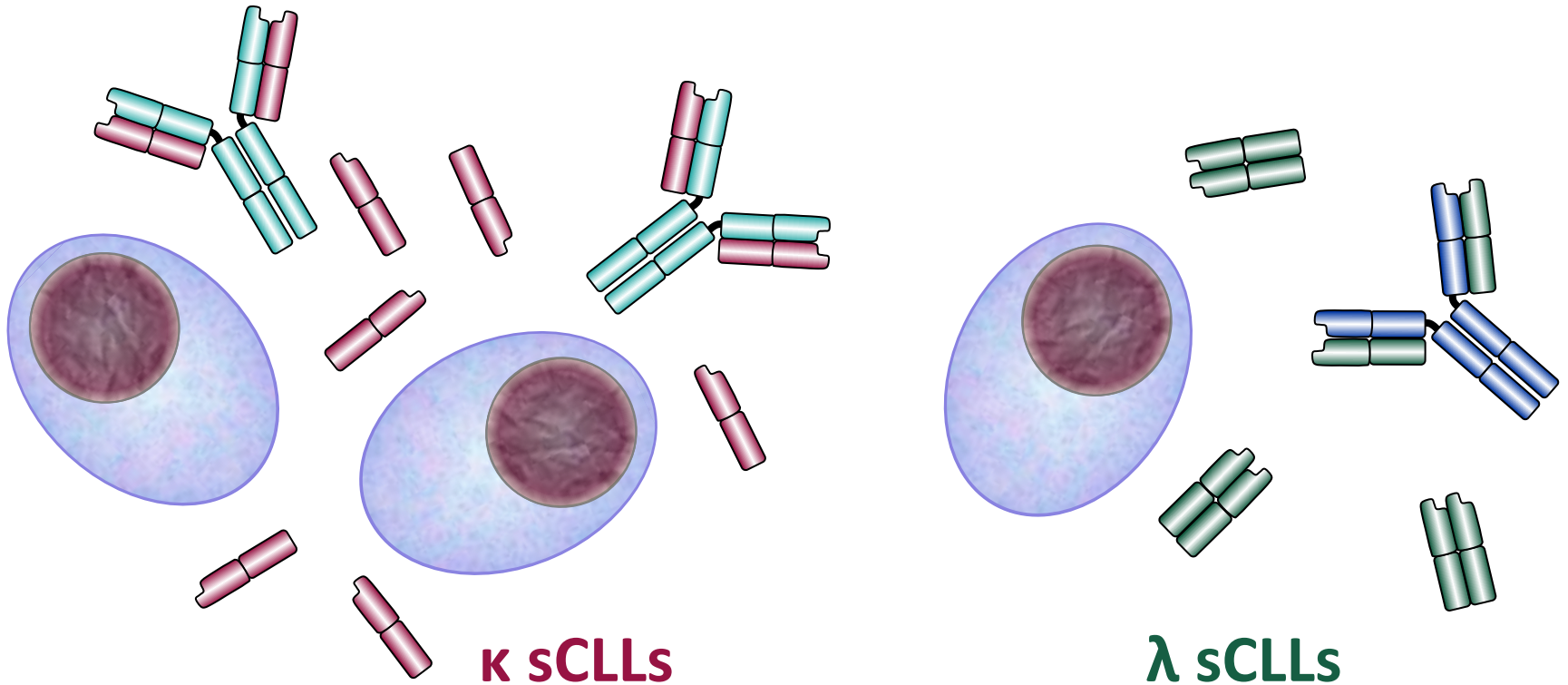
Cadenas Livianas Libres en LCR

Florencia Delgado PhD

Directora Científica – Latinoamérica

The Binding Site Group Ltd.

Producción de Inmunoglobulinas

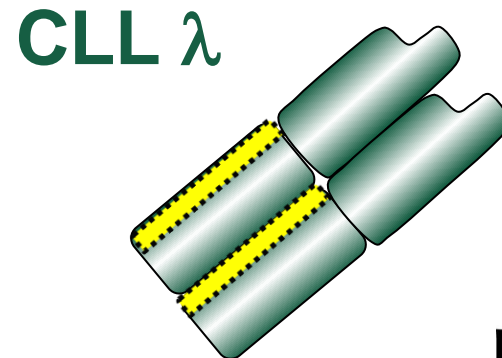
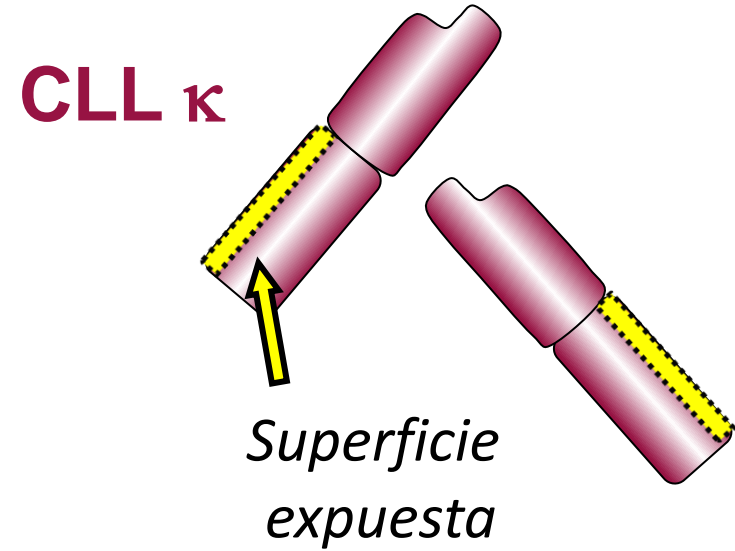
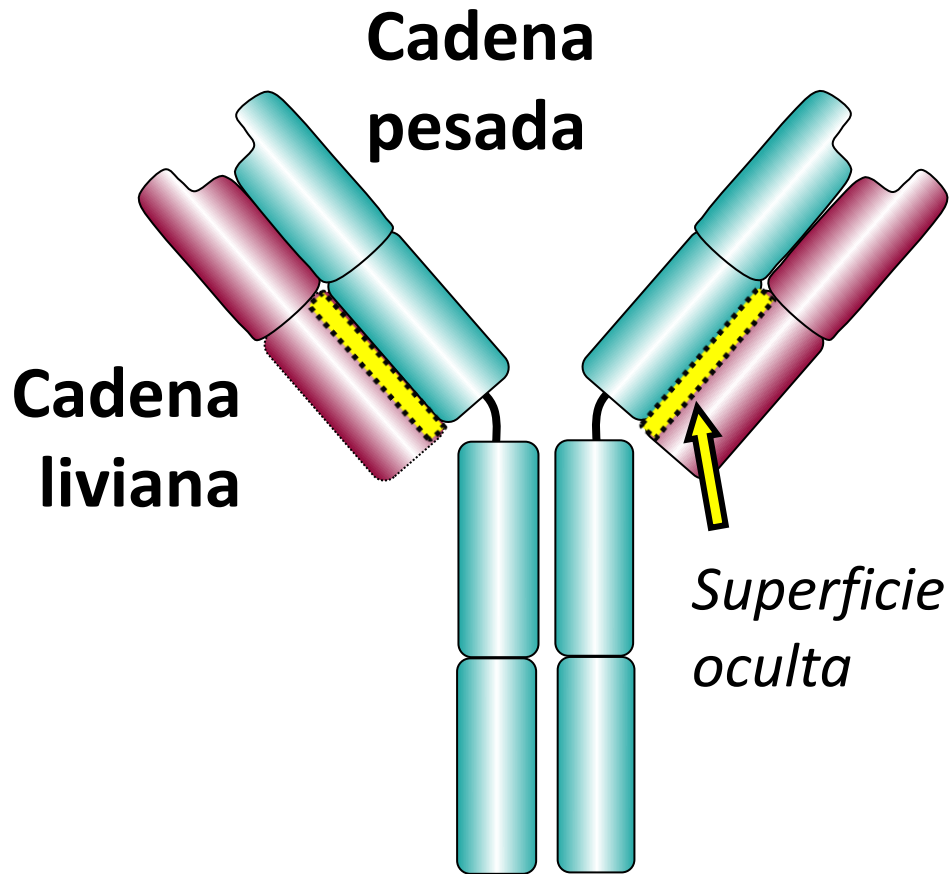


Rango normal: 3.3 – 19.4 mg/L

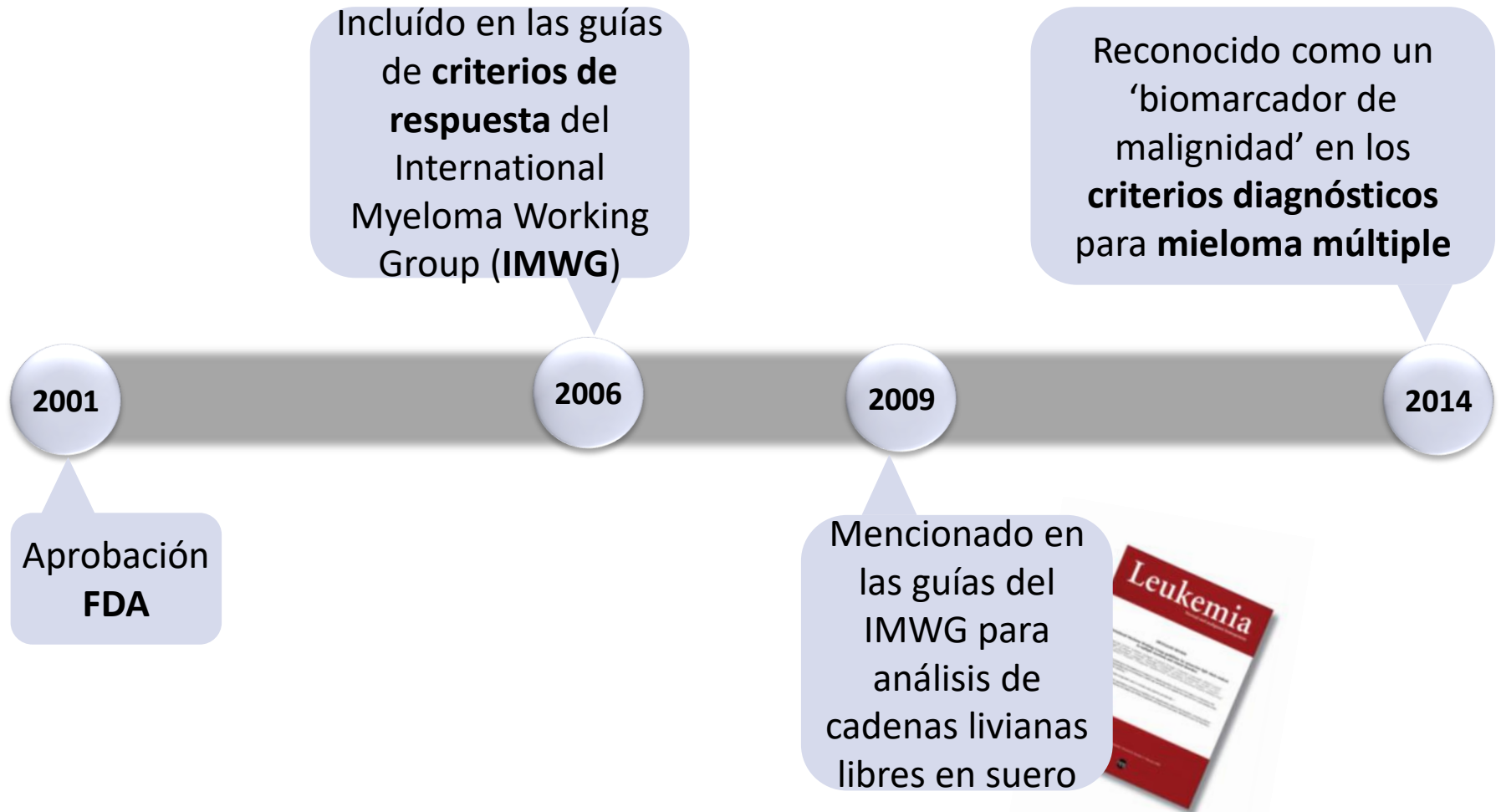
5.7 – 26.3 mg/L

Relación κ/λ : mediana = 0.6 (rango = 0.26 – 1.65)

Inmunoensayo Freelite



Freelite: un ensayo establecido

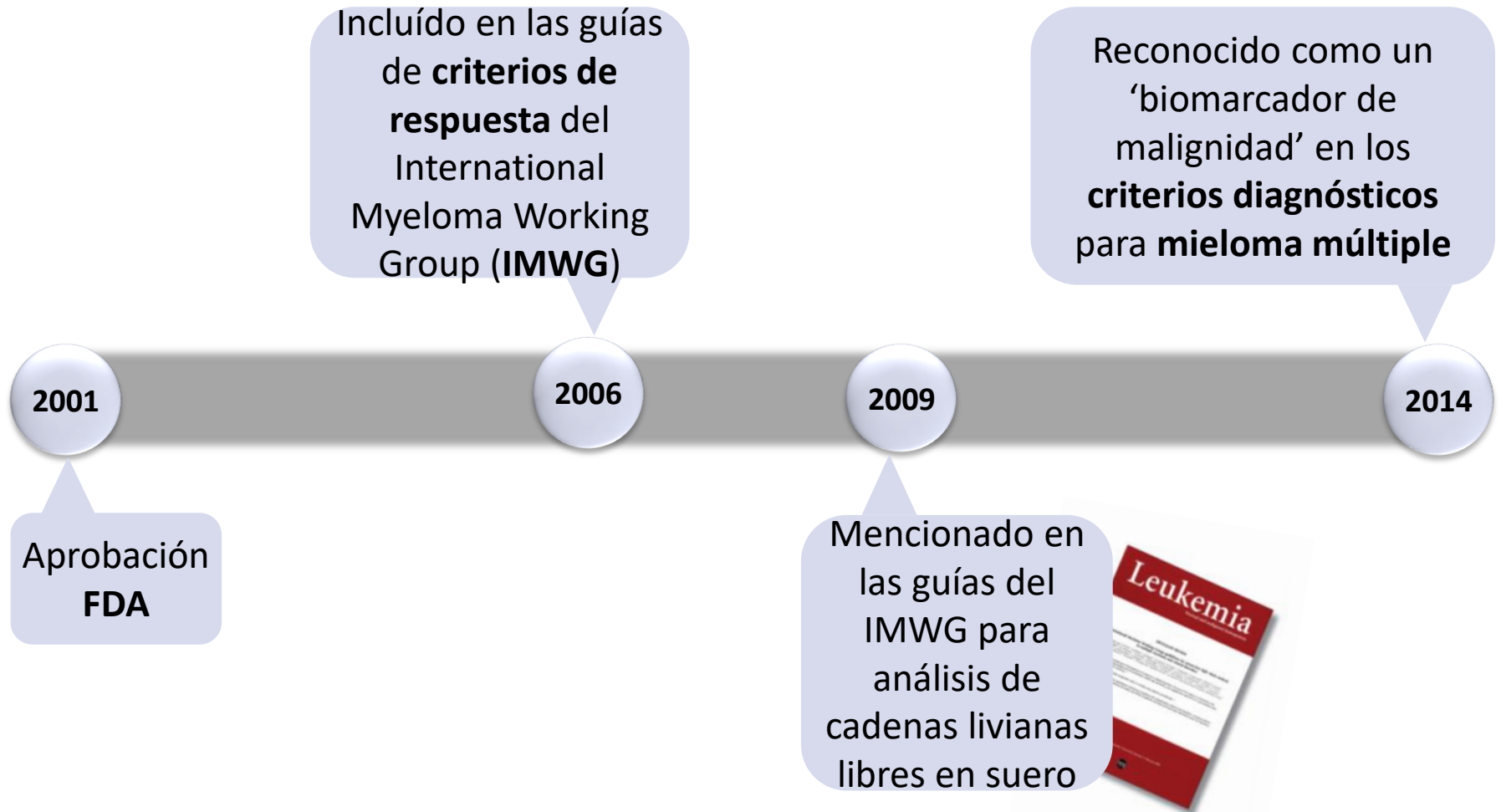


Durie Leukemia 2006;20:1467-73

Dispenzieri Leukemia 2009;23:215-24

Rajkumar Lancet Oncol. 2014;15:e538-48

Freelite: un ensayo establecido



Durie Leukemia 2006;20:1467-73

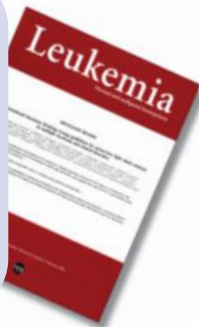
Dispenzieri Leukemia 2009;23:215-24

Rajkumar Lancet Oncol. 2014;15:e538-48

Freelite: un ensayo establecido

2009

Mencionado en las guías del IMWG para análisis de cadenas livianas libres en suero



Reconocido como un 'biomarcador de malignidad' en los **criterios diagnósticos** para **mieloma múltiple**

2014

Ensayos Freelite LCR CE-marked liberados para **SPAPLUS®**

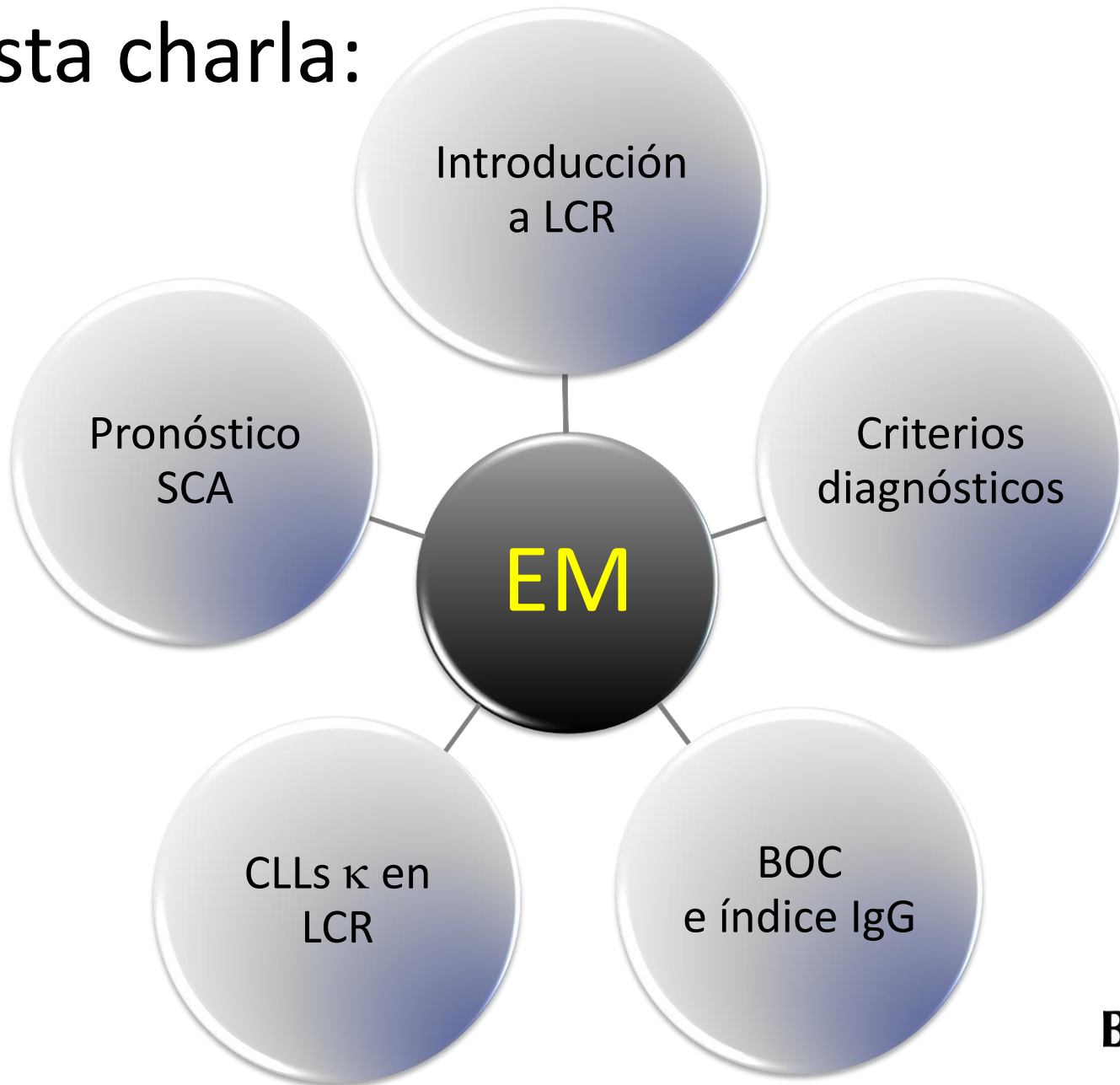
2014

2017

Ensayos Freelite LCR CE-marked liberados para **Optilite®**

Durie Leukemia 2006;20:1467-73
Dispenzieri Leukemia 2009;23:215-24
Rajkumar Lancet Oncol. 2014;15:e538-48

En esta charla:



Introducción a LCR

Líquido Cefalorraquídeo (LCR)

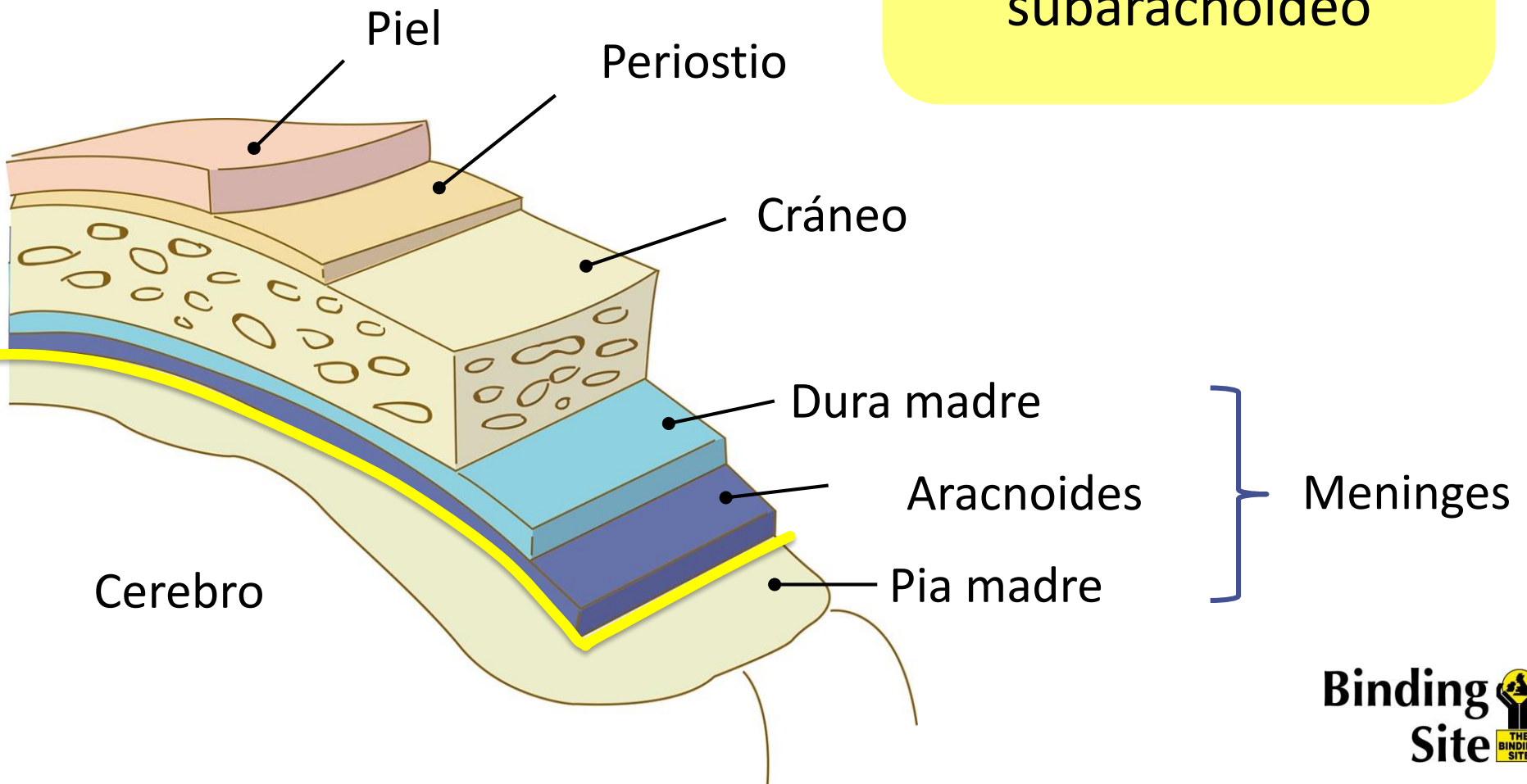


Funciones:

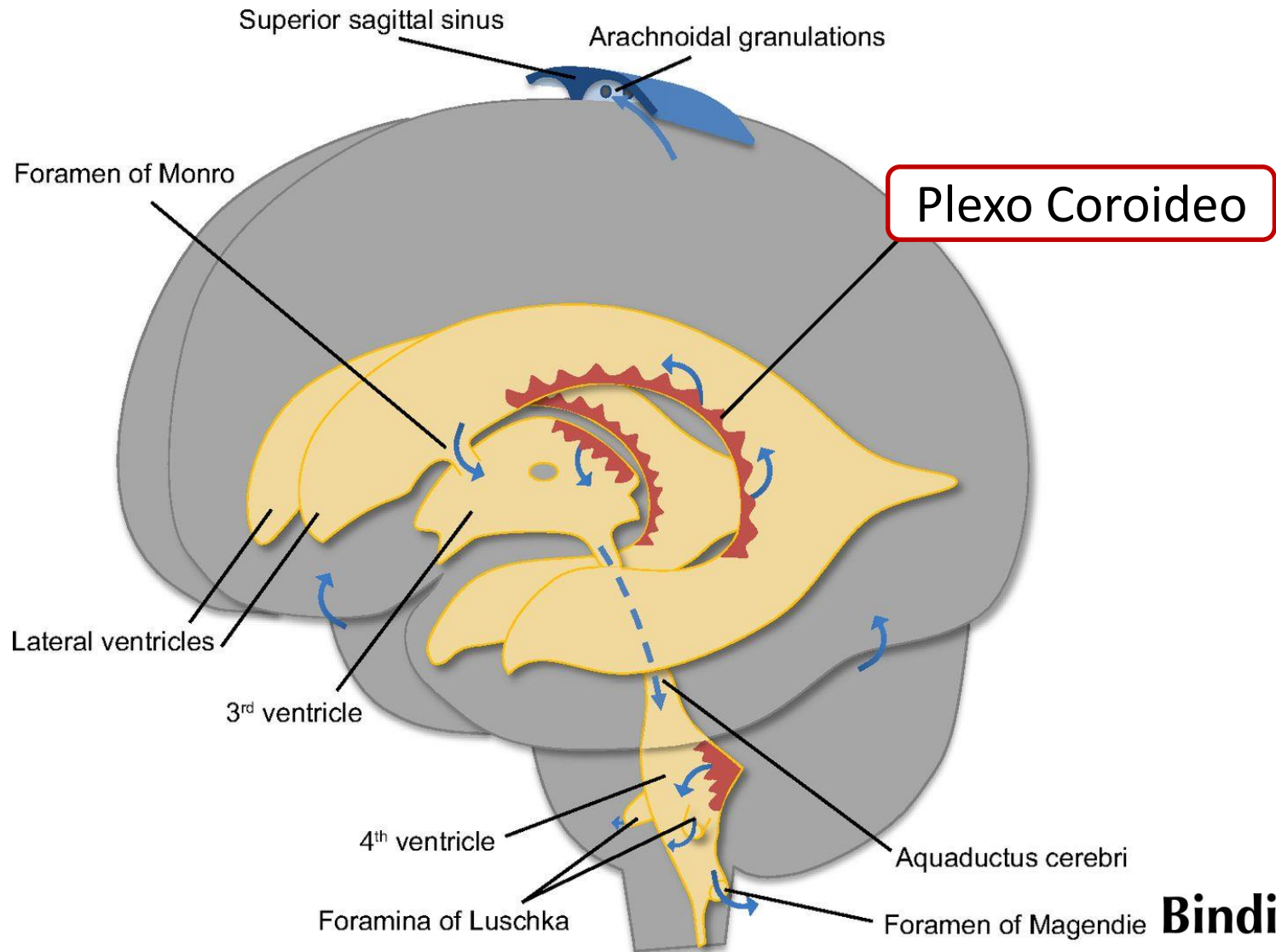
- **Amortiguación**
- Nutrición
- Eliminación de productos de desecho

¿Dónde está el LCR?

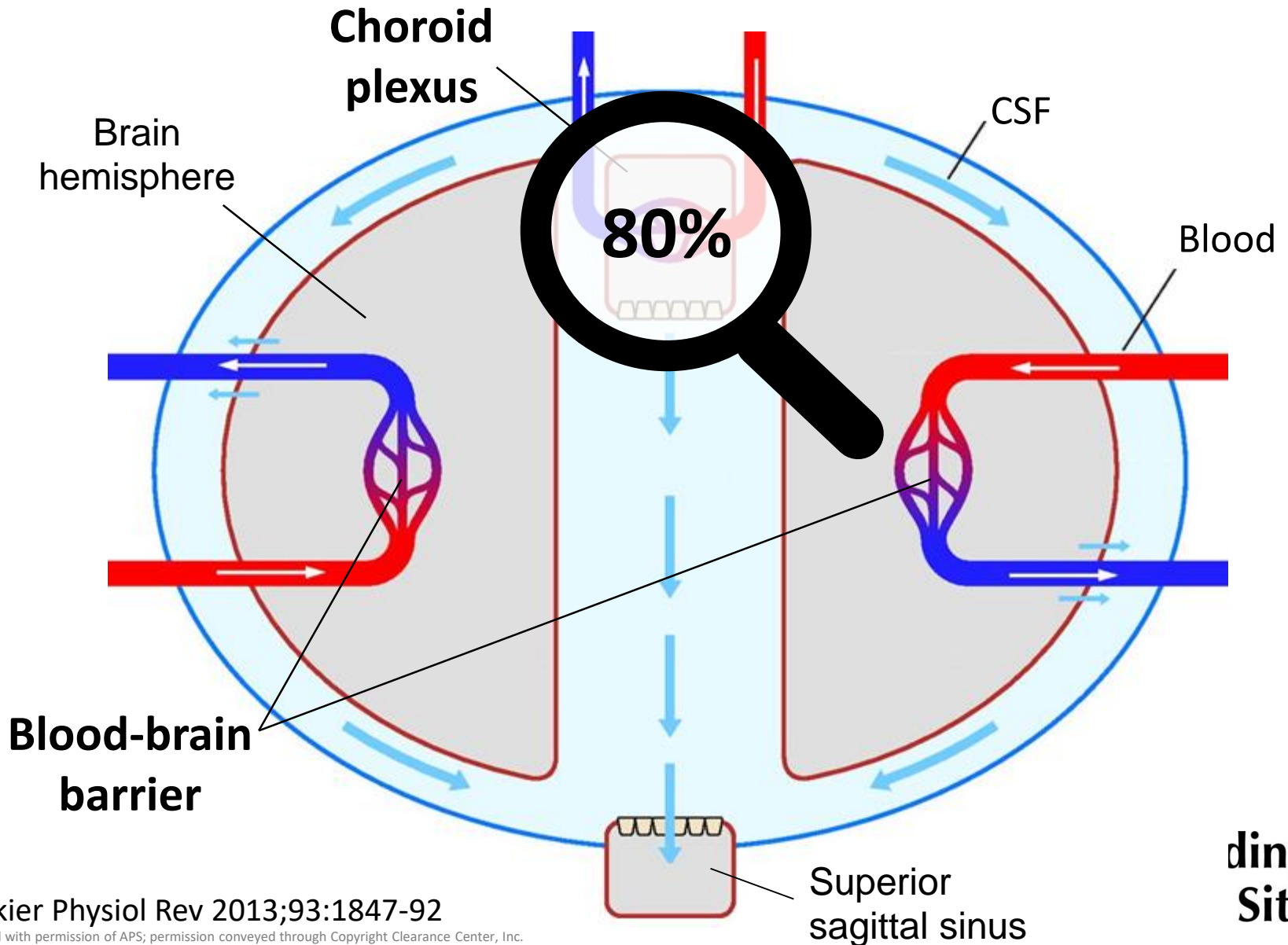
Intratecal:
Espacio
subaracnoideo



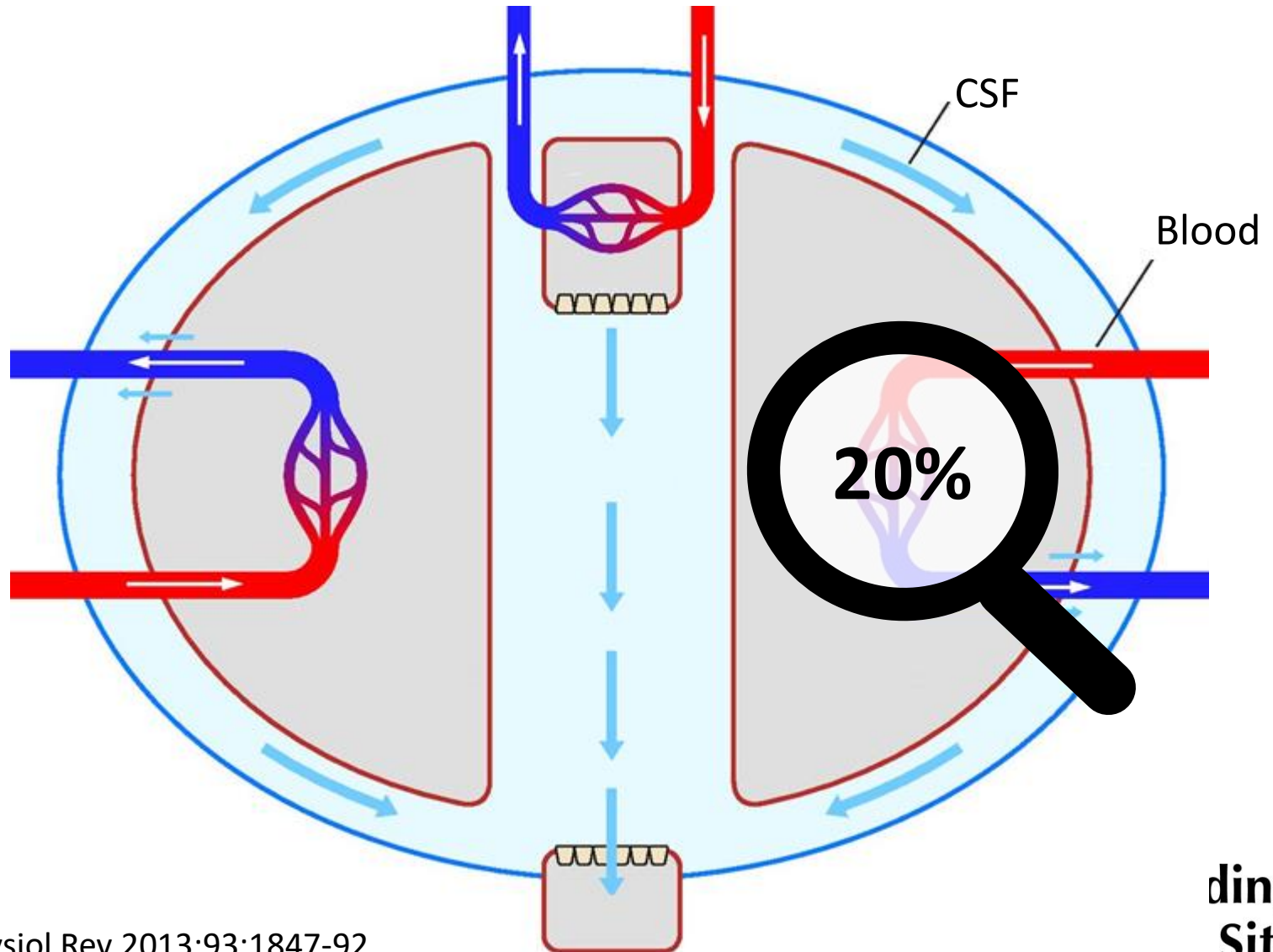
LCR es secretado por el plexo coroideo



Secreción de LCR, circulación y reabsorción



Secreción de LCR, circulación y reabsorción



Suero vs. LCR

	Serum	CSF
Na ⁺ (mmol/L)	136 – 145	136 - 150
Protein (g/L)	60 – 80	0.15 – 0.45
• Albumin (g/L)	33 – 47	0.177 – 0.251
• IgG (g/L)	6 - 16	0.008 – 0.042
• κ FLCs (mg/L)	3.3 – 19.4	0.13 – 0.22
• λ FLCs (mg/L)	5.7 – 26.3	0.13 – 0.20

Introducción a EM

¿Es Esclerosis Múltiple?



¿Es Esclerosis Múltiple?

Análisis
de LCR



Diagnóstico diferencial de otras condiciones

	Rango normal	Hallazgo típico en EM	Útil en el diagnóstico diferencial de...
Rto de leucocitos	<5 cells/ μ L	5-50 cells/ μ L	Infección bacteriana
Citología LCR			Tumor de SNC
Q_{Alb} (Albumin _{CSF} /Albumin _{serum})	<5-10x10 ⁻³	<10x10 ⁻³	Otras condiciones inflamatorias. X ej. Sarcoidosis

Detección de Igs intratecales – consistente con diagnóstico de EM

Bandas oligoclonales	≤1 band	>1 band	Otras condiciones inflamatorias x ej. SLE, neuromielitis óptica
Índice IgG	≤0.7	>0.7	

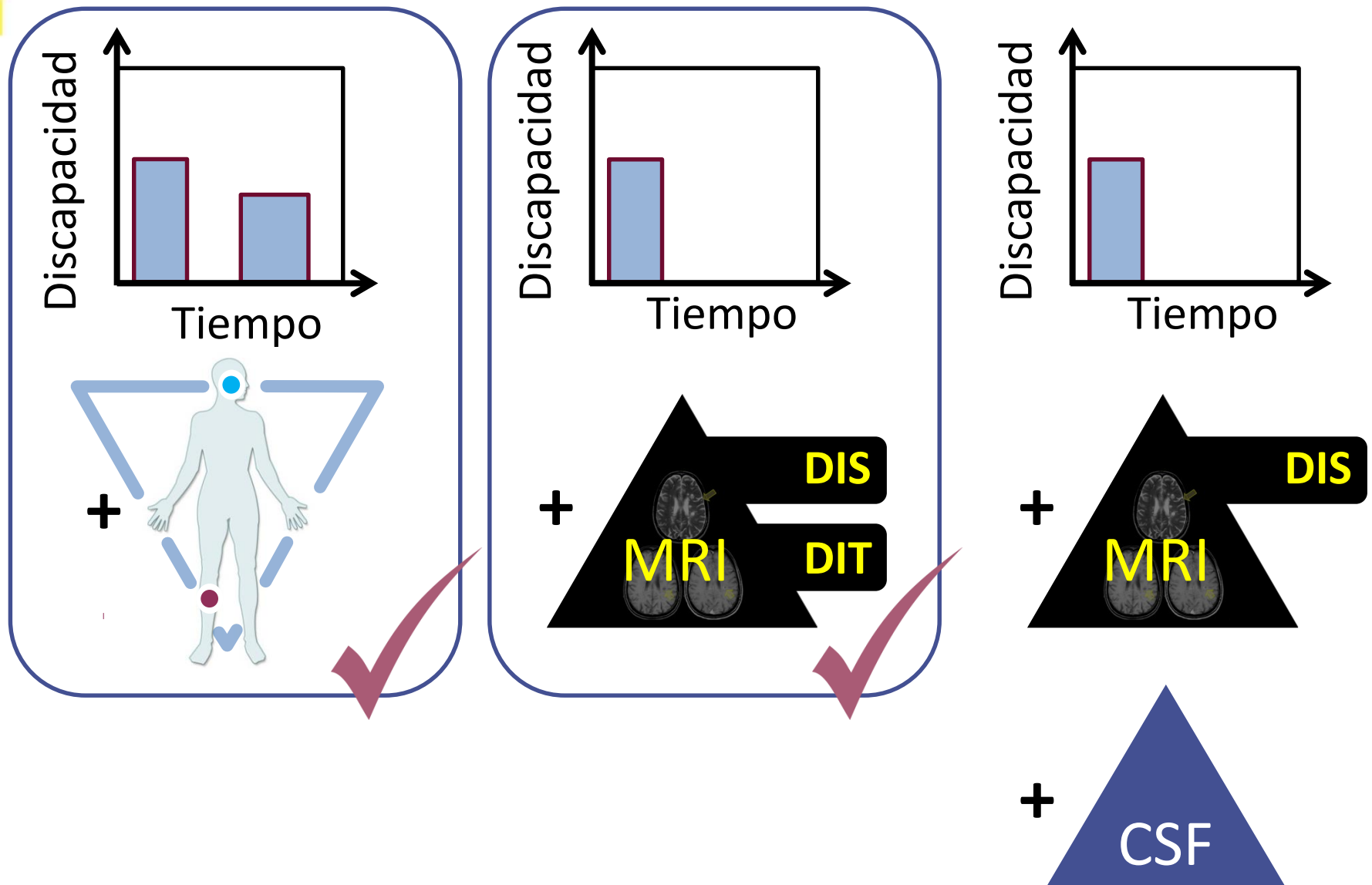
Criterios diagnósticos para EM

Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria

Alan J Thompson, Brenda L Banwell, Frederik Barkhof, William M Carroll, Timothy Coetzee, Giancarlo Comi, Jorge Correale, Franz Fazekas, Massimo Filippi, Mark S Freedman, Kazuo Fujihara, Steven L Galetta, Hans Peter Hartung, Ludwig Kappos, Fred D Lublin, Ruth Ann Marrie, Aaron E Miller, David H Miller, Xavier Montalban, Ellen M Mowry, Per Soelberg Sorensen, Mar Tintoré, Anthony L Traboulsee, Maria Trojano, Bernard M J Uitdehaag, Sandra Vukusic, Emmanuelle Waubant, Brian G Weinshenker, Stephen C Reingold, Jeffrey A Cohen

→ demostrar que el daño al sistema nervioso central está diseminado en **tiempo (DIT)** y **espacio (DIS)**

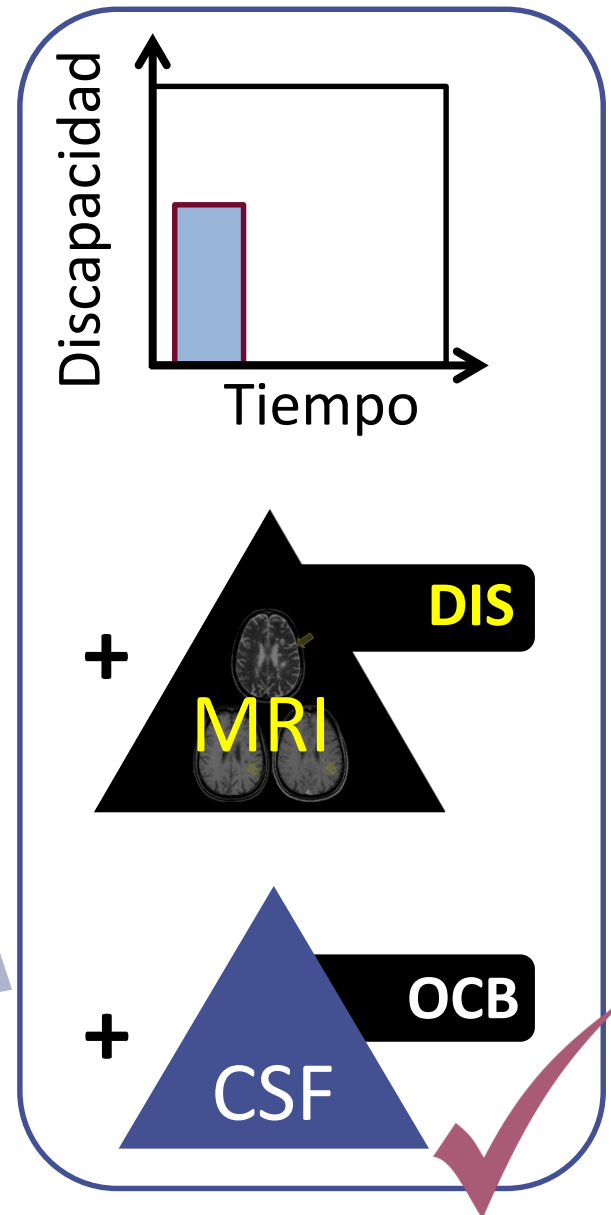
Criterios diagnósticos para EM



Criterios diagnósticos para EM

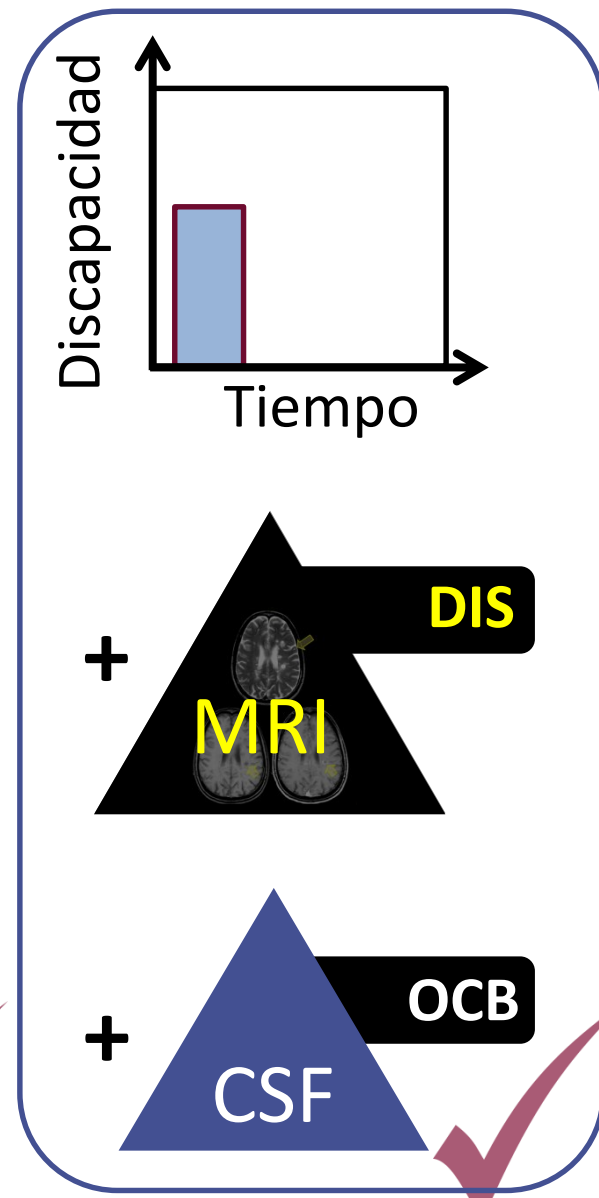
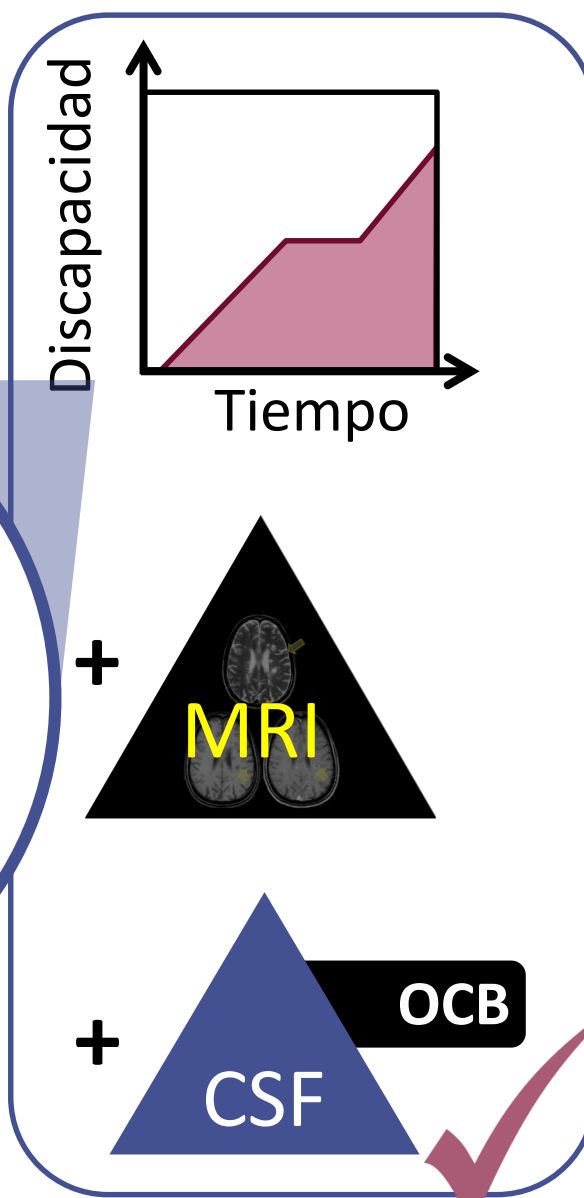
NUEVO

“...demostración de bandas oligoclonales en LCE en ausencia de hallazgos típicos permite que se realice un diagnóstico de esclerosis múltiple...”



Criterios diagnósticos para EM

“...el examen de LCR está fuertemente recomendado... cuando existe... un curso progresivo al inicio...”



Examen de LCR está fuertemente recomendado:

- Cuando la evidencia clínica y MRI es insuficiente para avalar el diagnóstico de EM
- Cuando la presentación es diferente a un SCA típico
por ej. curso progresivo al inicio
- Cuando la clínica, imágenes u otras características de laboratorio son atípicas para EM
- En población donde EM es menos frecuente
por ej. niños, individuos mayores, etc.

Detección de Igs intratecales



LCR

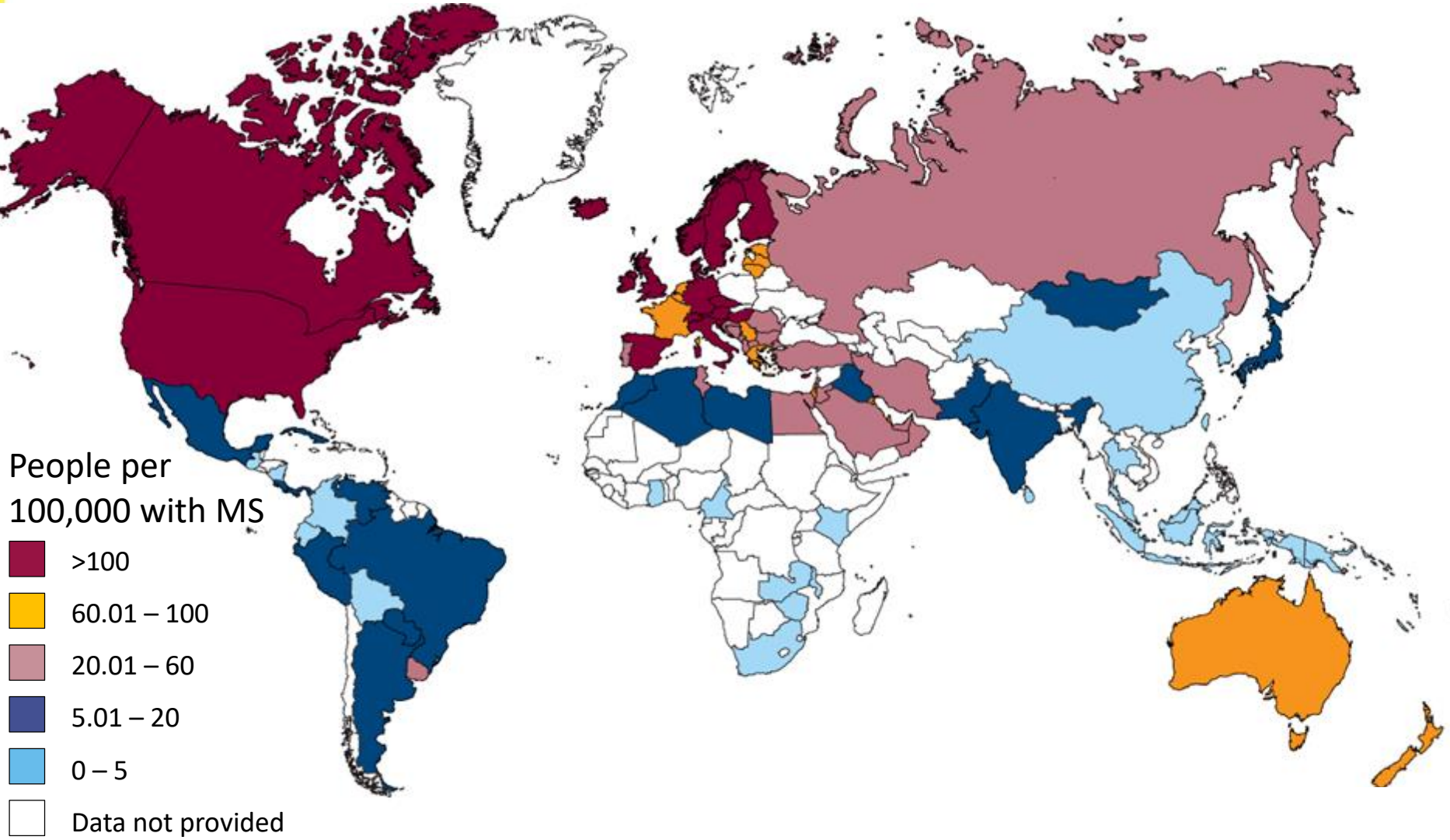


Población con EM

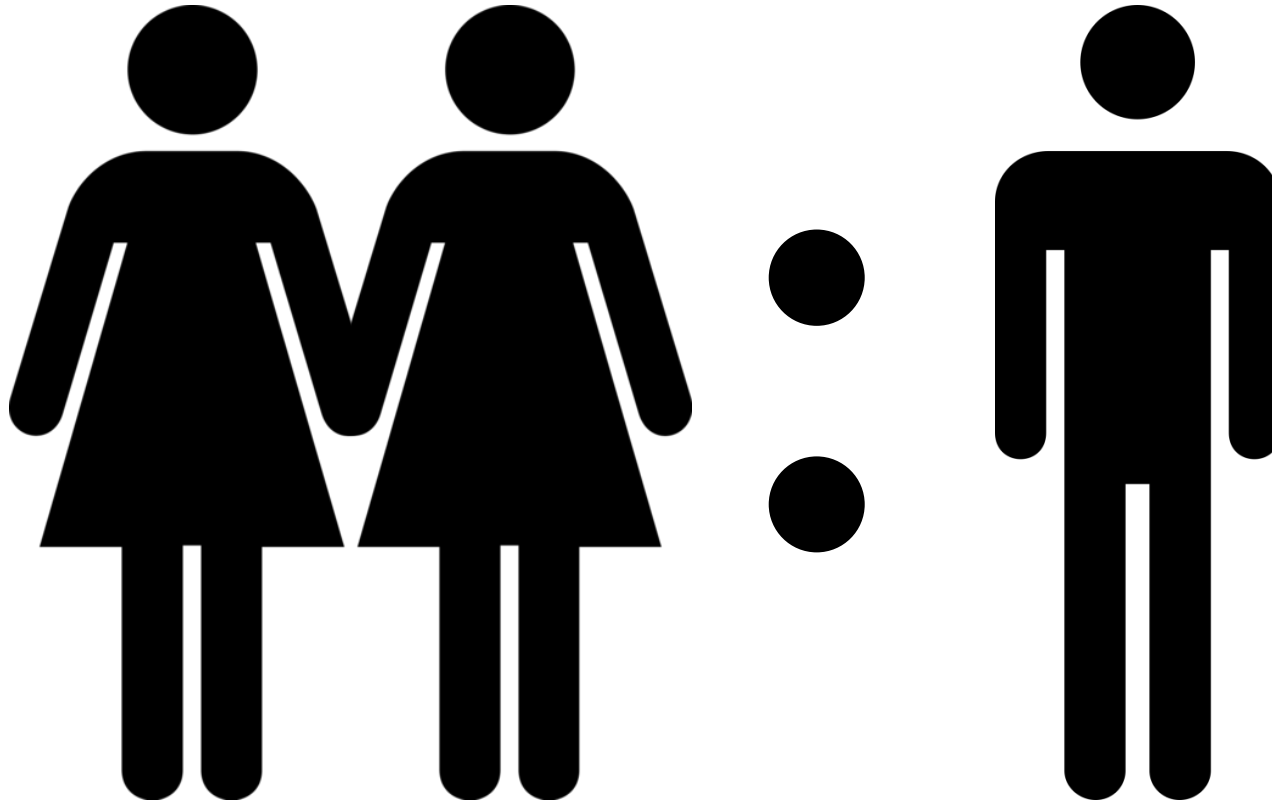


Total = 2.3 millones

Prevalencia EM (2013)



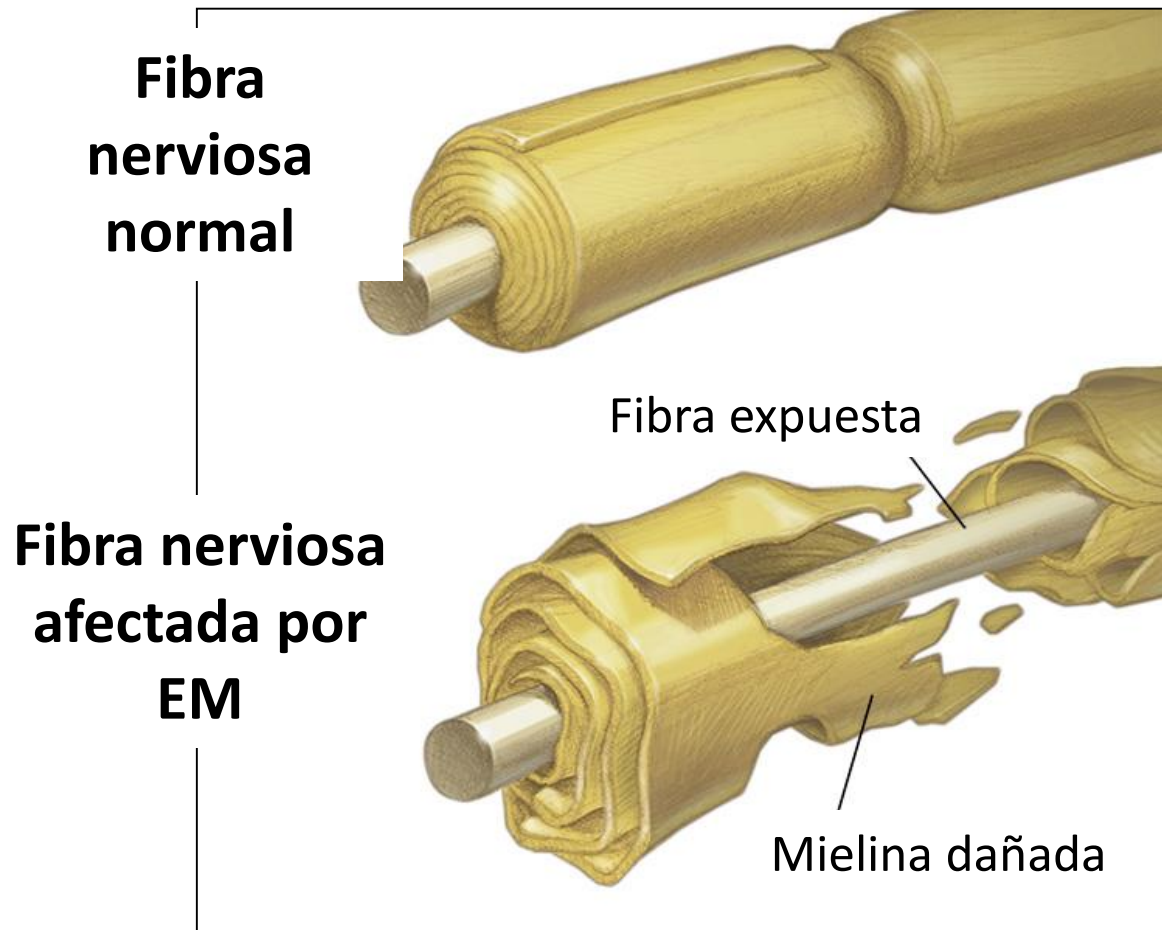
EM es 2 veces más frecuente en mujeres



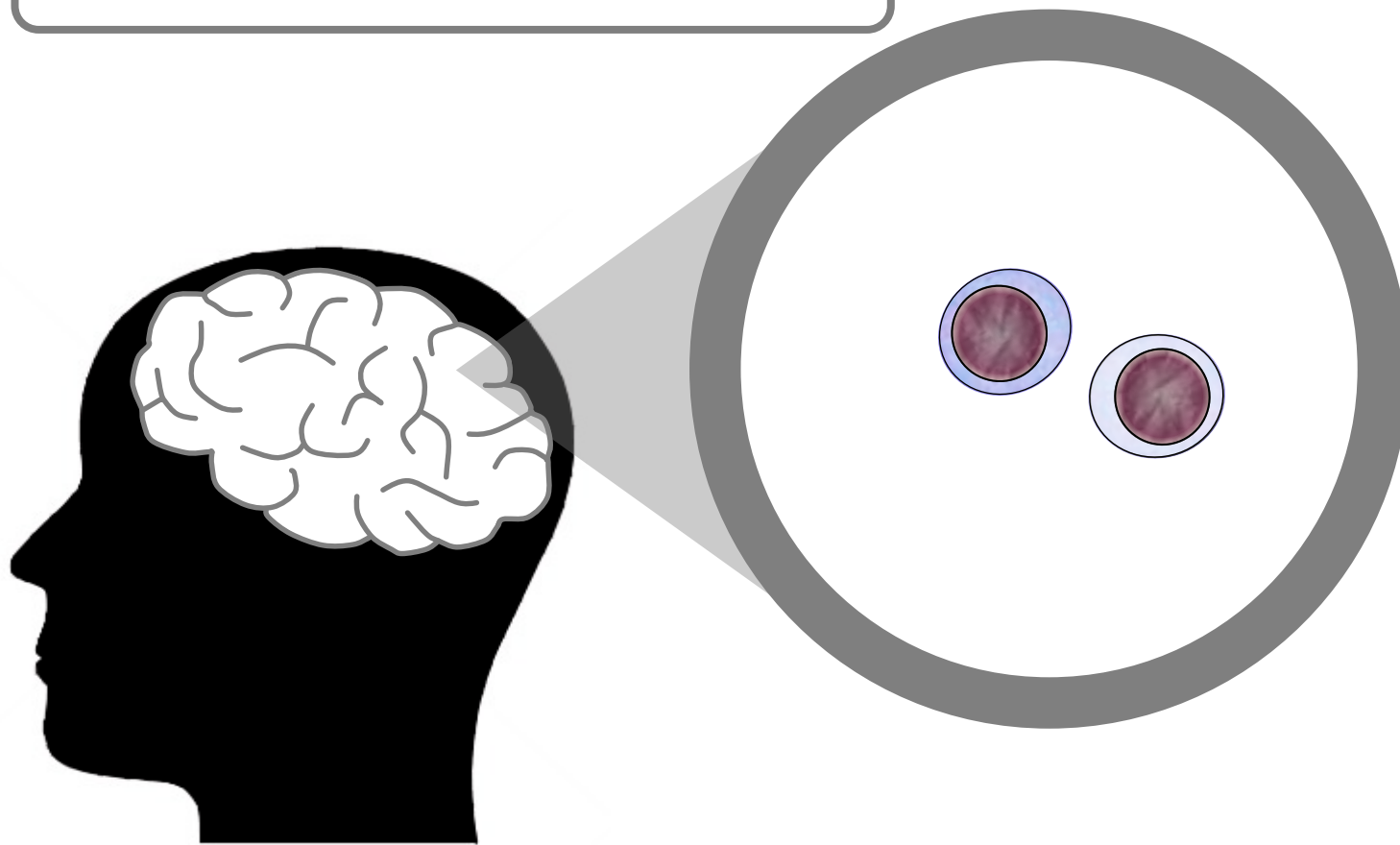
Edad promedio de presentación de EM

30 años

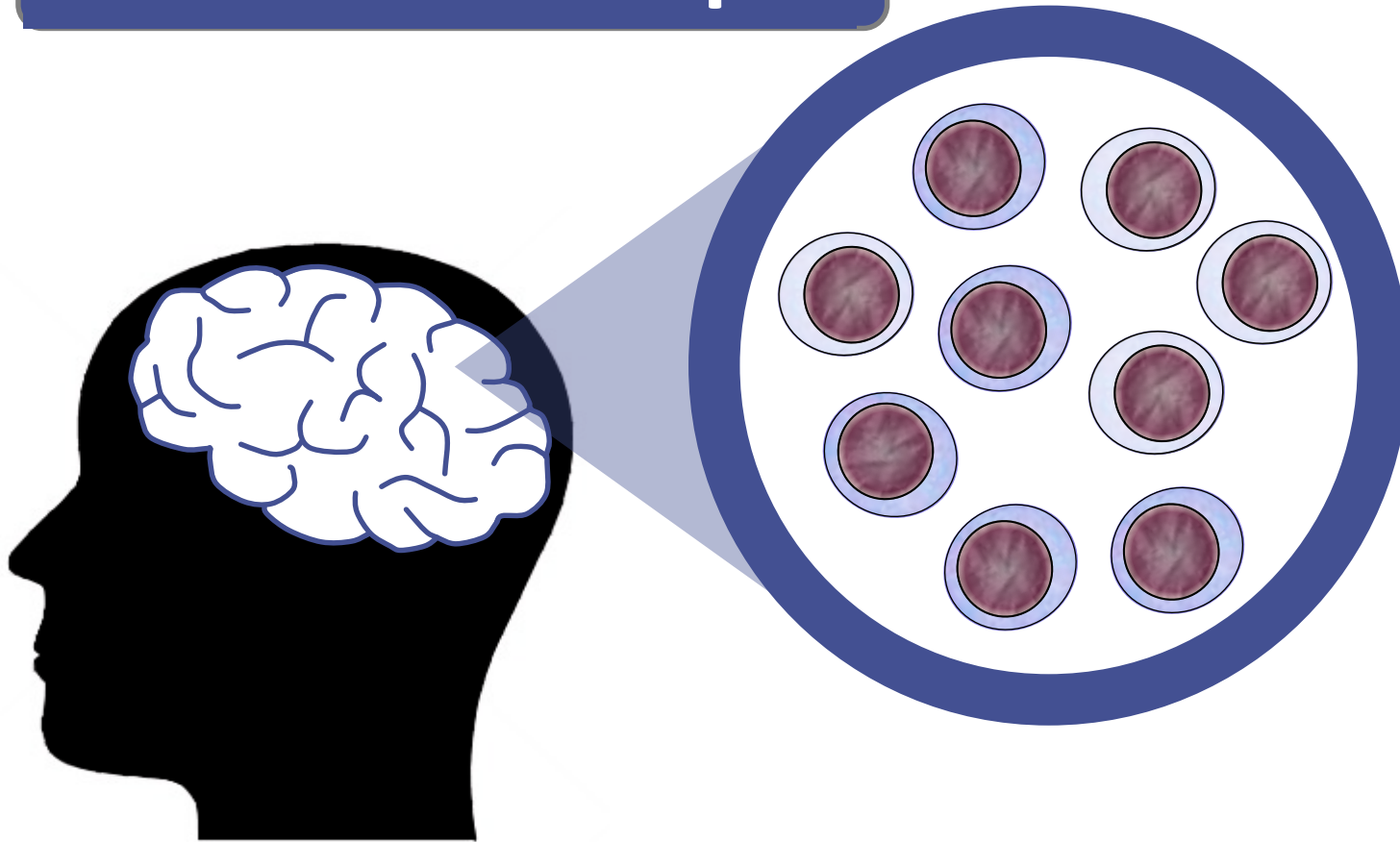
Daño a la vaina de mielina



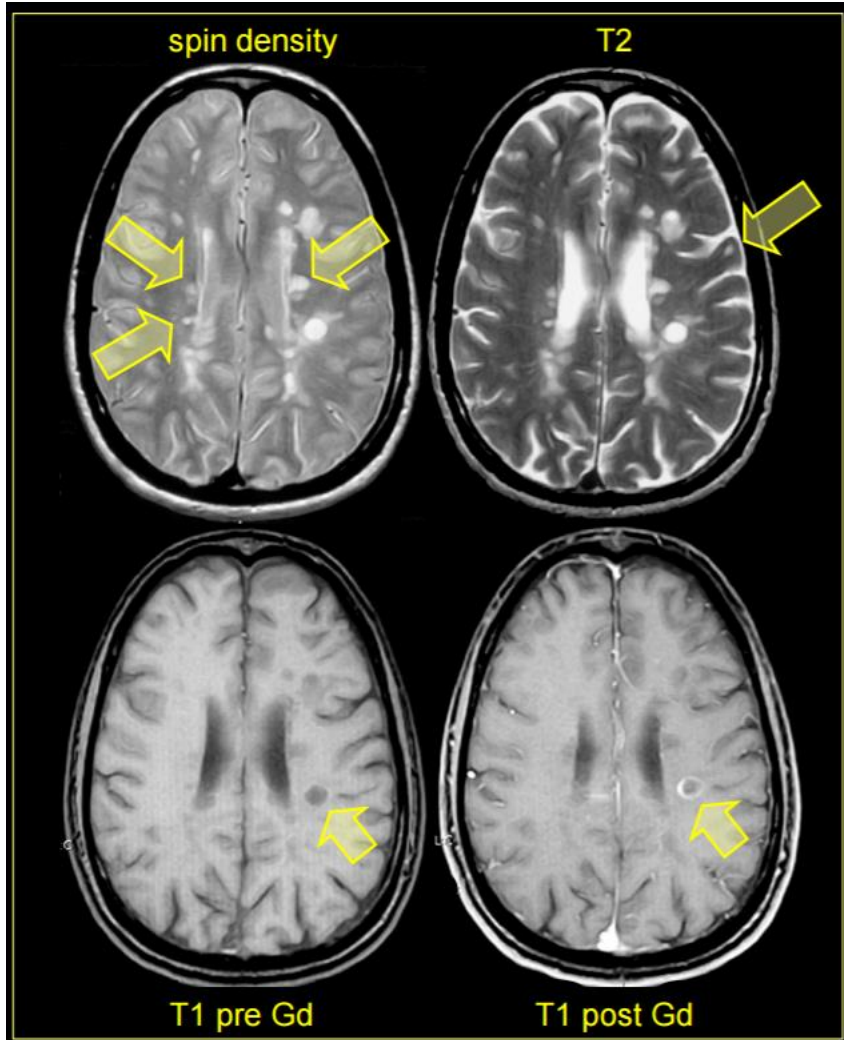
Individuos normales



Esclerosis múltiple



Imágenes cerebrales en EM



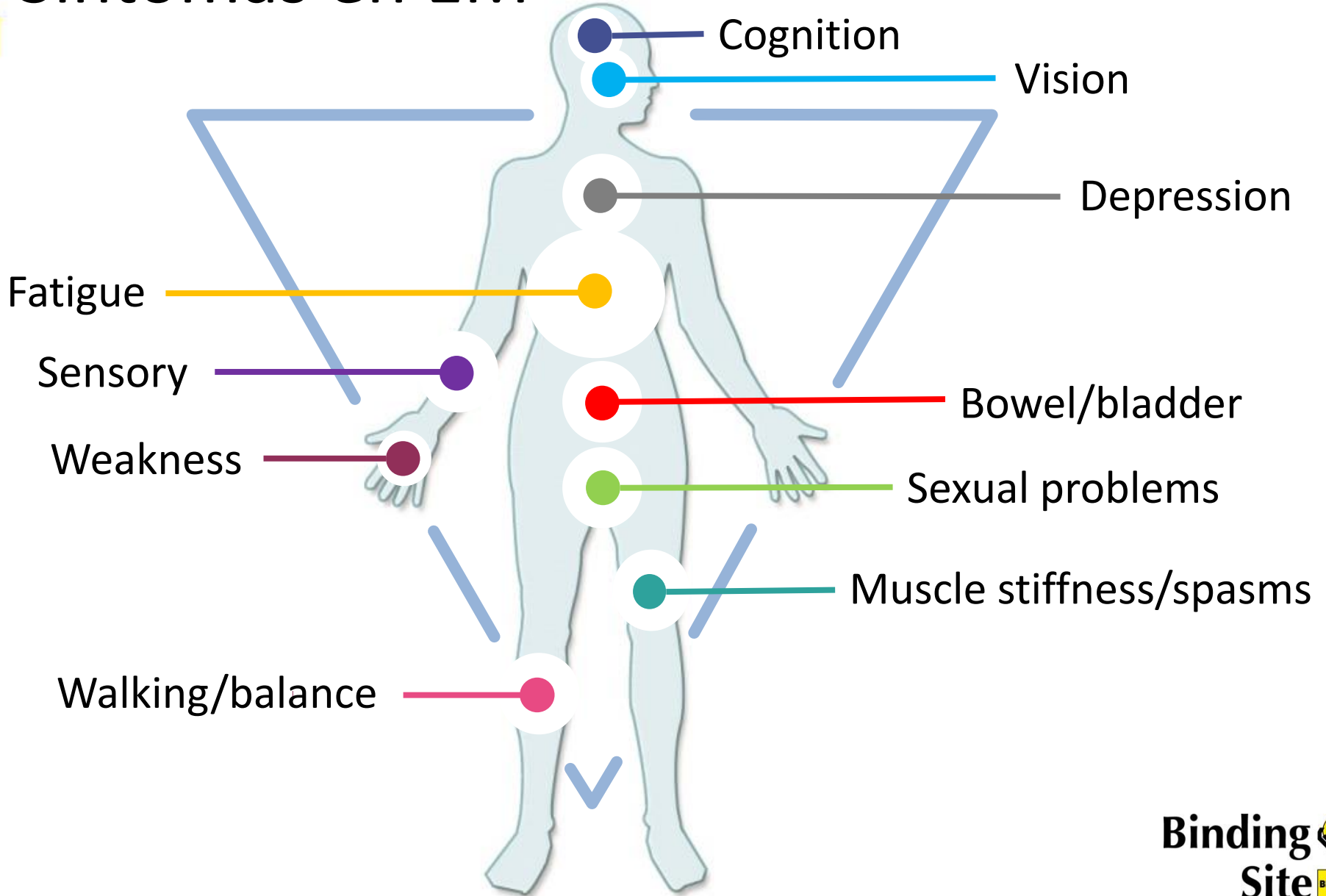
DIS

Diseminación en **espacio**

DIT

Diseminación en **tiempo**

Síntomas en EM



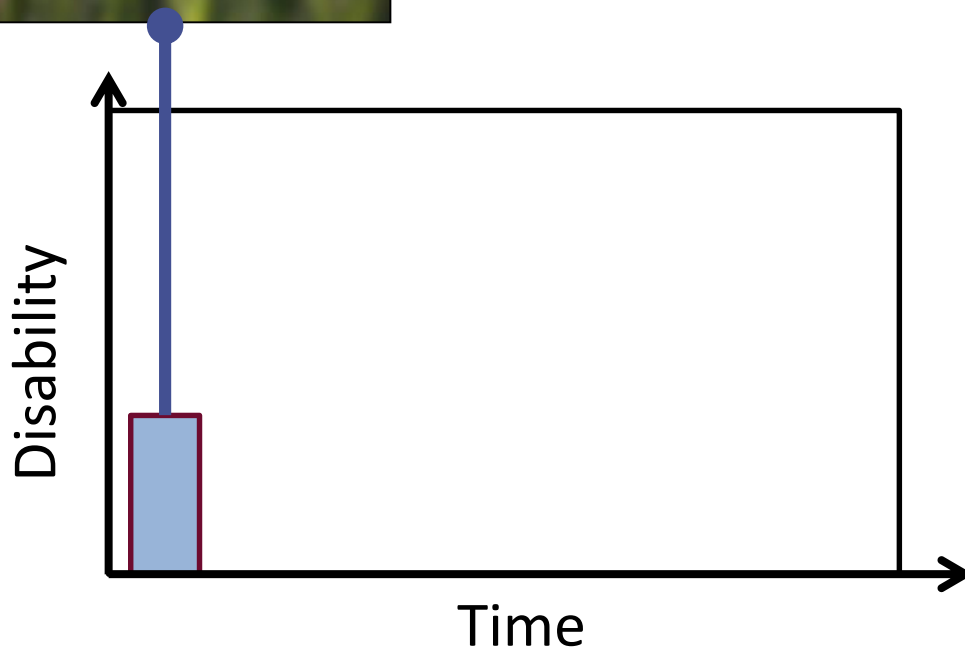
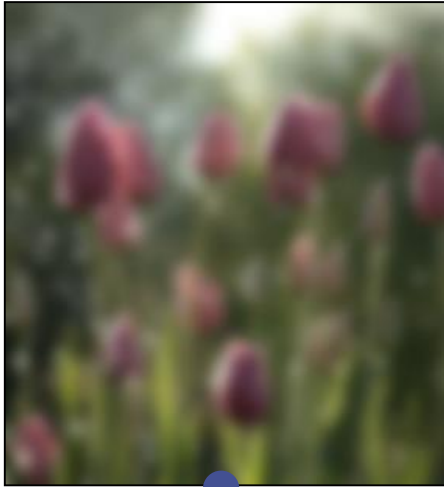
Criteria Diagnósticos para EM

Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria

Alan J Thompson, Brenda L Banwell, Frederik Barkhof, William M Carroll, Timothy Coetzee, Giancarlo Comi, Jorge Correale, Franz Fazekas, Massimo Filippi, Mark S Freedman, Kazuo Fujihara, Steven L Galetta, Hans Peter Hartung, Ludwig Kappos, Fred D Lublin, Ruth Ann Marrie, Aaron E Miller, David H Miller, Xavier Montalban, Ellen M Mowry, Per Soelberg Sorensen, Mar Tintoré, Anthony L Traboulsee, Maria Trojano, Bernard M J Uitdehaag, Sandra Vukusic, Emmanuelle Waubant, Brian G Weinshenker, Stephen C Reingold, Jeffrey A Cohen

‘The goal is to make a rapid and accurate diagnosis of MS...’

¿Es EM?

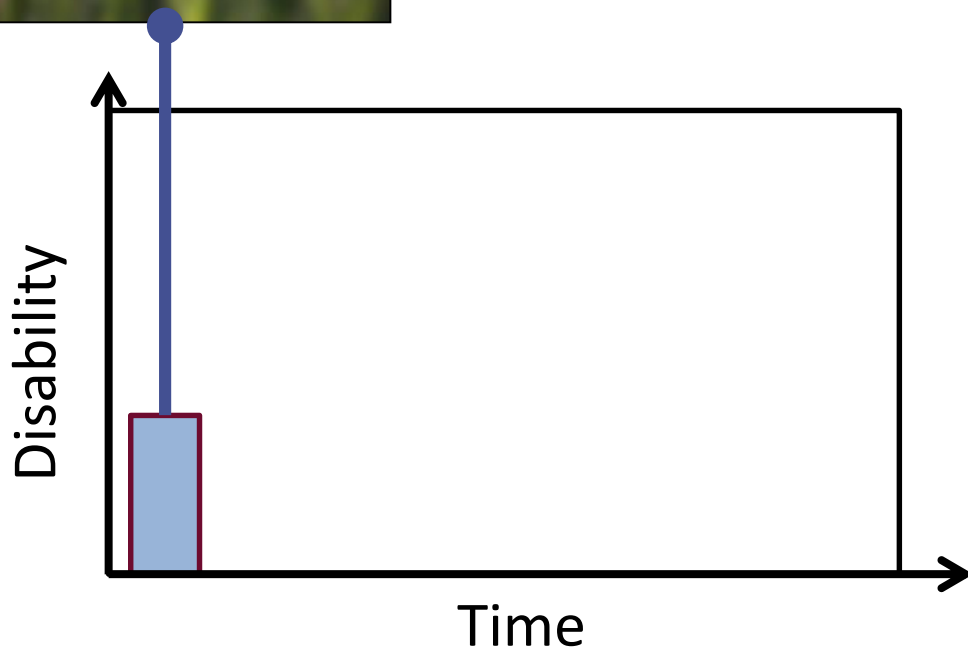


'Síndrome Clínico Aislado'

Síndrome Clínico Aislado

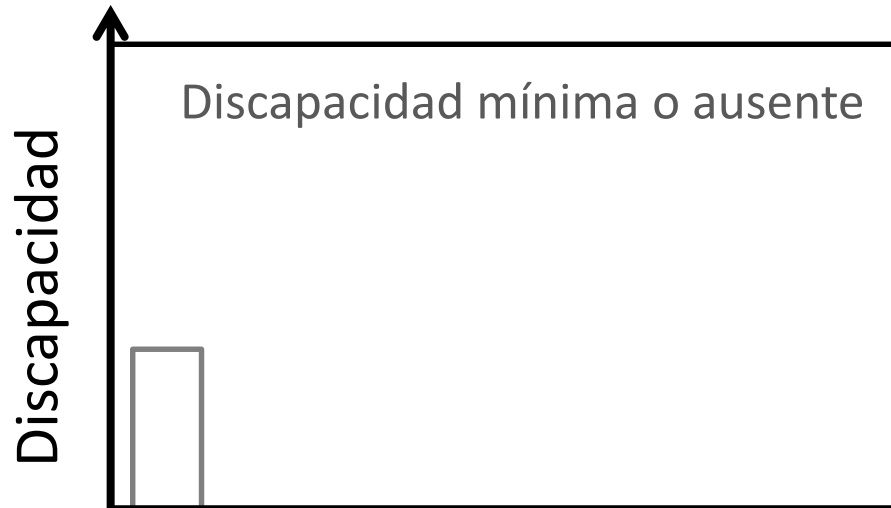


- Primer episodio de síntomas neurológicos que duran al menos 24 horas
- Causado por inflamación y desmielinización en el sistema nervioso central

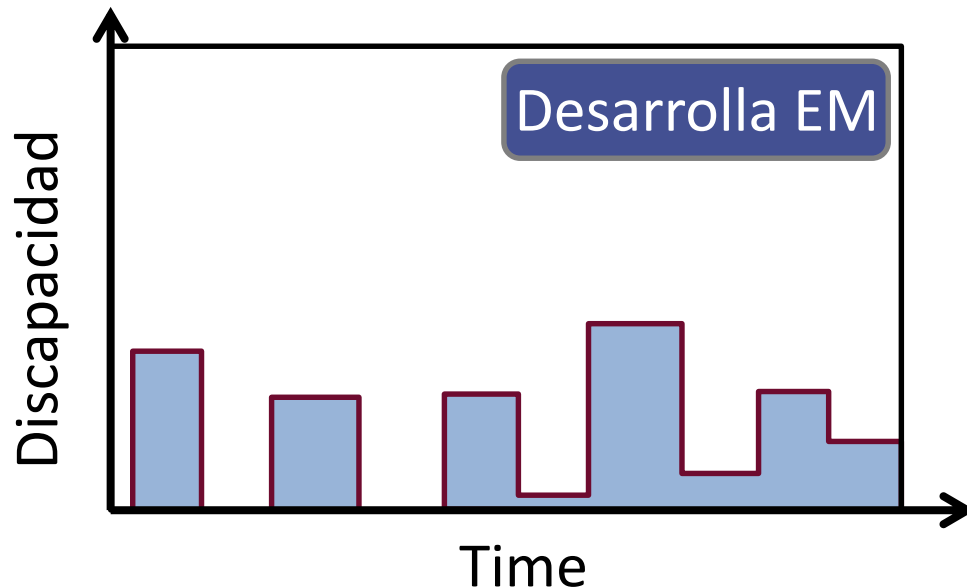


¿Es EM?

Luego de 20 años:



40%



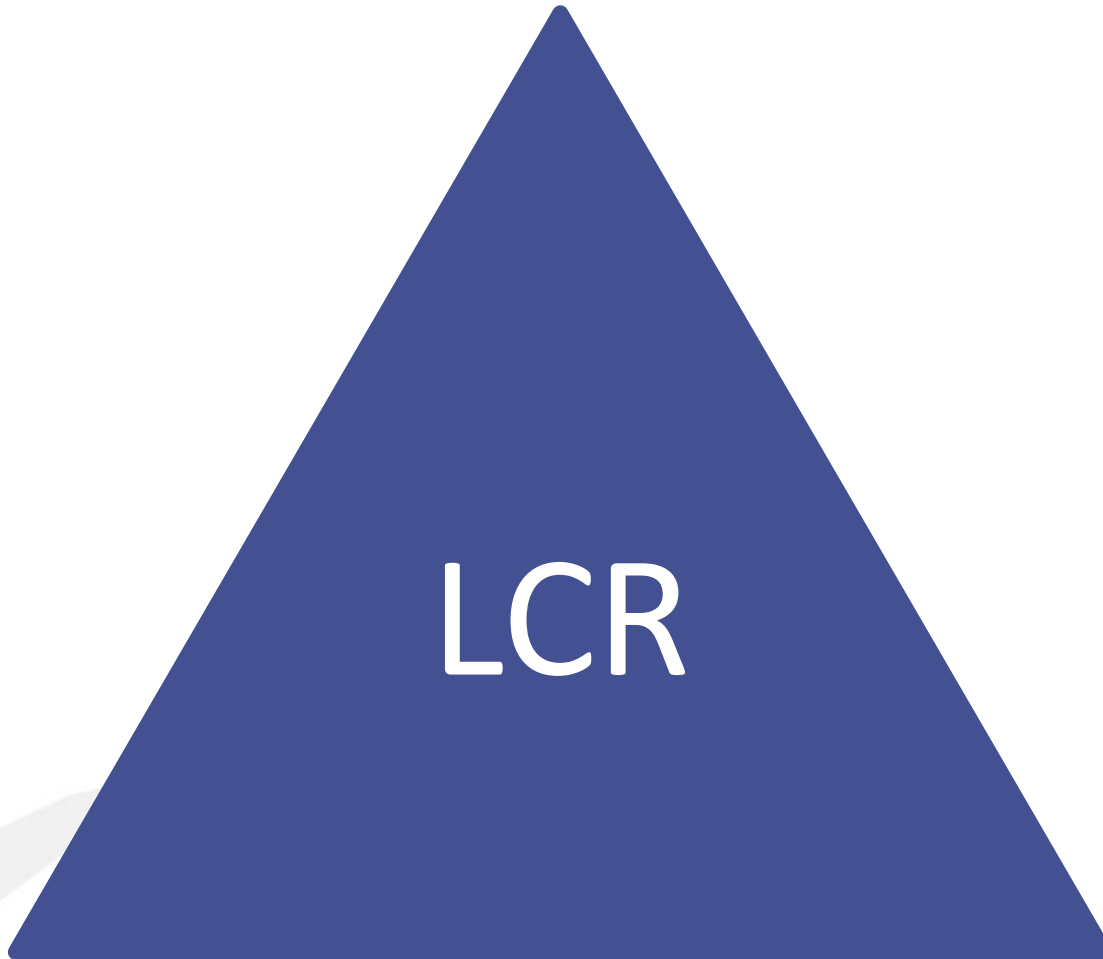
60%

¿Es EM?

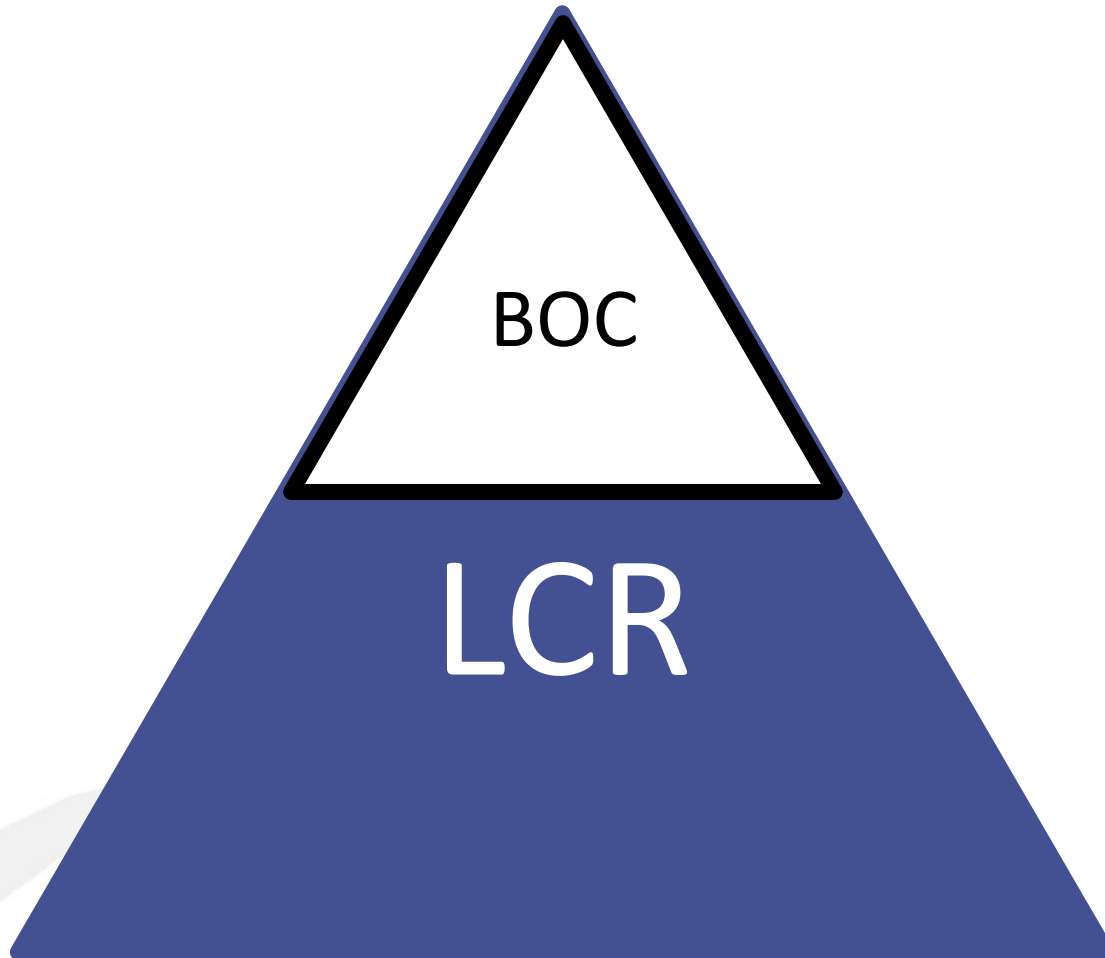


BOC e índice de IgG

Detección de Igs intratecales



Detección de Igs intratecales



Pruebas de laboratorio para detectar Igs intratecales

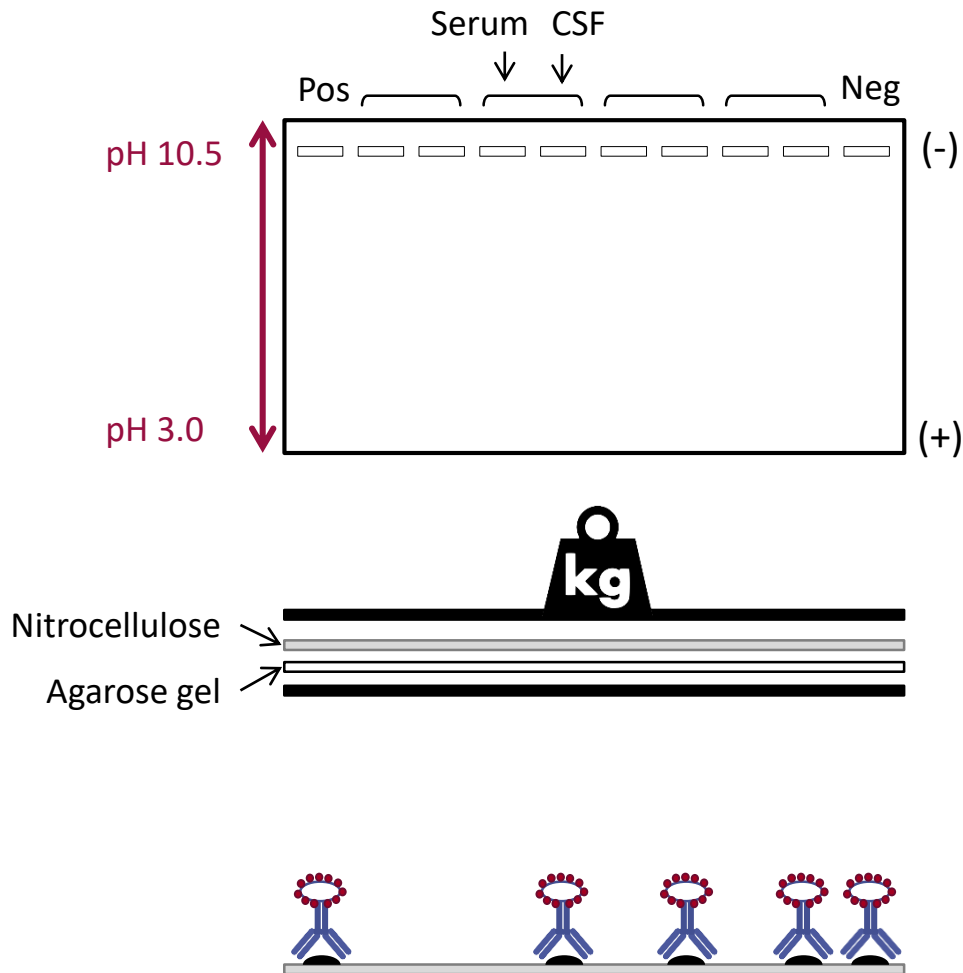
Técnica	Sensibilidad diagnóstica para EM	Especificidad diagnóstica
Bandas Oligoclonales	88 – 94%	92%

Presslauer J Neurol 2008;255:1508-14

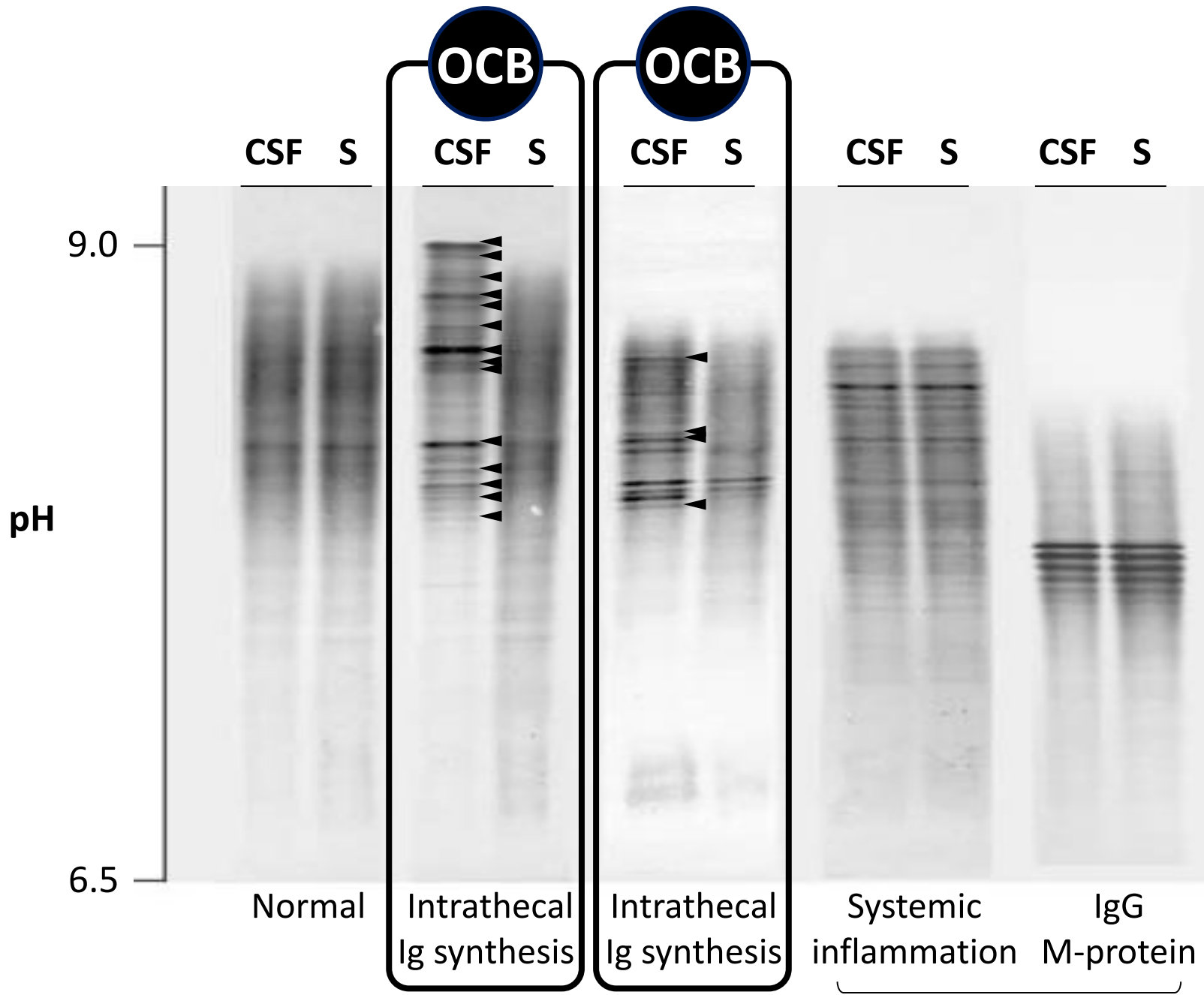
Dobson J Neurol Neurosurg Psychiatry 2013;84:909-14

Presslauer PLoS ONE 2014;9:e89945

Método casero para BOC



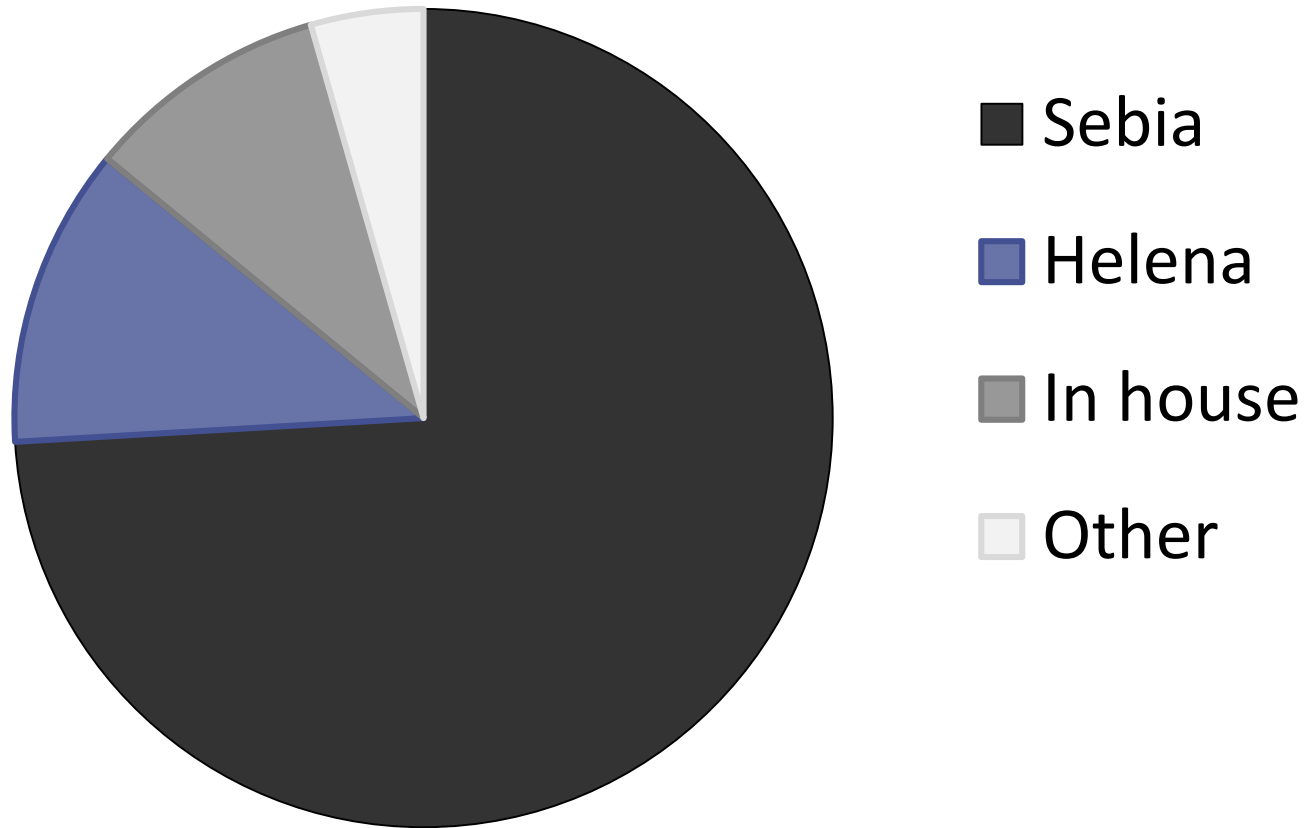
- Cast gel
- Dilute samples
- Load samples
- Run gel
- Transfer to nitrocellulose
- Add block solution
- Add anti-IgG peroxidase
- Add developer



Mirror pattern

Métodos BOC

UK and non-UK participants
n=135



○ Measure IgG, dilute samples

● Pre-migration

○ Load samples

● Isoelectric focusing

○ Apply anti-IgG peroxidase

○ Wash, rehydrate gel x2

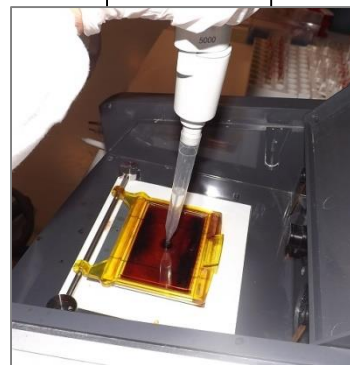
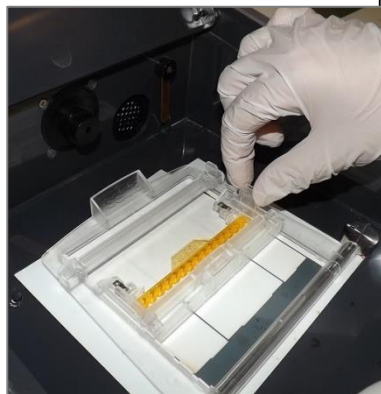
○ Apply developer solution

● Dry

○ Remove gel

● Wash

○ Interpret



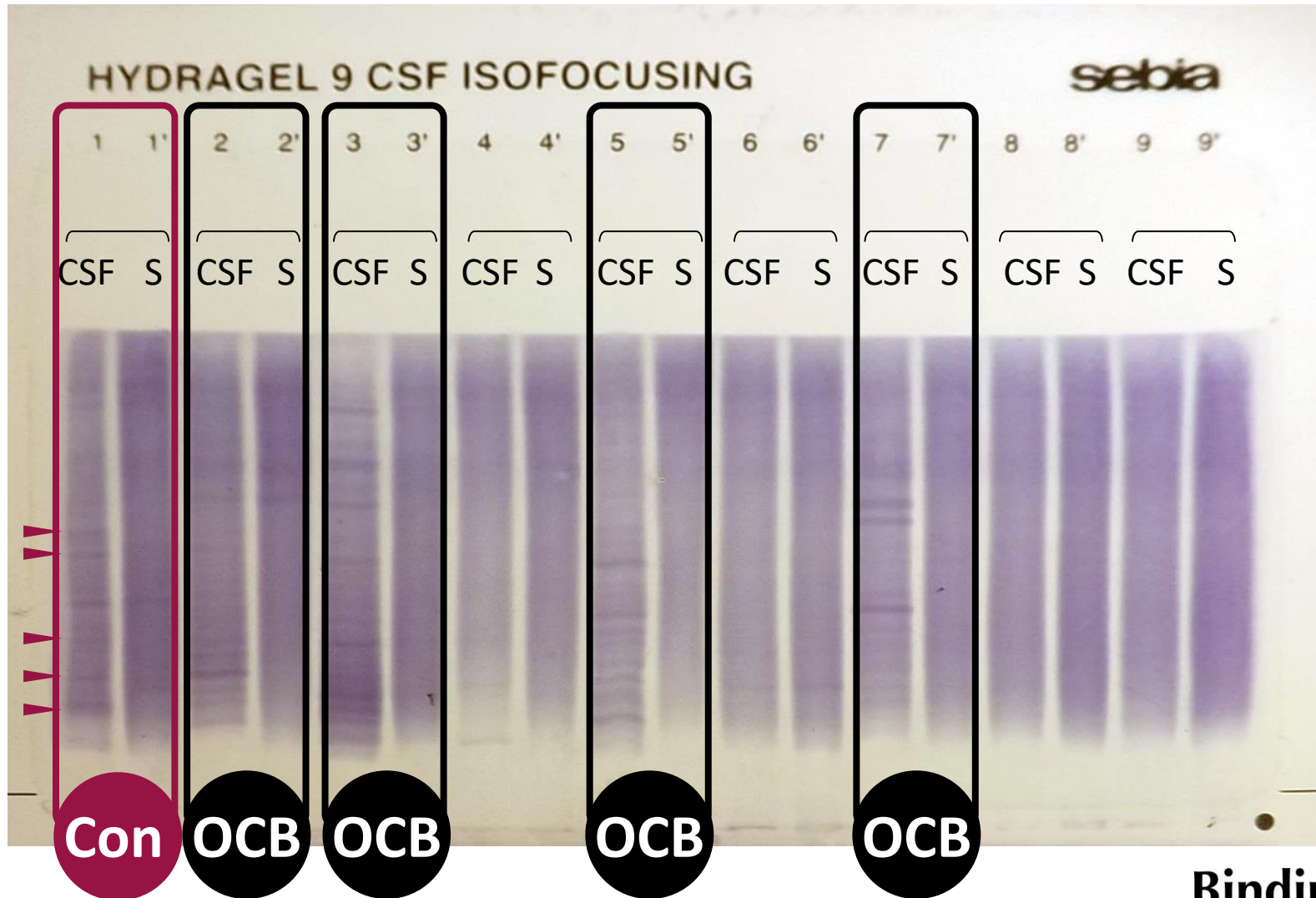
Start

1 hour

2 hours

3 hours

Ejemplo BOC Sebia

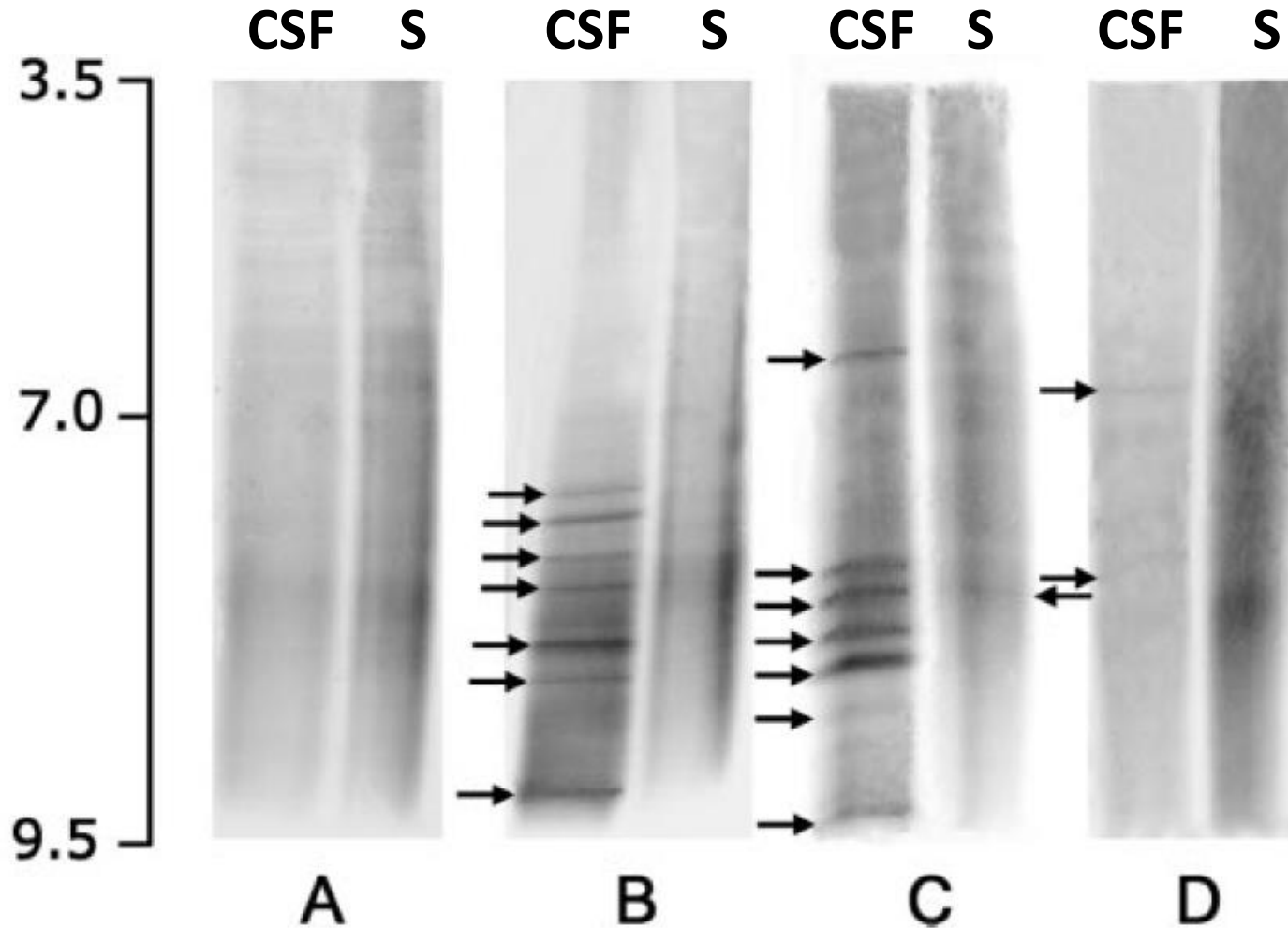


Reproducibilidad de BOC

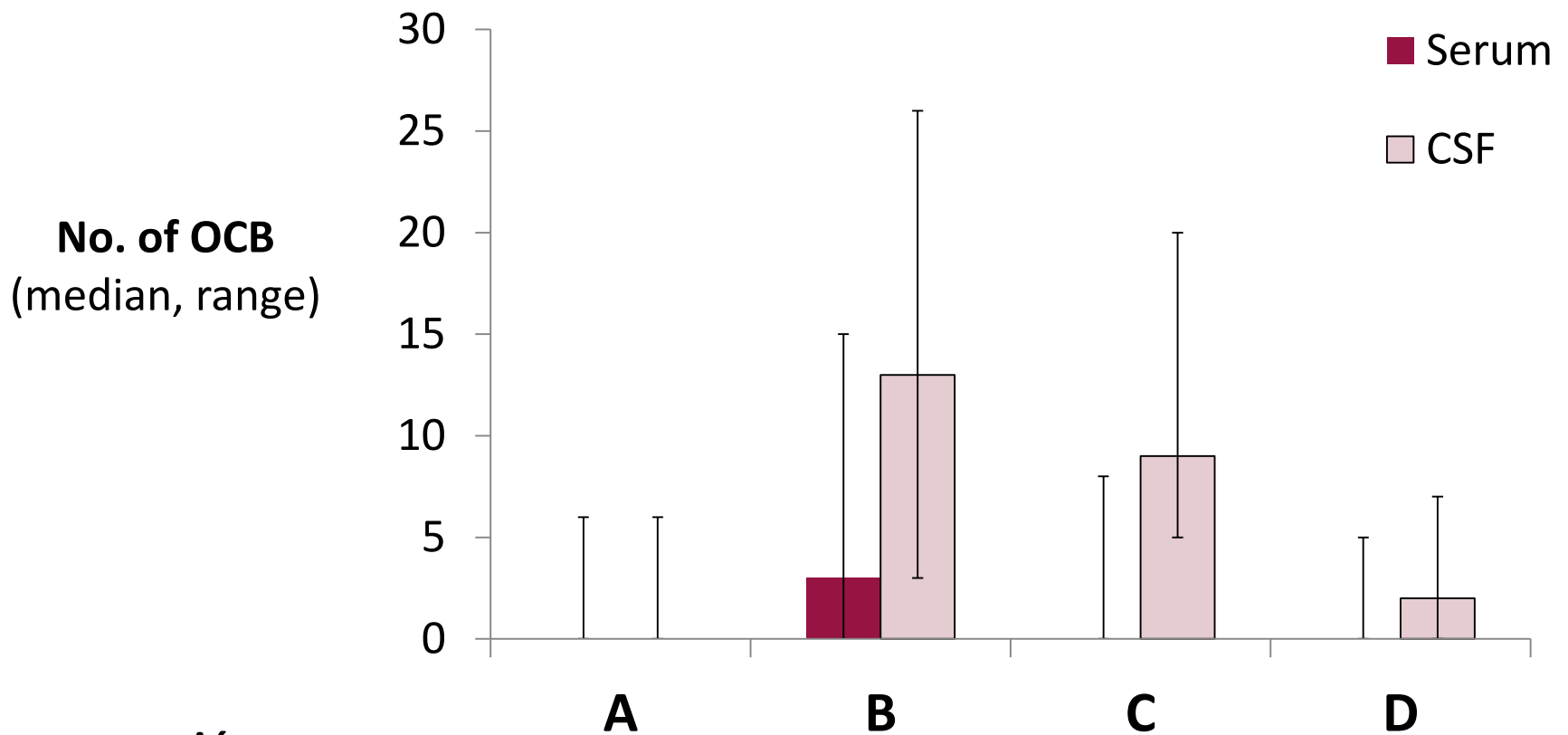
Asociación Italiana de Neuroinmunología

- 20 laboratorios
- Comparación de resultados de BOC para 4 pacientes con SCA

Reproducibilidad de BOC



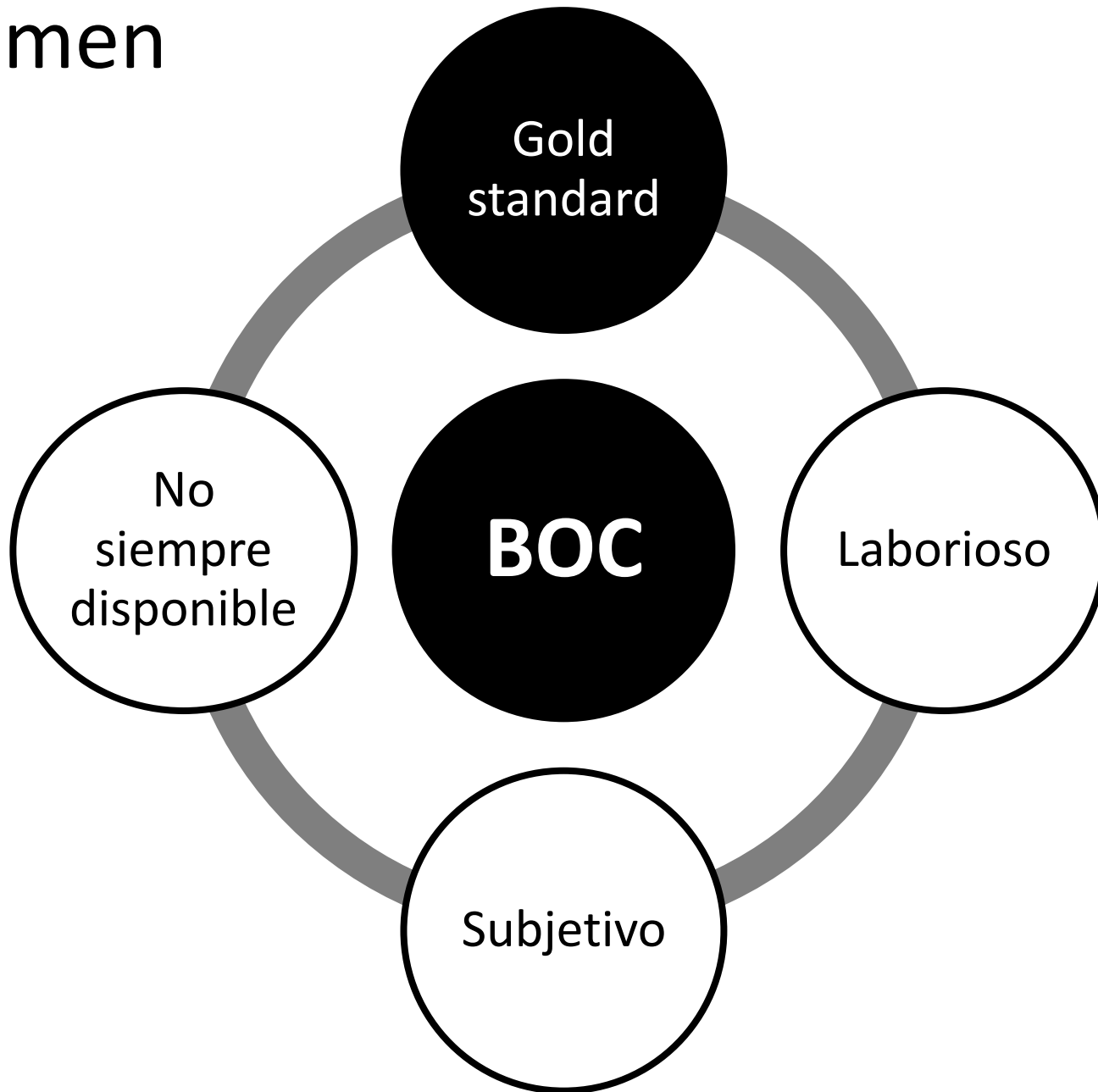
Reproducibilidad de BOC



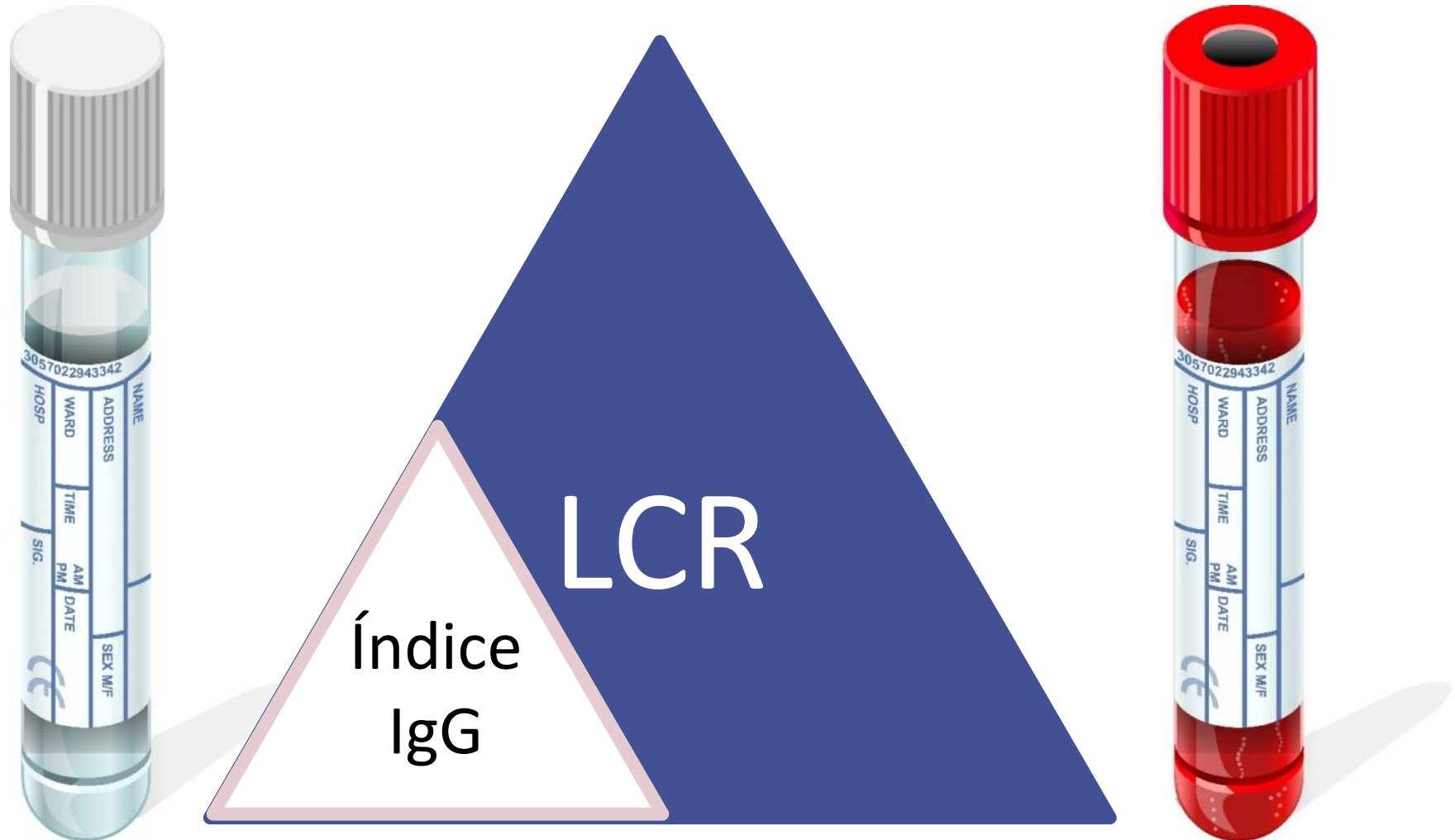
Interpretación

BOC pos		n=20	n=20	n=13
BOC neg	n=15			n=7
BOC neg, patrón espejo	n=5			

Resumen



Detección de Igs intratecales



$$\text{Índice IgG} = \frac{Q_{\text{IgG}}}{Q_{\text{Alb}}}$$

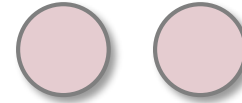
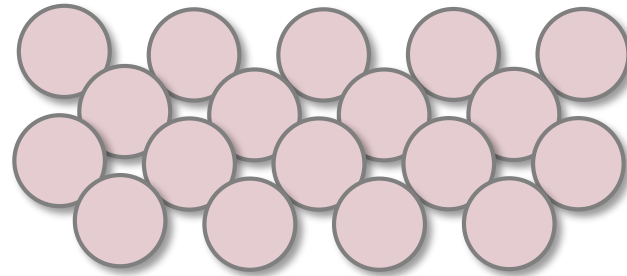
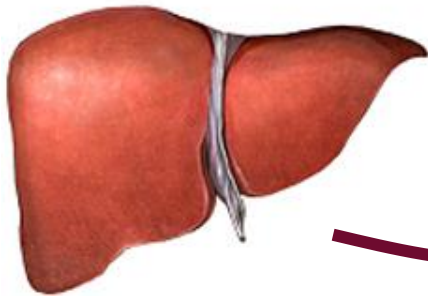
**Albúmina corrige en
base a la función de la
barrera
hematoencefálica**

Individuos normales

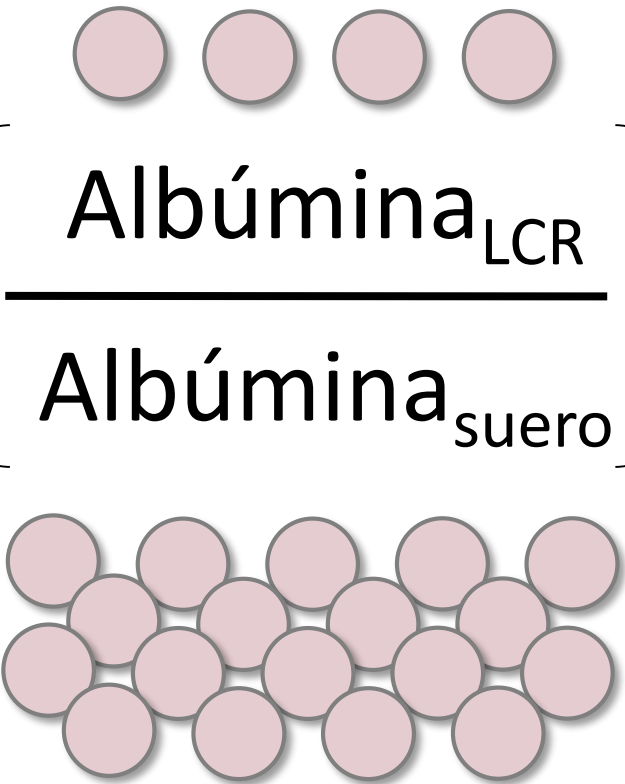
Q_{Alb}

=

$$\left[\frac{\text{Albúmina}_{\text{LCR}}}{\text{Albúmina}_{\text{suero}}} \right]$$

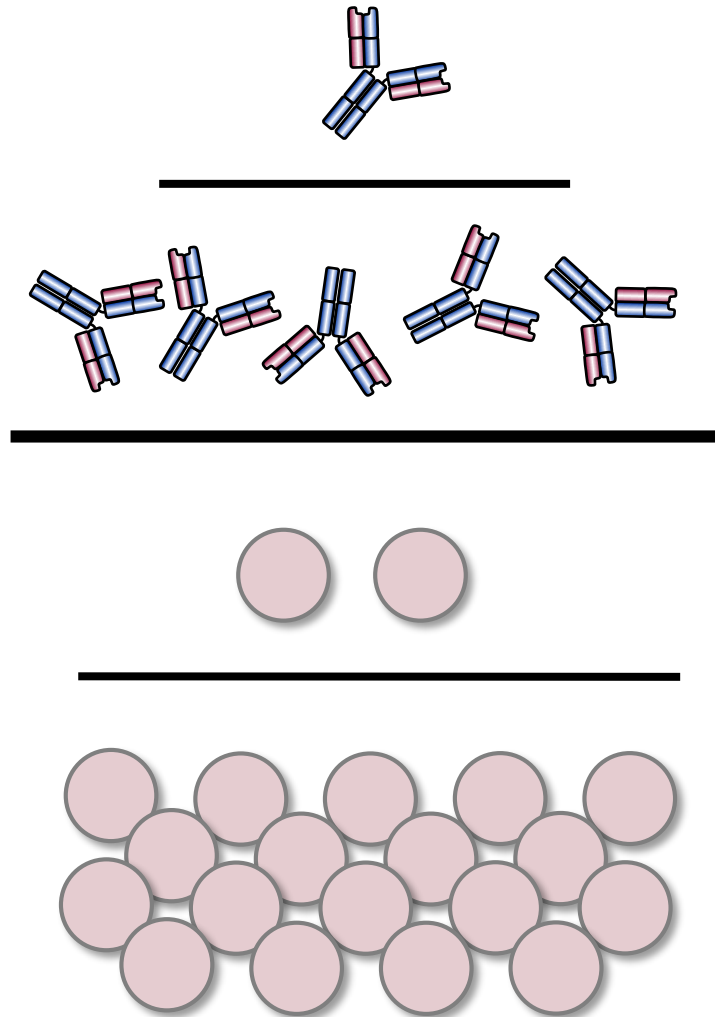


Barrera hematoencefálica dañada

$$Q_{\text{Alb}} = \frac{\text{Albúmina}_{\text{LCR}}}{\text{Albúmina}_{\text{suero}}}$$


$$\text{Índice IgG} = \frac{Q_{\text{IgG}}}{Q_{\text{Alb}}} = \frac{\left(\frac{\text{IgG}_{\text{LCR}}}{\text{IgG}_{\text{suero}}} \right)}{\left(\frac{\text{Albúmina}_{\text{LCR}}}{\text{Albúmina}_{\text{suero}}} \right)}$$

Individuos normales

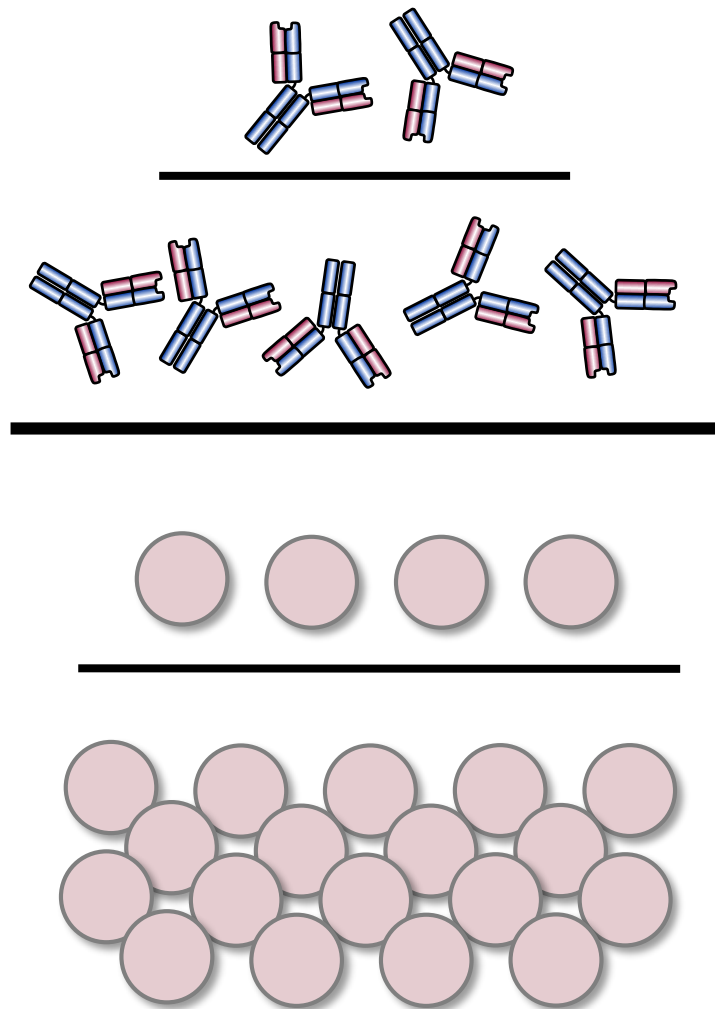


$$\left(\frac{\text{IgG}_{\text{LCR}}}{\text{IgG}_{\text{suero}}} \right)$$

=

$$\left(\frac{\text{Albúmina}_{\text{LCR}}}{\text{Albúmina}_{\text{suero}}} \right)$$

Barrera hematoencefálica dañada

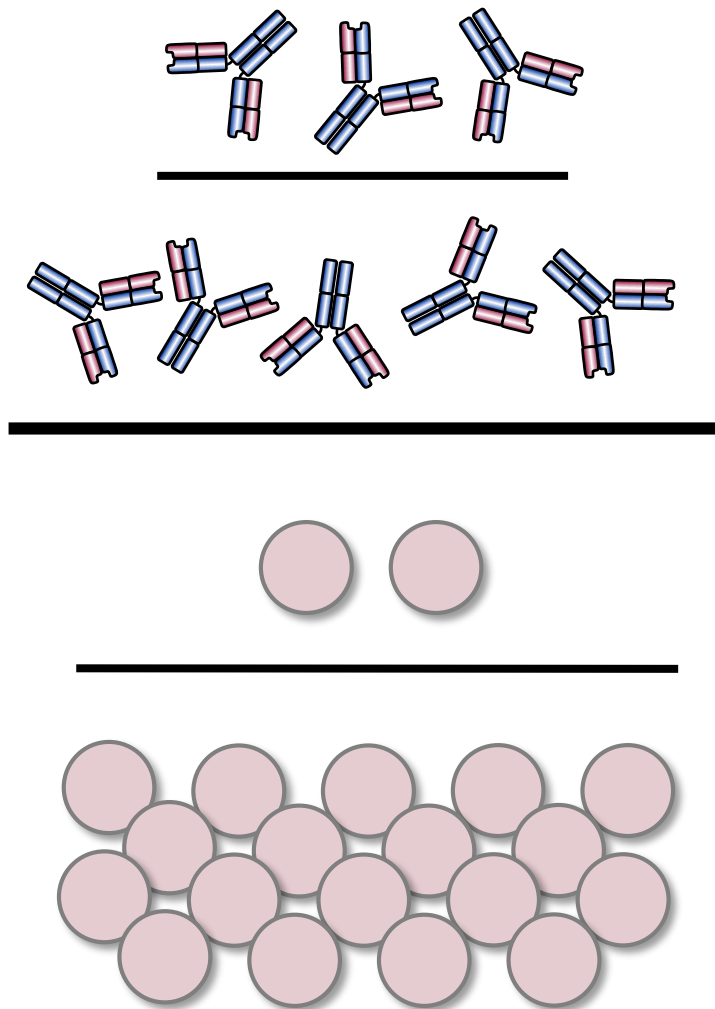


=

$$\left(\frac{\text{IgG}_{\text{LCR}}}{\text{IgG}_{\text{suero}}} \right)$$

$$\left(\frac{\text{Albúmina}_{\text{LCR}}}{\text{Albúmina}_{\text{suero}}} \right)$$

Esclerosis múltiple



=

$$\left(\frac{\text{IgG}_{\text{LCR}}}{\text{IgG}_{\text{suero}}} \right)$$

$$\left(\frac{\text{Albúmina}_{\text{LCR}}}{\text{Albúmina}_{\text{suero}}} \right)$$

Pruebas de laboratorio para detectar Igs intratecales

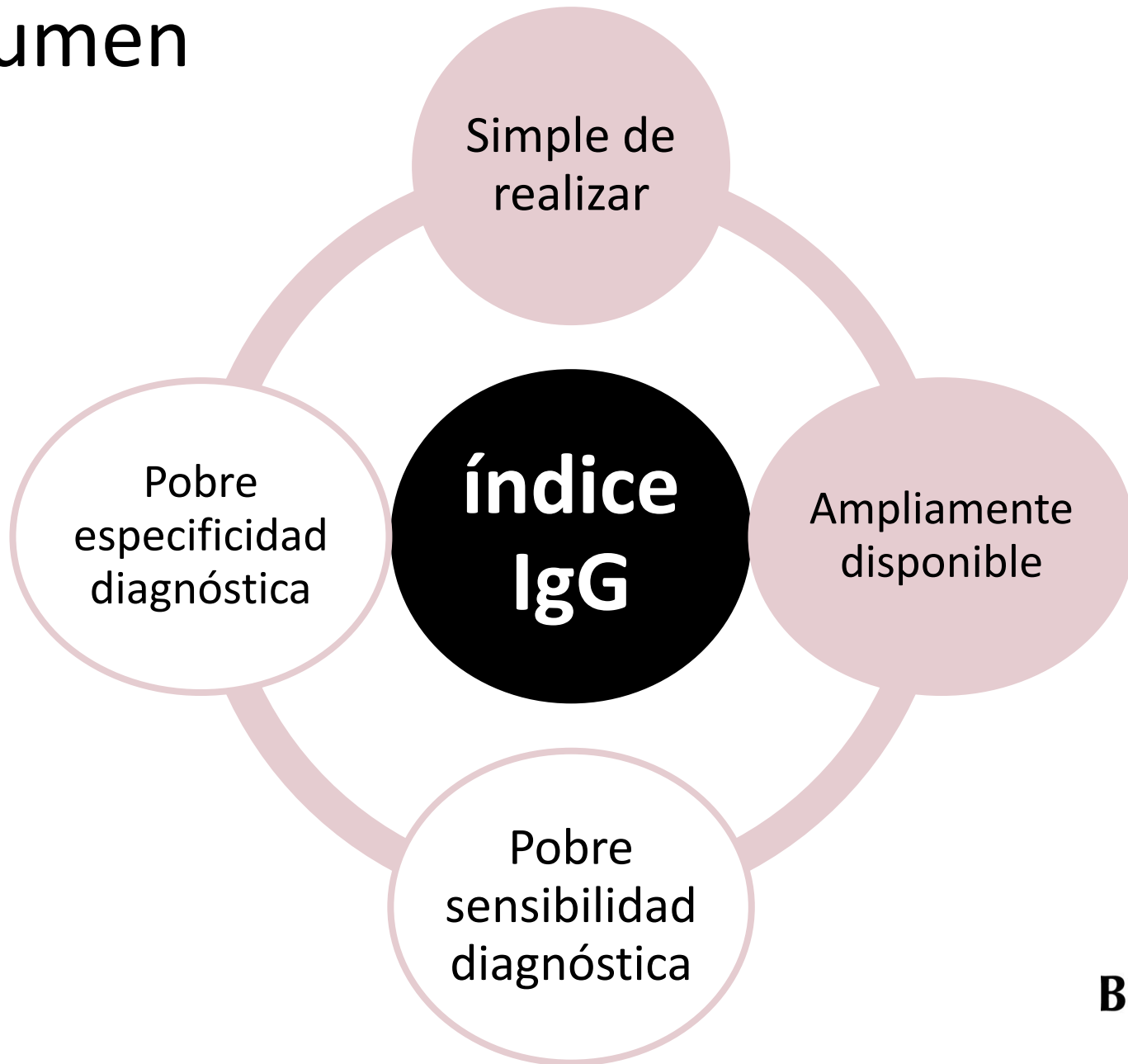
Técnica	Cuantitativa? Automatizada?	Sensibilidad diagnóstica para EM	Especificidad diagnóstica
Bandas oligoclonales	No cuantitativa, Puede ser automatizada	88 – 94%	92%
índice IgG	Si	75 – 85%	77%

Presslauer J Neurol 2008;255:1508-14

Dobson J Neurol Neurosurg Psychiatry 2013;84:909-14

Presslauer PLoS ONE 2014;9:e89945

Resumen



Pruebas de laboratorio para detectar Igs intratecales

Técnica	Cuantitativa? Automatizada?	Sensibilidad diagnóstica para EM	Especificidad diagnóstica
Bandas oligoclonales	No cuantitativa, Puede ser automatizado	88 – 94%	92%
Índice IgG	Si	75 – 85%	77%
CLLs...			

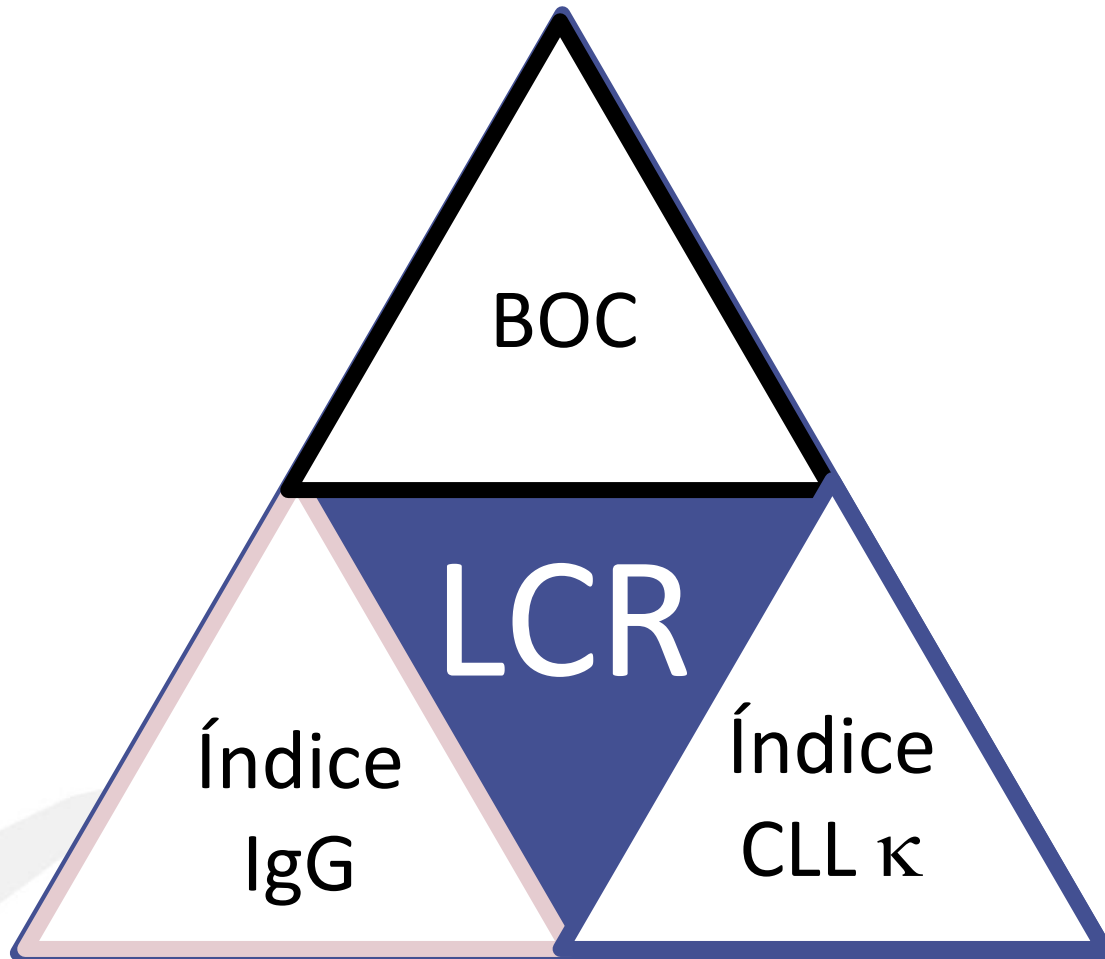
Presslauer J Neurol 2008;255:1508-14

Dobson J Neurol Neurosurg Psychiatry 2013;84:909-14

Presslauer PLoS ONE 2014;9:e89945

CLLs y diagnóstico de EM

Detección de Igs intratecales



Ensayo Freelite en LCR

- CE-marked Freelite Mx CSF kits para Optilite[®]
- CE-marked Freelite CSF kits para SPAPLUS[®]
 - Todos pueden ser usados también para suero (& orina)
- Rangos de medición extendida:

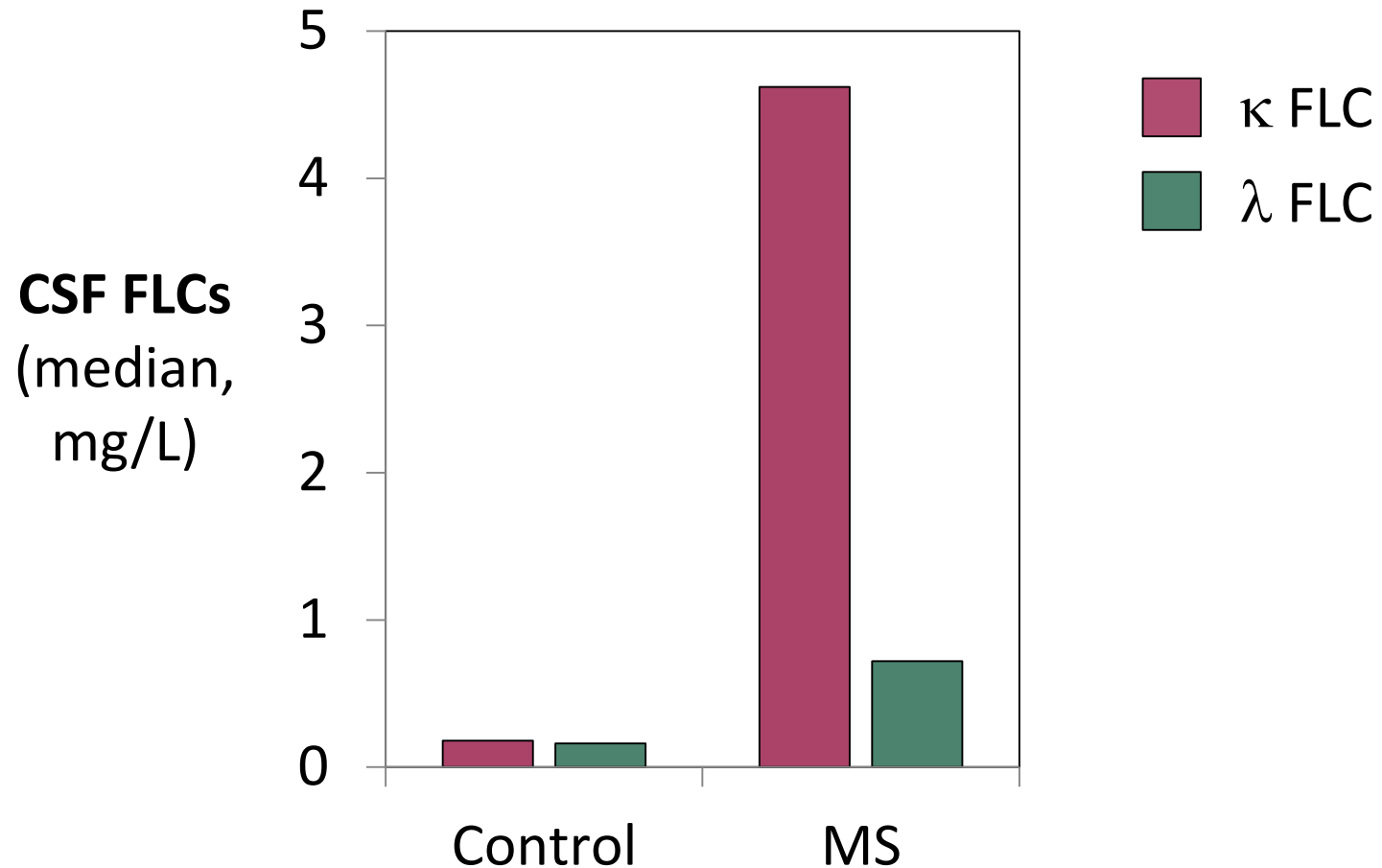
Optilite		Measuring range (mg/L)
κ Freelite	Serum	0.33 – 127,000
	CSF	0.33 – 12.7
λ Freelite	Serum	0.74 – 139,000
	CSF	0.74 – 17.4

Ensayo Freelite en LCR

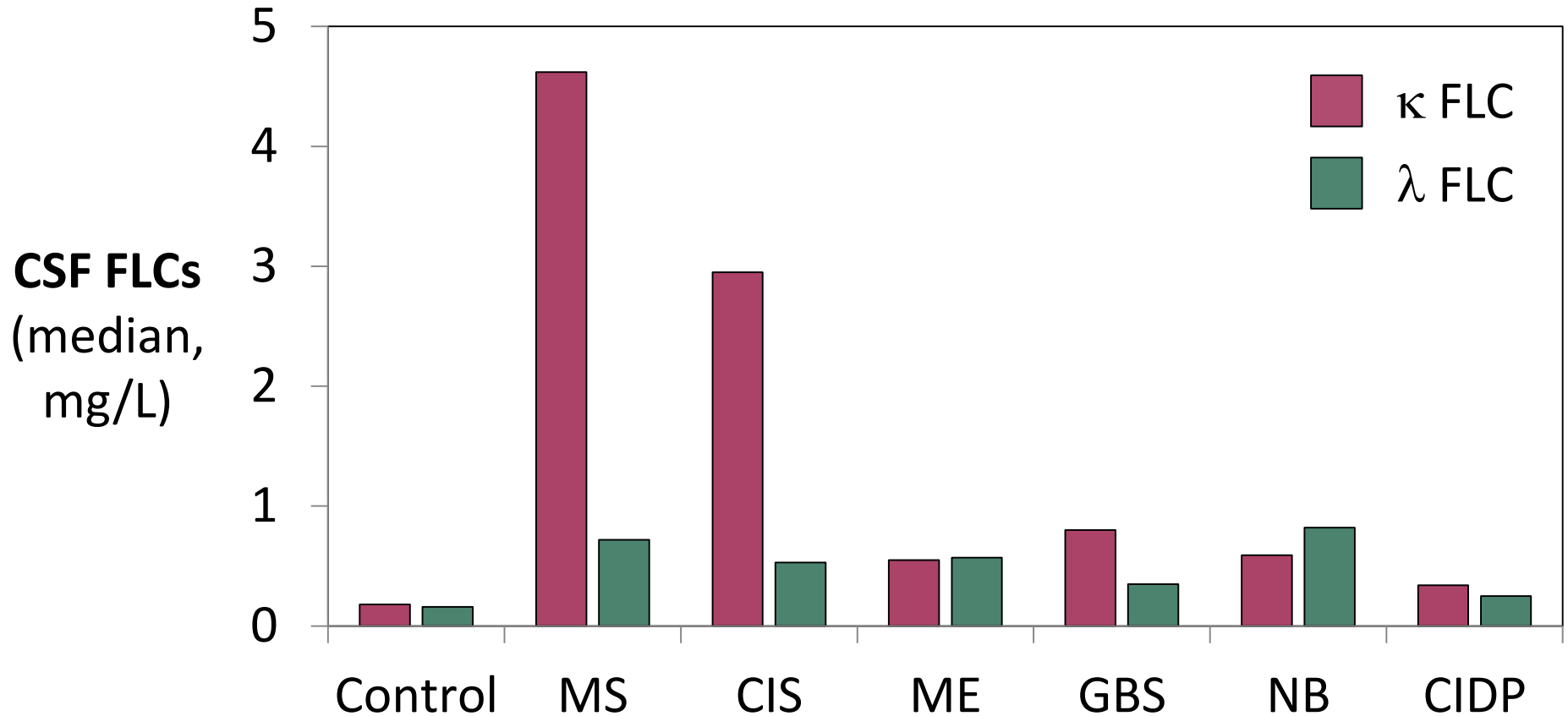
- CE-marked Freelite Mx CSF kits para Optilite[®]
- CE-marked Freelite CSF kits para SPAPLUS[®]
 - Todos pueden ser usados también para suero (& orina)
- Rangos de medición extendida:

SPAPLUS		Measuring range (mg/L)
κ Freelite	Serum	0.4 – 180,000
	CSF	0.1 – 180
λ Freelite	Serum	0.45 – 165,000
	CSF	0.1 – 165

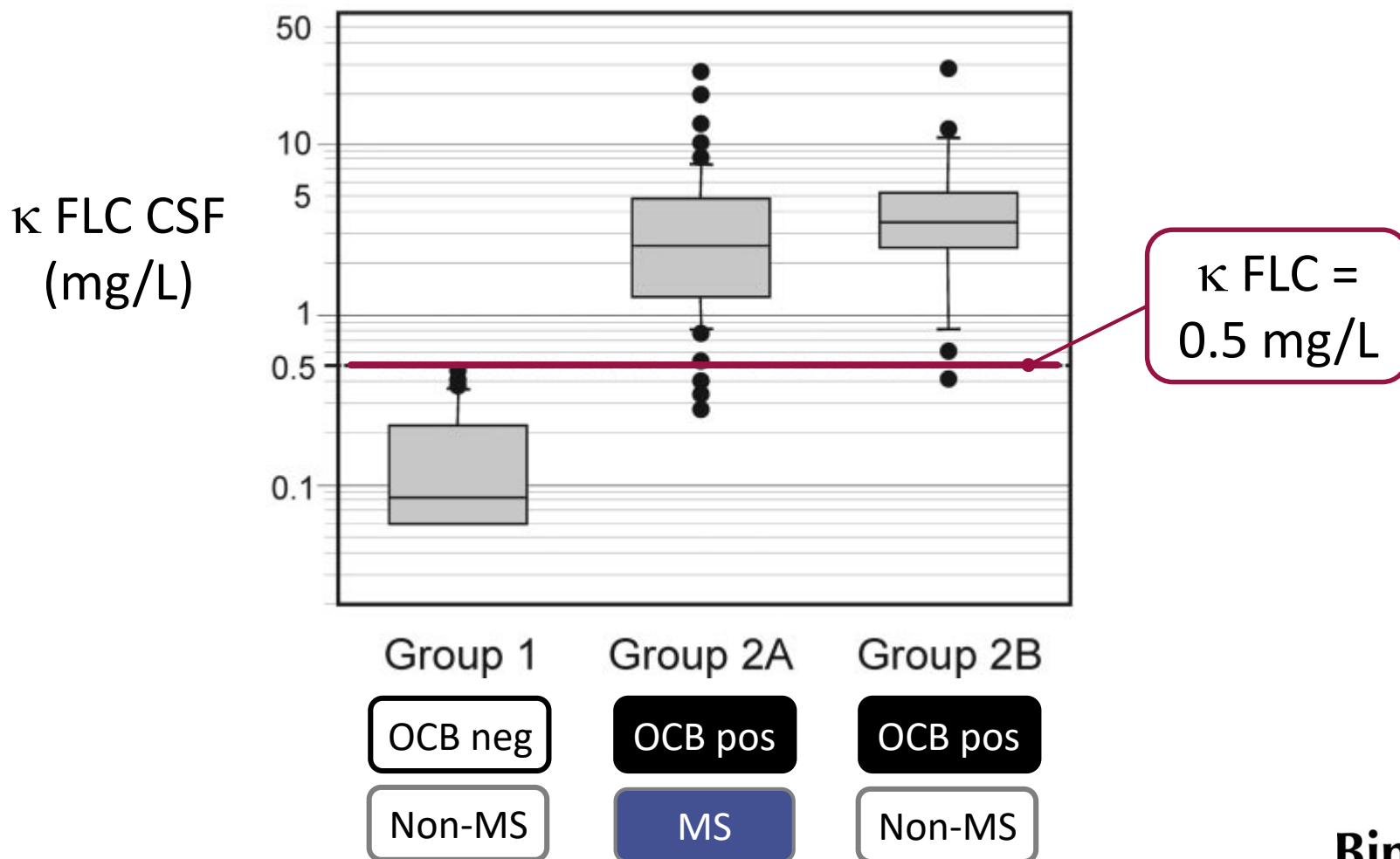
Concentraciones elevadas de CLL en LCR en EM




Concentraciones elevadas de CLL en LCR en EM



BOC positivas asociadas con niveles elevados de CLL κ en LCR



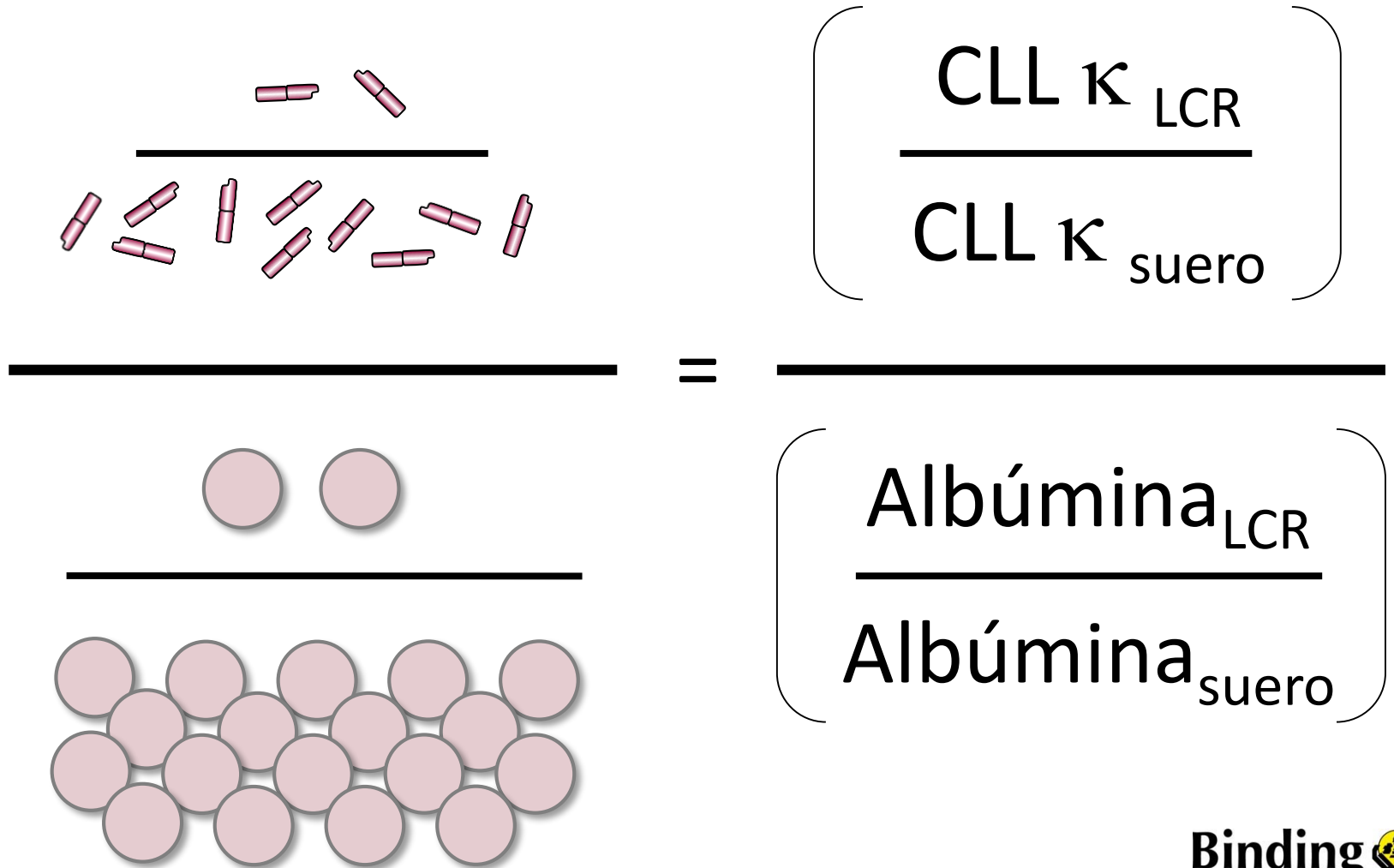

$$\text{Índice CLL } \kappa = \frac{Q_{\kappa \text{ FLC}}}{Q_{\text{Alb}}}$$

**Albúmina corrige en
función de barrera
hematoencefálica**

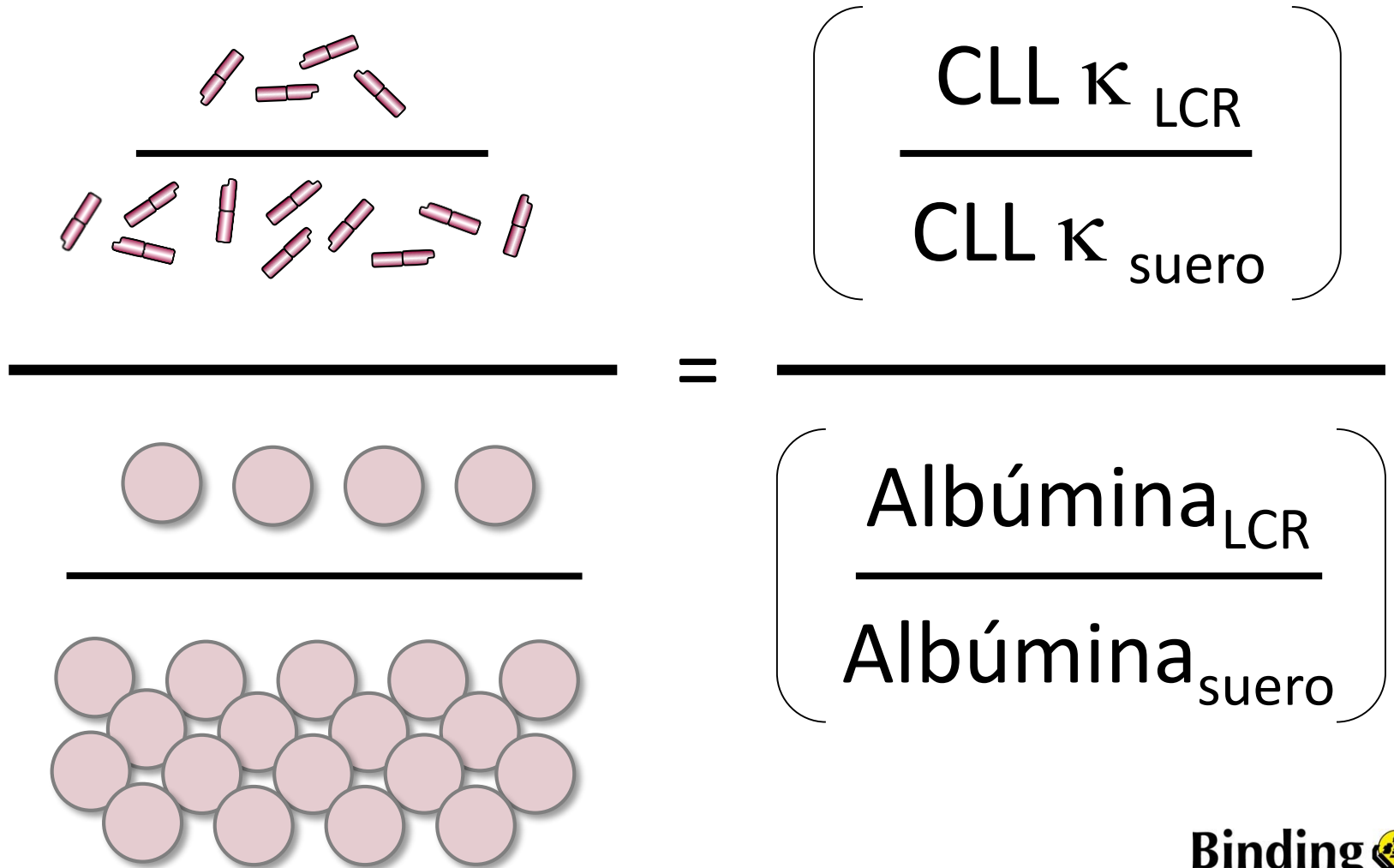


$$\text{Índice CLL } \kappa = \frac{Q_{\kappa \text{ FLC}}}{Q_{\text{Alb}}} = \frac{\left(\frac{\text{CLL } \kappa_{\text{LCR}}}{\text{CLL } \kappa_{\text{suero}}} \right)}{\left(\frac{\text{Albúmina}_{\text{LCR}}}{\text{Albúmina}_{\text{suero}}} \right)}$$

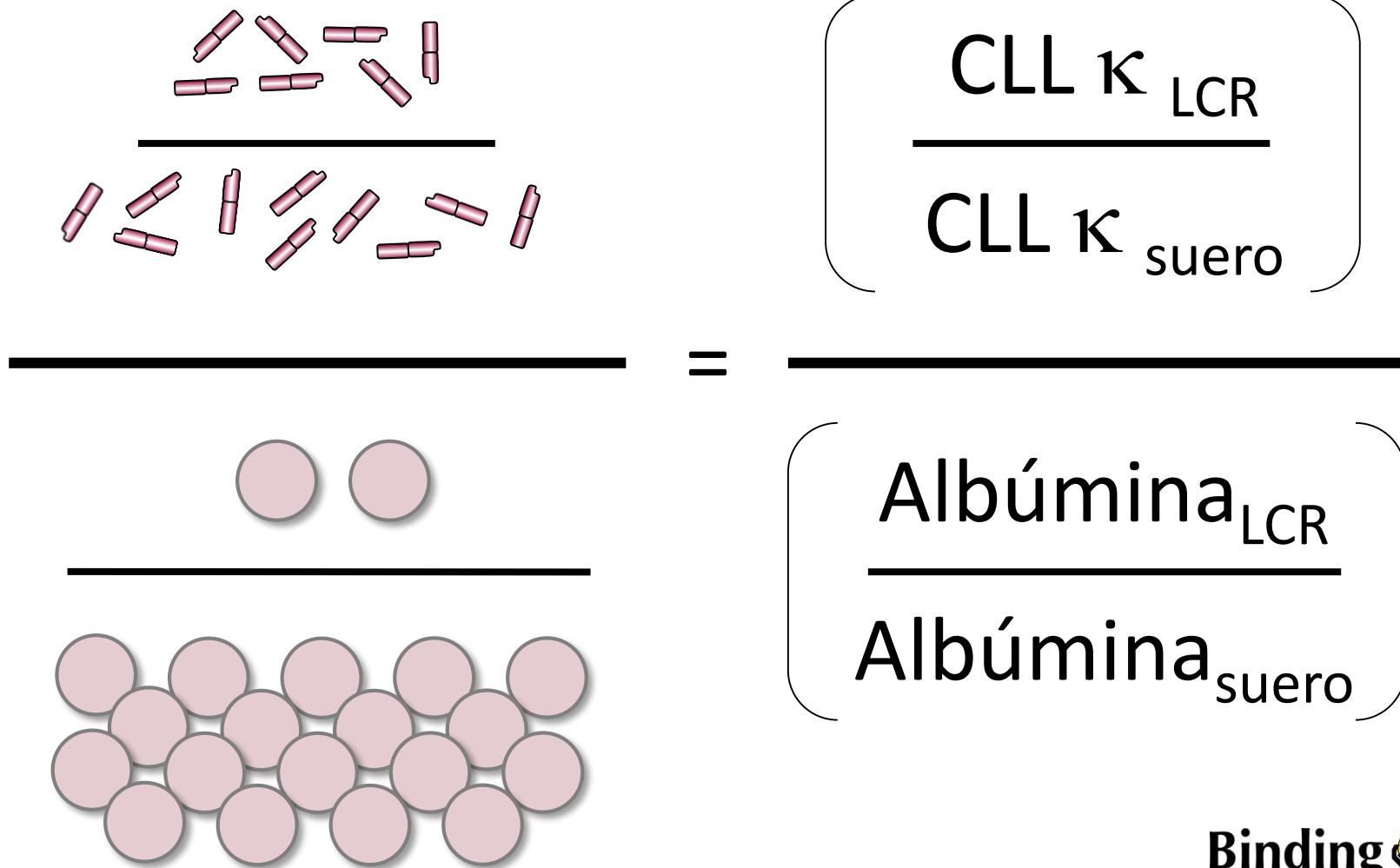
Individuos normales



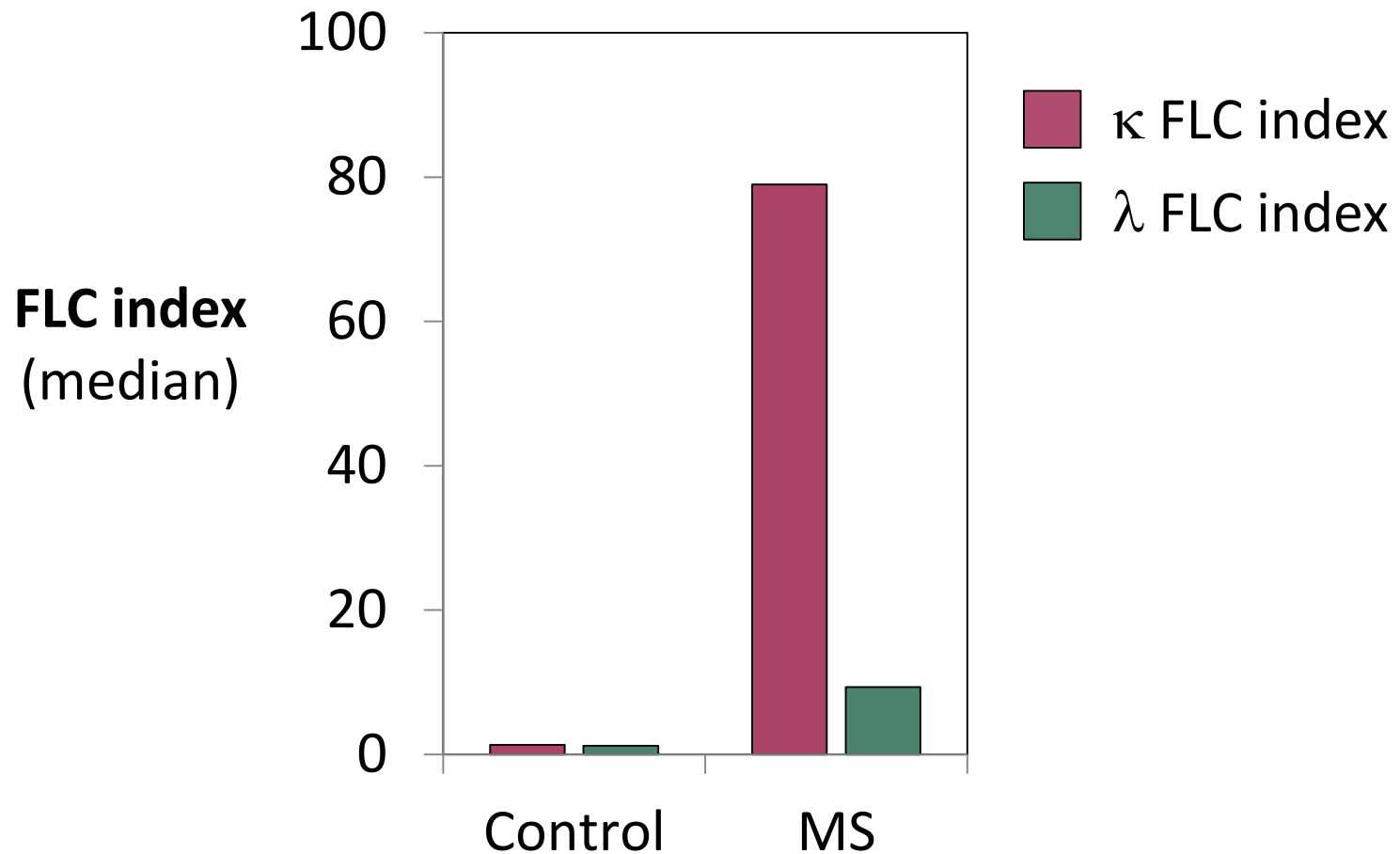
Barrera hematoencefálica dañada



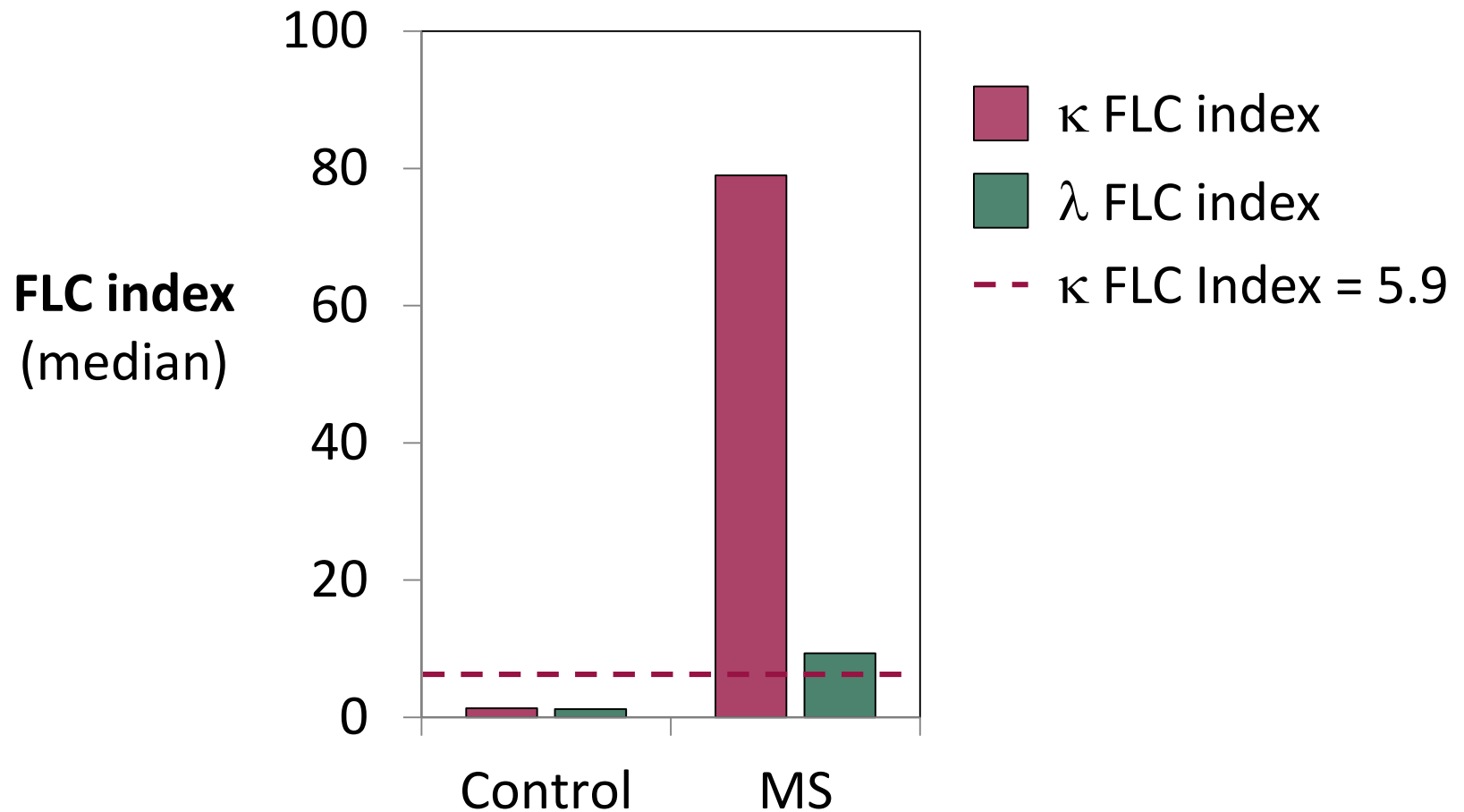
Esclerosis múltiple



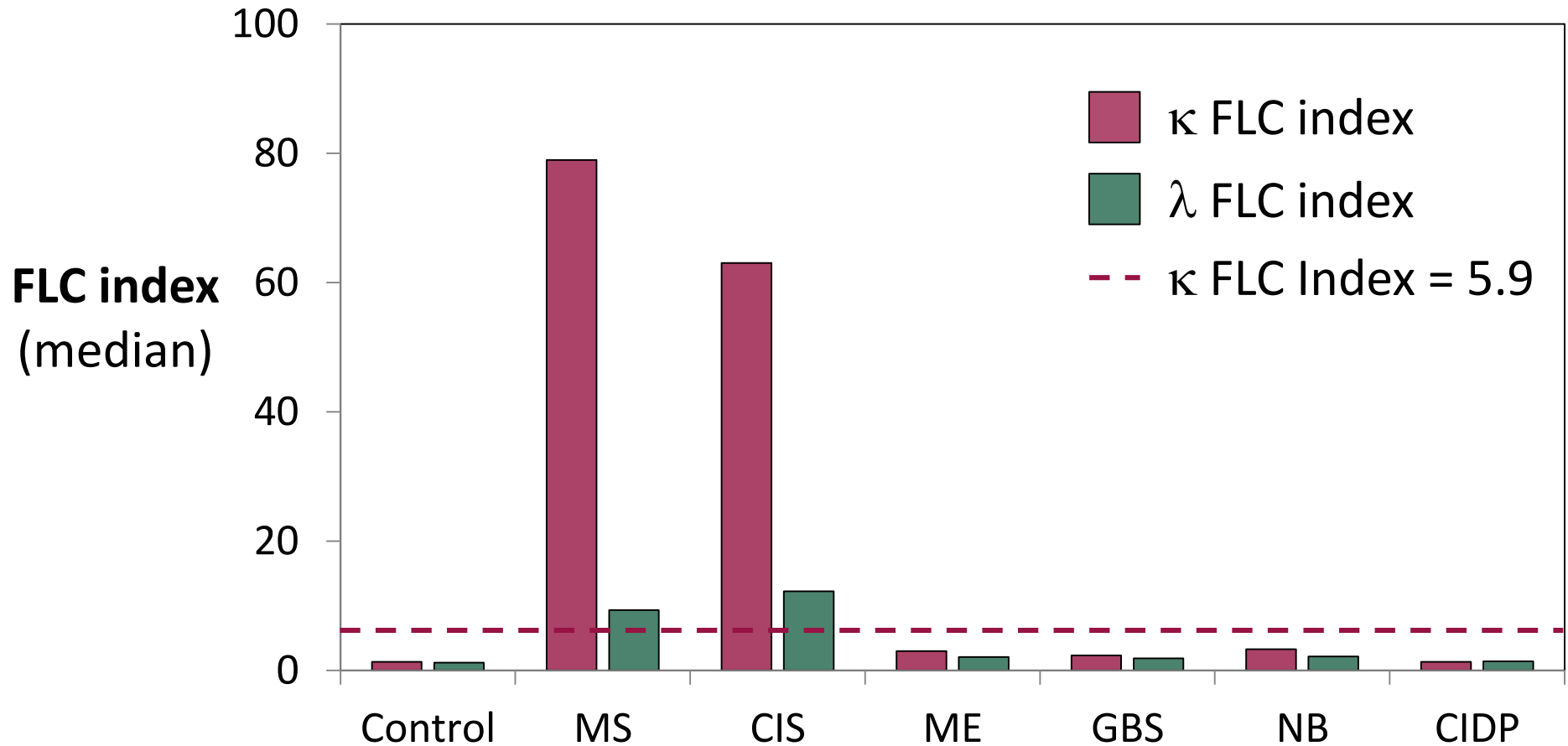
Índice de CLL elevado en EM



Índice de CLL elevado en EM



Índice de CLL elevado en EM



Pruebas de laboratorio para detectar Igs intratecales

Técnica	Cuantitativa? Automatizada?	Sensibilidad diagnóstica para EM	Especificidad diagnóstica
Bandas oligoclonales	No-cuantitativa, Puede ser automatizada	88 – 94%	92%
Indice IgG	Si	72 – 85%	77%
Indice κ CLL (≥ 5.9)	Si	96%	86%

Presslauer J Neurol 2008;255:1508-14 Presslauer Mult Scler 2016;22:502-10

Dobson J Neurol Neurosurg Psychiatry 2013;84:909-14 Presslauer PLoS ONE 2014;9:e89945

Comparación de índice κ CLL y BOC

	No. of patients			κ FLC index			OCB	
	MS (CIS)	ONID	NIND	Cut-off	Sensitivity	Specificity	Sensitivity	Specificity
Presslauer	41 (29)	78	45	≥ 5.9	96	86	91	92
Leurs	297 (274)	204		6.3	94	83	86	92
Bernadi	24	0	51	7.82	95	98	100	96
Gurtner	62 (3)	104	78	≥ 8.868	88.1	88.7	94.0	84.0
Menendez-Valladares	29	14	56	10.62	93.1	95.7	96.5	98.6
Bayart	59	44	39	> 12.45	78.0	77.1	78.0	79.5
Valencia-Vera	37	85		≥ 2.91	83.78	85.88	89.19	81.18

Presslauer J Neurol 2008;255:1508-14

Leurs Presented at ECTRIMS 2017:P1131a

Bernardi Biochimica Clinica 2013;37:389-94

Gurtner Clin Chem Lab Med 2018;56:1071-80

Menendez-Valladares Multiple Sclerosis J - Exp Transl Clin 2015

Bayart Acta Neurol Scand 2018;138:352-8

Valencia-Vera Clin Chem Lab Med 2017;56:609-613

Editorial

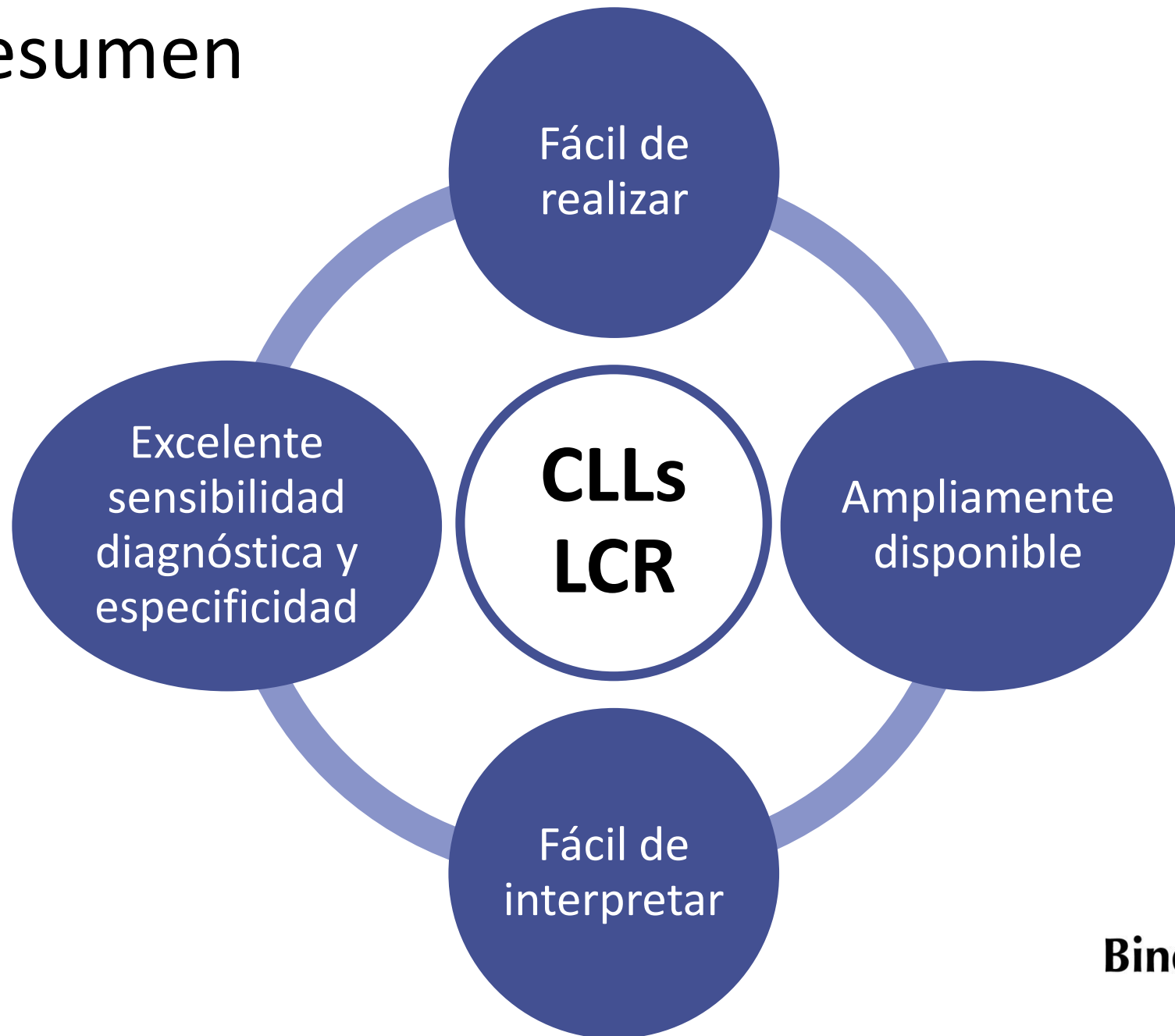
David Zeman

Free light chains in the cerebrospinal fluid. Do we still need oligoclonal IgG?

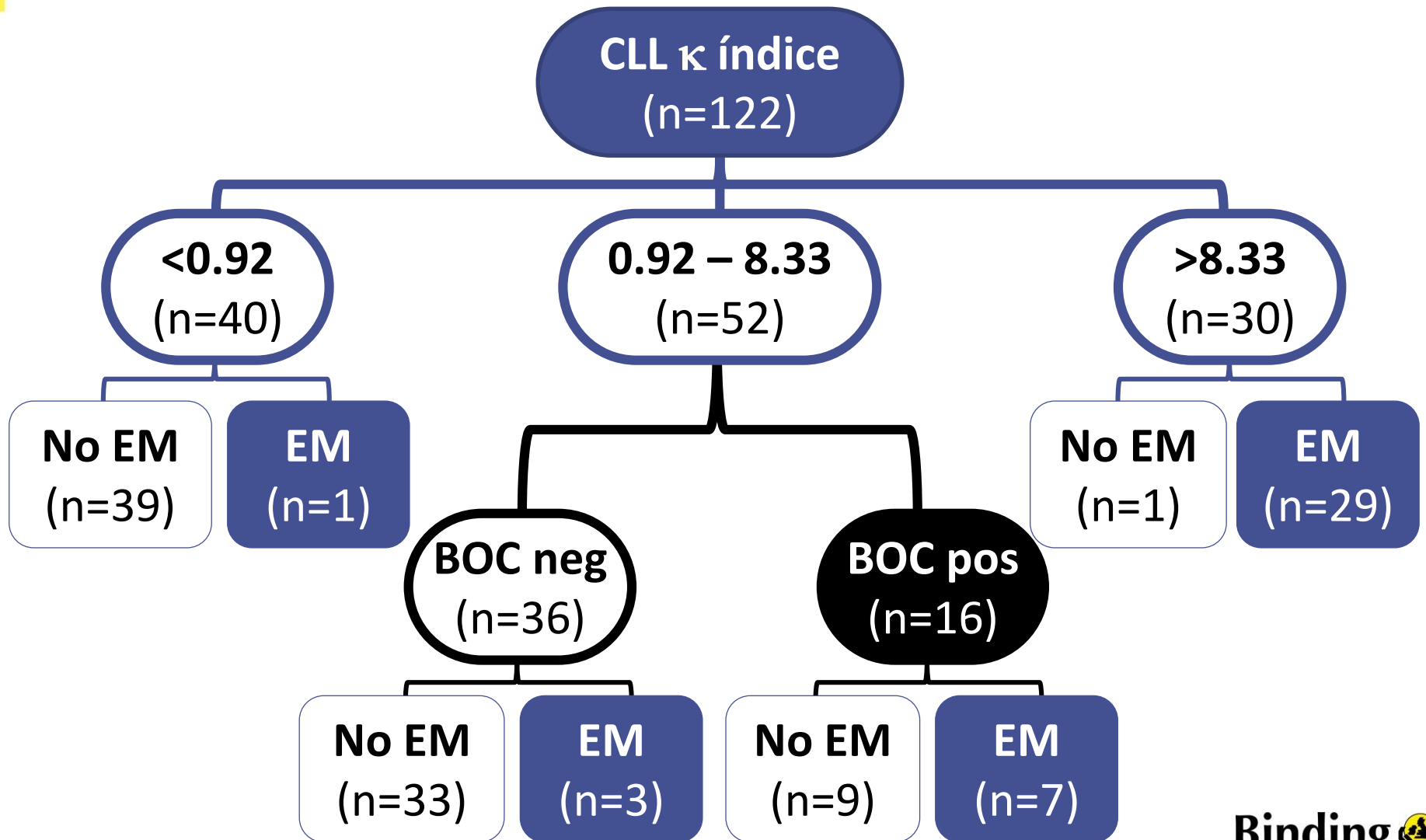
“...quantitative CSF κ FLC analysis has just entered clinical routine.”

“Perhaps the next revision of McDonald criteria could mention intrathecal κ FLC synthesis as a suitable alternative to oligoclonal IgG.”

Resumen

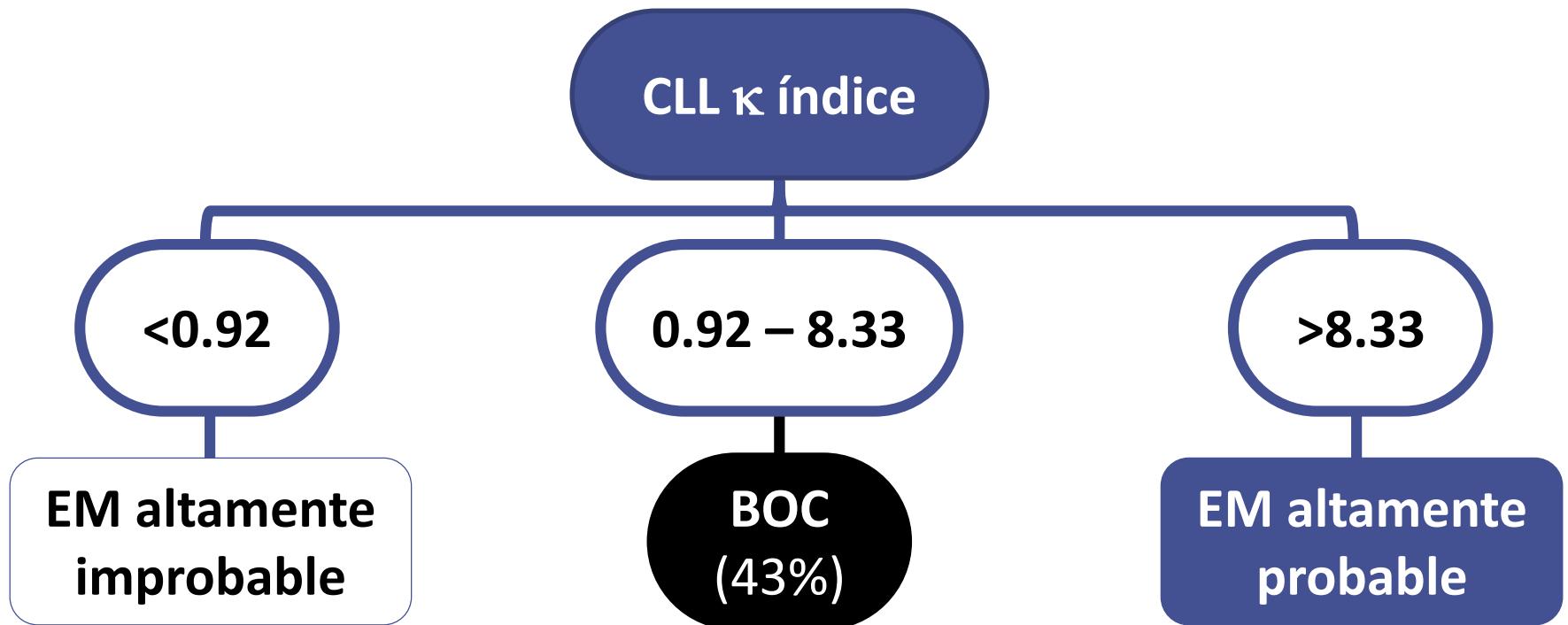


Algoritmo de screening para sospecha de EM



Algoritmo de screening para sospecha de EM

“...we propose κ FLC determination as a screening tool to select which CSF samples should be candidates for OCB tests.”



Resumen:

CLLs en EM y SCA

- CLLs κ están altamente elevadas
- CLL λ están moderadamente elevadas

Evidencia de aval al diagnóstico de EM

- Un índice IgG elevado
- ≥ 2 bandas oligoclonales
- **Un índice CLL κ elevado**

Marcadores
prognóstico para
predecir conversión
SCA

- ≥ 2 bandas oligoclonales
- **Un índice CLL κ elevado**

¿Preguntas?

florencia.delgado@bindingsite.com

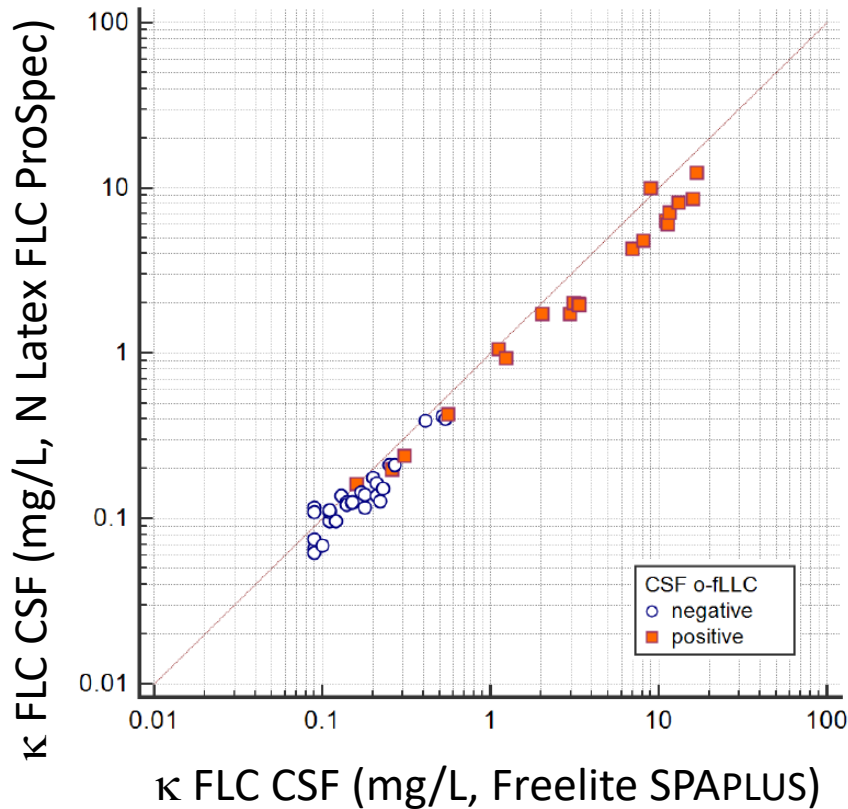
Wikilite
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Chapter	
36	Cerebrospinal fluid and free light chains

Appendix

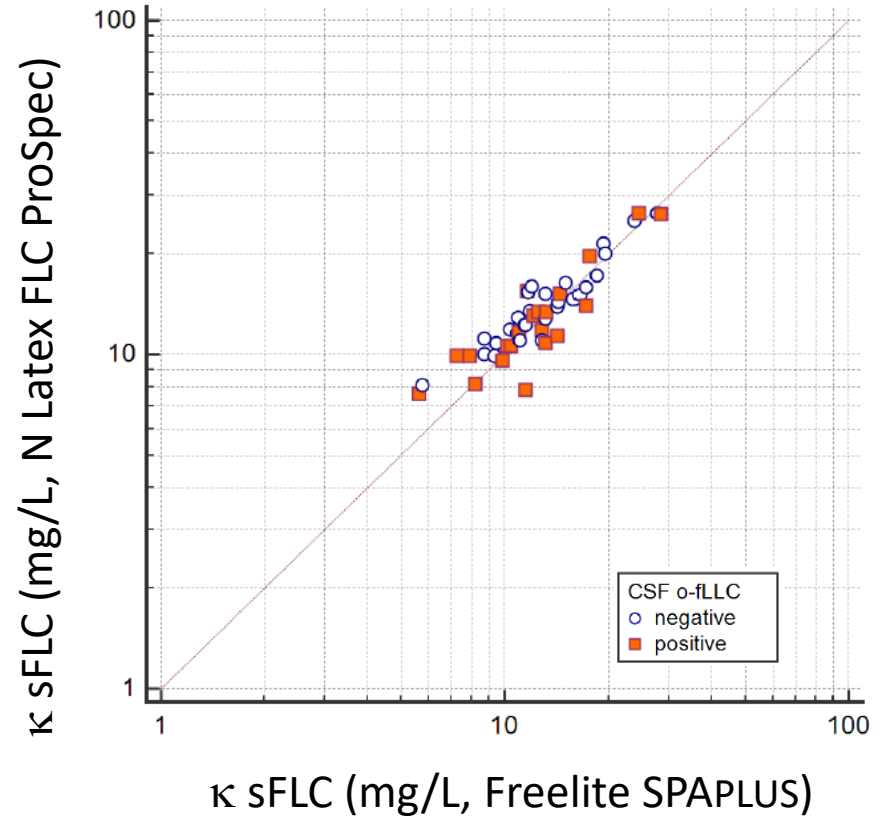
Comparison study: κ FLCs

CSF



$$y=0.63x + 0.03$$

Serum



$$y=0.92x + 1.70$$

Comparison study: κ FLCs

	n (MS/non-MS)	Cut-off	Sensitivity (%)	Specificity (%)
IgG OCB	28/95	≥ 2 OCB	85.7	88.4
κ FLC index Freelite	26/92	>4.12	92.3	85.7
κ FLC index N Latex FLC	5/27	>19.8	80.0	96.3

Choosing a method: practical considerations

‘...we chose the **Freelite** turbidimetric assay on SPAPLUS analyser, which is economic and user-friendly...’

‘...[**N Latex FLC**] results in a high consumption of reagents...
...which is a major drawback...’

Each method as well as each analyser has its own limits and advantages. Finally, we chose the Freelite turbidimetric assay on SPA_{PLUS} analyser, which is economic and user-friendly; manual dilution of the CSF sample is rarely required for fKLC and almost never for fLLC. Nevertheless, ELISA based assays might be better applicable in some laboratories worldwide where access to automated instruments is limited. In addition, diluted CSF is used in the ELISA assay, which leads to a much lower amount of sample required for analysis. Although the sample volume in the automatised assays is low (SPA_{PLUS} requires 10 µl for fKLC and 6.5 µl for fLLC analysis, i.e. 21.5 µl for both measurements, while 25 µl for both measurements were required for the analysis on BN ProSpec), we still require a sample volume that is slightly above 100 µl in both cases. Nephelometric methods (either Freelite or N Latex FLC) might be best suited for scientific purposes. Freelite has a marginally better sensitivity for fKLC (about 0.05 mg/L versus 0.1 mg/L on the BN ProSpec instrument), in consequent dilutions it strives to achieve a signal in the sample that is in the middle of the calibration curve where the measurement can be expected to be most precise. On the other hand, this results in a high consumption of reagents for samples with high CSF fLLC concentrations, which is a major drawback of this method.