NMOSD and MOGAD: Laboratory diagnostic

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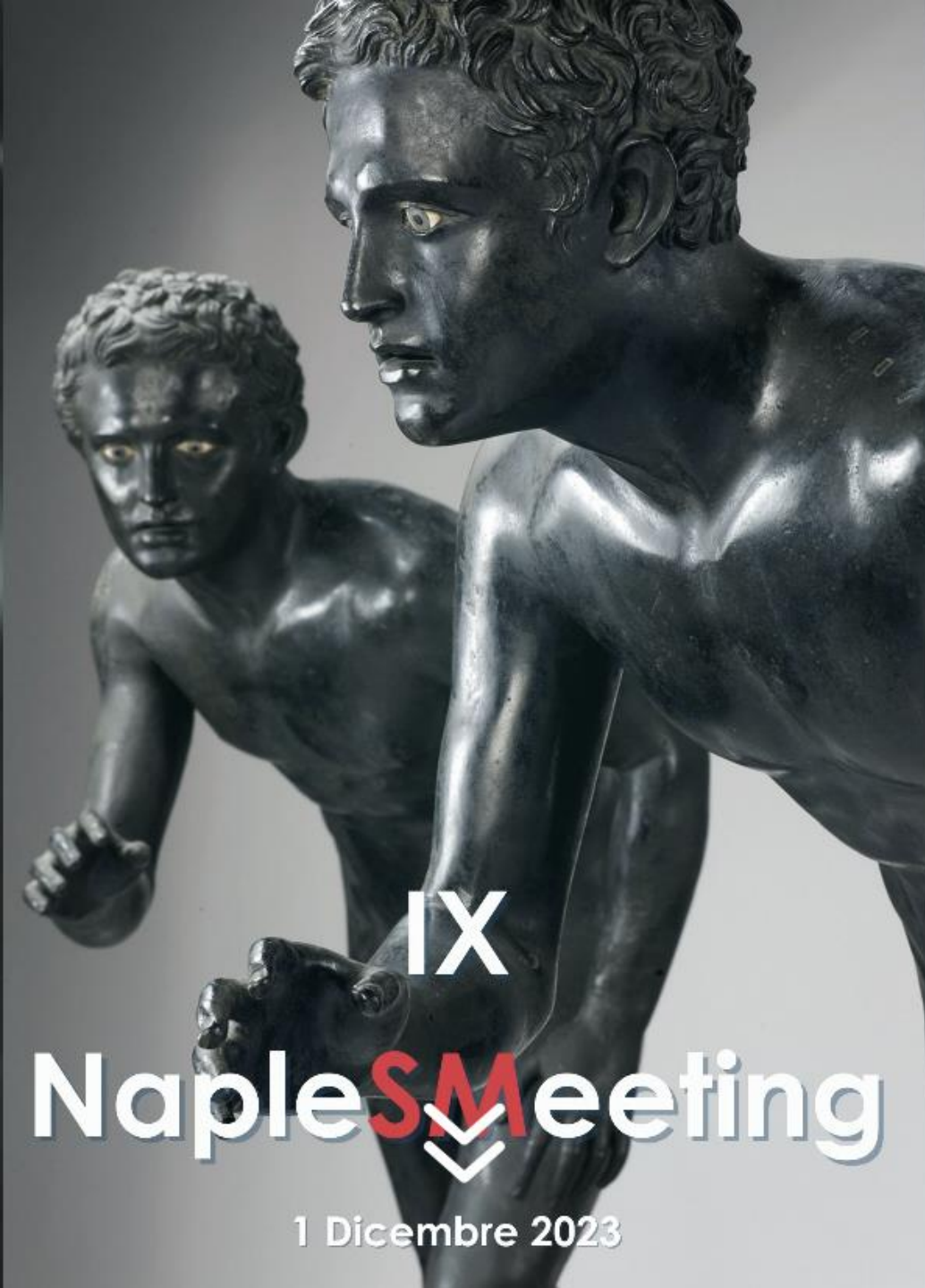
Summary

NMOSD

MOGAD

Neuromyelitis optica spectrum disorder

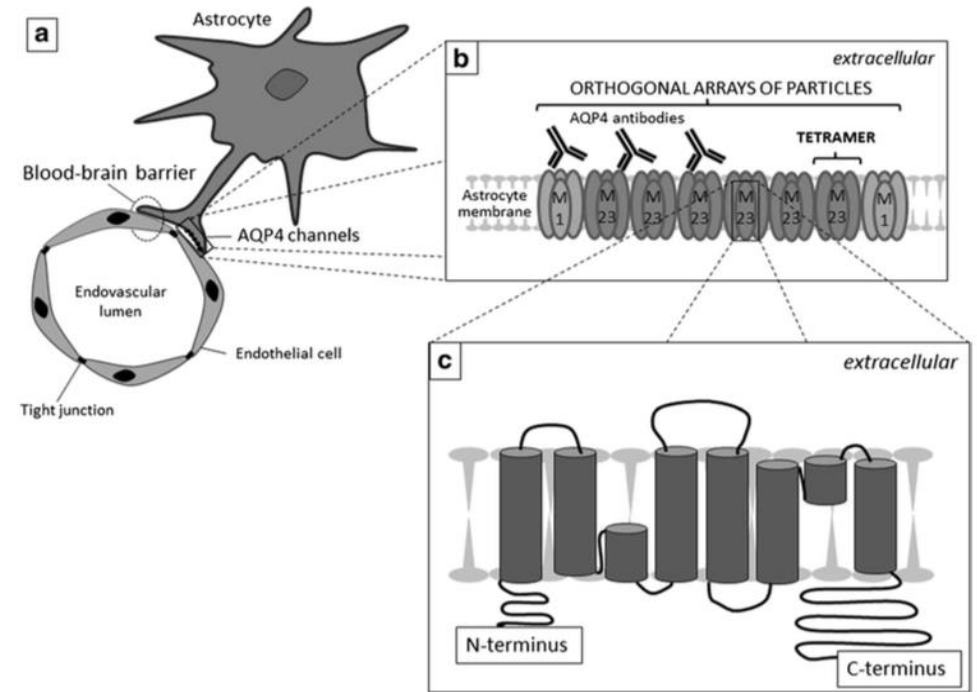A serum autoantibody marker of neuromyelitis optica: distinction from multiple sclerosis

Vanda A Lennon, Dean M Wingerchuk, Thomas J Kryzer, Sean J Pittock, Claudia F Lucchinetti, Kazuo Fujihara, Ichiro Nakashima, Brian G Weinshenker

AQP4 antibodies
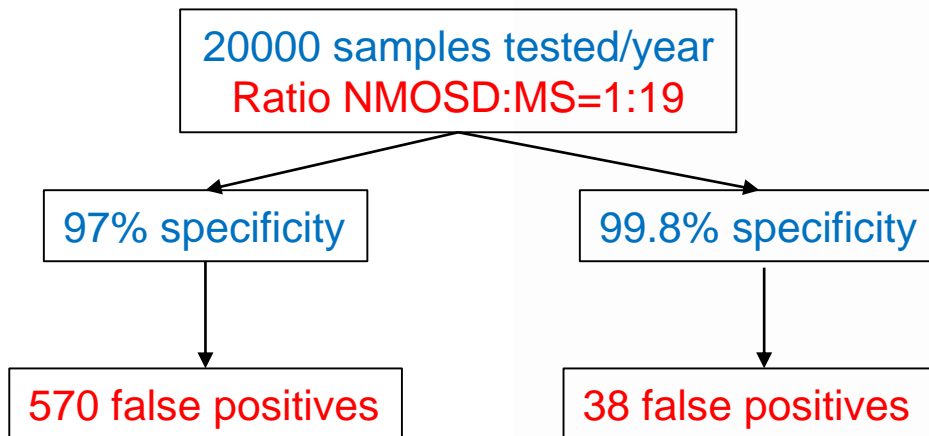
Pial layer

Virkow Robin's spaces

Lennon, 2007; Gastaldi, 2017
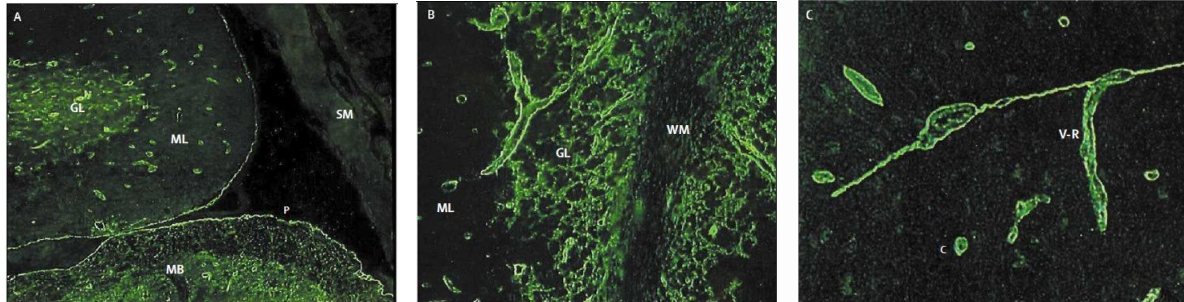
Laboratory testing for AQP4 antibodies

Priorities in AQP4 abs testing

- Most patients tested do not have NMOSD (low prevalence)
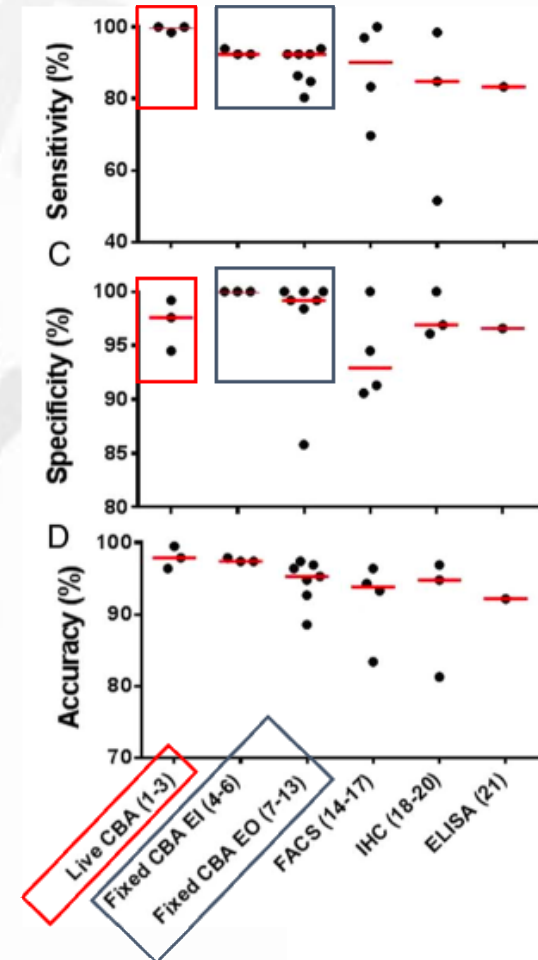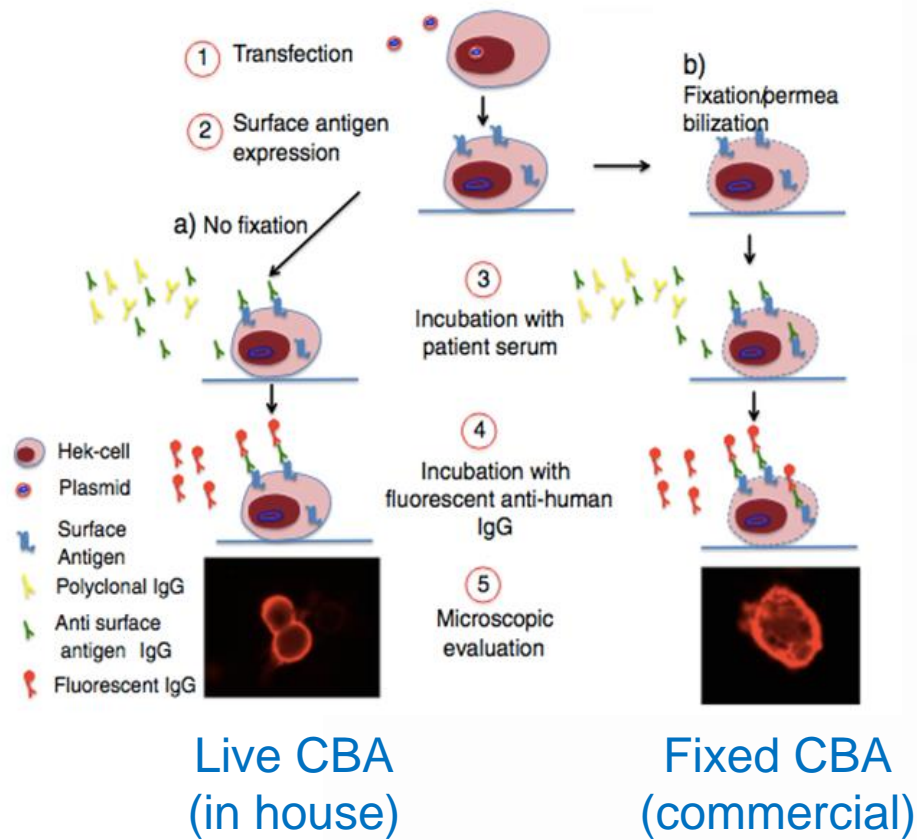- Small differences in specificity have a relevant impact

20000 samples tested/year
Ratio NMOSD:MS=1:19

97% specificity

99.8% specificity

570 false positives

38 false positives

Highly specific assay are required when the positive rate is low

CBAs for the detection of AQP4 antibodies

Cell Based Assay (CBA)

Live CBA
(in house)

Fixed CBA
(commercial)

Gastaldi, Neurotherapeutics 2016; Waters, Clin Exp Neuroimmunol 2014

Live vs Fixed CBA for anti-AQP4 antibodies

1882 consecutive samples sent for AQP4 testing
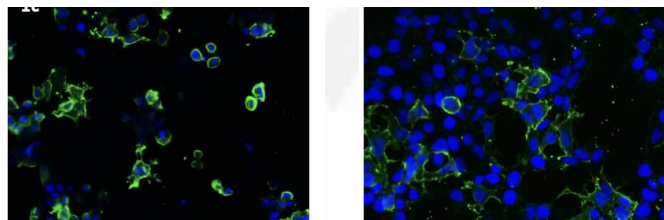
Live CBA + Fixed CBA

104 samples positive

13/104 had discrepant results

	LCBA	CCBA	LCBA+CCBA
Specificity	0.99	0.99	1.0
PPV	0.89	0.94	0.99

Diagnosis of NMOSD (%)

100%	50%	44%
(n 87)	(n 4)	(n 9)
CCBA+/LCBA+	CCBA+/LCBA-	CCBA-/LCBA+
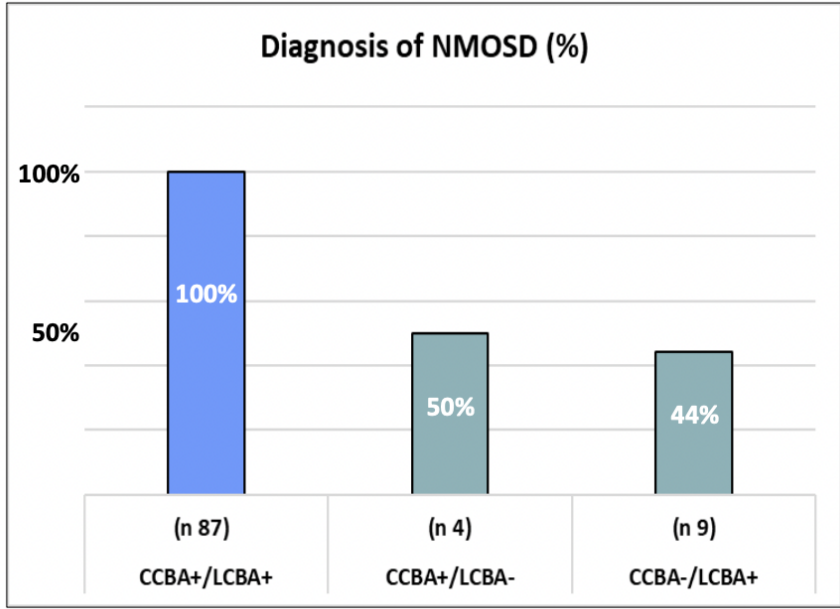
Both Live and Fixed CBA are reliable alternatives for AQP4 testing

Risi et al, in preparation

AQP4 testing perfomance in "real life"
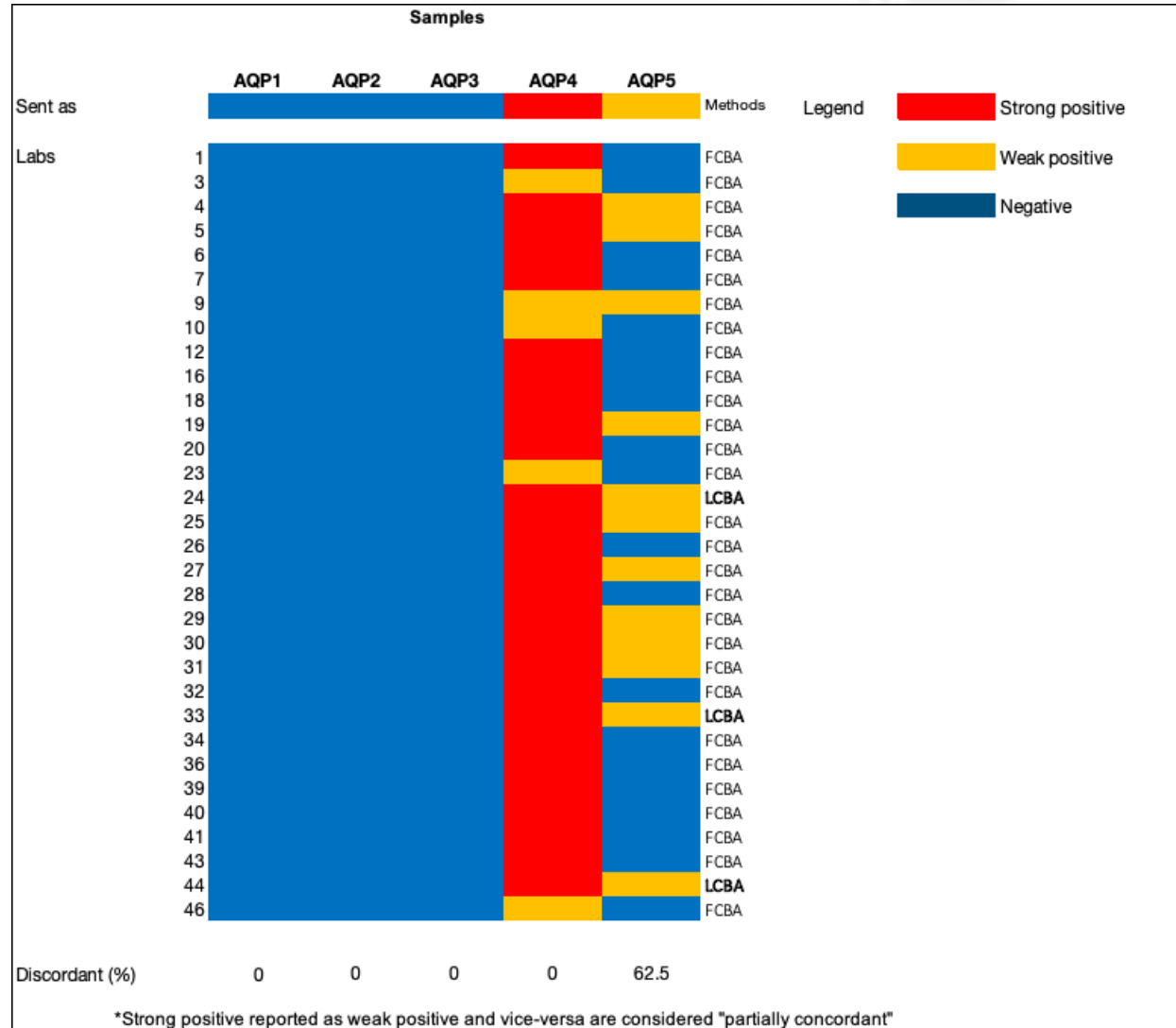
AQP4 Testing remains critical in routine practice

www.nina.aini.it

AQP4 antibodies: CSF testing

- 616 patients with paired serum/CSF
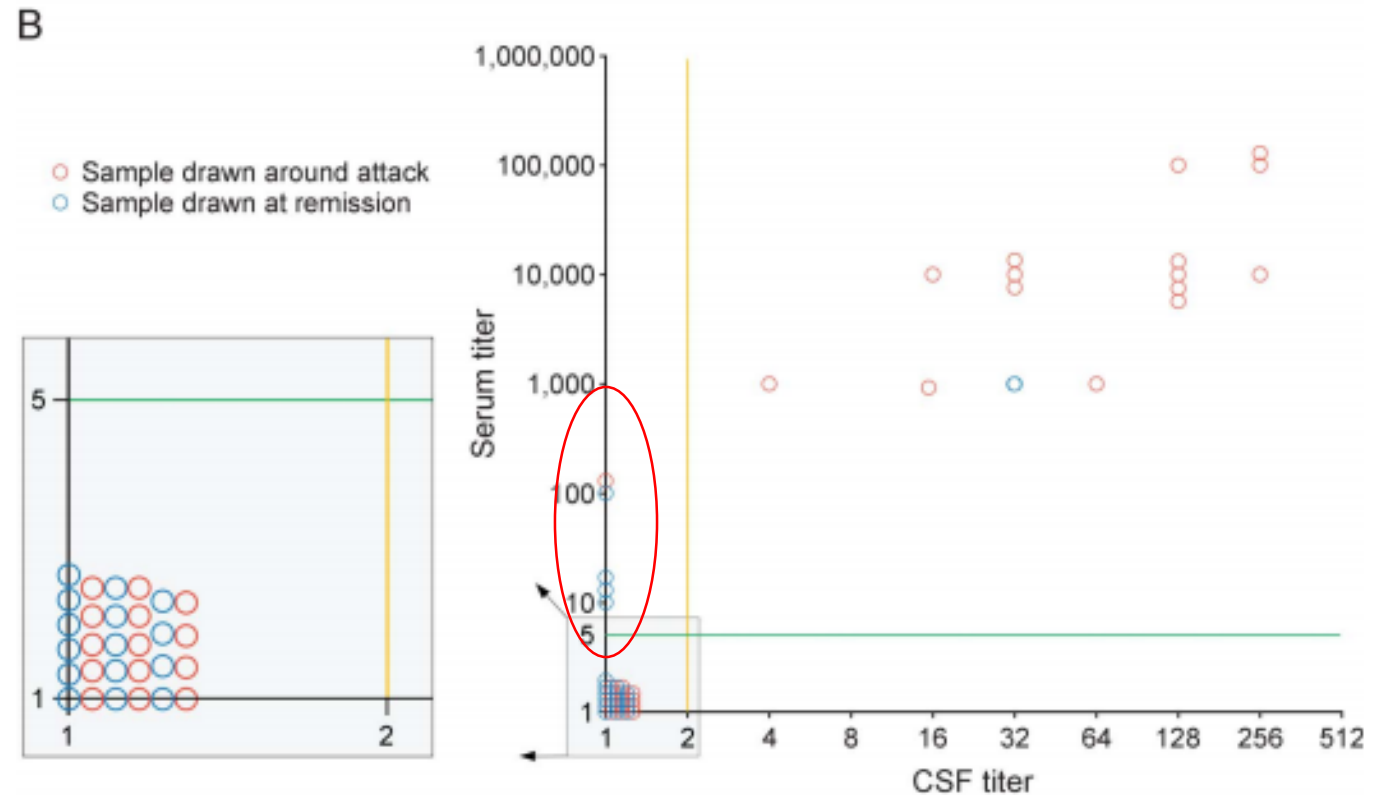- 58 patients with NMOSD
- No patient was pos in CSF and neg in serum
- CSF had lower sensitivity (5/58 were positive on serum only)

Mayed, 2016

Serum is the specimen of choice for AQP4 abs

Idots for the detection of AQP4 antibodies

JAMA Neurology | Original Investigation

Rapid Immunodot AQP4 Assay for Neuromyelitis Optica Spectrum Disorder

Ying Fu, PhD; Jin Bi, MD; Yaping Yan, PhD; Xiaobo Sun, MS; Ke Li, PhD; So Yeon Kim, MSc; Sang-Min Han, PhD; Luyao Zhou, MD; Rui Li, MD; Qiao Huang, MD; Ning Wang, MD; Aiyu Lin, MD; Ho Jin Kim, MD, PhD; Wei Qiu, MD
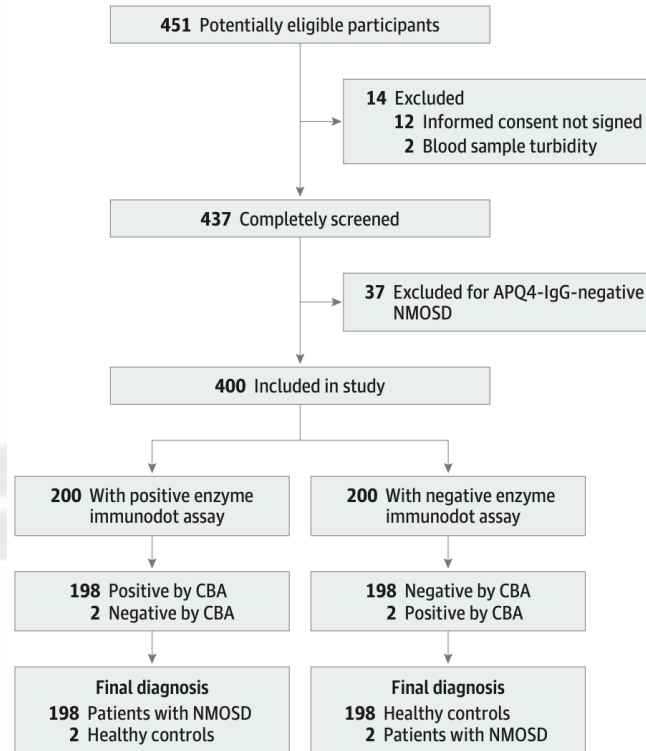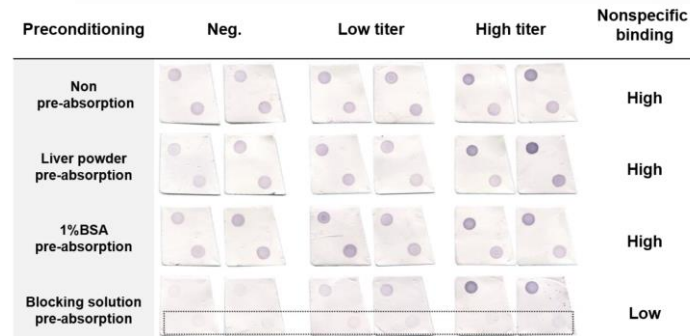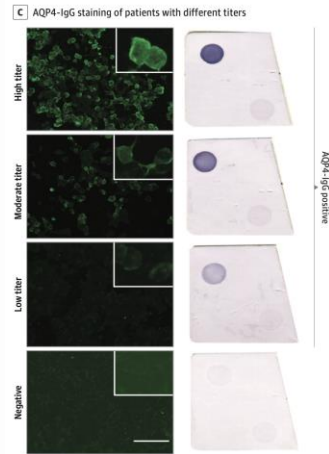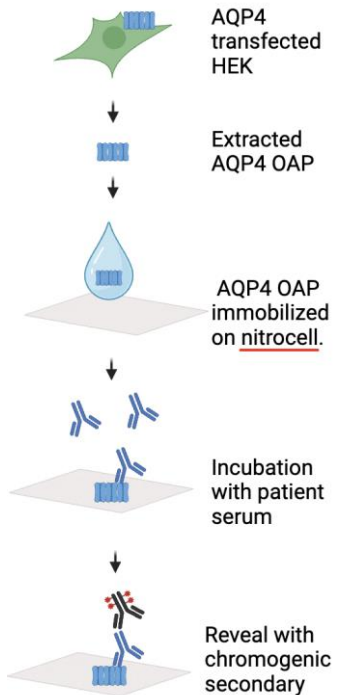
China Cross validation
31 AQP4+ samples

Korea Cross validation
47 AQP4+ samples
13 MOG+ samples
13 AQP4/MOG-

Additional controls
116 MS samples
50 SLE samples
49 MG samples
22 HAM samples
7 other samples

Immunodot assay results	Cell-based assay results			Sensitivity, % (95% CI)	Specificity, % (95% CI)
	Positive	Negative	Total		
Positive	330	4	334		
Negative	2	500	502	99.4 (97.8-99.9)	99.2 (98.0-99.8)
Total	332	504	836		

- Samples excluded if they had turbidity (selection according to pre-analytical conditions)

- Controls have been pre-selected (200 were HC)
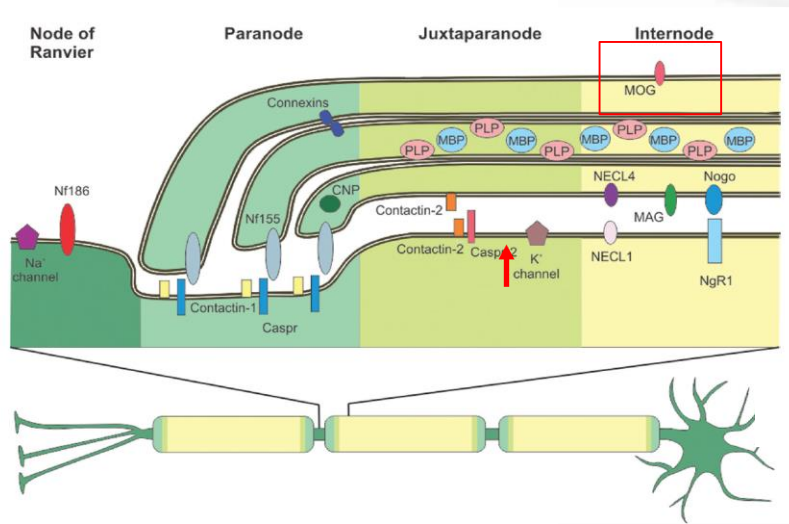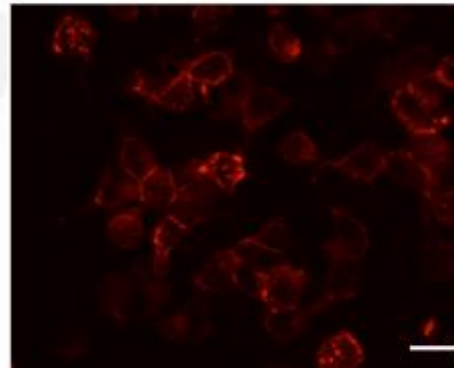
- Not tested yet in routine diagnostic

Fu et al, 2023

Summary

NMOSD

MOGAD

MOG-IgG in non MS demyelination

Node of Ranvier | Paranode | Juxtaparanode | Internode
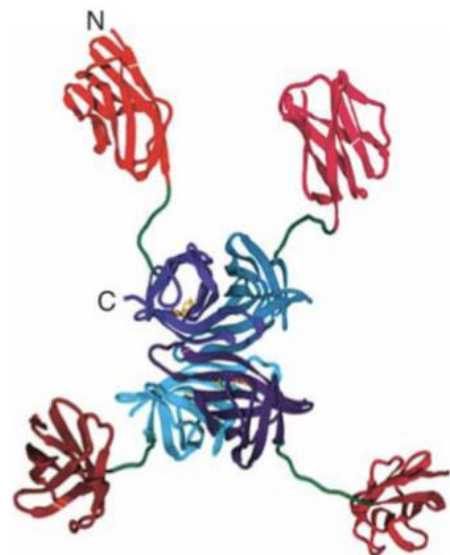
O'Connor 2007

Anti-Human IgG (H-L)

A. Myelin oligodendrocyte glycoprotein (MOG)

Myelin oligodendrocyte glycoprotein antibodies are associated with a non-MS course in children

OPEN

Hacohen et al, 2015
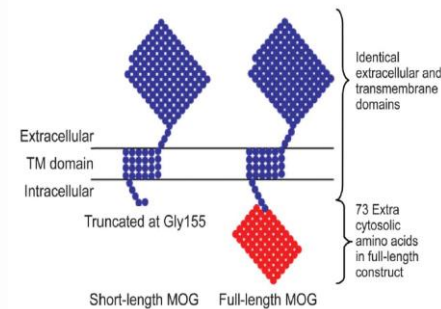
MOG cell-based assay detects non-MS patients with inflammatory neurologic disease

OPEN

Waters et al, 2015

MOG Abs detection strategies

Secondary antibody

Anti-H+L

Anti-FC
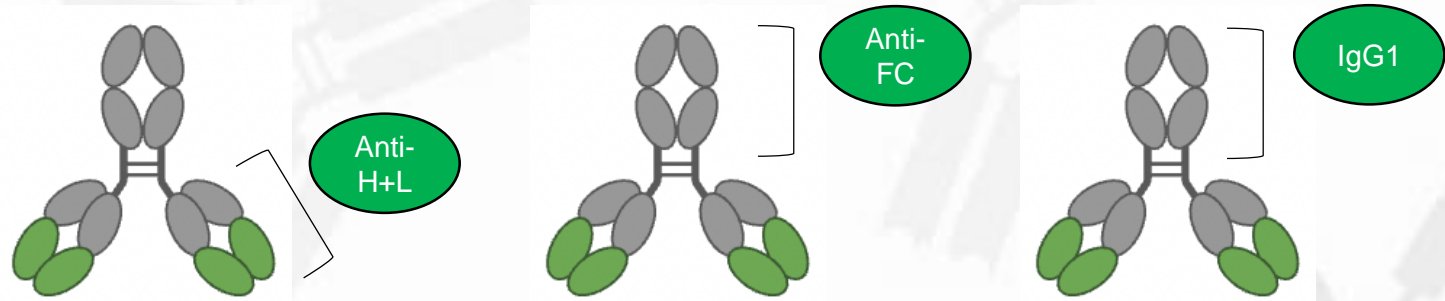
IgG1

Cell status
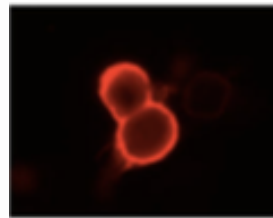
Live cells
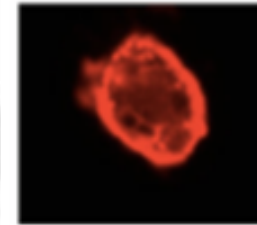
Fixed cells

Readout
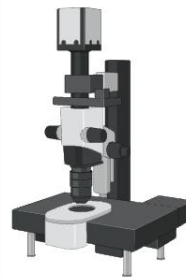
Microscope
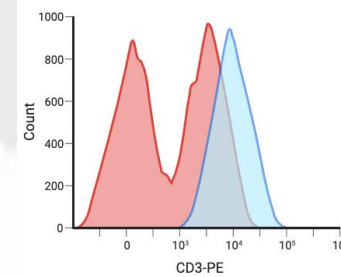
Flow-cytometry

Lack of standardization

Live vs Fixed CBA for anti-MOG antibodies

International multicenter examination of MOG antibody assays

Live CBA are the gold standard for MOG diagnostic

Gastaldi, 2020; Reindl 2021

Testing MOG-IgG1 increases specificity

Waters, 2015; Gastaldi, 2021
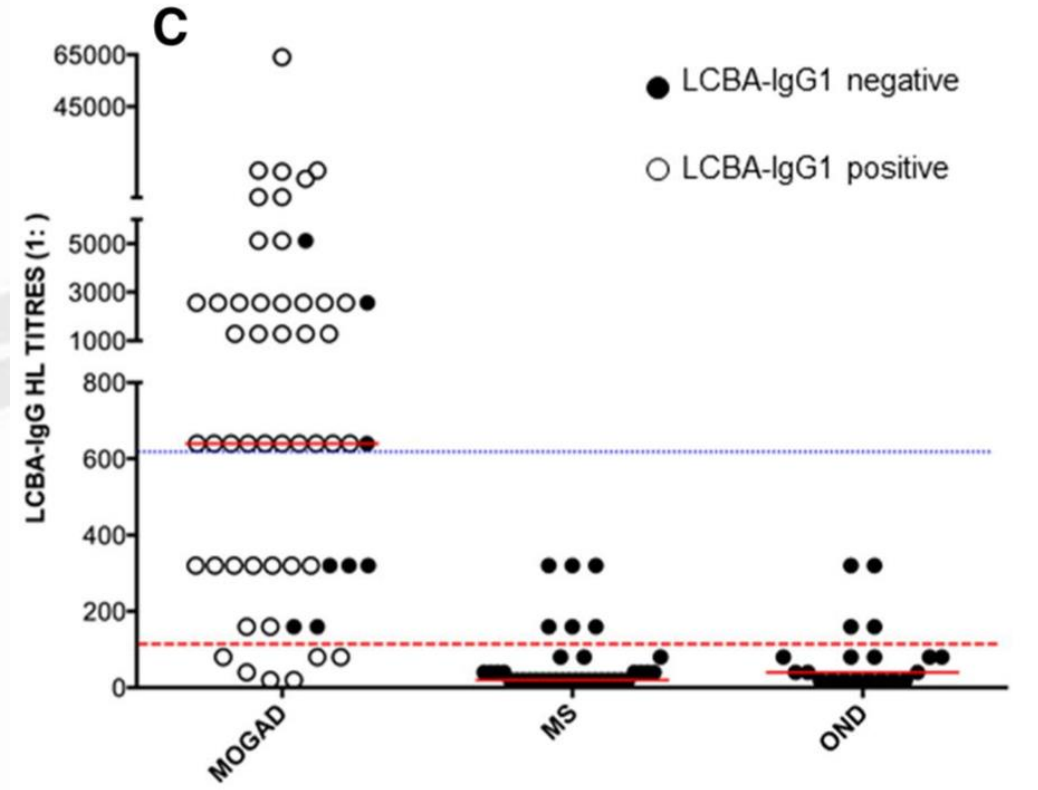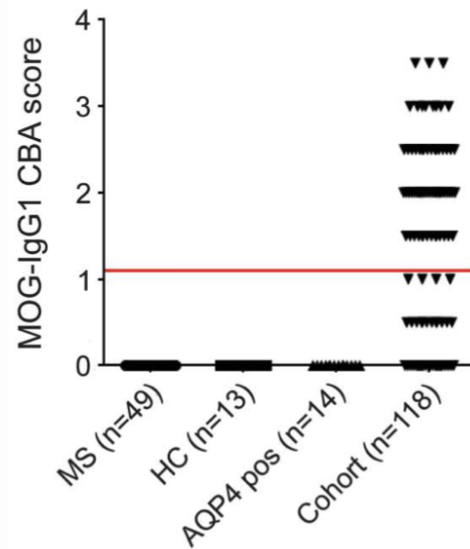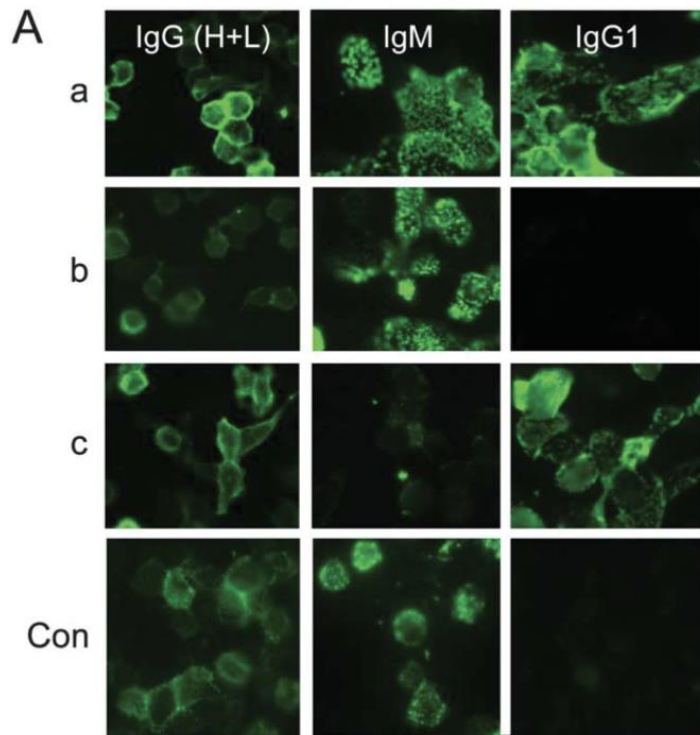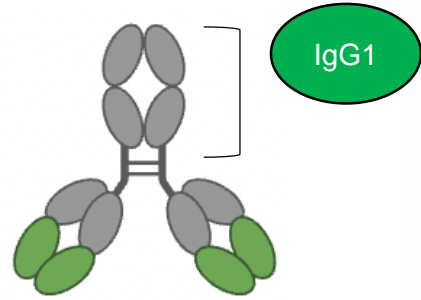
Positive predictive value of MOG-IgG is titre dependent

Positive Predictive Value of Myelin Oligodendrocyte Glycoprotein Autoantibody Testing

Elia Sechi, MD; Marina Buciuc, MD; Sean J. Pittock, MD; John J. Chen, MD, PhD; James P. Fryer, MS; Sarah M. Jenkins, MS; Adrian Budhram, MD; Brian G. Weinshenker, MD; A. Sebastian Lopez-Chiriboga, MD; Jan-Mendelt Tillema, MD; Andrew McKeon, MD; John R. Mills, PhD; W. Oliver Tobin, MB BCh, BAO, PhD; Eoin P. Flanagan, MD

o MOG-IgG can occur in patients without MOGAD (False positives)
o Positive predictive value increases with titres

Patients to be tested should be selected to increase pre-test probability

Gastaldi, Jneurol 2021; Sechi, Jama Neurol, 2023

Fixed CBA titres and MOGAD diagnosis

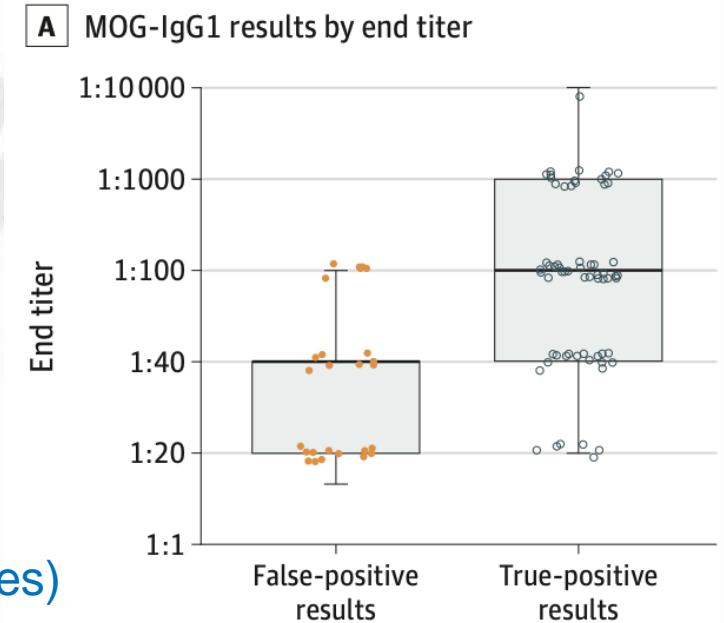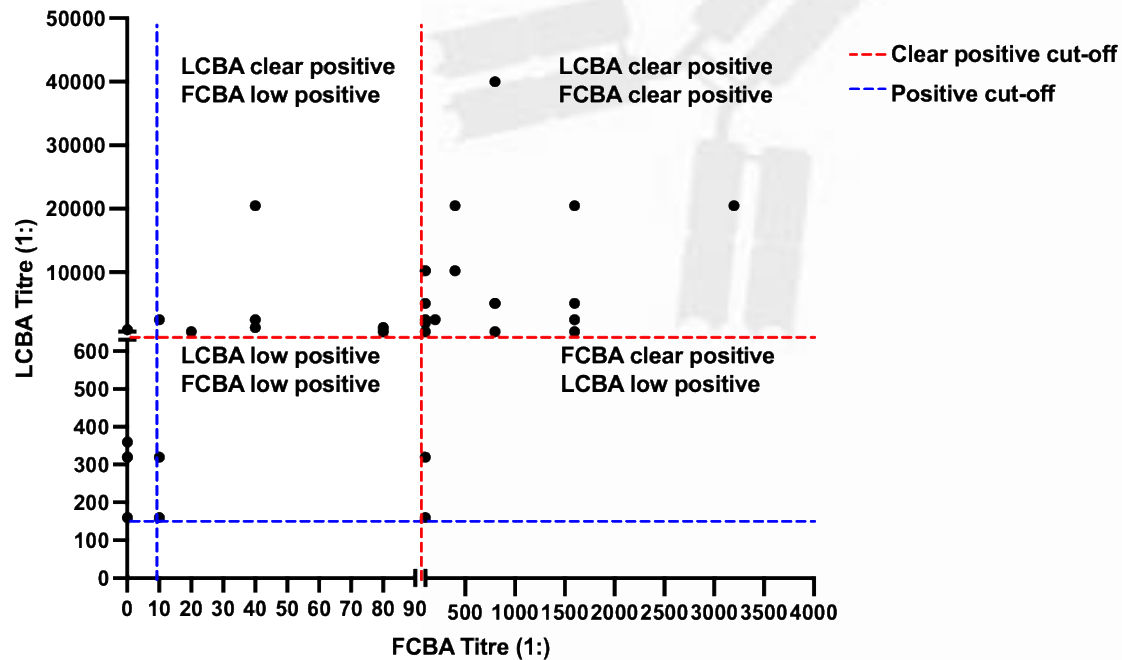| Patient N	FCBA (qualitative)	FCBA (Titration)	LCBA	Supporting features	Diagnosis
1			1:1280	Yes	ON
2			1:160	Yes	TM
3			1:320	Yes	TM
4			1:320	Yes	ON
5			1:320	Yes	ON
6		1:10	1:2560	No	ON
7		1:80	1:1280	No	TM
8		1:100	1:5120	No	ON
9		1:100	1:10240	No	ON
10		1:400	1:10240	No	ON
11		1:400	1:10240	No	ON
12		1:100	1:640	Yes	ADEM
13		1:100	1:2019	Yes	ON
14		1:100	1:2560	Yes	TM
15		1:100	1:10240	Yes	ON
16		1:200	1:2560	Yes	ON
17		1:400	1:20480	Yes	CE
18		1:800	1:640	Yes	ADEM
19		1:800	1:5120	Yes	ON
20		1:800	1:5120	Yes	ON
21		1:800	1:5120	Yes	ON
22		1:800	1:40000	Yes	CE
23		1:1600	1:640	Yes	ON
24		1:1600	1:2560	Yes	ON
25		1:1600	1:5120	Yes	ADEM
26		1:1600	1:20480	Yes	BE
27		1:3200	1:20480	Yes	ON
28		1:20	1:640	Yes	ADEM
29		1:40	1:1280	Yes	ADEM
30		1:40	1:2560	Yes	ON
31		1:40	1:20480	Yes	TM
32		1:80	1:640	Yes	ON
33		1:80	1:640	Yes	CE
34		1:80	1:1280	Yes	TM
35		1:100	1:160	Yes	ON
36		1:100	1:320	Yes	BE
37		1:10	1:160	Yes	ON
38		1:10	1:320	Yes	TM

Legend:
- Red — Clear+/MOGAD
- Orange — Low+/ Supporting criteria+ (MOGAD)
- Blue — Low+/Supporting criteria- (no MOGAD)
- Black — Negative (no MOGAD)

Banwell, 2023

Diagnosis of MOGAD (requires fulfilment of A, B, and C)		
Optic neuritis* Myelitis† ADEM‡ Cerebral monofocal or polyfocal deficits§ Brainstem or cerebellar deficits¶ Cerebral cortical encephalitis often with seizures‖		
Cell-based assay: serum**	Clear positive††	No additional supporting features required
	Low positive‡‡	• AQP4-IgG seronegative AND • ≥1 supporting clinical or MRI feature
	Positive without reported titre	
	Negative but CSF positive§§	

Scatter plot — FCBA Titre (1:) vs LCBA Titre (1:)

- LCBA clear positive / FCBA low positive
- LCBA clear positive / FCBA clear positive
- LCBA low positive / FCBA low positive
- FCBA clear positive / LCBA low positive

- Clear positive cut-off (red dashed)
- Positive cut-off (blue dashed)

Risi et al, submitted

Non-IgG1 MOG subclasses

| Case	Sex	Age	Onset	Final diagnosis	MOG antibody titer (1:)				
					IgG (H + L)	IgG1	IgG2	IgG3	IgG4
1	M	20	ON	MS	0	40	0	0	0
2	M	31	MY	CIS	0	40	0	0	0
3	F	33	ON	CIS	0	20	0	0	0
4	F	35	ON + MY	CIS	40	80	0	0	0
5	F	65	Brainstem	NMOSD	0	20	0	0	0
6	F	30	MY	MY	40	40	0	0	0
7	M	36	ON	ON	80	20	0	0	0
8	M	49	MY	CIS	0	20	0	0	0
9	F	55	MY	MY	0	0	0	20	0
10	F	31	MY	MY	80	0	0	40	0
11	F	28	MY	CIS	40	0	0	20	0
12	M	63	MY	MY	80	0	20	0	0

MOG cell-based assay detects non-MS patients with inflammatory neurologic disease

OPEN

One pt with MOG IgG3 (isolated)- probable MS

Waters, 2015; Mariotto, 2017; Gastaldi, 2021
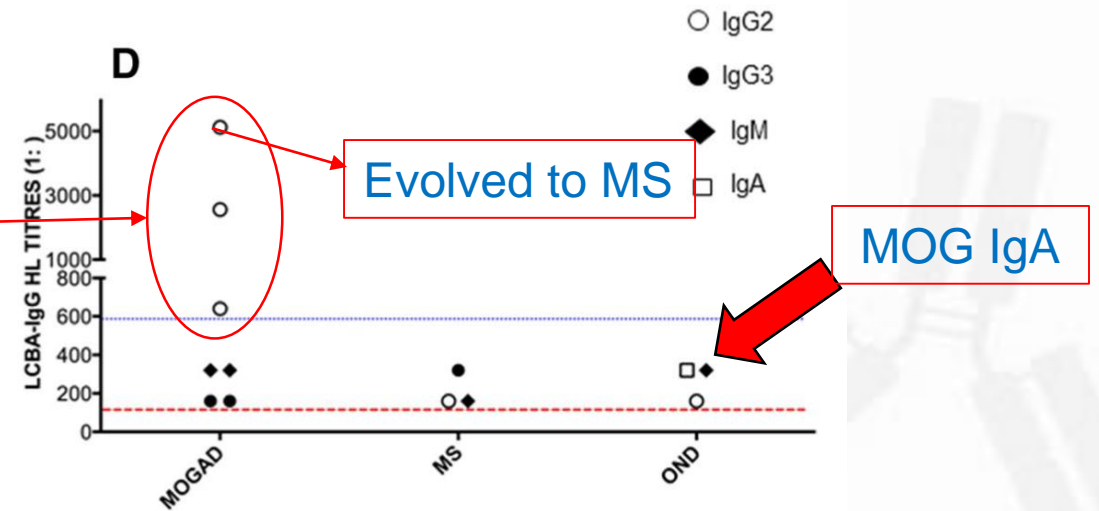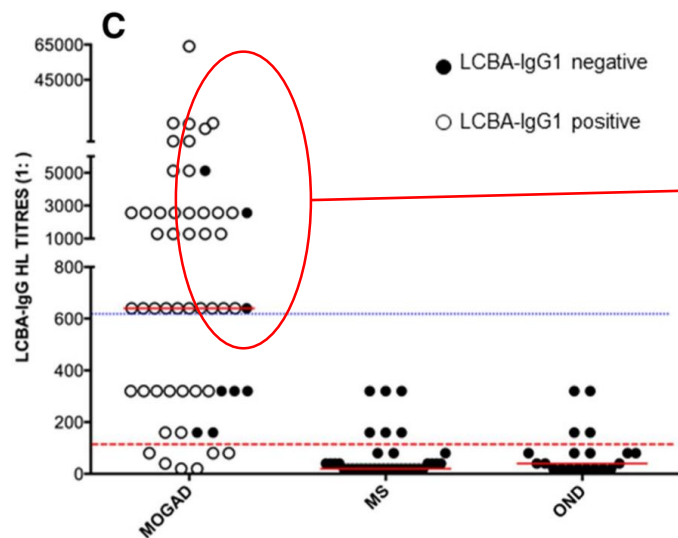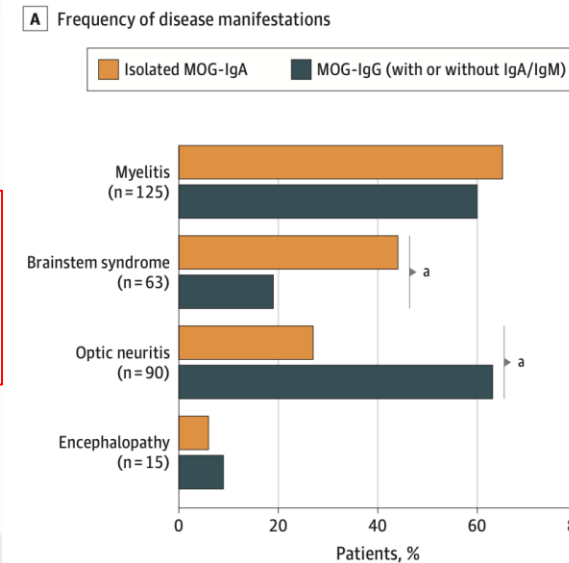
MOG IgA: clinical relevance
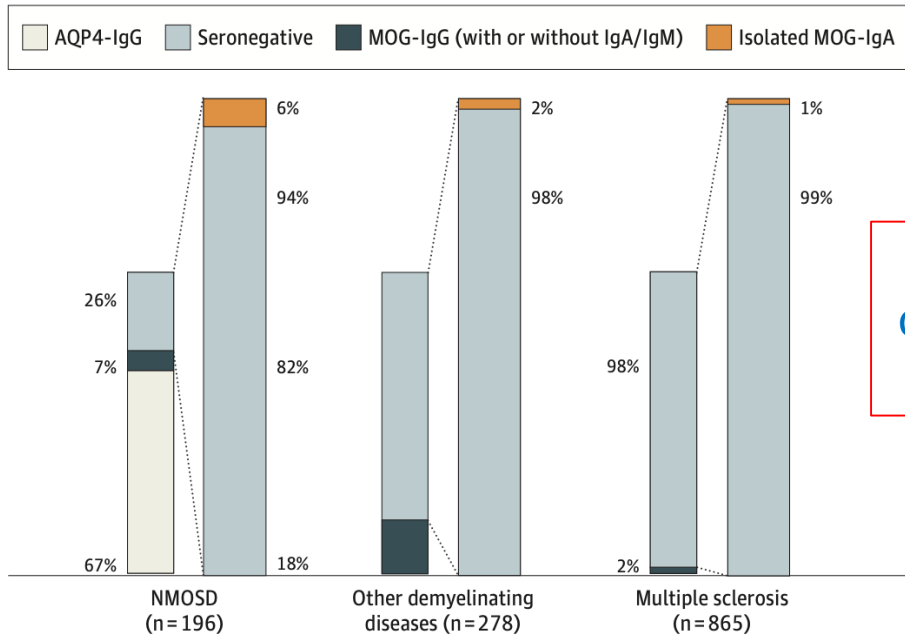

15/81 (19%) of MOG-IgG+ patients
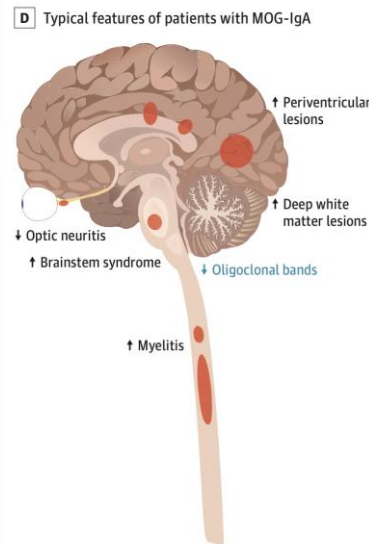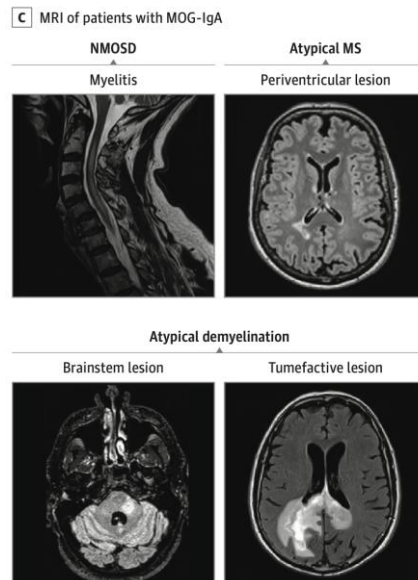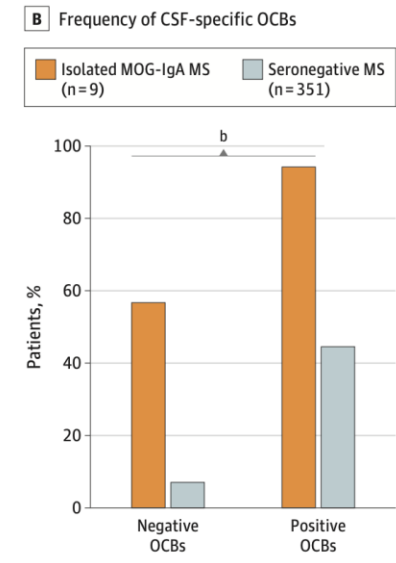
- o MOG-IgG are identified in patients with demyelinating syndromes and not controls (110 HC, no disease controls)

- o MOG-IgA are identified in patients with diverse demyelinating conditions (MOGAD, NMOSD, MS)
  → no relevance as diagnostic biomarker

- o MOG-IgA associate with "specific" features in MOGAD and with "atypical" MS (how is "atypical" MS defined?)
  → Potential role as clinical marker (to be confirmed)

Gomes et al, JAMA Neurology, 2023

CSF MOG antibodies

Significance of Myelin Oligodendrocyte Glycoprotein Antibodies in CSF
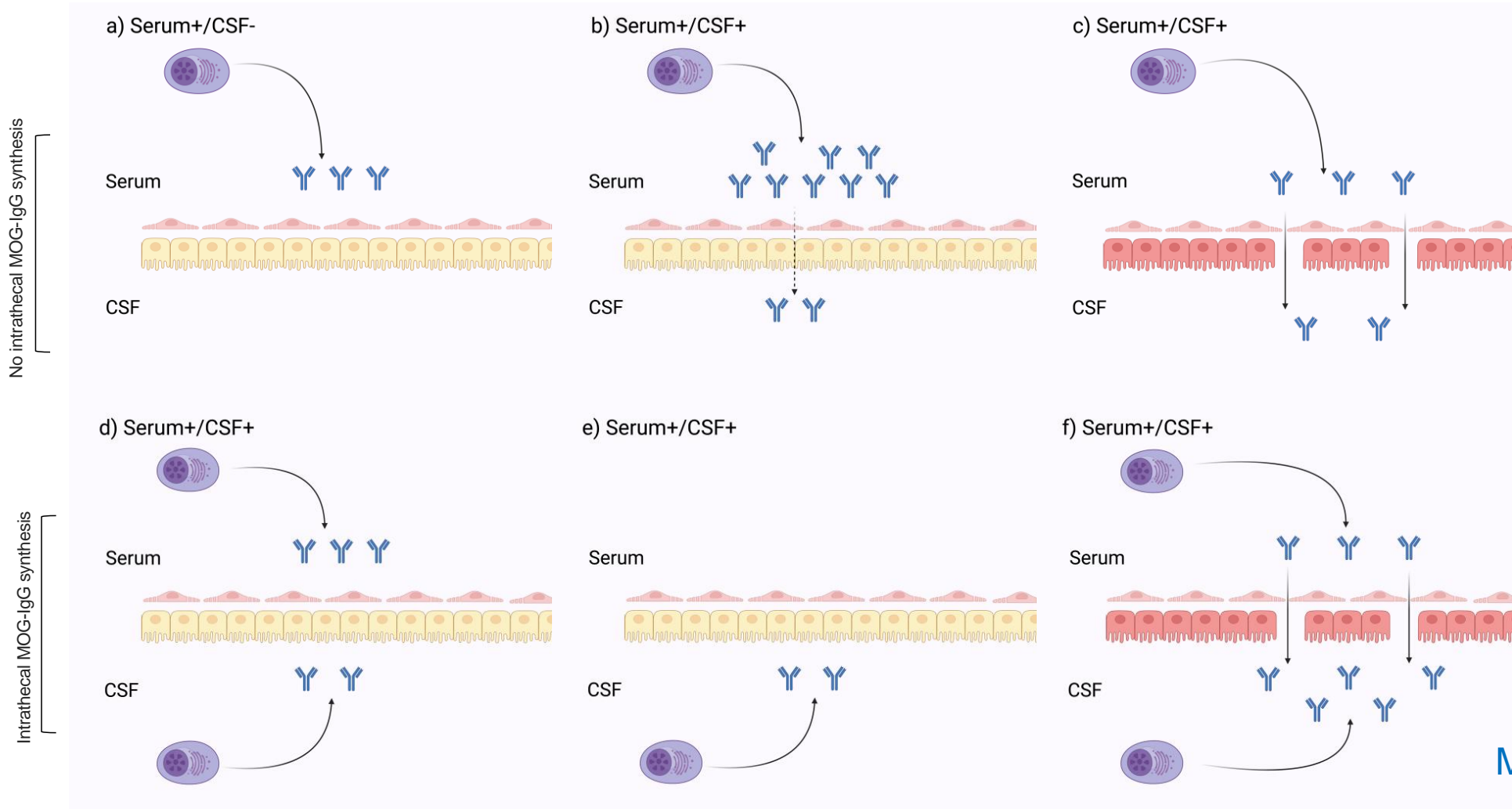
A Retrospective Multicenter Study

- 145/255 (56.8%) had CSF antibodies

- 31/255 (12.1%) had CSF only antibodies

- CSF-MOG patients had a higher EDSS score and sphyncteric symtoms

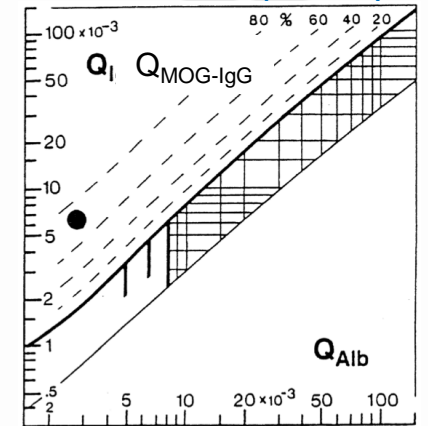- CSF MOG positivity has been included as a supporting criterion in the recent MOGAD criteria
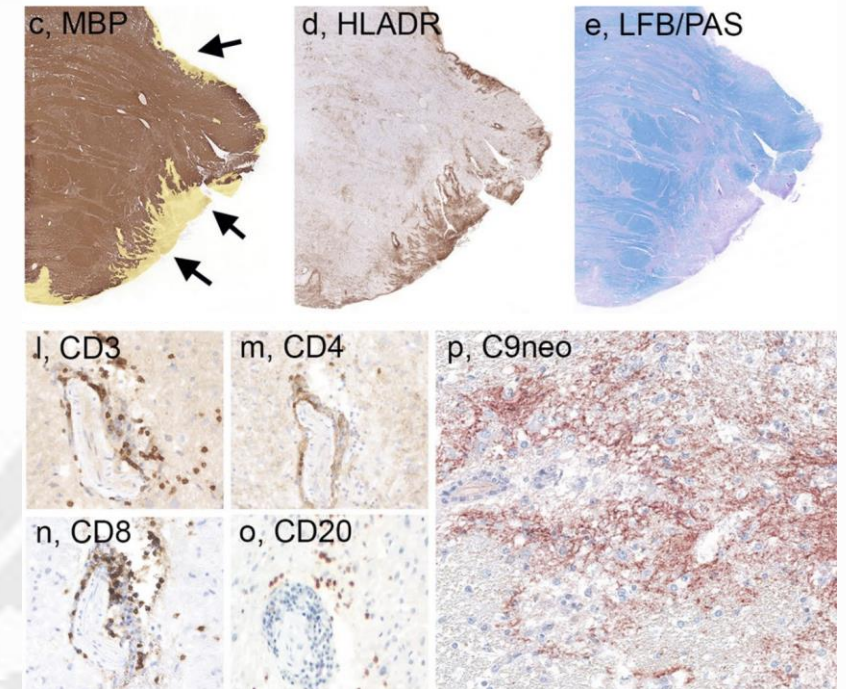
OPEN ISSUES
- Intrathecal synthesis should be systematically investigated
- CSF only MOG can occur in non-MOGAD phenotype at a variable percentage (0-30%)

Carta et al, 2020; Carta et al, 2023

MOG IgG intrathecal synthesis or CSF positivity

a) Serum+/CSF-

b) Serum+/CSF+

c) Serum+/CSF+

d) Serum+/CSF+

e) Serum+/CSF+

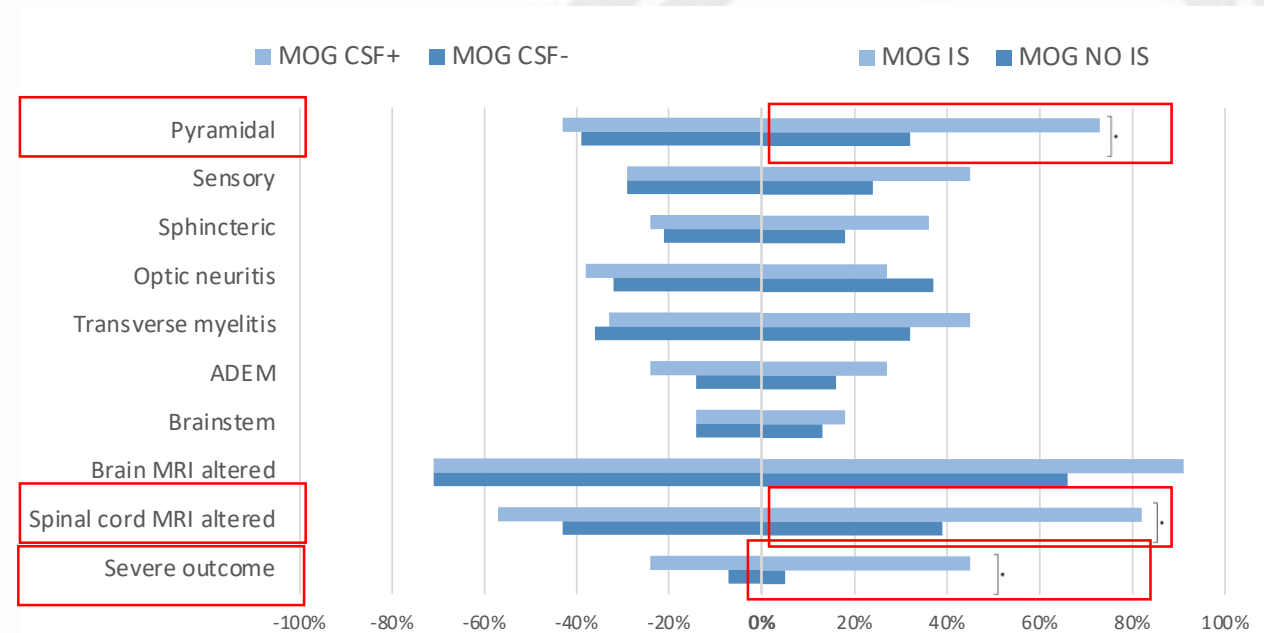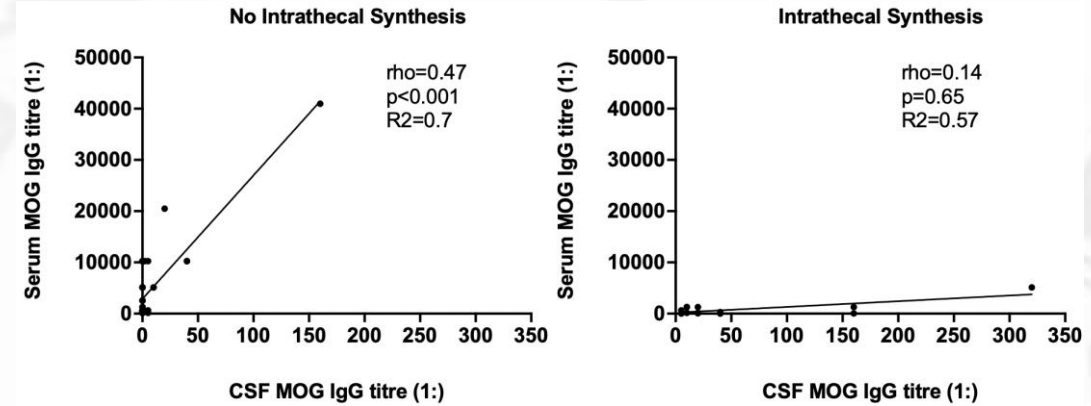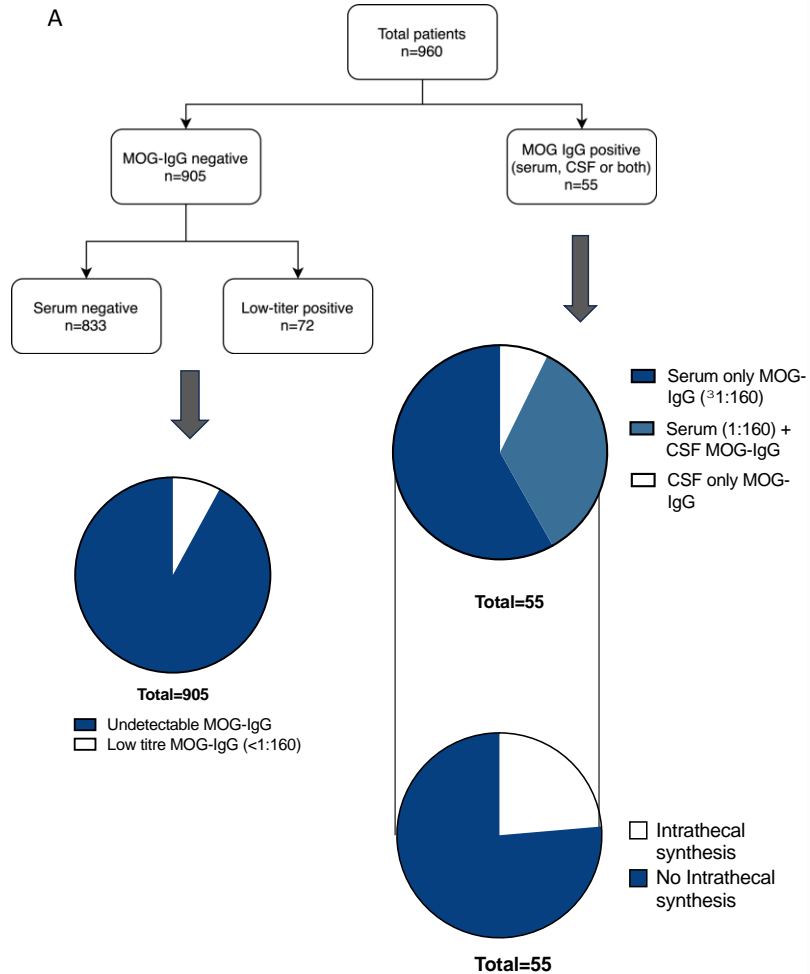f) Serum+/CSF+

No intrathecal MOG-IgG synthesis

Intrathecal MOG-IgG synthesis

Serum

CSF

MOG-IgG serum titre
MOG-IgG CSF titre
B-CSF-B (Qalb)

MOG-IgG antibody Index>4
=
MOG-IgG Intrathecal synthesis

Greco et al, in preparation

Relevance of MOG IgG intrathecal synthesis in MOGAD

Greco et al, in preparation

Summary

- CBAs are the gold standard for AQP4 abs

- FCBA and LCBA have robust performances, but real life data reveal frequent inaccurate results

- Serum is the sample of choice

- Novel tests (idots) on their way

- CBAs are the gold standard for MOG abs

- LCBA has a better perfomance (despite lack fo standardization

- MOG-IgG can be found (especially at low titre) in non-MOGAD patients (pre-test probability!)

- Non-IgG1 subclasses and IgA: still pending judgment

- CSF MOG-IgG: watch out for intrathecal synthesis!

Diagnostic project for second opinion on critical samples for AQP4, MOG, ACHR and MUSK

www.nina.aini.it; ninaflow@aini.it

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Network Italiano
Neurologia Autoimmune

Thanks for your attention!

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