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A multicenter comparison of MOG-IgG cell-based assays

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Abstract

Objectives

To compares 3 different myelin oligodendrocyte glycoprotein–immunoglobulin G (IgG) cell-based assays (CBAs) from 3 international centers.

Methods

Serum samples from 394 patients were as follows: acute disseminated encephalomyelitis (28), seronegative neuromyelitis optica (27), optic neuritis (21 single, 2 relapsing), and longitudinally extensive (10 single, 3 recurrent). The control samples were from patients with multiple sclerosis (244), hypergammaglobulinemia (42), and other (17). Seropositivity was determined by visual observation on a fluorescence microscope (Euroimmun fixed CBA, Oxford live cell CBA) or flow cytometry (Mayo live cell fluorescence-activated cell sorting assay).

Results

Of 25 samples positive by any methodology, 21 were concordant on all 3 assays, 2 were positive at Oxford and Euroimmun, and 2 were positive only at Oxford. Euroimmun, Mayo, and Oxford results were as follows: clinical specificity 98.1%, 99.6%, and 100%; positive predictive values (PPVs) 82.1%, 95.5%, and 100%; and negative predictive values 79.0%, 78.8%, and 79.8%. Of 5 false-positives, 1 was positive at both Euroimmun and Mayo and 4 were positive at Euroimmun alone.

Conclusions

Overall, a high degree of agreement was observed across 3 different MOG-IgG CBAs. Both live cell-based methodologies had superior PPVs to the fixed cell assays, indicating that positive results in these assays are more reliable indicators of MOG autoimmune spectrum disorders.

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Glossary

ADEM = acute disseminated encephalomyelitis; **AQP4** = aquaporin-4; **CBA** = cell-based assay; **DMA** = disease-modifying agent; **IDD** = inflammatory demyelinating disorder; **IgG** = immunoglobulin G; **IIF** = indirect immunofluorescence; **MOG** = myelin oligodendrocyte glycoprotein; **MS** = multiple sclerosis; **NMO** = neuromyelitis optica; **PPV** = positive predictive value.

There is accumulating evidence that CNS inflammatory demyelinating disorders (IDDs), including forms of neuromyelitis optica (NMO) spectrum disorders, acute disseminated encephalomyelitis (ADEM), optic neuritis (recurrent more than single episode), and transverse myelitis are commonly associated with immunoglobulin G (IgG) targeting aquaporin-4 (AQP4) or myelin oligodendrocyte glycoprotein (MOG).[1–5] Until their relatively recent discovery, patients with these disorders were commonly misdiagnosed as having multiple sclerosis (MS), yet contemporary findings show that MS, MOG-IgG, and AQP4-IgG–associated IDDs have clinical, radiologic, pathologic, and prognostic differences.[5,6]

MOG-IgG–associated IDDs may have a higher prevalence in children and are often relapsing, commonly manifesting as optic neuritis. Attacks may be associated with accumulating neuronal injury and functional impairment. MOG-IgG may be transient or persistent, and its role as a predictor of relapse remains a focus of ongoing study. While MOG antibody has had a checkered past as a biomarker because of a lack of any specific disease association, contemporary methodologies using cell-based assays (CBAs) now define an autoimmune oligodendroglyopathy with a preferential response to immunosuppressants rather than disease-modifying agents (DMA) commonly used in MS.[4–8] Early initiation and prolonged administration of such drugs may prevent relapses and reduce disability accrual, although randomized clinical trials have not yet been undertaken. MOG-IgG also provides important prognostic information. Hence, accurate serologic diagnosis is imperative to optimize clinical care.

A recent review article published in 2017 by key opinion leaders in the field stated that "methods for detecting MOG antibodies have improved substantially, with cell based assays (CBAs) being state of the art."[1] In this blinded study, 3 different MOG-IgG CBAs from 3 international centers were compared.

Methods

Standard protocol approvals, registrations, and patient consents

All patients in our study consented to the use of their medical records for research purposes. The study was approved by the Institutional Review Board of Mayo Clinic, Rochester, MN (No. 08-007846).

Serum samples from 394 patients and controls were tested: 91 patients were classified as having a MOG-IgG–like clinical phenotype and included ADEM (28), AQP4-IgG seronegative NMO (27, fulfilling Wingerchuk diagnostic criteria for NMO, either 1999 or 2006 [excluding antibody status]), optic neuritis (21 single, 2 relapsing), or longitudinally extensive transverse myelitis (10 single, 3 recurrent). The control samples were collected from patients with MS (244, selected from the Olmsted County MS population-based cohort), hypergammaglobulinemia (42), and other (17, encephalitis, glioma, Creutzfeldt-Jakob disease, glaucoma). Sensitivity was calculated as the percentage of positives cases within the MOG-IgG–like clinical phenotype cohort. Specificity was calculated as the percentage of positive cases in the MS cohort and those with other neurologic presentations inconsistent with an MOG-related clinical phenotype. Positive predictive value (PPV) was calculated as the percentage of positive test results in patients with MOG-IgG–like clinical phenotypes of all positive test results and estimates the reliability of a positive test result. In contrast, the negative predictive value is the percentage of negative test results in patients without an MOG-IgG–like clinical phenotype of all negative test results and is an estimate of how reliably a negative test result rules out the disease. This study was approved by the Mayo Clinic Institutional Review Board.

All samples were stored at −80°C at the Mayo Clinic central laboratory. They were divided into aliquots and provided frozen as coded samples to the 3 neuroimmunology laboratories: Mayo Clinic; Oxford, UK; and Euroimmun, Germany. All samples were tested by investigators blinded to the clinical information. Methodologies of the 3 assays are shown in table 1, and staining of cells considered positive and negative by all 3 assays is illustrated in the figure

Data availability statement

The dataset used and analyzed during the current study is available from the corresponding author on reasonable request.

Results

Of the 25 case samples positive by any methodology, 21 were concordant on all 3 assays, 2 were positive by the Oxford assay and Euroimmun assays, and 2 were positive only by the Oxford assay.

Clinical specificity, as measured using a cohort of 244 patients with MS and 17 patients with disorders clearly outside of the autoimmune MOG spectrum, was 98.1% for Euroimmun, 99.6% for Mayo, and 100% for Oxford. The corresponding PPVs were 82.1%, 95.5%, and 100%, respectively. Negative predictive values were 79.0%, 78.8%, and 79.8%. Of the 5 false-positive findings in this cohort, 1 was positive by both

Table 1 CBA methodologies

Center	Assay type	Transfected cell type	Methodology	Determination of serostatus (see figure)
Mayo Clinic	Live cell: binding MOG-IgG1 determined by flow cytometry (FACS)	HEK-293 cells transiently transfected with a recombinant expression vector (pIRES2-MOG-AcGFP).	Bound patient's antibodies are detected using an AlexaFluor-647 anti-human IgG1-specific secondary antibody. The median fluorescence intensity associated with AlexaFluor-647 (indicating the presence of bound human antibody) is determined for both the nontransfected and transfected cell populations.[9]	A ratio of these 2 values gives the MOG-IgG binding index. A MOG-IgG binding index ≥2.5 is considered positive for MOG-IgG antibodies.[9]
Oxford	Live cell: binding of MOG-IgG1 determined by visual observation	HEK293T cells transiently transfected with plasmid DNA encoding full-length human MOG with glucose and PEI.	After 24-30 h, serum diluted 1:20 in 250 μL DMEM with 20 mmol/L HEPES buffer and 1% BSA was incubated with the MOG-expressing cells for 1 h at room temperature. Cells were washed and incubated with AlexaFluor-488 goat anti-human IgG1 at 1:500 in the same buffer for 45 mins at RT. After fixation and washing, the cells were embedded in mounting medium that contained DAPI (1:1,000) to counterstain the nuclei.	A sample was considered positive if it showed a typical surface stain on cells transfected with MOG and no stain on cells transfected with a control antigen
Euroimmun	Fixed cell: binding MOG-IgG determined by visual observation	HEK-293 transiently transfected with full-length human MOG protein and fixed with formaldehyde	In parallel, HEK cells were transfected with an empty vector and processed similarly. These 2 populations form the test substrate, which is incubated with sera (1:10 dilution) for 30 min at room temperature. After washing, the substrate is incubated for 30 min at RT with goat anti-human IgG. After washing and embedding, slides were evaluated manually by 2 observers on a fluorescence microscope with a ×200 magnification.	A sample was considered positive if it showed a typical surface stain on cells transfected with MOG and no stain on cells transfected with a control antigen.

Abbreviations: AcGFP = acetylated green fluorescent protein; BSA = bovine serum albumin; CBA = cell-based assay; DMEM = Dulbecco modified Eagle medium; FACS = fluorescence-activated cell sorting assay; IgG = immunoglobulin G; HEK = human embryonic kidney; HEPES = 4-[2-hydroxyethyl]-1-piperazineethanesulfonic acid; MOG = myelin oligodendrocyte glycoprotein; PEI = polyethylenimine; RT = room temperature.

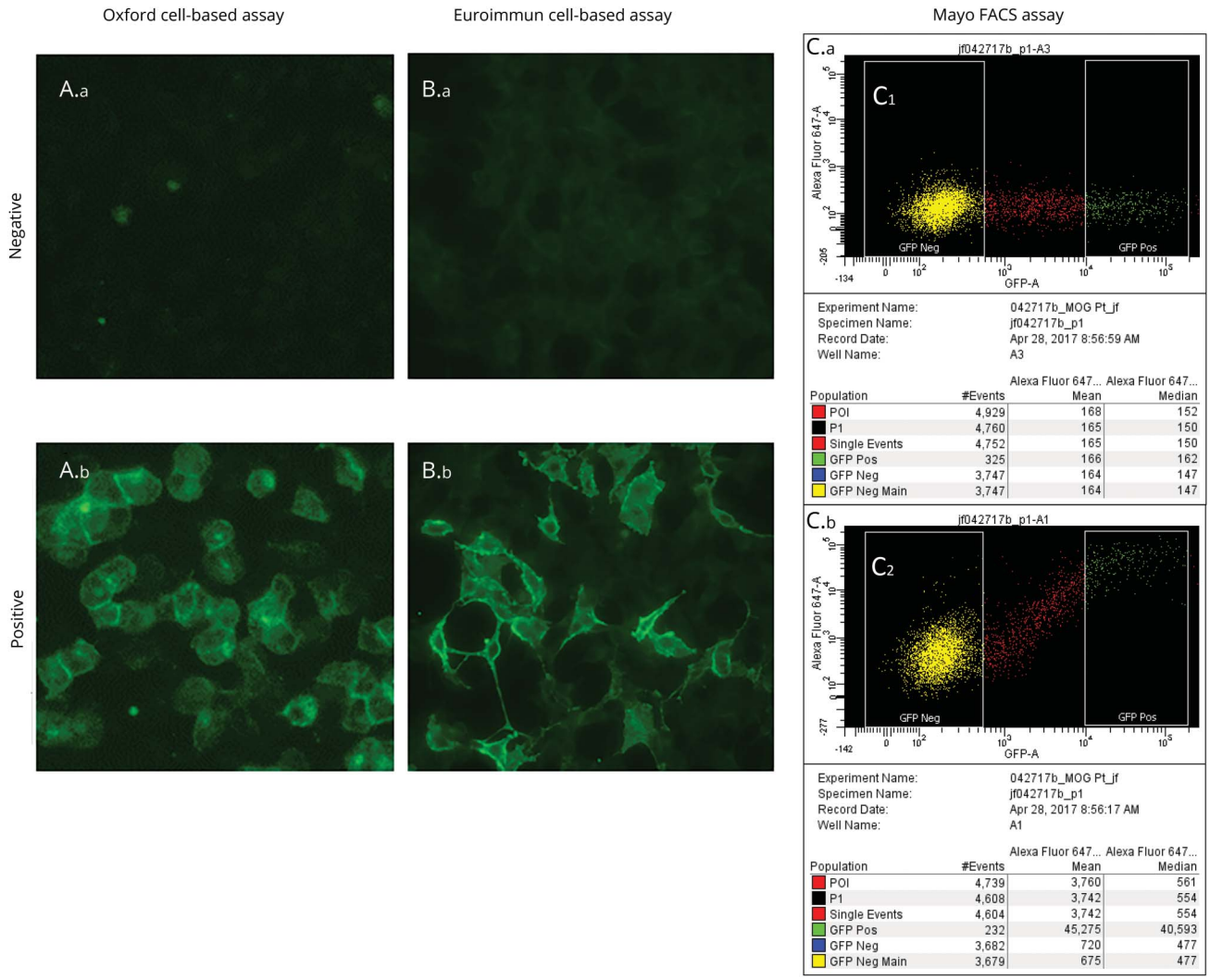
the Euroimmun and Mayo assays (table 2). The additional 4 false-positive results were limited to the Euroimmun CBA. Analytical specificity was high for all 3 assays; no false-positives were identified in a cohort of 42 patients with hypergammaglobulinemia. The results of this multicenter method comparison study of MOG-IgG testing are summarized in table 2. All pairwise comparisons revealed good interassay reliability with κ values >0.8 indicating a high degree of agreement across methods (Cohen κ statistic). Therefore, despite different methodologies and testing locations, the majority of samples achieved the same results across platforms. This is critical for the initial deployment of MOG-IgG–based assays because it provides confidence in the reliability of a positive result but also indicates that detection of MOG-IgG antibodies is robust and that these assays are inherently well standardized.

Discussion

Both live cell–based methodologies, distinct assays performed at different centers, had superior PPVs to the fixed assays, indicating that positive results in these assays are more reliable indicators of MOG spectrum disorders. ELISA is not a reliable methodology for MOG-IgG detection. MOG-IgG–related diseases may benefit from early and ongoing immunotherapies. Often, inflammatory idiopathic CNS disease such as ADEM, optic neuritis, and transverse myelitis are treated similarly to those with glial antibodies in the acute setting (steroids or plasmapheresis). However, for maintenance immunotherapy, patients without a glial antibody may be less likely to be treated with longer-term immunosuppressants, and longer treatment regimens are associated with fewer relapses in MOG-IgG–related diseases. A false-negative result would often result in a misdiagnosis of MS and consequent treatment with DMAs, which have been reported to worsen AQP4-IgG–positive IDDs, although some are effective for both disorders (anti-CD20 treatments). Data on DMAs exacerbating MOG-IgG disease are currently lacking.

Another concerning consequence of diagnostic inaccuracies is the detection of a false-positive result. Because MOG-IgGs will likely be commonly ordered in the clinical evaluation of a suspected demyelinating event, a false-positive result in a patient with a clinical diagnosis of MS might result in the selection of an immunosuppressant drug (e.g., mycophenolate mofetil, cellcept) rather than a Food and Drug Administration–approved DMA. In this study, 5 of 27 (18.5%) positive results in the commercial test were in control samples, giving a relatively poor PPV (82.1% vs 95.5%–100%). The test is simpler to run in routine diagnostic laboratories, but

Figure Comparison of positive and negative controls for Oxford, Euroimmun, and Mayo Clinic MOG-IgG assays



Oxford cell-based assay (CBA) (A.a) negative and (A.b) positive result; Euroimmun CBA (B.a) negative and (B.b) positive result; and Mayo fluorescence-activated cell sorting assay (FACS) (C.a) negative and (C.b) positive result. For the Mayo FACS assay, 2 cell populations are used to obtain a median fluorescent intensity. The green fluorescent protein (GFP)–negative population represents nontransfected cells, and the GFP-positive population represents cells that express both acetylated GFP and myelin oligodendrocyte glycoprotein (MOG) protein. The AlexaFluor-647 median intensity is an indicator of bound human serum antibodies. As shown in panel (C.b), the positive control has a median AlexaFluor-647 intensity of 40,593 for the GFP-positive population, and the GFP-negative population is 477. The negative control (C.a) has a median 647 intensity of 162 for the GFP-positive population, and the GFP-negative population is similar at 147. These statistical values are used to calculate the immunoglobulin G (IgG) binding index, which is a ratio of the GFP-positive value over the GFP-negative value.

it has to be fixed to allow transport and storage. The fixation may generate cryptic epitopes, which could explain the clearly positive binding. These discrepancies have also been described in AQP4 assay comparisons. Future studies should address this issue in their design, which may help with a better understanding of this kind of discrepancy.

Study funding

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Disclosure

P. Waters and the University of Oxford hold patents and receive royalties and revenue for performing antibody assays in neurologic diseases. L. Komorowski is an employee of Euroimmun AG, a company that develops, produces, and manufactures immunoassays for the detection of disease-associated antibodies. M. Woodhall reports no disclosures relevant to the manuscript. S. Lederer is an employee of Euroimmun AG, a company that develops, produces, and manufactures immunoassays for the detection of disease-associated antibodies. M. Majed, J. Fryer, and J. Mills report no disclosures relevant to the manuscript. E. Flanagan receives research support as a site principal investigator for Medimmune on a study of MEDI551 in NMO spectrum disorders. S. Irani and the University of Oxford hold patents and receive royalties and revenue for performing antibody assays in neurologic diseases. A. Kunchok reports no disclosures relevant to the manuscript. A. McKeon has a patent pending for MAP1B antibody as a marker of

Table 2 Distribution of MOG-IgG result by 3 assays in serum samples of cases and controls

	Oxford	Mayo	Euroimmun
Sensitivity (95% CI)	27.5 (18.6–37.8)	23.1 (14.9–33.1)	25.3 (16.8–35.5)
Specificity (95% CI)	100 (98.6–100)	99.6 (97.9–100)	98.1 (95.6–99.4)
PPV (95% CI)	100	95.5 (74.1–99.4)	82.1 (64.2–92.2)
NPV (95% CI)	79.8 (77.7–81.8)	78.8 (76.8–80.6)	79.0 (76.9–80.9)
IDDs considered within the autoimmune MOG spectrum, n (%)			
Total (91)	25 (27)	21 (23)	23 (25)
ADEM (28)	7 (25)	6 (21)	7 (25)
AQP4 seronegative NMO (27)	16 (59)	13 (48)	14 (52)
ON (23)			
Single (21)	0	0	0
Relapsing (2)	1 (50)	1 (50)	1 (50)
LETM (13)			
Single (10)	1 (10)	1 (10)	1 (10)
Relapsing (3)	0	0	0
IDDs not considered within the autoimmune MOG spectrum			
MS (244)	0	1 (0.4%)	5 (2%)
RRMS (157)	0	1 (0.6%)	2 (1.2%)[a]
SPMS (54)	0	0	2 (3.7%)[b]
PPMS (15)	0	0	1 (6.7%)[c]
CIS/RIS (18)	0	0	0
Other disorders			
Other (17)	0	0	0
Encephalopathy (14)	0	0	0
Glioma (2)	0	0	0
Glaucoma (1)	0	0	0
Nonneurologic controls			
HGGN (42)	0	0	0

Abbreviations: ADEM = acute disseminated encephalomyelitis; AQP4 = aqaporin-4; CI = confidence interval; CIS = clinically isolated syndrome; HGGN = hypergammaglobulinemia; IDD = inflammatory demyelinating disorder; IgG = immunoglobulin G; LETM = longitudinally extensive transverse myelitis; MOG = myelin oligodendrocyte glycoprotein; MS = multiple sclerosis; NMO = neuromyelitis optica; NPV = negative predictive values; ON = optic neuritis; PPMS = primary progressive multiple sclerosis; PPV = positive predictive values; RIS = radiologically isolated syndrome; RRMS = relapsing remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis.
[a] These 2 patients fulfilled McDonald 2017 criteria for MS.
[b] These 2 patients fulfilled McDonald 2017 criteria for MS and Lorscheider et al.[10] definition of SPMS.
[c] Patient had primary progressive course with progressive left hemiparesis with spasticity and neurogenic bladder. MRI of the cervical spine showed 2 short-segment T2-hyperintense lesions (left C2-3 and right C6-7).[2]

neurologic autoimmunity and paraneoplastic disorders; consulted for Grifols, Medimmune, and Euroimmun; and received research support from Medimmune and Euroimmun but has not received personal compensation. S. Pittock holds patents that relate to functional AQP4/NMO-IgG assays and NMO-IgG as a cancer marker; has a patent pending for MPA1B antibody as a marker of neurologic autoimmunity and paraneoplastic disorders; consulted for Alexion and Medimmune; and received research support from Grifols, Medimmune, and Alexion. All compensation for consulting activities is paid directly to Mayo Clinic. Go to Neurology.org/N for full disclosures.

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Mark Woodhall, PhD	Oxford Autoimmune Neurology Group, Nuffield Department of Clinical Neurosciences, UK	Author	Acquisition, analysis, and interpretation of data; critical revision of the manuscript
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Masoud Majed, MD	Department of Neurology, Mayo Clinic, Rochester, MN	Author	Drafting of manuscript; acquisition, analysis and interpretation of data; statistical analysis; critical revision of the manuscript
Jim Fryer, MS	Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN	Author	Acquisition, analysis, and interpretation of data; critical revision of the manuscript
John Mills, PhD	Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN	Author	Acquisition, analysis, and interpretation of data; statistical analysis; critical revision of the manuscript

Appendix *(continued)*

Name	Location	Role	Contribution
Eoin P. Flanagan, MB, BCh	Departments of Neurology and Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN	Author	Acquisition, analysis, and interpretation of data; critical revision of the manuscript
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Amy Kunchok	Department of Neurology, Mayo Clinic, Rochester, MN	Author	Acquisition, analysis, and interpretation of data; critical revision of the manuscript
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Sean J. Pittock, MD	Departments of Neurology and Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN	Author	Study design and conceptualization; drafting of manuscript; acquisition, analysis, and interpretation of data; critical revision of the manuscript

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