

e-Methods

MOG antibody testing:

Live Cell-based Assays by indirect Immunofluorescence (CBA-IIF):

Serum from all patients (n=160) and available paired CSF samples (n=127) were tested for MOG-IgG using an in-house CBA with HEK293 cells transfected with full-length MOG, C-terminally fused to EGFP as previously described.¹ The live cells were trypsinized overnight at 37°C in a p100 plate. They were then transferred to a p60 plate with the coverslips and transfected with the MOG DNA overnight at 37°C. The next day, the cells were incubated at 37 °C with serum (1:80) or CSF (1:2) diluted in DMEM, for 35 min at 37°C. The cells were washed 3 times with PBS and then fixed with 4% PFA for 10 min. After 7 wash cycles cells (3 with PBS, 1 with 0.3% Triton, and 3 with PBS), the cells were immunolabeled with an Alexa Fluor 594 secondary antibody against IgG-Fc γ fragment-specific (1:1000) for 35 min at room temperature, protected from light. The coverslips were then washed 3 times with distilled water and mounted with DAPI-containing medium (Vectashield) and visualized under a fluorescence microscope.

The specificity of the serum 1:80 cutoff was confirmed by analyzing 429 samples from patients with inflammatory neurological disorders, including 373 with multiple sclerosis, in which found no false positives were identified.²

For MOG-IgM and IgA, the same procedure was followed using Alexa Fluor 594 against IgM-Fc μ (1:4000) or Alexa Fluor 594 against IgA α chain specific (1:1000) as secondary antibody.³

In patients showing myelin staining on rat brain tissue immunohistochemistry, CBA-IIF was performed using the same procedure, but with rodent MOG DNA, as previously described.⁴

Live CBA by Fluorescence-Activated Cell Sorting (CBA-FACS):

All serum samples negative for MOG-IgG by CBA-IIF (but not CSF, due limited sample availability and lower sensitivity), were additionally examined for MOG-IgG using an in house live CBA-FACS with HEK293 cells transfected with full-length

MOG C-terminally fused to EGFP. The Live cells were trypsinized, washed and incubated at 37 °C with serum (1:160 diluted in DMEM) for 30 min in a 96-well plate at a 1×10^5 concentration/well. Cells were washed 3 times with PBS and then fixed with 4% PFA for 10 min. After 3 wash cycles cells were immunolabeled with Alexa Fluor 647 secondary antibody against IgG-Fc γ fragment-specific (1:500) for 1 hour at room temperature. Cells were washed 3 times and analyzed in Cytoflex flow cytometer.

MOG IgG positivity was determined with MOG ratio = Fluorescence of MOG+ cells/Fluorescence of MOG- cells with a threshold of MOG ratio >2. IgG MOG Ratio between 2-4 was considered low positive and IgG MOG Ratio > 4 considered clear positive. Samples with values between 2-4 were confirmed with IIF-CBA at a serum dilution 1:80.

Standard Protocol Approvals, Registrations and Data availability

We used the STROBE cohort reporting guidelines.⁵ The data have been included in a centralized database anonymously complying with the current confidentiality and data protection regulations of personal nature (European Union regulation 2016/679) and is available from the corresponding author upon reasonable request. Samples are stored in the Neuroimmunology Collection (registered in the ISCII with number C0000051 and in the Biobank of IDIBAPS with the number R091217-012).

References:

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