DETECTION OF ANTI MOG ANTIBODIES IN DEMYELINATING DISORDERS OF THE CENTRAL NERVOUS SYSTEM (CNS)

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POSTER 395

INTRODUCTION:

Myelin-oligodendrocyte glycoprotein (MOG) is a CNS-specific antigen expressed on the surface of myelin sheaths. Anti MOG antibodies (Abs) have been recently described as diagnostic marker of acquired demyelinating diseases of the CNS different from Multiple Sclerosis (MS) and in particular in seronegative Aquaporin-4 Neuromyelitis Optica spectrum disorders , pediatric ADEM or very early onset pediatric MS. At the moment there is not a standardized protocol to detect MOG-Abs but it is known that the most specific method is an cell based assay (CBA). Here we present our work to set up the FACS procedure to detect the MOG-Abs in routine.

FACS METHOD:

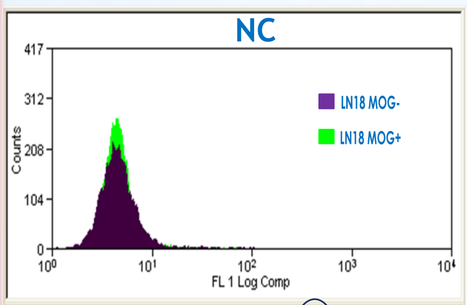
Glial LN18 cell line untrasfected and stably transfected with full-length MOG were provided by Hemmer laboratory in Monaco. Briefly, we incubated both Glial LN18 cell lines with patients' sera diluted 1:200; then cells were washed and incubated with goat anti-human IgG, or anti human IgG subclasses, conjugated with secondary antibody Alexa Fluor 488 diluted 1:100 and analyzed immediately by FACS.

INTERNAL CONTROLS

In each test we consider 3 different internal controls that are stored at -20°C in small aliquots sufficient to performe a single test in order to avoid the variations due to the repeated thawing. They are: 1 healthy commercial serum (NC), 1 purified anti MOG Ab commercial control, 1 human IgG anti-MOG+ patient serum. We expressed the levels of Abs titers as the difference in mean fluorescence intensity (ΔMFI) between the MOG-transfected and untransfected LN18 cell lines. Our cut-off assay is ΔMFI = 23 based on the average ΔMFI plus 6 SD of 42 healthy control. Each serum with value up to ΔMFI =23 was tested one more time adding the IgG subclasses evaluation.

INTERNAL TEST CONTROLS

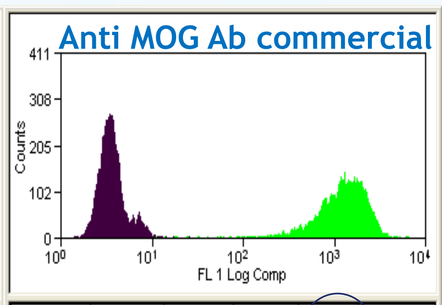
NC



Region	Count	% Hist	% All	Mean	Median	Std Dev.
Total	5000	100.00	100.00	7.45	4.56	97.18
Total	5111	100.00	100.00	6.08	4.56	13.12

Δ MFI= 6,08 - 7,45 = -1,37

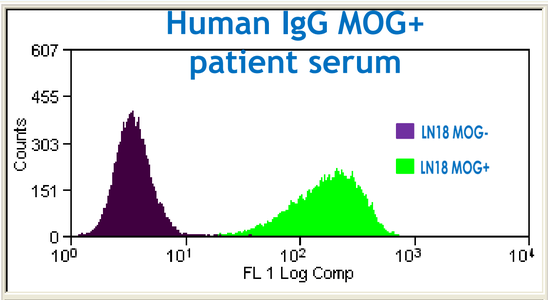
Anti MOG Ab commercial



Region	Count	% Hist	% All	Mean	Median
Total	5000	100.00	100.00	10.43	3.54
Total	5000	100.00	100.00	1442.98	1323.03

Δ MFI= 1443 - 10,43 =1432,5

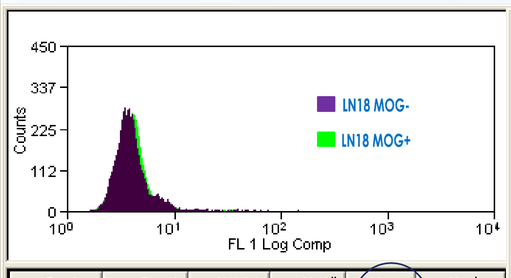
Human IgG MOG+ patient serum



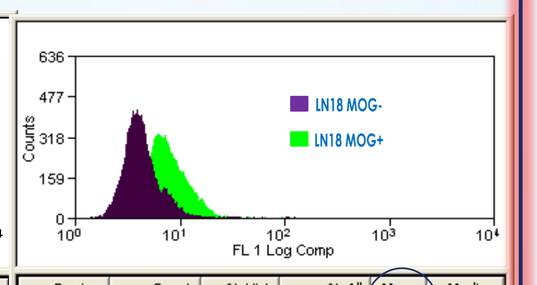
Region	Count	% Hist	% All	Mean	Median
Total	9131	100.00	100.00	4.68	3.41
Total	9461	100.00	100.00	206.45	181.48

Δ MFI= 206.45 - 4.68 = 201.77

MOG- patient serum



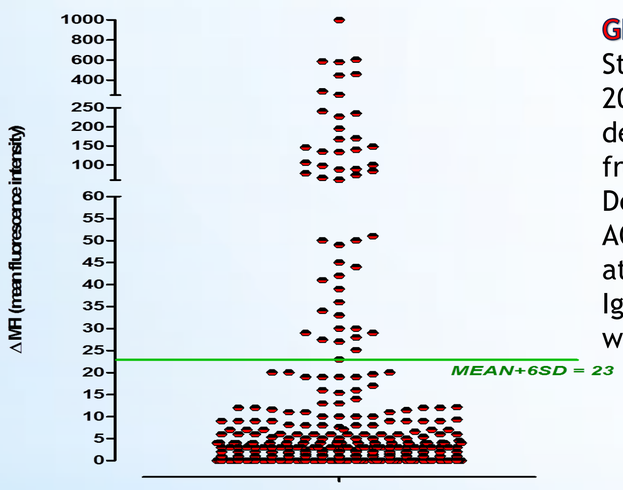
Region	Count	% Hist	% All	Mean	Median
Total	5000	100.00	100.00	5.48	3.81
Total	9370	100.00	100.00	5.04	3.95



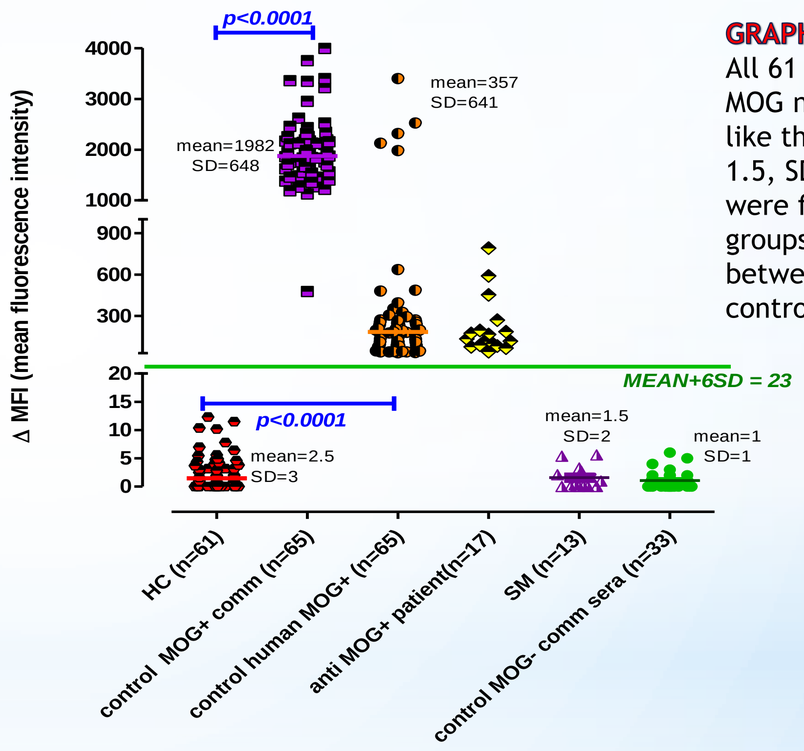
Region	Count	% Hist	% All	Mean	Median
Total	9157	100.00	100.00	6.07	4.09
Total	9520	100.00	100.00	9.07	7.29

RESULTS (update until April 2017):

The low and high limit detection of CBA was verified using serial dilution up to 1:2560 of both controls commercial and human serum MOG+ (R^2= 0.99 for both regression curve). Medium values of positive controls were 1982 for purified anti-MOG Ab and 357 for IgG anti-MOG+ human sample.

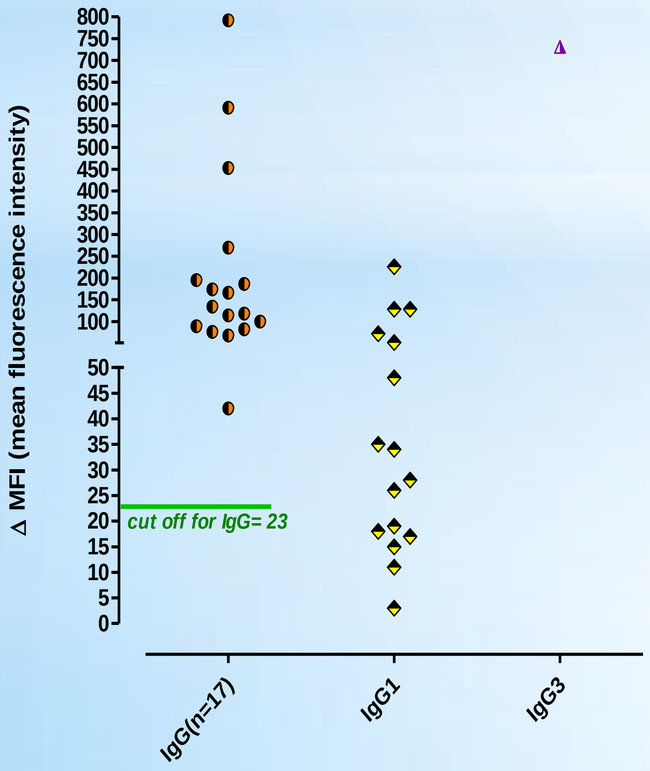


GRAPH 1:
Starting from June 2015 until April 2017 we performe 570 anti MOG detection on 488 patients coming from different Italian Neurology Department. Cross reactivity to AQP4 and MOG was never detected at the same time. We identifying 21 IgG MOG+ patients (4.3%) consistent with literature data.



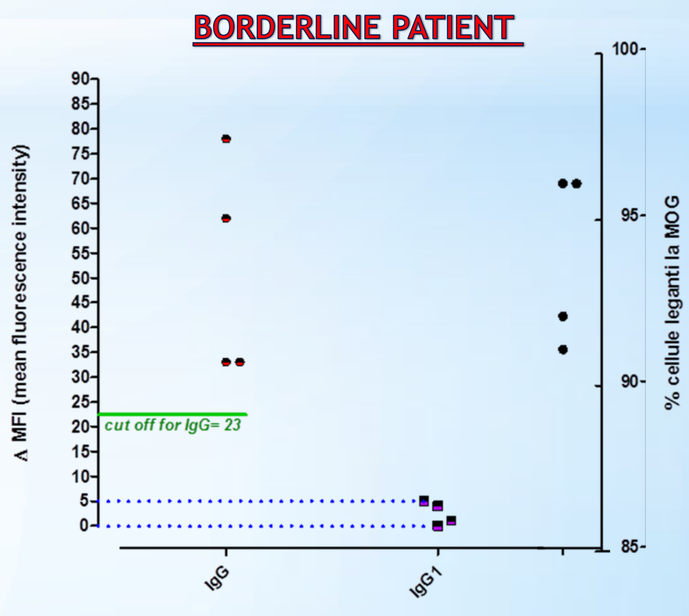
GRAPH 2:
All 61 healthy controls (HC) were MOG negative (mean 2.5, SD 3), like the 13 MS sera patients (mean 1.5, SD 2). No statistical differences were found between HC and MS groups while the difference between HC and both positive controls was significant (p<0.0001).

IgG1 MOG+ PATIENT



GRAPH 3 and 4:
The majority of IgG MOG+ patients are IgG1, only one is IgG3. In this group we identifying 4 BORDERLINE PATIENTS with no caracterization of IgG subclasses and Ab titre around the threshold, but surprisingly with a very high values of % binding MOG cells (96%) like the IgG1 MOG+ patients. In this case we suggest to retest the serum patient after 3 months. In fact, 1 of these patients became IgG1 anti-MOG+ 3 months later.

BORDERLINE PATIENT



CONCLUSION:

Our CBA test is reliable and reproducible and do not provide false positive results. We are the only one Italian laboratory offering a CBA for diagnostic routine analyze and not only for research. We receive samples coming from different Italian neurology department. The MOG detection represents a new diagnostic tool to discriminate from different inflammatory CNS diseases.

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