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# Intrathecal Anti-MOG Antibody Production is Elevated in Multiple Sclerosis

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## Abstract

**Objective—**Evaluate antibodies (Ab) to myelin oligodendrocyte glycoprotein (MOG) in the serum and cerebrospinal fluid (CSF) of multiple sclerosis (MS) subjects and controls.

**Design—**Prospective case control series.

**Setting—**Academic referral center.

**Subjects—**Twenty-six controls with non-inflammatory neurologic disease (NIND) and 35 MS subjects donated serum and CSF for rMOG Ab determination.

**Main Outcome Measures—**Serum and CSF rMOG Ab and albumin levels were used to calculate an "rMOG index". Clinical disability, CSF markers, and magnetic resonance (MR) metrics were correlated to rMOG index.

**Results—**rMOG index was elevated in MS subjects compared to controls (p=0.012). Progressive MS subjects exhibited elevated rMOG indices compared to relapsing remitting MS (RRMS)

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Dr. O'Connor contributed to the manuscript through data analysis and manuscript preparation.
Dr. Cross contributed to the manuscript through subject recruitment, data collection, data analysis and manuscript preparation.

The corresponding author takes full responsibility for the data, the analyses and interpretation, and the conduct of the research. The corresponding author guarantees the accuracy of the references. The corresponding author has full access to all the data and has the right to publish any and all data, separate and apart from the attitudes of the sponsor.
The Methods section includes a statement that IRB approval as been obtained for the use of human subjects for this study.
All authors have agreed to conditions noted on the Authorship Agreement Form.

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(p=0.041). rMOG index was inferior to IgG index in differentiating MS subjects from controls. However, 7 of 16 MS subjects with normal IgG indices had an elevated rMOG index. rMOG index did not correlate with clinical disability, other CSF markers, or radiographic outcome measures.

**Conclusions—**rMOG index, a marker of intrathecal MOG Ab production, may provide complementary information to routine CSF testing in the diagnosis of MS. Furthermore, intrathecal anti-MOG Ab production may be more pronounced in progressive than relapsing forms of MS.

## INTRODUCTION

Multiple sclerosis (MS) is a demyelinating neurologic disorder of the central nervous system (CNS) with a hypothesized autoimmune etiology and a clinical course that is often unpredictable at disease onset.[1] Discovering a pathologic biomarker to help accurately make the MS diagnosis or predict disease activity and progression would be very useful. Humoral immunity may play a role in MS pathogenesis as suggested by cerebrospinal fluid (CSF) oligoclonal IgG[2] and by the presence of antibodies (Abs) and complement in association with myelin damage in MS plaques.[3, 4] Various antigens have been proposed as targets of the autoantibody response.[5, 6] Myelin oligodendrocyte glycoprotein (MOG) is one candidate target self-antigen. This protein is a small component of myelin exclusive to the CNS located on the outer surface of the myelin sheath and hence accessible to Ab attack.[7, 8] MOG is used to induce experimental autoimmune encephalomyelitis (EAE) in many species.[9, 10] Although anti-MOG Abs alone cannot induce EAE, they enhance demyelination in some rodent and primate EAE models.[10, 11] In humans, the pathogenic role of anti-MOG Abs is less clear. The potential of anti-MOG Abs as diagnostic and/or prognostic biomarkers is also unknown. Previous studies have shown that MOG-specific Abs and T cells are present in healthy controls as well as in MS patients,[12] suggesting that the presence of serum anti-MOG Abs will not be useful to diagnose MS. However, the level and specific target of serum Abs to MOG may be important.[13-18] For example, serum autoantibodies that targeted extracellular MOG in its native conformation were shown to be lytic *in vitro*, supporting a potential pathogenic role of these Abs in MS.[19] Controversy surrounds whether serum Abs against recombinant MOG may predict a second MS relapse in clinically isolated syndrome (CIS) patients.[20-25] Some of the contradictory evidence to date is likely the result of methodological differences between studies. In another CIS study, anti-myelin Abs were associated with intrathecal IgG production, CSF pleocytosis, and T2 lesion load.[26] Other studies suggest MOG Ab levels are elevated in CSF of MS patients compared to non-inflammatory neurologic disease (NIND) controls.[27, 28]

The present study was undertaken to further explore the relationship between serum and CSF anti-MOG Abs and MS diagnosis, clinical course and activity. Ab levels were quantified by ELISA using recombinant human extracellular MOG that adopted the native conformation and was glycosylated. To determine whether intrathecal production of anti-MOG Abs (e.g. in CSF) might be important, an “rMOG Index” was calculated.

## METHODS

### Subjects

This study was performed at Washington University (St. Louis MO) with Institutional Review Board (IRB) approval. Written consent was obtained from all participants. CSF and serum samples were collected concurrently during diagnostic procedures from 26 NIND subjects and 35 MS subjects (Table 1). NIND group included subjects with headache, seizure disorder and stroke or small vessel disease. All NIND subjects had either a normal

brain magnetic resonance imaging (MRI) or evidence of small vessel disease or stroke, and CSF analyses without evidence of CNS inflammation or autoimmune processes such as intrathecal immunoglobulin (Ig) production or presence of oligoclonal Ig. All subjects with MS fulfilled the McDonald criteria for MS diagnosis.[29] Patients were classified by their MS specialist physician based on previously published criteria for MS clinical subtypes.[30] Of the MS subjects, 22 had relapsing remitting MS (RRMS), five had secondary progressive MS (SPMS) and eight had primary progressive MS (PPMS).

Routine CSF studies, IgG index, and oligoclonal bands were determined for each subject. All CSF analyses were performed at Barnes-Jewish Hospital in St. Louis, MO, except oligoclonal band determination which was determined by isoelectric focusing with IgG immunoblotting at Mayo Medical Laboratories in Rochester, MN.[31] Relapse or remission status was determined at the time of lumbar puncture, prior to performing the rMOG assays. Expanded Disability Status Scale (EDSS)[32] and the Multiple Sclerosis Severity Score (MSSS)[33] were determined at the time of CSF analyses. Seventeen MS subjects had magnetic resonance (MR) determination of gadolinium enhancement number and volume, T1 hypointensity number and volume, and T2 hyperintensity number and volume as part of a separate study, performed at the time of CSF analysis.

### Recombinant human myelin oligodendrocyte glycoprotein (rMOG)

The recombinant MOG (rMOG) protein consisting of the 120 amino acid extracellular domain of MOG was produced using a baculovirus and insect cell-mediated expression system, then purified as previously described.[34] The recombinant protein was partially glycosylated as demonstrated after treatment for 3h at 37°C with the enzymatic protein deglycosylation kit (Sigma-Aldrich, St Louis, MO), according to manufacturer's protocol, to remove N-linked and O-linked carbohydrates (data not shown). To confirm that rMOG folded and that conformational determinants were present in the recombinant product, an independent laboratory measured binding to the rMOG with a monoclonal Ab (mAb 2B7) directed against a conformation-dependent epitope. The mAb 2B7 recognized the rMOG in a solid-phase dissociation-enhanced lanthanide fluorescence immunoassay (DELFIA) performed as previously described, confirming that the rMOG was folded and conformation-dependent epitopes were present.[35]

### ELISAs to measure Abs to human myelin oligodendrocyte glycoprotein (MOG)

Ig levels (all isotypes) to rMOG in serum and CSF samples were semi-quantitatively measured by ELISA as previously described.[36] Briefly, rMOG was coated at 10 μg/ml in bicarbonate buffer overnight. Standards of human gamma globulin (Jackson ImmunoResearch West Grove, PA) at varying concentrations (from 570 ng/ml to 9 ng/ml) in duplicate wells were included in each assay. Next, plates were washed with PBS four times and PBS–3% BSA was added for 2 h at 22°C. Plates were again washed, after which diluted serum samples (1:250 and 1:500) were added to the wells in duplicate for 1 h at 22°C. In contrast to serum samples, CSF samples were assayed undiluted and incubated overnight at 4°C. Known positive and known negative samples were included with each assay to confirm interassay consistency. After incubation of the serum/CSF samples plates were washed and goat anti-human poly-valent Ig-HRP (1:3500; Sigma, St. Louis, MO) was applied for 1 h at 22°C. Plates were then washed four times, and 100μl freshly prepared tetramethylbenzidine substrate (BD Biosciences, San Diego) was added per well for 30 min at 22°C, protected from light. The reaction was stopped by addition of 100μl of 2.5M sulfuric acid (LabChem Inc. Pittsburgh). Absorbance was read within 30 min at 450nm on a BioTek ELX800 ELISA plate reader and data analyzed with KC junior software (BioTek). To consider a serum sample to be positive, two criteria were required: a dilution effect (i.e., absorbance at 1:500 must be approximately 1/2 of that at 1:250), and absorbance must be ≥ 0.1.

The rMOG index is a formula that accounts for blood-brain barrier integrity and indicates the level of Ab to rMOG made within the CNS. This formula is similar to the method of IgG index calculation, but uses the rMOG absorbances in place of IgG concentrations. The rMOG index was calculated by the following formula:

$$\text{rMOG index} = \frac{\text{CSF rMOG Ab} / \text{Serum rMOG Ab}}{\text{CSF albumin} / \text{Serum albumin}}$$

### Statistical Analysis

rMOG indices of two independent samples were compared using Student's t-test or Mann Whitney U tests based on sample size and distribution. Correlation of rMOG indices with CSF indices, disability level and MR metrics were made with Pearson or Spearman correlation as appropriate. Receiver operator characteristic (ROC) curves were generated using the statistical program SPSS 16.0 (SPSS Inc.)

## RESULTS

Antibodies to rMOG were assayed in 35 MS subjects and 26 controls, with similar demographics (Table 1). No statistical differences existed between controls and MS subjects for serum rMOG Abs (mean absorbance 0.443 ± 0.383 vs. 0.341 ± 0.309; p=0.436) (Figure 1a) or CSF rMOG Ab (mean absorbance 0.194 ± 0.222 vs. 0.264 ± 0.262; p=0.271) (Figure 1b). However, the rMOG index showed a significant difference between controls and MS subjects (0.376 ± 0.247 vs. 0.696 ± 0.663; p=0.012) (Figure 1c). Range of the rMOG index for controls was 0.073-0.973 while range for MS subjects was 0.147-3.051. 25.7% of MS subjects had rMOG index >2 SD above the normal control mean rMOG index.

An rMOG index positive threshold of 0.7 yielded the optimal sensitivity and specificity as a diagnostic test, displaying a sensitivity of 0.37, a specificity of 0.92, a positive predictive value of 0.87, and a negative predictive value of 0.52 for MS. To further assess the usefulness of the rMOG index in the diagnosis of MS, sensitivity and specificity values for rMOG index and IgG index were compared. Area under the ROC curve for IgG index was 0.891 while it was 0.685 for rMOG index (Figure 3). Thus, the IgG index was a superior for differentiating controls versus MS subjects in this cohort. Nonetheless, 7 subjects with normal IgG index had elevated rMOG index >0.7.

Antibodies to rMOG were compared among different MS clinical subtypes. Due to low sample sizes, the SPMS and PPMS groups were combined into a single progressive MS group for statistical comparison to the RRMS group. When comparing serum and CSF anti-MOG Abs between RRMS and progressive MS (SPMS and PPMS), no statistical difference was observed (p=0.864 and p=0.120 respectively). However, a higher mean rMOG index was found in progressive MS subjects (n=13) compared to RRMS subjects (n=22) (mean 0.899 vs. 0.576 respectively; p=0.041) (Figure 2). On the other hand, no difference in IgG index between RRMS and progressive MS was observed (0.988 vs. 0.901 respectively; p=0.442).

Interestingly, no correlations between rMOG index and other CSF markers were found in this cohort. rMOG index and IgG index correlated poorly with one another ($R^2$=0.014). rMOG index did not differ between MS subjects with or without oligoclonal bands (p=0.107). The CSF white blood cell count did not correlate with serum rMOG Abs (p=0.507), CSF rMOG Abs (p=0.835), or the rMOG index (p=0.814).

There was no clear correlation of rMOG index with other clinical and radiographic outcomes. No significant difference in rMOG index in subjects in the midst of a relapse or in remission was observed, although the comparison was limited in that only five subjects were in a relapse at the time of lumbar puncture. Level of disability at the time of lumbar puncture as measured by EDSS or MSSS did not correlate with rMOG index (p=0.559 and p=0.881 respectively). In subjects with quantitative brain MR measures, there was no association of rMOG index with gadolinium enhancement (p=0.746), T1 hypointensity number (p=0.269) or volume (p=0.363), or T2 lesion number (p=0.925) or volume (p=0.918).

## COMMENT

The present study expands upon prior studies by calculating the rMOG index as a reflection of the intrathecal synthesis of Abs to rMOG. Interestingly, the rMOG index was higher in MS subjects than controls, whereas CSF anti-MOG Abs showed no difference. Moreover, serum anti-MOG Abs did not differentiate controls from MS subjects in the present study, corroborating several other published studies.[15, 17, 18, 37]

This study also adds to existing literature by examining a different cohort of MS subjects, including several with advanced disease and including subjects with progressive clinical subtypes of MS. Progression can be defined as accumulation or advancement of disability, not in the setting of acute clinical relapse. In the present cohort, rMOG index was higher in subjects with SPMS and PPMS when compared to RRMS, while serum and CSF anti-MOG Abs individually demonstrated no difference. A previous study examining MS subtypes did not detect differences in CSF anti-MOG Ab levels between RRMS and PPMS, while SPMS subjects were not included.[18] In another study, higher levels of anti-MOG Abs in SPMS compared to RRMS subjects were reported.[38] Ectopic B-cell follicles have been detected in the meninges of SPMS subjects and were associated with an aggressive clinical course. These ectopic lymphoid follicles may be a source of MOG Ab production within the CNS.[39, 40] Antibodies directed against conformational epitopes of extracellular MOG are known to have demyelinating potential[41, 42] and might be implicated in degenerative changes characteristic of the progressive clinical subtypes of the disease. One might hypothesize that rMOG index elevation suggests the degree of CNS damage as opposed to IgG index which is non-antigen specific. More study is needed to determine whether the rMOG index may predict progression of disability outside of relapses. Additionally, longitudinal studies would help determine if rMOG Ab status changes over time within individual patients and in relation to changing disease phase.

The present study indicates that the rMOG index was inferior to the IgG Index for diagnosis of MS. However, employing the positive rMOG index threshold of 0.7 identified an additional seven of the sixteen MS subjects with a normal IgG index. Moreover, the two indices did not correlate with one another. Sampling error may explain why the number of MS subjects with normal IgG indices is greater than previously published sensitivities of the test in isolation.[43] Employing the rMOG index increased the sensitivity of CSF testing from 80% to 91%. This may indicate that the rMOG index can provide important information distinct from that provided by total IgG intrathecal production (Table 2A and 2B). Another study also demonstrated that intrathecal synthesis of anti-MOG Abs was occurring in MS patients when total IgG intrathecal synthesis was not elevated.[38]

The clinical role (diagnostic and prognostic) of anti-MOG Abs in MS remains controversial. Differences reported in the literature could be due to the varied techniques used to detect MOG Abs (ELISA, immunoblot, immunohistochemistry, RIA and FACS-based assays) as well as different MOG protein preparations used as antigen. Extensive studies in EAE models have shown that only Abs that recognize native extracellular MOG protein are

pathogenic, whereas Abs that bind to denatured protein or short synthetic peptides fail to induce demyelination.[44-46] Natural MOG is glycosylated. Previous reports found that MS patients had serum reactivity against a synthetic glycosylated MOG fragment, but not against non-glycosylated fragments.[47] In the present study, recombinant human MOG-extracellular portion was produced in insect cells, resulting in a preparation which is partially glycosylated (data not shown) with the conformational structure of native mammalian extracellular MOG protein.

A large number of published studies have examined the significance of Abs to MOG in the CIS and MS populations.[20, 21] Several of these studies examined the ability of Abs to predict a second relapse in CIS. In one study of 103 CIS subjects, the presence of serum antibodies to rMOG predicted shortened time to second attack.[20] In another study of 133 subjects with CIS, it was reported that serum anti-MOG and anti-myelin basic protein Abs correlated with intrathecal IgG production, CSF pleocytosis, and T2 lesion load,[26] but did not predict future development of clinically definite MS. On the other hand, in the present study, which included only subjects with established MS, correlation between rMOG index or serum rMOG Ab levels and other CSF and radiographic measures was absent. Differences in results between studies may reflect differences in patient populations and methodological differences.

Limitations of this study include small sample size, particularly of the progressive MS subgroups. Additionally, quantitative brain MR imaging was conducted on only about half the MS subjects limiting the power to make correlations. The control group of this study did not include other inflammatory neurologic diseases (OIND), which may demonstrate increased anti-MOG Ab levels.[27] Furthermore, CIS patients, a group that might benefit from improved diagnostic testing, were not included in this study. A final limitation is that antibody levels were determined by direct absorbance due to the lack of suitable reagents to construct a standard curve specific to rMOG antibodies.

The rMOG index has potential to serve as an additional diagnostic tool for MS, since it identified 7 subjects with MS in this cohort that had normal IgG indices. While inferior to the established diagnostic IgG index test, rMOG index may complement markers of intrathecal immunoglobulin production. Moreover, the rMOG index was associated with progressive disease subtypes in our small cohort. Thus, further studies are needed to evaluate the diagnostic and prognostic utility of the rMOG index, including its utility in predicting evolution from clinically isolated syndrome to clinically definite MS, and to confirm its predictive value for progressive subtypes of MS.

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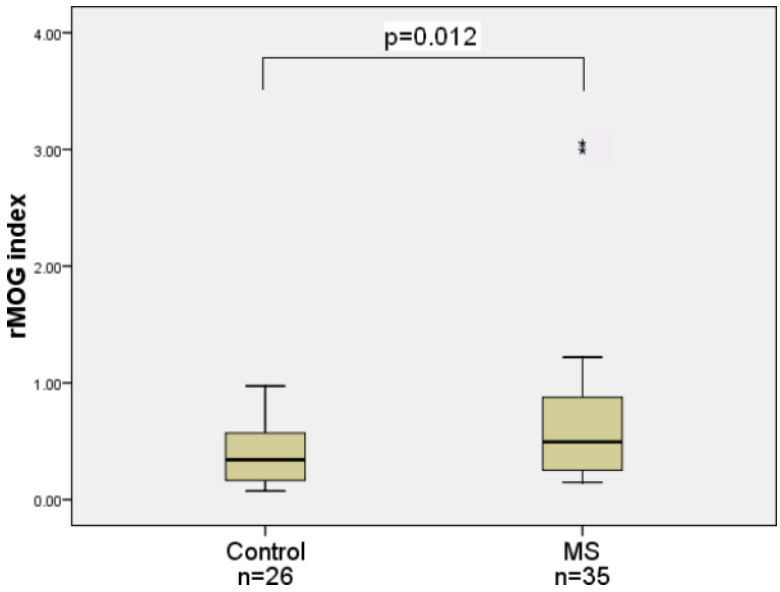


**Figure 1.**
rMOG index in controls versus MS. The box plot compares rMOG indices between 26 control subjects and 35 MS subjects. MS subjects have elevated rMOG indices compared to controls (p=0.012).

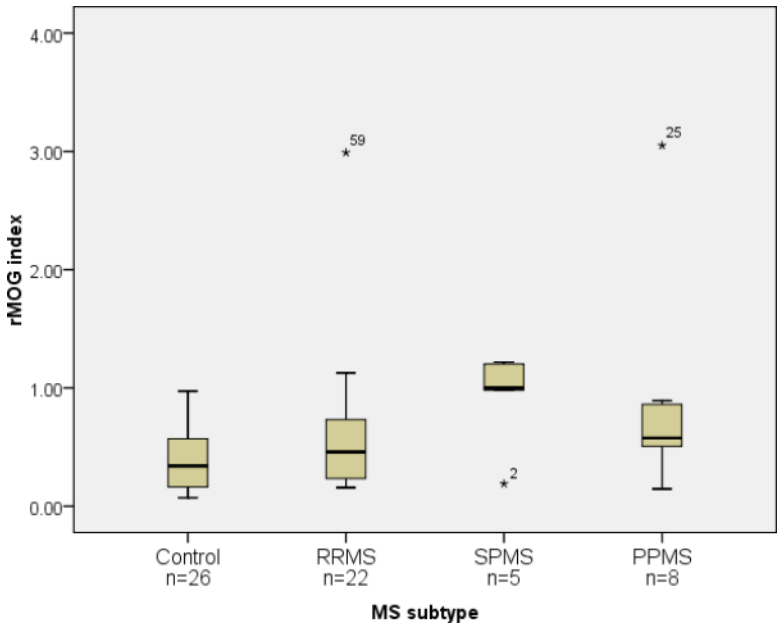


**Figure 2.**

rMOG indices of MS subtypes. A box plot of controls, and MS subjects separated by disease subtype (RRMS, SPMS, PPMS) reflects elevated rMOG index in progressive MS subjects (p=0.041).

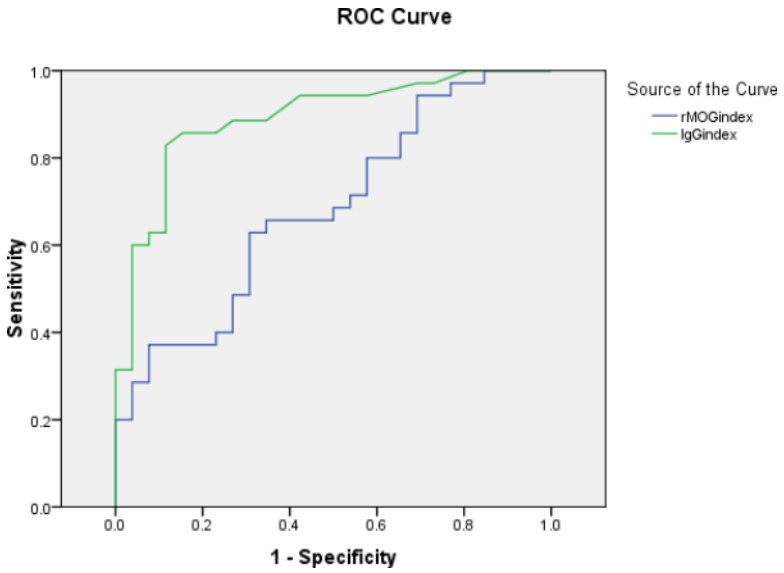


**Figure 3.**
Receiver Operator Curve (ROC) of rMOG index and IgG index. A larger area under the curvereflects a better diagnostic test. TheROC demonstrates superiority of IgG index (area under the curve 0.891) over rMOG index (0.685) as a diagnostic test for differentiating MS from controls.

## Table 1

Demographics of control and MS subjects.

| | Controls | MS |
|---|---|---|
| Number | n=26 | n=35 |
| Subtype | N/A | RRMS; n=22<br>SPMS; n=5<br>PPMS; n=8 |
| Mean age +/- SD | 43.5 +/- 14.4 | 44.0 +/- 8.7 |
| Gender (F:M) | 20:6 | 20:15 |
| Ethnicity (C:AA ) | 23:3 | 32:3 |
| Disease duration | N/A | 9.7 +/- 7.3 |
| Disease Modifying Therapy | N/A | None; n=13<br>IFN-β1a IM; n=5<br>IFN-β1a SC; n=4<br>IFN-β1b SC; n=9<br>GA; n=4 |
| Median EDSS (range) | N/A | 6.0 (1.0-7.0) |
| Median MSSS (range) | N/A | 6.74 (2.56-9.59) |

C = Caucasian; AA = African American; N/A = not applicable; RRMS = relapsing remitting MS; SPMS = secondary progressive MS; PPMS = primary progressive MS; IFN = Interferon; GA = Glatiramer acetate; EDSS = Expanded Disability Status Scale; MSSS = Multiple Sclerosis Severity Score.

## Table 2A

Control CSF profile. IgG index, IgG synthesis rate, oligoclonal band and rMOG index status are reported for control subjects. Elevated levels are highlighted in yellow. Control subject #25 is a 26 year old female with cerebral autosomal dominant arteriopathy with subcortical infarcts and and leukoencephalopathy (CADASIL). Control subject #26 is an 82 year old male with cryptococcal meningitis.

| Control # | IgG Index (0.00-0.68) | IgG Synthesis Rate (0.0-2.8) | Oligoclonal Bands (0-3) | rMOG index (0.00-0.69) |
|---|---|---|---|---|
| 1 | 0.37 | <0.0 | Negative | 0.10 |
| 2 | 0.38 | <0.0 | Negative | 0.37 |
| 3 | 0.38 | <0.0 | Negative | 0.39 |
| 4 | 0.41 | <0.0 | Negative | 0.12 |
| 5 | 0.41 | <0.0 | Negative | 0.46 |
| 6 | 0.44 | <0.0 | Negative | 0.22 |
| 7 | 0.44 | <0.0 | Negative | 0.69 |
| 8 | 0.46 | <0.0 | Negative | 0.30 |
| 9 | 0.47 | <0.0 | Negative | 0.15 |
| 10 | 0.47 | <0.0 | Negative | 0.50 |
| 11 | 0.47 | <0.0 | Negative | 0.57 |
| 12 | 0.48 | <0.0 | Negative | 0.15 |
| 13 | 0.48 | N/A | N/A | 0.33 |
| 14 | 0.49 | <0.0 | Negative | 0.20 |
| 15 | 0.49 | <0.0 | Negative | 0.35 |
| 16 | 0.52 | <0.0 | Negative | 0.07 |
| 17 | 0.52 | <0.0 | Negative | 0.60 |
| 18 | 0.53 | <0.0 | Negative | 0.17 |
| 19 | 0.53 | <0.0 | Negative | 0.22 |
| 20 | 0.54 | <0.0 | Negative | 0.86 |
| 21 | 0.55 | <0.0 | Negative | 0.69 |
| 22 | 0.56 | 10.7 | Negative | 0.15 |
| 23 | 0.57 | <0.0 | Negative | 0.97 |
| 24 | 0.64 | <0.0 | Negative | 0.37 |
| 25 | 0.66 | 0.5 | Positive | 0.16 |
| 26 | 0.92 | 16.3 | Positive | 0.62 |

N/A=not available

## Table 2B

IgG index, IgG synthesis rate, oligoclonal band and rMOG index status of MS subjects. Elevated levels are highlighted in yellow.

| MS # | IgG Index (0.00-0.68) | IgG Synthesis Rate (0.0-2.8) | Oligoclonal Bands (0-3) | rMOG index (0.00-0.69) |
|---|---|---|---|---|
| 1 | 0.44 | <0.0 | Negative | 0.87 |
| 2 | 0.47 | <0.0 | Positive | 0.24 |
| 3 | 0.52 | <0.0 | Negative | 1.13 |
| 4 | 0.52 | <0.0 | Negative | 1.22 |
| 5 | 0.54 | <0.0 | Positive | 0.20 |
| 6 | 0.57 | 0.3 | Negative | 0.48 |
| 7 | 0.58 | <0.0 | Negative | 0.88 |
| 8 | 0.59 | 2.7 | Positive | 0.45 |
| 9 | 0.59 | 1.7 | Positive | 0.98 |
| 10 | 0.61 | 0.7 | Positive | 0.19 |
| 11 | 0.61 | 4.3 | Positive | 0.35 |
| 12 | 0.62 | 17.0 | Positive | 0.84 |
| 13 | 0.63 | 0.3 | Negative | 0.15 |
| 14 | 0.65 | 3.7 | Positive | 1.00 |
| 15 | 0.68 | 15.0 | Positive | 0.16 |
| 16 | 0.68 | <0.0 | Negative | 0.48 |
| 17 | 0.72 | 7.4 | Positive | 0.56 |
| 18 | 0.80 | 14.1 | Positive | 0.89 |
| 19 | 0.80 | N/A | Positive | 3.05 |
| 20 | 0.81 | 2.4 | Positive | 0.19 |
| 21 | 0.81 | 3.2 | Positive | 0.59 |
| 22 | 0.85 | 5.5 | Positive | 0.49 |
| 23 | 0.90 | 5.5 | Positive | 0.83 |
| 24 | 0.90 | <0.0 | Negative | 2.99 |
| 25 | 1.04 | 5.6 | Positive | 0.30 |
| 26 | 1.10 | 16.6 | Positive | 0.47 |
| 27 | 1.10 | 68.3 | Positive | 1.20 |
| 28 | 1.28 | 8.9 | Positive | 0.55 |
| 29 | 1.33 | 14.5 | Positive | 0.51 |
| 30 | 1.45 | 25.9 | Positive | 0.19 |
| 31 | 1.49 | 20.0 | Positive | 0.26 |
| 32 | 1.60 | 26.5 | Positive | 0.21 |
| 33 | 1.65 | 39.8 | Positive | 0.24 |
| 34 | 2.15 | 33.7 | Positive | 0.73 |
| 35 | 2.60 | 60.9 | Positive | 0.46 |

N/A=not available