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Background

There is an unmet need to develop specific serum based biomarkers for the diagnosis and prognosis of Relapsing Remitting MS (RRMS). We have reported that elevated levels of serum anti-Glc(α 1,4)Glc(α) (GAGA4) IgM antibodies exist in RRMS patients in comparison to patients with other neurological diseases (OND)¹. We have also reported the ability to discern which patients with CIS (clinically isolated syndrome or first neurological event) convert to RRMS vs. OND patients with 36% sensitivity, and 91% specificity². In this study, we further investigated whether other anti glucose based IgM antibodies may improve the RRMS prediction for CIS patients.

1. Schwarz M, et. al. Serum Anti-Glc(α 1,4)Glc(α) Antibodies as a Biomarker for Relapsing Remitting Multiple Sclerosis. J Neurol Sci. 2006;244(1-2):59-68.

2. Freedman et al. Multiple Sclerosis 2005; 11:S180

Aim

To evaluate the predictive value of IgM antibodies against various glucose based saccharides: Glc(α 1,2)Glc(α) (GAGA2), Glc(α 1,3)Glc(α) (GAGA3), Glc(α 1,4)Glc(α) (GAGA4), Glc(α 1,6)Glc(α) (GAGA6), α-GlcNAc (GNa) and P64 (proprietary glucose based structure) for identifying patients with CIS that will evolve to RRMS, or will have a more active disease.

Anti glycan IgM antibodies in RRMS vs OND patients.

Methods:

Study population: Retrospective analysis on 88 frozen sera taken from CIS patients presented for diagnostic work-up and followed for a minimum of 4 years. Forty four patients were subsequently confirmed to have RRMS. Control group included 44 OND patients (other inflammatory (OIND), n=23, or non-inflammatory neurological disease (ONIND), n=21). The RRMS and control groups were matched for gender composition, age and total IgM.

Immunoassay: Sera were diluted 1:1200 and levels of GAGA6, GAGA4 and GNa IgM Ab measured by enzyme immunoassay and normalized to IgM levels.

Table 1: Demographic and clinical characteristics of study population RRMS vs OND

	RRMS	OND (total)	ONIND	OIND
N	44	44	23	21
Age, mean (SD), years	37.6 (9.0)	38.5 (9.5)	36.5 (7.8)	39.8 (11.1)
Women, n (%)	38 (86)	33 (75)	17 (73)	16 (76)
Total IgM, Mean (SD), RFU*10 ⁶	2.10 (0.80)	1.94 (0.68)	1.91 (0.71)	1.93 (0.69)

Results:

1) Levels of anti-GAGA6 and anti-GAGA4 IgM are depicted in Figure 1A-B.

Significantly higher levels of anti-GAGA6 IgM (p=0.01) and anti-GAGA4 IgM (p=0.005) Ab were observed in CIS patients who converted to RRMS as opposed to OND. The difference for anti-GNa IgM levels was not significant.

2) Using the OND sample set and a cut-off of mean + 2SD for anti GAGA6 and GAGA4, we have found that 17/44 (39%) converting CIS patients were positive for at least one of the markers, whereas 42/44 (95%) OND patients were negative for both Ab, corresponding to a sensitivity of 39%, a specificity of 95%, positive predictive value of 89%, and negative predictive value of 61%. See Table 2 and figure 2 for correlation between anti-GAGA4 and anti-GAGA6 levels.

Table 2: Differentiation between RRMS patients and OND patients based on anti-GAGA4, anti-GAGA6, and combined score

Anti glycan antibodies (cutoff)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive Value (%)	Efficiency (%)
Anti-GAGA4 (0.53)	34.1	90.9	78.9	58.0	62.5
Anti-GAGA6 (0.21)	22.7	97.7	90.0	55.3	59.8
Positive for Anti-GAGA4 or Anti-GAGA6	38.6	95.6	89.5	60.9	67.0

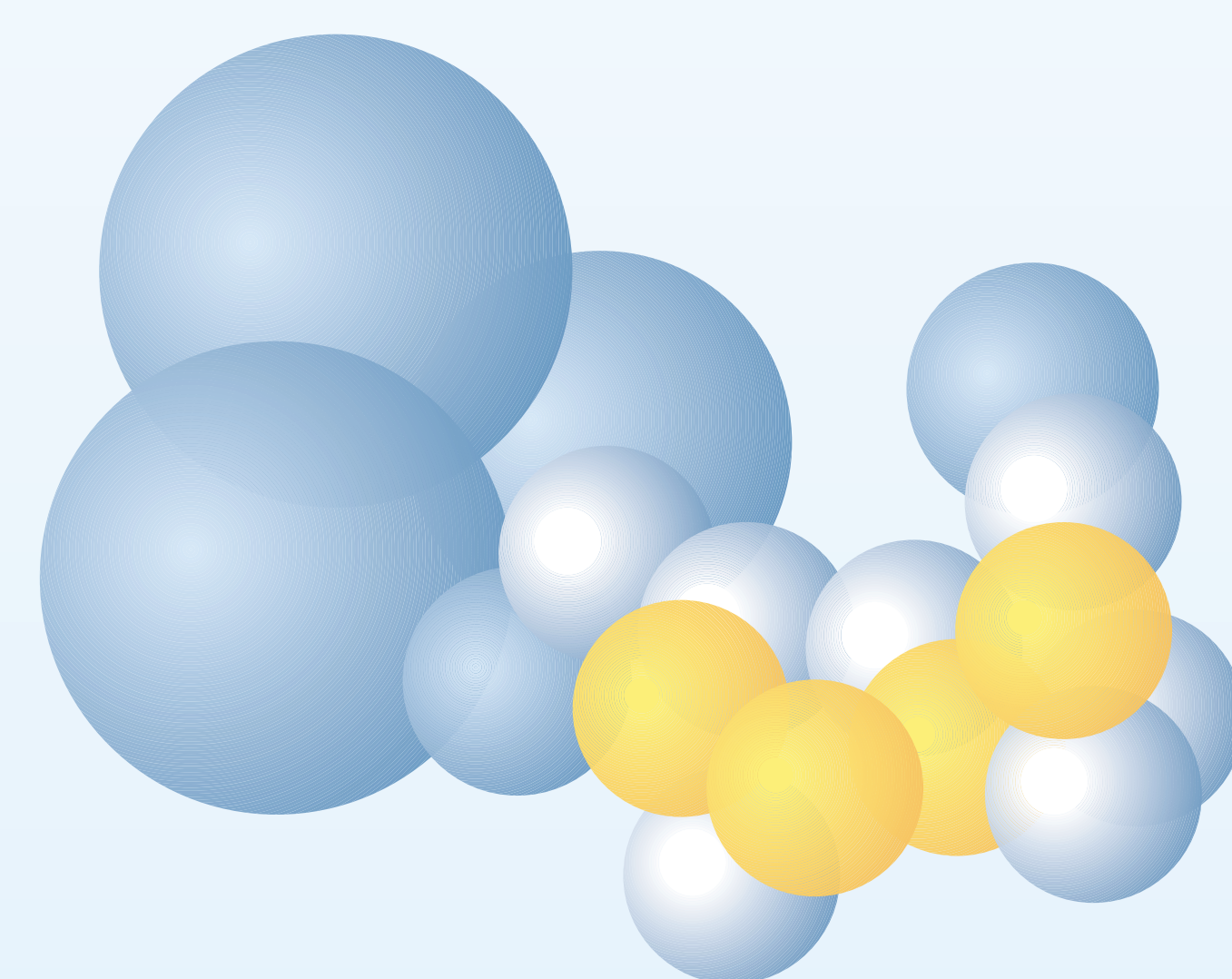


Figure 1 –levels of A) Anti-GAGA6, and B) anti-GAGA4

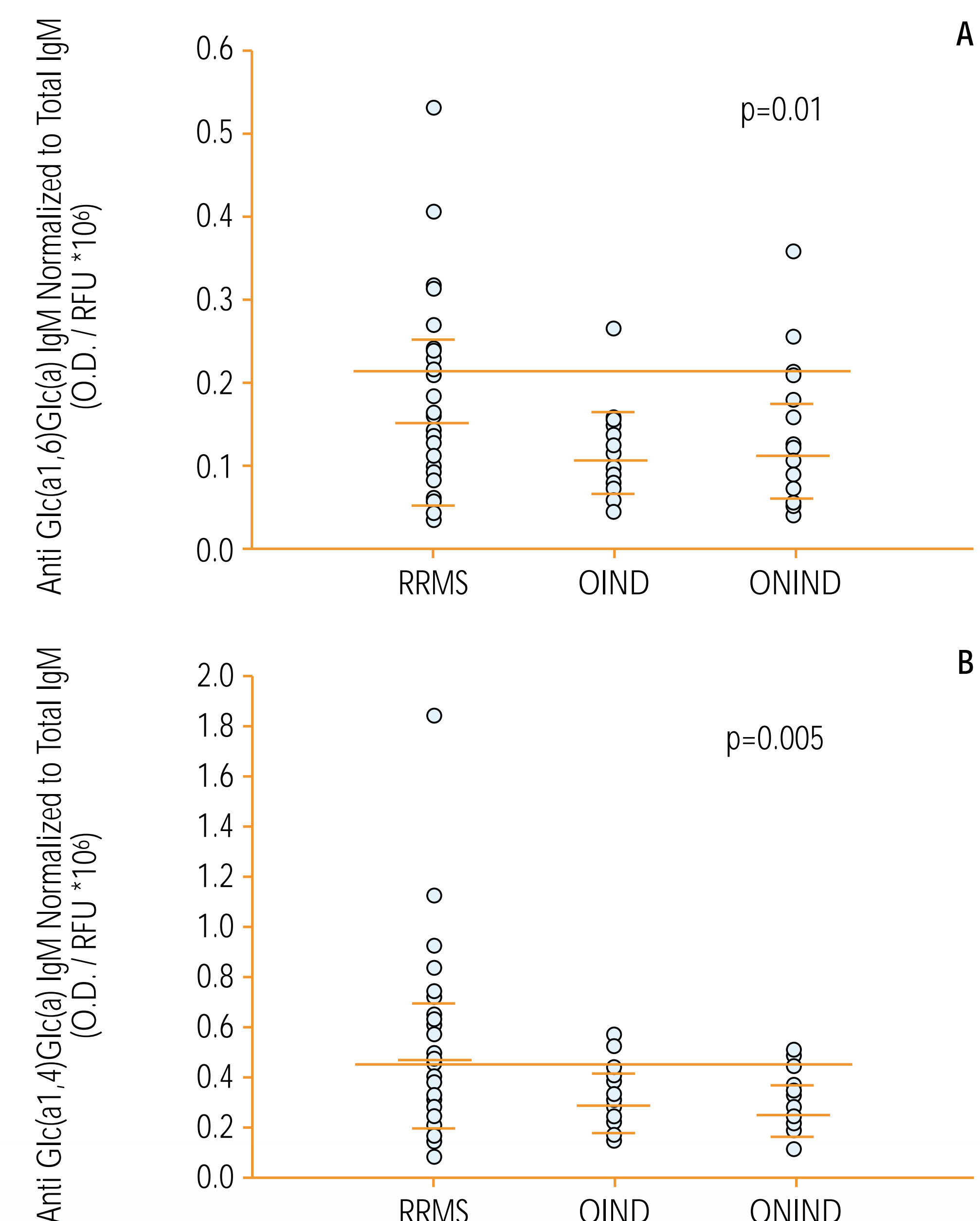
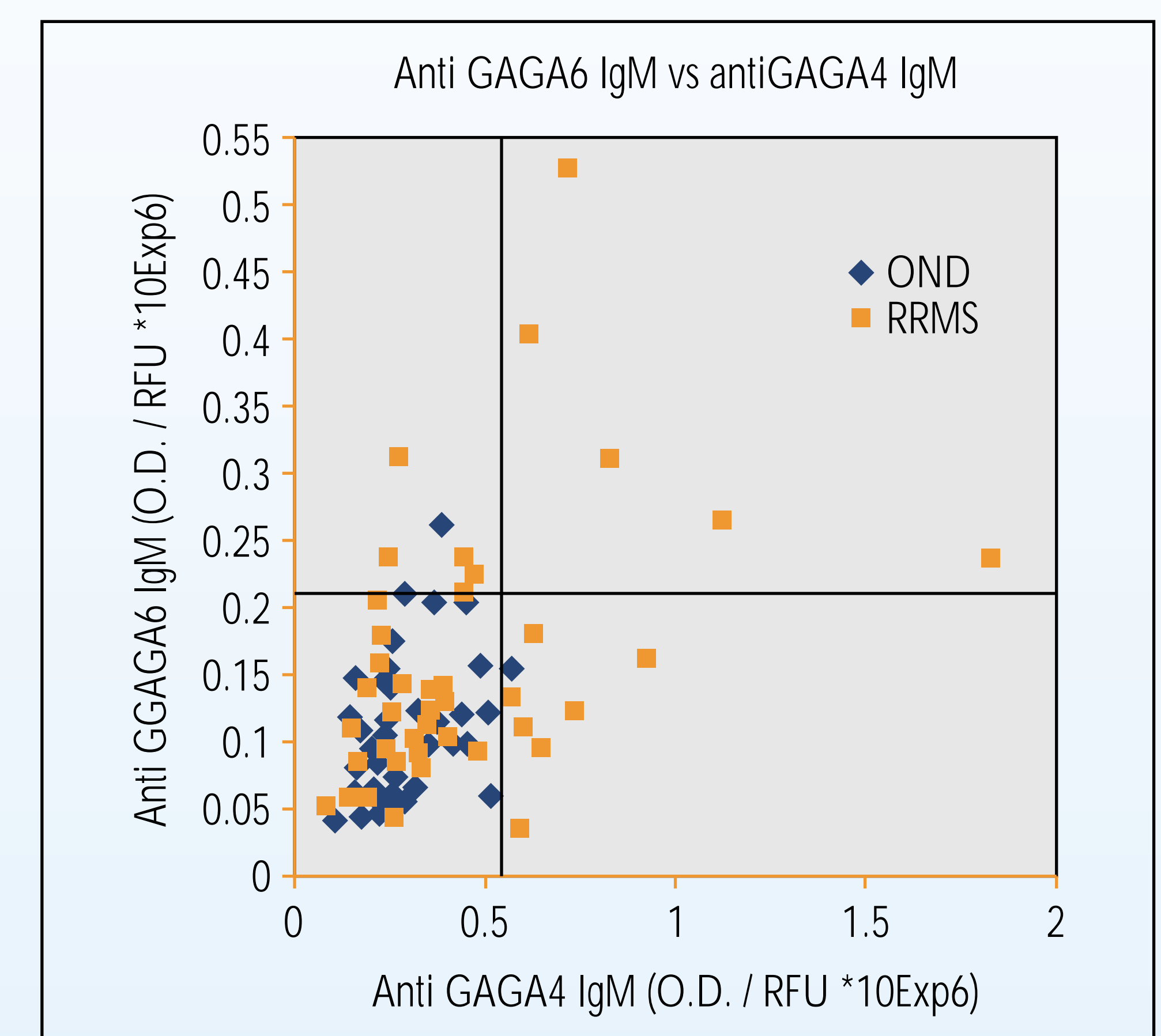


Figure 2: Comparison between anti-GAGA4 and anti-GAGA6 levels in patients who become RRMS and patients who become OND. Bar represents cut of values.



Levels of anti glycan antibodies panel in RRMS patients having early (<24 month) vs. late (>24) next attack

Methods:

Study population: Retrospective analysis of 100 frozen sera samples taken from RRMS patients at the time they were presented for diagnostic work-up, patients were followed up clinically and time to second relapse was recorded.

Immunoassay: Levels of anti-GAGA2, GAGA3, GAGA4, GAGA6, GNa and P64 IgM antibodies were measured by fluorescence immunoassay as described¹. Calibrator sera sample, considered as 1 unit, was included in each run. Antibodies levels were normalized according to calibrator and pronounced in arbitrary units. Patients were considered positive if at least one of the markers was above cutoff.

Table 4: Demographic and clinical characteristics of RRMS patients (n=100) having early (<24 month) vs Late (>24) next attack.

	Early relapse, n=58 (<24 months)	Late relapse, n=42 (>24 months)
Mean age, years (SD)	34.9 (10.9)	36.2(8.2)
Females (%)	43/58(74)	29/42(69)
Mean time to next relapse, months (SD)	11.0(7.0)*	46.8(17.1)

* p<0.001 T test

Results:

Levels of IgM anti-GAGA2, anti-GAGA3, anti-GAGA4, anti-GAGA6, anti-GNa, anti-P64 and time to next relapse for each patient are depicted in Table 5. Patients were considered positive if were above cut of values in at least one of the markers, see cutoff values in Table 5.

Significantly high number of patients having early relapse vs. patients having late relapse were positive for one of the markers. (8/42 vs 32/58 (p=0.001, chi square test), Odds Ratio 5.2 (95% 2.1-13.2), enabling differentiation between the groups in 81% specificity, 55% sensitivity, and 80% positive predictive value.

Diagnostics characteristics for differentiation between patients having early vs. late relapse are based on a panel of anti glycan antibodies described in table 6.

Table 5: Levels of IgM anti-GAGA2, anti-GAGA3, anti-GAGA4, anti-GAGA6, anti-GNa, anti-P64 (arbitrary units) and time till next relapse (months) for each patient. CO - Cutoff for each marker.

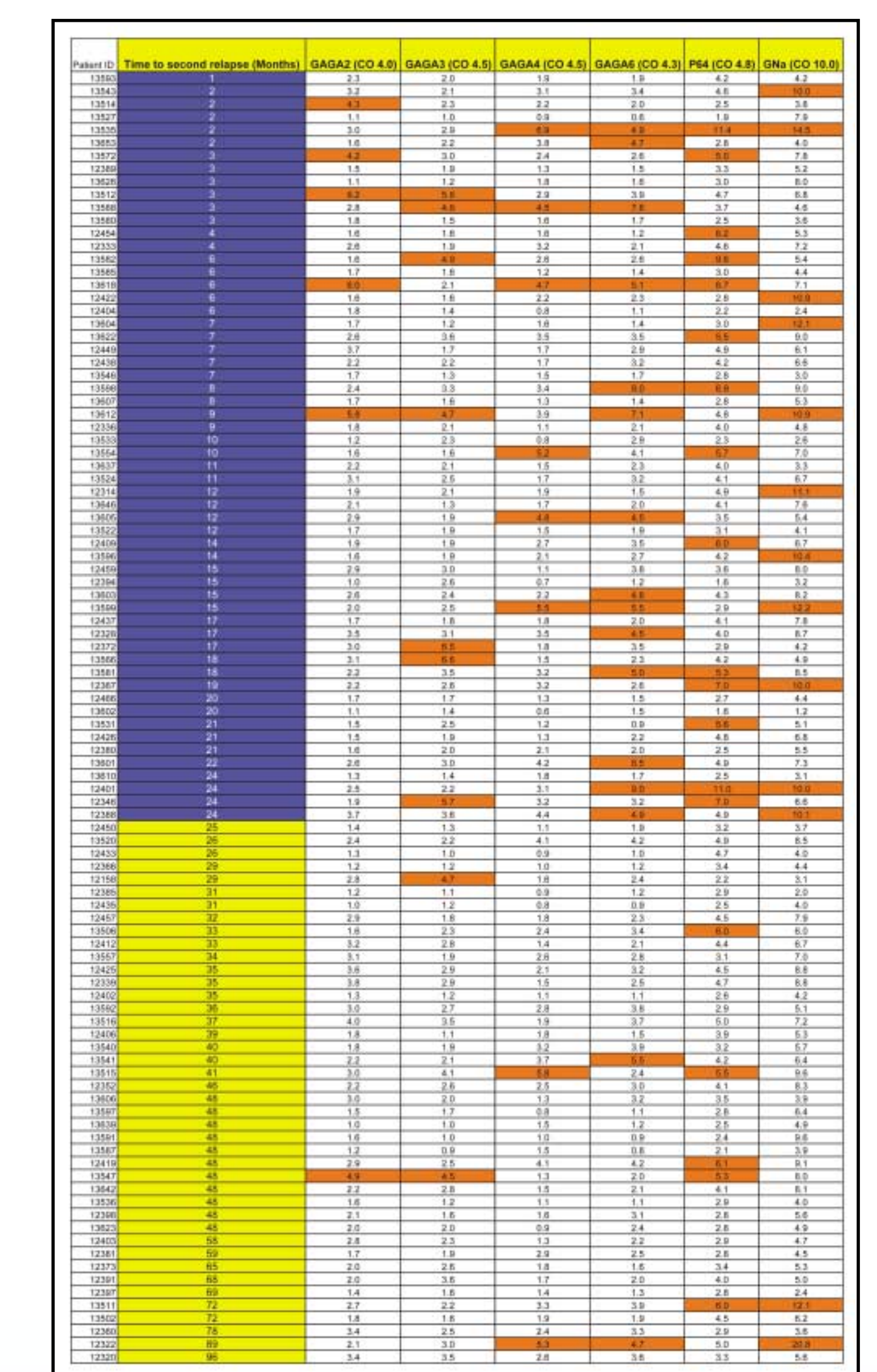


Table 6: Diagnostics characteristics of the panel of anti glycan antibodies

	Early Relapse (<24m)	Late Relapse (>24m)	Total
Positive in one of markers	32	8	40
Negative in all markers	26	34	60
Total	58	42	100

	90% CI	
Sensitivity	55.2%	43.6% to 66.4%
Specificity	81.0%	68.2% to 90.2%
Positive predictive value	80.0%	
Negative predictive value	56.7%	
Efficiency	66.0%	

8/42 vs 32/58 (p=0.001, chi square test), Odds Ratio 5.2 (95% 2.1-13.2).

Conclusions:

- Measuring Anti-GAGA4 together with Anti-GAGA6 IgM yields higher sensitivity (39%), specificity (95%) and predictive value (89%) of CIS patients evolving to RRMS.
- Higher levels of IgM antibodies to panel of anti glucose based disaccharide predicts at first neurological event which patients will have imminent attacks.