



# A Successful Validation of Anti-GAGA4 IgM Antibody Assay to Differentiate Multiple Sclerosis Patients from Other Neurological Disease Patients in a German Cohort, a Cross Sectional Retrospective Analysis

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## BACKGROUND

The diagnosis of multiple sclerosis (MS) is often a long process, even with MRI scans, many patients are defined with tentative MS. Thus, additional tests, such as blood tests based on serum biomarkers can aid in the diagnosis and work-up of MS.

Several previous studies demonstrated that levels of the IgM antibody serum biomarker, anti-GAGA4 [(Glc(α1,4)Glc(α)] are specific for MS patients; and significantly higher in MS compared to other neurological disease (OND) patients. Results demonstrated anti-GAGA4 differentiates RRMS from OND patients, Sensitivity: 30%-60%, Specificity: 85%-97%, PPV: 83.0%-92.3% and NPV: 52.3%-60.0%.<sup>1,2</sup>

## OBJECTIVES

Validate the ability of anti-GAGA4 IgM immunoassay to differentiate relapsing/remitting MS (RRMS) from OND patients in a German cohort.

Secondary objective was to observe anti-GAGA4 IgM levels of primary progressive MS patients (PPMS).

## METHODS

**Study Type:** Cross-sectional retrospective analysis of frozen sera.

**Study Population:** German (18yrs to 65yrs):

A.	RRMS* (n=83, 65%F)	OND (n=35, 37%F)	PPMS (n=8, 50%F)
Age yrs, mean (SD)	48.5 (11.8)	53.5 (10.2)	42.5 (12.2)

B.	GBS	Borreliosis	CIDP
OIND (n=15)	5	6	4
	NPH	Dementia	ALS
ONIND (n=20)	1	4	15

**Table 1. A.** Patient demographics; **B.** OND controls makeup: other inflammatory neurological disease (OIND, n=15) and other non-inflammatory neurological diseases (ONIND, n=20). \*RRMS vs. OND p<0.0001, Mann Whitney.

**Total IgM (T-IgM) Levels:** Measured on Roche Modular P800 Automated analyzer using the Roche Tina-quant IgM Gen.2 Turbidometric method; reported in mg/mL.

**Immunoassay:** Sera with masked identity were diluted 1:1200 and units (EU) of anti-GAGA4 IgM were measured by *gMS*<sup>®</sup> Dx Immunoassay (Glycominds, Lod, Israel) and normalized by dividing to square root of total IgM levels (T-IgM mg/mL serum)<sup>0.5</sup>.

**Age Correction Formula:** Results from another cohort<sup>3</sup> determined that anti-GAGA4 IgM (EU)/T-IgM(mg/mL)<sup>0.5</sup> levels decrease by age, though till the age of 20yrs IgM levels increase; so Anti-GAGA4 levels were corrected to age according the following formula:

$$\text{Anti-GAGA4(EU)/T-IgM(mg/mL)}^{0.5} + 0.455(\text{age}-20\text{yrs}).$$

**Cutoff for Anti-GAGA4 Positivity:** According to previous<sup>3</sup> results anti-GAGA4 status (positive or negative antibody) was set at 57 EU/(mg /mL)<sup>0.5</sup>.

## RESULTS

**Anti-GAGA4 EU Comparison:** RRMS patients had higher levels (Table 2, Figure 1) of anti-GAGA4 IgM and T-IgM than OND controls (P=0.0001, Mann-Whitney U). It seems PPMS patients (Table 2) have relatively low anti-GAGA4 and T-IgM, however the population was too small for statistical analysis.

**ROC Curve Analysis:** Anti-GAGA4 IgM assay has the ability to discern RRMS from OND (Figure 2).

**Proportion of Positive Patients:** RRMS patients had proportionally higher number of positives (Figure 3) for anti-GAGA4 IgM than OND patients (p=0.01, Fisher's exact).

**Diagnostic Test:** Anti-GAGA4 [57 EU/(mg/mL)<sup>0.5</sup> cutoff] can be used for differentiating RRMS patients from OND patients (Table 3).

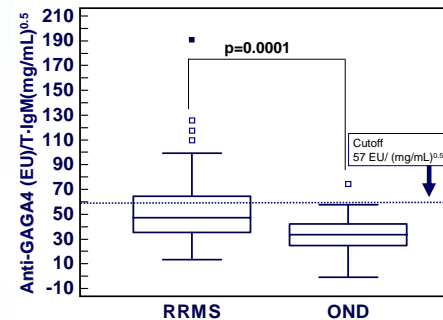
**Table 2: Antibody Levels**

	Median (95%CI)		
	RRMS (n=83)	OND (n=35)	PPMS (n=8)
Anti-GAGA4 (EU)	50.7 <sup>†</sup> (36.4-60.1)	22.2 (11.4-27.2)	34.8 (11.6-61.7)
T-IgM (mg/mL)	1.4 <sup>†</sup> (1.3-1.8)	1.2 (0.9-1.5)	0.98 (0.61-1.6)
Anti-GAGA4 EU/(mg/mL) <sup>0.5</sup> + age correct	48.0 <sup>‡</sup> (39.2 - 55.0)	33.3 (27.0 - 38.8)	35.2 (14.6-84.3)

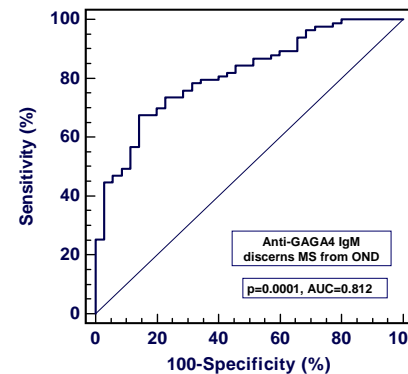
\* RRMS vs. OND, p<0.0001, Mann Whitney U.

† RRMS vs. OND, p=0.005, Mann Whitney U.

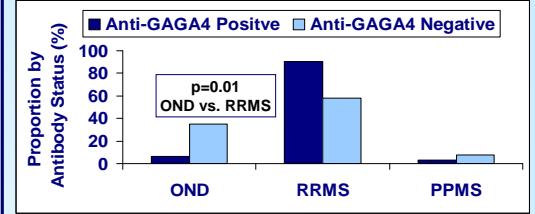
‡ RRMS vs. OND, p=0.0001, Mann Whitney U.



**Figure 1:** Box plots of normalized and age corrected anti-GAGA4 levels showing median values, 25% / 75% quartile, outliers and cutoff for anti-GAGA4 positivity.



**Figure 2:** ROC curve analysis plot of normalized and age corrected anti-GAGA4.



**Figure 3 :** Total proportion of patients positive or negative for normalized and age corrected anti-GAGA4 (Cutoff 57 EU/(mg/mL)<sup>0.5</sup>).

**Diagnostic Accuracy: RRMS (n=83) vs. OND (n=35)**

<b>Sensitivity (%)</b>	<b>33.7 (95%CI 23.7-45.0)</b>
<b>Specificity (%)</b>	<b>94.3 (95%CI 80.8-90.1)</b>
<b>PPV (%)</b>	<b>93.3 (95%CI 77.9-99.0)</b>
<b>NPV(%)</b>	<b>37.5 (95%CI 27.4-48.5)</b>

**Table 3:** Diagnostic accuracy test of normalized and age corrected anti-GAGA4 IgM (Cutoff 57 EU/(mg/mL)<sup>0.5</sup>).

## CONCLUSIONS

We validated that the *gMS*<sup>®</sup> Dx, an anti-GAGA4 IgM immunoassay is specific for MS patients with PPV of over 90%, and therefore can be used in the aid of RRMS diagnosis.

## REFERENCES

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