

Performance of An Anti-GAGA4 IgM Assay in Distinguishing MS Patients from Control Groups in a US Cohort: A Cross Sectional Retrospective Study

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BACKGROUND

Multiple sclerosis (MS) diagnosis is based on a classic presentation, CSF and MRI findings. However, additional tests, such as blood tests based on serum biomarkers can aid in the diagnosis and work-up of MS.

We previously found relapsing remitting MS (RRMS) patients have higher blood levels of IgM antibodies to Glc(α1,4)Glc(α) (GAGA4) than patients with other neurological disease (OND) and healthy controls (HC).^{1,2} Moreover, diagnostic accuracy tests demonstrated that anti-GAGA4 can discern MS patients from OND patients, with a sensitivity in the range of 26%-57% and a specificity in the range of 85% to 97%.^{1,2}

OBJECTIVES

Assess the performance of anti-GAGA4 IgM enzyme immune assay (EIA) to differentiate diagnosed MS patients from HC and OND patients positive for other neurological related antibodies in a United States cohort.

METHODS

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

Study Population: USA cohort: serum from 640 MS (48.5 ±11.8yrs), 100 HC (39.9±12.3yrs) and 31 OND controls (61.2±16.4yrs) positive for various anti-neuronal autoantibodies (MAG, Amphiphysin, Hu, Ri, Yo, Gangliosides).

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.

Enzyme Immunoassay: Sera with masked identity were diluted 1:1200 and EIA units (EU) of anti-GAGA4 IgM were measured by gMS[®] Dx Immunoassay (Glycominds, Lod, Israel) and normalized by dividing to square root of total IgM levels (T-IgM mg/mL serum)^{0.5}.

Cutoff Determination: Anti-GAGA4 IgM cutoff level for determining antibody status (positive/negative) was set at the upper 15 percentile of non-MS controls.

RESULTS

Age Correction Formula: Anti-GAGA4 IgM (EU)/T-IgM(mg/mL)^{0.5} levels decrease by age (Fig. 1), though till the age of 20yrs IgM levels increase; so Anti-GAGA4 levels are:

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

Diagnostic Performance: Anti-GAGA4 IgM has the ability to discern MS from HC and MS from OND controls (Fig. 2 and 3; Table 1).

Anti-GAGA4 EU Comparison: MS patients had (Fig. 4) higher levels of anti-GAGA4 IgM (median, 49.0 EU/(mg/mL)^{0.5} than both HC (median, 35.5 EU/(mg/mL)^{0.5} and OND controls (median, 31.8 EU/(mg/mL)^{0.5}.

Cut-off for Anti-GAGA4 Positivity: The upper 15 percentile of non-MS controls was 57 EU/ (mg/mL)^{0.5}.

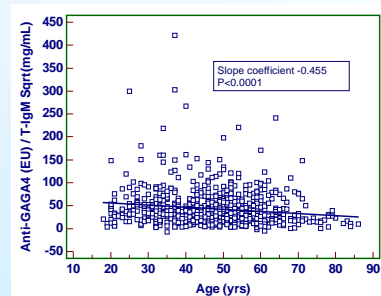


Figure 1: Linear regression of all populations.

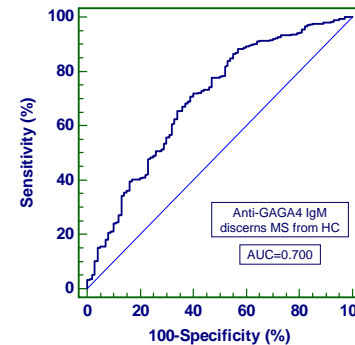


Figure 2: ROC curve analyses of MS patients and healthy controls.

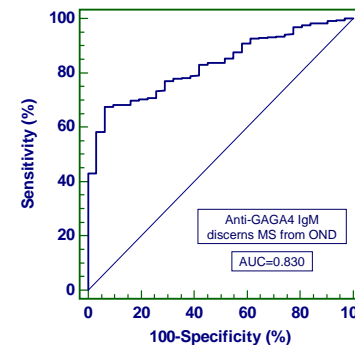


Figure 3: ROC curve analyses of MS patients and OND controls.

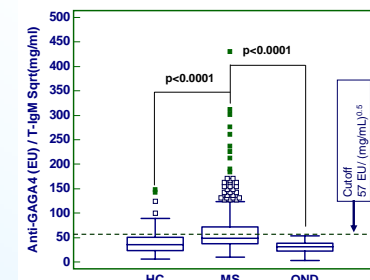


Figure 4: Box plots of anti-GAGA4 levels showing median values, 25% / 75% quartiles and outliers.

Population	Diagnostic Accuracy	
MS (n=640)	Sensitivity (%)	38.8 (95%CI 35.0-42.7)
HC (n=100)	Specificity (%)	84.0 (95%CI 75.3-90.6)
OND (n=31)	Specificity (%)	100.0 (95%CI 86.7-100.0)

Table 1: Diagnostic accuracy of anti-GAGA4 IgM

Anti-GAGA4 IgM Age Increase: Interestingly, anti-GAGA4 IgM levels were slightly increased with longer duration of MS.

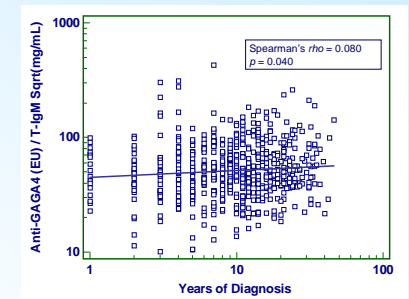


Figure 5: Linear regression of MS population.

CONCLUSIONS

➤ In this US cohort, the anti-GAGA4 IgM EIA was able to differentiate MS patients from those with autoantibodies associated with other neurological diseases.

➤ Anti-GAGA4 IgM serum marker has the potential to be an adjunct diagnostic tool for MS.

REFERENCES

- Schwarz M, Spector L, Gortler M, Weishaus O, Glass-Marmor L, Karni A, Dotan N, Miller A. Serum anti-Glc(α1,4)Glc(α) antibodies as a biomarker for relapsing-remitting multiple sclerosis. J Neurol Sci 2006;244:59-68.
- Freedman MS, Miller, Schwarz M, Weisshaus O, Altstock RT, Dukler A, Dotan A, and Sindic C. A panel of anti-glycan IgM antibodies for predicting the development of relapsing-remitting multiple sclerosis after the first neurological event. Mult Scler 2006;12:S167.

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