

Abstract

There is an unmet need to develop specific biomarkers for multiple sclerosis (MS) to improve the management of patients and the monitoring of the effectiveness of treatment.

We have screened serum from 107 patients with relapsing–remitting MS (RRMS) against a library of glycans on a glycan chip, and have found significantly higher levels of IgM anti-Glc(α1,4)Glc(α) (anti-Ga4Ga) antibodies than in 77 control patients suffering from other neurological diseases (OND, $p < 0.001$), a trend toward significance relative to other autoimmune diseases (OAD, $p = 0.06$), and similar levels to primary progressive MS (PPMS, $p = 0.25$). The differences in the levels of anti-glycan IgM antibodies did not result from differences in the level of total IgM. Analysis of covariance, adjusting for total IgM in a subset of subjects from the MS, OND and OAD groups, found significant differences in anti-glycan IgM among the groups ($p < 0.001$) and only a weak positive relationship to total IgM ($p = 0.20$). Furthermore, a trend of higher levels of IgM anti-Ga4Ga antibodies was detected in sera from patients with RRMS in relapsing than in remitting state ($p = 0.11$), and in patients with a relatively active disease course (hyperactive, HA) than in non-HA patients with MS ($p = 0.4$). We suggest that the level of anti-Ga4Ga antibodies is a specific biomarker for MS, with potential clinical utility for MS disease management.

1. Introduction

There is an unmet need to develop specific biomarkers for multiple sclerosis (MS) to improve the management of patients and the monitoring of the effectiveness of treatment. Molecular markers can potentially be useful to assess a range of issues in MS, such as susceptibility to development of MS; risk of developing definite MS after a first clinical episode; disease activity; disease progression; prognosis and response to treatment. Currently, in addition to accepted clinical scales, two para-clinical tools are in regular use for the diagnosis of MS: Brain or spinal magnetic resonance imaging (MRI), or oligoclonal bands (OCB) of IgG in cerebrospinal fluid (CSF). Both of them cannot be used for prognosis disease activity or help in routine disease management.

2. Patients and Methods

2.1. Patients

Table 1. (A) Patient Characteristics

	RRMS	Relapsing MS	Recurring MS	RRMS	Stroke	OND	ONIND	OND	OAD	RA	Cherny
N	107	41	66	16	9	50	30	11	27	15	12
Age, mean(SD) years	37(11)	35(10)	38(11)	50(10)	50(20)	35(11)	56(11)	49(7)	40(10)	44(9)	37(10)
Women, n (%)	31(26)	32(78)	49(74)	9(56)	3(33)	22(44)	14(47)	3(25)	21(76)	12(79)	9(75)
Medication											
Copaxone®, n (%)	15(14)	4(10)	11(17)	-	-	-	-	-	-	-	-
Betaseron®, n (%)	10(9)	5(12)	5(8)	-	-	-	-	-	-	-	-
Rubiflo®, n (%)	16(15)	6(15)	10(15)	-	-	-	-	-	-	-	-
Axoness®, n (%)	17(16)	6(15)	11(17)	-	-	-	-	-	-	-	-
Total (N/A), n (%)	43(40)	17(41)	26(39)	-	-	-	-	-	-	-	-
Total treated, n (%)	58(54)	21(51)	37(56)	-	-	-	-	-	-	-	-
Untreated, n (%)	49(46)	20(49)	29(44)	16(100)	-	-	-	-	-	-	-

aMS, multiple sclerosis; OAD, other autoimmune diseases; ONIND, other inflammatory neurological diseases; OND, other neurological diseases; ONIND, other non-inflammatory neurological diseases; PPMS, primary progressive multiple sclerosis; RA, Rheumatoid arthritis; RRMS, relapsing–remitting multiple sclerosis.

Table 1. (B) Make-Up of the OND Group

Chronic inflammatory (OIND)	(OIND) No. of cases
Meningitis 6	6
Guillain-Barré syndrome 2	2
Myositis/gravis 2	2
Epilepsy 1	1
Chronic non-inflammatory (ONIND) No. of cases	Chronic non-inflammatory (ONIND) No. of cases
Parkinson's disease 4	4
Leak 2	2
Stovemet disorder 1	1
Dystonia 3	3
Migra the headache 8	8
Alzheimer's disease 2	2
Amotrophic Lateral sclerosis (ALS) 2	2
Huntington's disease 3	3
Acute	No. of cases
Stroke 9	9

2.2. GlycoChip® preparation and sample processing

Anti-glycan antibodies in all serum samples were detected using the GlycoChip® (Glycominds Ltd., Lod, Israel) as previously described (Schwarz M, Glycobiology 13:749, 2003). Sera were incubated on the glycans spotted glass slides in a automated hybridization system. The detection of bound IgM antibodies was done with secondary fluorescence antibodies. Total IgM assay was done in a 96 well plate using immuno-fluorescence detection.

Figure 1

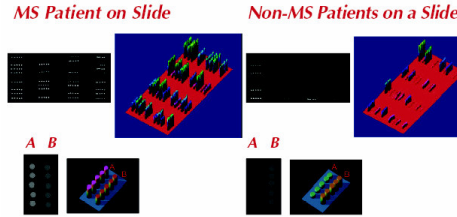


Figure 1. Laser scanner images of two slides exposed to serum samples from an MS patient and from a non-MS patient. A-5 spots lane of Glc(α1,4)Glc(α), B-5 spots lane of anti human IgM. Bar presentation of signals intensity.

3. Results

3.1. IgM Anti-Glycan Antibody Levels in RRMS and Control Populations Results

Results (Figure 2 and Table 2) reveal significantly higher levels of IgM anti-Ga4Ga in RRMS as compared with OND ($p < 0.001$), and showed a trend toward significance relative to Other Autoimmune Diseases (OAD, $p = 0.06$). Differentiation between MS and OND patients can be done in 90% specificity and 72% sensitivity by using combination of anti-Ga4Ga and anti-Ga.

Figure 2A

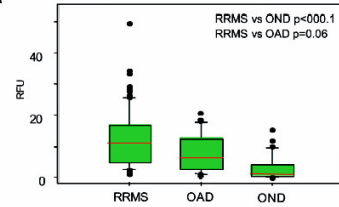


Figure 2B

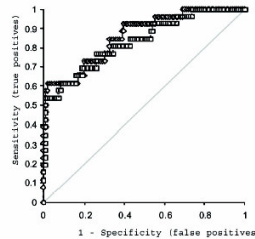


Figure 2. A - Box plot describing distribution of anti-Ga4Ga antibodies in the different groups investigated: OAD, other autoimmune diseases; OND, other neurological diseases; RRMS, relapsing–remitting MS. The box includes signals of 50% of the population. The line in the box represents the median value. The boundary of the box closest to zero indicates the 25th percentile, and the boundary of the box furthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 90th and 10th percentiles. B – ROC curve for discrimination between RRMS and OND based on anti-Ga4Ga and anti-Ga IgM antibodies.

Table 3. Descriptive Statistics for anti-glycan antibody levels in RRMS and control groups

Sugar Ligand	Signal	RRMS	Relapsing MS	Remitting MS	PPMS	OAD	OND
	Median, RFU	10,583,221	11,965,877	9,903,854	8,237,277	6,473,243	1,146,109
Glc(α1,4)Glc(α) SD		8,769,550	9,609,488	8,127,044	6,412,262	6,058,448	6,560,037
	p-value vs RRMS				0.25	0.06	>>0.001

3.2. Binding Specificity of anti Glc(α1,4)Glc(α)

In order to demonstrate that the antibody binding observed is specific for the attached sugars, a depletion experiment was performed in which an MS patient sera pool was preincubated with glycoFrac™ resin containing specific glycans. Figure 2A demonstrates that IgM antibodies specific to Glc(α1,4)Glc(α) and -Glc are practically depleted from the serum after incubation with the Glc(α1,4)Glc(α)-containing beads, while binding to α-Gal and α-RNA remains intact. The antibodies bound to the Glc(α1,4)Glc(α)-containing beads were specifically eluted with Glc(α1,4)Glc(α) and applied to a GlycoChip®. Figure 2B shows that these affinity-purified antibodies react specifically with glucose-based structures such as Glc(α1,4)Glc(α) and α-Glc (glycan numbers 16–18 and 17), and other sugars containing free glucose residues (glycan numbers 18–21, 52, 54, 55).

Figure 1

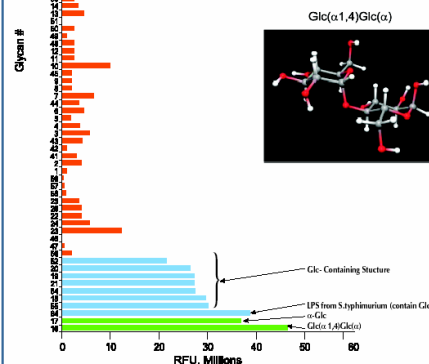
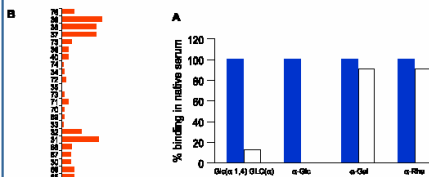


Figure 3. Demonstration of binding specificity of anti-Ga4Ga antibodies in a depletion experiment. (A) Binding levels of four serum anti-glycan antibodies after incubation of serum with Glc(α1,4)Glc(α)-containing beads (empty bars), relative to binding in whole serum (filled bars). (B) Binding profile of antibodies bound to Glc(α1,4)Glc(α)-containing beads and eluted with Glc(α1,4)Glc(α). Green bars represent antigens present on the beads used for affinity purification (Glc(α1,4)Glc(α) and Glc(α1,4)Glc(α)). Blue bars represent other glycan antigens containing free glucose residues. Orange bars represent glycans different from or lacking free Glc-residues. Binding is measured in relative fluorescent units (RFU). (C) Three dimensional model of Glc(α1,4)Glc(α).

3.3. Total IgM

The differences in the levels of anti-glycan IgM antibodies did not result from differences in the level of total IgM. Analysis of covariance, adjusting for total IgM in a subset of subjects from the MS, OND and OAD groups, found significant differences in anti-glycan IgM among the groups ($p < 0.001$) and only a weak positive relationship to total IgM ($p = 0.20$). (Figure 4)

Figure 4

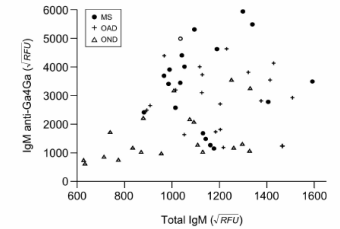


Figure 4. Specific anti-Ga4Ga IgM antibody levels versus total IgM in MS and control groups in a representative subset of each OND, other neurological diseases; RRMS, relapsing–remitting MS.

3.4. Anti-Ga4Ga Levels in Patients with RRMS

No difference in anti-Ga4Ga level was observed in patients receiving disease-modifying drugs (DMDs) in comparison with untreated patients (not shown). Anti-Ga4Ga levels are somewhat ($p = 0.14$), but consistently, higher in patients with relapsing than patients with remitting MS (Figure 4A). Hyperactive (HA) patients, defined as either having three or more relapses per year in the last two consecutive years or a change in EDSS score of 2 or more points per year in the last two consecutive years, have a higher level of antibodies in comparison to other patients with RRMS (Figure 4B). An even larger difference was found between HA patients and non-HA patients in remission (4C). This may suggest that these anti-glycan antibodies can serve as markers for disease activity and progression.

Figure 5A

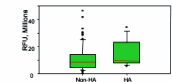


Figure 5B

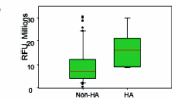


Figure 5. Box plot describing distribution of anti-Ga4Ga antibodies in different MS patient groups. (A) Hyperactive (HA; $n = 11$) and non-hyperactive (non-HA; $n = 96$) patients with RRMS (relapsing and remitting). (B) HA ($n = 5$) and non-HA ($n = 25$) patients in remission. The box includes signals of 50% of the population. The line in the box represents the median value. The boundary of the box closest to zero indicates the 25th percentile, and the boundary of the box furthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 90th and 10th percentiles.

4. Summary and Conclusions

- Using the unique GlycoChip technology we have found significantly higher levels of anti-Glc(α1,4)Glc(α) IgM in patients with RRMS as compared with patients with OND.
- The antibodies bind specifically to Glc(α1,4)Glc(α).
- The differences in anti Glc(α1,4)Glc(α) IgM is not attributed to total IgM differences between MS and OND Groups.
- A trend towards higher levels of Glc(α1,4)Glc(α) IgM in hyper-active (HA) RRMS Patients.
- Large scale retrospective study based on an existing sera and clinical data bank is performed this days for validation of specificity and the prognostics value of the marker for disease activity.
- We are looking for further collaborations with researchers having sera and clinical data banks of MS patients for further validation.