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High and Persistent Anti-GM1 Antibody Titers Are Associated With Poor Clinical Recovery in Guillain-Barré Syndrome

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Abstract

Background and Objectives

Guillain-Barré syndrome (GBS) is an acute immune-mediated polyradiculoneuropathy that may follow a preceding infection inducing a cross-reactive antibody response to glycosphingolipids in peripheral nerves. The immune response in GBS is considered to be short lasting, explaining its monophasic clinical course. However, the disease course varies between patients, and residual deficits frequently occur. The duration of the antibody response has not been defined extensively in GBS, and the persistence of these antibodies may impair clinical recovery. The aim of this study was to determine the titer course of serum antibody titers to the ganglioside GM1 in relation to clinical course and outcome in patients with GBS.

Methods

Acute-phase sera from patients with GBS included in previous therapeutic trials were screened for anti-GM1 IgG and IgM antibodies in ELISA. Anti-GM1 antibody titers were determined in sera collected at entry and during a 6-month follow-up. Clinical course and outcomes were compared between groups based on the titer course.

Results

Anti-GM1 antibodies were detected in 78 (20.7%) of 377 included patients. The anti-GM1 IgG and IgM antibody titer course was highly variable between patients. A subset of anti–GM1-positive patients had persistent anti-GM1 antibodies at 3 months (n = 27/43 [62.8%]) and 6 months (n = 19/41 [46.3%]). Patients with a high anti-GM1 IgG and IgM titer at entry recovered more slowly and less complete than anti–GM1-negative patients (IgG: $p = 0.015$, IgM: $p = 0.03$). High vs low IgG titers were independently associated with poor outcome after correcting for known prognostic factors ($p = 0.046$). Among patients with a high anti-GM1 IgG titer at entry, a slow titer decline was associated with poor outcome at 4 weeks ($p = 0.003$) and 6 months ($p = 0.032$). Persistent high IgG titers at 3 and 6 months were associated with poor outcome at 6 months (3 months: $p = 0.022$, 6 months: $p = 0.004$).

Discussion

High anti-GM1 IgG and IgM antibody titers at entry and persistent high anti-GM1 IgG antibody titers are associated with poor outcome in patients with GBS. Antibody persistency indicates ongoing antibody production long after the acute disease state in GBS. Further research is required to determine whether antibody persistency interferes with nerve recovery and is a target for treatments.

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Glossary

GBS = Guillain-Barré syndrome; **IQR** = interquartile range; **IVIg** = IV immunoglobulin; **LOS** = lipooligosaccharide; **MP** = methylprednisolone; **PE** = plasma exchange.

Guillain-Barré syndrome (GBS) is an acute immune-mediated polyradiculoneuropathy with an onset of rapidly progressive weakness followed by a slow recovery.[1,2] The immune response causing the nerve damage is considered to be short lasting, as clinical nadir is generally reached within 2–4 weeks.[1] Because of this clinical course, patients are usually treated only in the early phase of the disease with IV immunoglobulin (IVIg) or plasma exchange (PE).[1,2] However, despite immunomodulatory treatment, the clinical course and outcome of GBS remain highly variable, and many patients show incomplete recovery.[1,3] This subgroup in particular could potentially benefit from more effective treatment strategies, but early identification of these patients remains difficult.

Campylobacter jejuni is the predominant preceding infection in GBS and triggers an immune response to peripheral nerves by molecular mimicry.[1,4] Lipooligosaccharides (LOSs) of *C jejuni* elicit the production of cross-reactive antibodies to structurally resembling gangliosides such as GM1.[4,5] Gangliosides are sialylated glycosphingolipids that occur throughout the peripheral nervous system, forming lipid rafts in plasma membranes and playing a role in nerve cell function, homeostasis, and repair.[6,7] In animal models, antiganglioside antibodies have been shown to induce complement-mediated injury to axons and myelin and inhibit nerve repair.[4,6,8,9]

Anti-GM1 antibodies have been extensively investigated in relation to the clinical course and outcome in GBS, demonstrating associations with axonal and pure motor variants.[4,6,7,10-12] Yet, only few studies have explored the anti-GM1 antibody titer course in GBS.[13-23] Thus far, these studies showed that the serum anti-GM1 antibody titers usually show a rapid decline, although a subset of patients has a prolonged antibody response in which antibodies may persist for months. Our hypothesis was that the persistency of anti-GM1 antibodies in patients with GBS may result in more severe deficits and slower clinical recovery. The aim of this study was to determine the titer course of the anti-GM1 antibodies in relation to the clinical course and outcome during a follow-up of 6 months in patients with GBS.

Methods

Study Population

This study was conducted using a cohort of patients who fulfilled the diagnostic criteria for GBS and were previously included in various therapeutic trials.[24-28] Patients were eligible for inclusion in these trials if they were hospitalized within 2 weeks from onset of weakness and if they were unable to walk 10 m independently (GBS disability score ≥3). Exclusion criteria were age <4 years, a previous episode of GBS, severe concurrent disease, a previous severe allergic reaction to matched blood products, a known selective IgA deficiency, immune-mediated disease other than well-regulated diabetes mellitus, treatment with immunosuppressive agents, steroids, antacids, or drugs interfering with the enterohepatic circulation, contraindications for steroid treatment, pregnancy, breastfeeding, or foreseeable difficulties precluding follow-up. All patients were treated with either IVIg (0.4 g/kg/d) for 5 consecutive days or PE (200–250 mL/kg in 5 sessions) in 7–14 days. Within the previously conducted therapeutic trials, subsets of patients were additionally treated with IV methylprednisolone (MP; 500 mg/d for 5 consecutive days) or oral mycophenolate mofetil (CellCept, Roche, Welwyn, United Kingdom; 1,000 mg/d for 6 consecutive weeks). In 2 clinical trials, treatments were randomly allocated.[25,27] Baseline characteristics, disease severity, and anti-GM1 positivity did not differ between treatment groups in all studies.

Clinical data were acquired from existing databases. Electrophysiologic subtyping was performed according to Hadden criteria.[29] Serum samples were taken during clinical trials at study entry and at 2, 4, 12, and 26 weeks of follow-up. Samples were stored at −80°C.

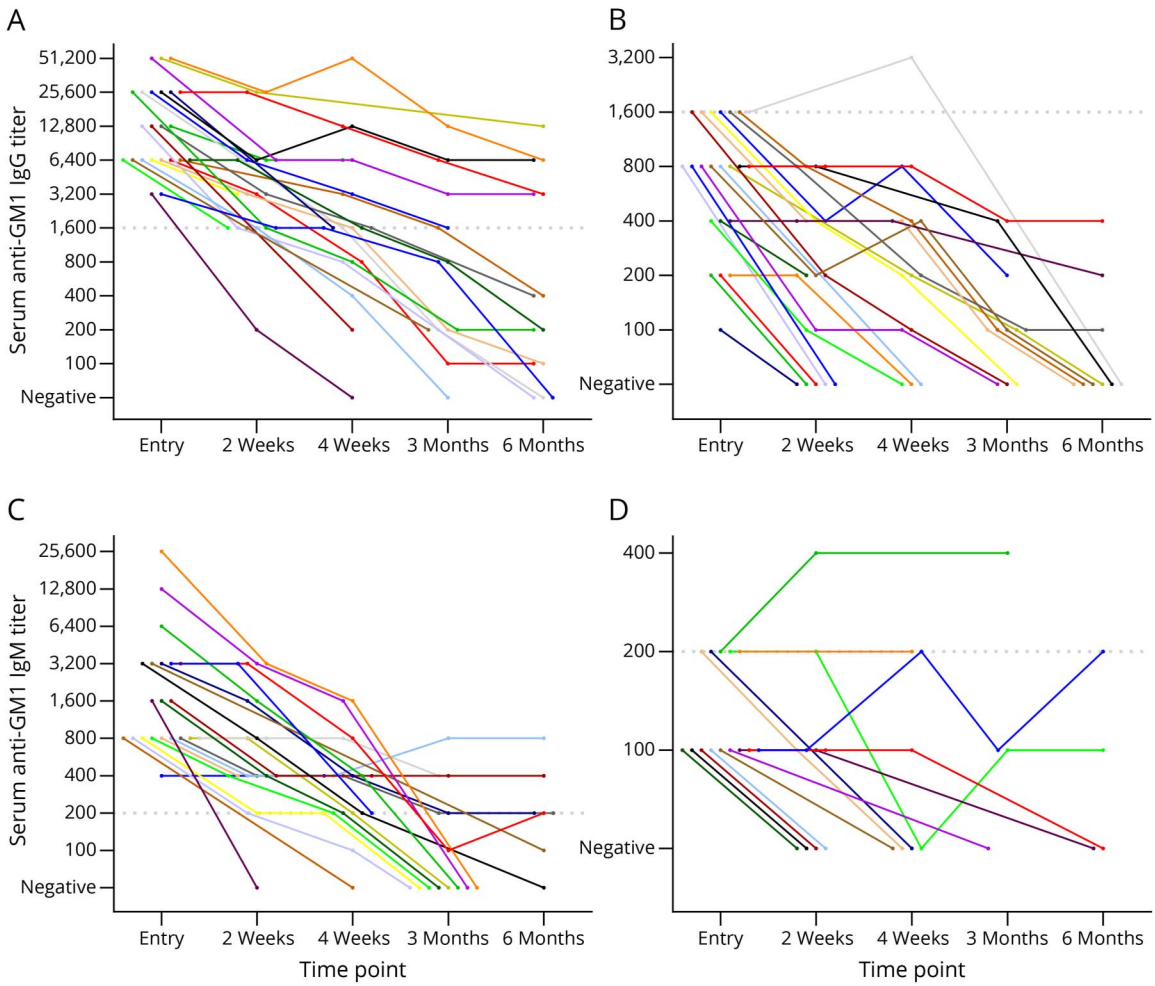
Detection of Serum Anti-GM1 Antibodies

Screening for and titration of serum anti-GM1 antibodies were performed batchwise in a single laboratory using the same Inflammatory Neuropathy Cause and Treatment group standard ELISA.[30,31] Serum samples that were tested positive during screening were subsequently tested in serial 2-fold dilutions starting from 1:100 to a maximum of 1:51,200. The anti-GM1 antibody titer was defined as the highest dilution that resulted in a delta optical density higher than the cutoff value (0.20 for IgG and 0.30 for IgM).[30]

Statistical Analyses

Poor clinical outcome was defined as the inability to walk 10 m independently at 6 months of follow-up (GBS disability score ≥3). Patients positive for anti-GM1 antibodies were dichotomized based on the presence of a high (> median titer) or low (≤ median titer) IgG and IgM titer at entry. A persistent antibody titer was defined as the presence of anti-GM1 antibodies at entry and at 3 or 6 months. In case antibody titers at 3 or 6 months were higher than the median IgG or IgM titer at entry, this was defined as a persistent high antibody titer. Among patients with a high antibody titer at entry, a rapid decline was defined as a reduction of ≥50% in the number of 2-fold dilution steps at 4 weeks or 6 months

Figure 1 Variation in Individual Serum Anti-GM1 IgG and IgM Antibody Titer Courses



The variation in individual serum anti-GM1 titer courses during 6 months of follow-up is shown in separate panels for patients with a high (A) or low (B) serum anti-GM1 IgG titer at entry and a high (C) or low (D) serum anti-GM1 IgM titer at entry. Each colored line depicts an individual patient. High titers at entry were defined as titers higher than the median titer at this time point, and low titers were defined as titers equal to or lower than the median titer. Median titers are indicated with a horizontal dotted line.

(e.g., from a titer of 12,800 [dilution step 8] at entry to a titer of ≤800 [≤ dilution step 4] at week 4), and a slow decline was defined as a reduction of <50%.

Normality was assessed using the Shapiro-Wilk test. Comparisons were made with the Mann-Whitney *U* test, Kruskal-Wallis test, χ^2 test, Fisher exact test, and ordinal logistic regression analyses. Time to reach the ability to walk 10 m independently (GBS disability score ≤2) was analyzed with Kaplan-Meier curves and log-rank tests. Correction of survival analyses for known prognostic factors (age, preceding diarrhea, and MRC sum score at entry) was performed with Cox regression. Data were presented as median (interquartile range [IQR]) or number (percentage). A 2-sided p value < 0.05 was considered statistically significant. Bonferroni corrections were applied for multiple comparisons, when indicated. Data were analyzed in Statistical Package for the Social Sciences version 25 and GraphPad Prism 9. Missing data were not imputed.

Standard Protocol Approvals, Registrations, and Patient Consents

Study approvals were obtained from the Institutional Review Board at the Erasmus MC, and informed consent was obtained from all participants before inclusion.

Data Availability

Data supporting the findings of this study are available on reasonable request, if in accordance with the privacy regulations.

Results

Anti-GM1 IgG and IgM Titers in GBS

Acute-phase sera from 377 patients were screened for the presence of anti-GM1 antibodies. A flowchart with the number of patients available for each analysis is provided in eFigure 1, links.lww.com/NXI/A823. Anti-GM1 antibodies

Table Clinical Associations of Anti-GM1 Positivity, IgG Positivity, and IgM Positivity

Variable	Anti-GM1 neg. (n = 276)	Anti-GM1 pos. (n = 74)	*p* Value	IgG pos. (n = 51)	*p* Value	IgM pos. (n = 44)	*p* Value	IgG only (n = 30)	*p* Value	IgG + IgM both (n = 21)	*p* Value
Demographics											
Age at onset	55 (34–67)	49 (34–61)	n.s.	51 (39–62)	n.s.	45 (30–58)	0.015	53 (45–65)	n.s.	47 (25–60)	n.s.
Sex (males)	146 (52.9)	45 (60.8)	n.s.	30 (58.8)	n.s.	30 (68.2)	n.s.	15 (50.0)	n.s.	15 (71.4)	n.s.
Clinical characteristics											
Preceding diarrhea	48/275 (17.5)	32/73 (43.8)	<0.001	29/51 (56.9)	<0.001	19/43 (44.2)	<0.001	13/30 (43.3)	<0.001	16/21 (76.2)	<0.001
Preceding URTI	105/275 (38.2)	24/73 (32.9)	n.s.	15/51 (29.4)	n.s.	13/43 (30.2)	n.s.	11/30 (36.7)	n.s.	4/21 (19.0)	n.s.
***C jejuni* infection**	67/275 (24.4)	39/72 (54.2)	<0.001	32/50 (64.0)	<0.001	25/43 (58.1)	<0.001	14/29 (48.3)	0.006	18/21 (85.7)	<0.001
CMV infection	45/274 (16.4)	3/72 (4.2)	0.007	1/50 (2.0)	0.007	3/43 (7.0)	n.s.	0/29 (0)	0.012	1/21 (4.8)	n.s.
Sensory deficits at entry	194/275 (70.5)	35/71 (49.3)	<0.001	18/49 (36.7)	<0.001	24/43 (55.8)	n.s.	11/28 (39.3)	<0.001	7/21 (33.3)	<0.001
CNI at entry	121 (43.8)	20/72 (27.8)	0.013	8/50 (16.0)	<0.001	14/43 (32.6)	n.s.	6/29 (20.7)	0.016	2/21 (9.5)	0.002
MRC sum score at entry (0–60)	44 (35–48)	37 (29–46)	<0.001	34 (27–44)	<0.001	40 (31–46)	0.016	35 (20–44)	0.001	34 (29–44)	0.012
MRC sum score at nadir (0–60)	38 (24–46)	34 (14–44)	0.026	32 (12–42)	0.009	32 (16–46)	n.s.	34 (6–43)	n.s.	26 (14–42)	n.s.
MRC sum score at 2 w (0–60)	47 (34–54)	44 (25–52)	n.s.	44 (20–52)	n.s.	40 (25–52)	n.s.	48 (20–53)	n.s.	34 (19–50)	0.015
MRC sum score at 4 w (0–60)	52 (39–58)	48 (27–56)	n.s.	48 (23–57)	0.049	47 (27–56)	n.s.	52 (30–58)	n.s.	39 (17–56)	0.033
MRC sum score at 3 m (0–60)	58 (51–60)	55 (40–60)	0.036	53 (31–60)	0.008	54 (41–59)	0.030	57 (39–60)	n.s.	45 (26–58)	0.001
MRC sum score at 6 m (0–60)	60 (57–60)	58 (48–60)	0.009	58 (42–60)	0.005	58 (45–60)	0.013	59 (52–60)	n.s.	55 (34–60)	0.002
GBS-DS at nadir (0–6)	4 (4–5)	4 (4–4)	0.032	4 (4–4)	0.031	4 (4–4)	n.s.	4 (4–4)	N.A.	4 (4–4)	N.A.
GBS-DS at 4 w (0–6)	3 (2–4)	4 (2–4)	N.A.	3 (2–4)	N.A.	4 (2–4)	N.A.	2 (2–4)	N.A.	4 (2–4)	N.A.
GBS-DS at 6 m (0–6)	1 (1–2)	1 (1–3)	N.A.	1 (0–3)	N.A.	1 (1–3)	N.A.	1 (0–3)	N.A.	2 (1–4)	N.A.
Walking unaided at 6 m	229/274 (83.6)	54/72 (75.0)	n.s.	36/50 (72.0)	n.s.	32/42 (76.2)	n.s.	22 (73.3)	n.s.	14/20 (70.0)	n.s.
Mechanical ventilation	88 (31.9)	15 (20.3)	n.s.	8 (15.7)	0.020	9 (20.5)	n.s.	6 (20.0)	n.s.	2 (9.5)	0.032
Treatment-related fluctuation	49 (17.8)	12/72 (16.7)	n.s.	8/50 (16.0)	n.s.	7/43 (16.3)	n.s.	5/29 (17.2)	n.s.	3/21 (11.1)	n.s.
EMG classification											
Normal	5/250 (2.0)	0/64 (0)	n.s.	0/42 (0)	n.s.	0/40 (0)	n.s.	0/24 (0)	n.s.	0/18 (0)	n.s.
Demyelinating	127/250 (50.8)	19/64 (29.7)	0.003	6/42 (14.3)	<0.001	17/40 (42.5)	n.s.	2/24 (8.3)	<0.001	4/18 (22.2)	0.019

Continued

Table Clinical Associations of Anti-GM1 Positivity, IgG Positivity, and IgM Positivity *(continued)*

Variable	Anti-GM1 neg. (n = 276)	Anti-GM1 pos. (n = 74)	*p* Value	IgG pos. (n = 51)	*p* Value	IgM pos. (n = 44)	*p* Value	IgG only (n = 30)	*p* Value	IgG + IgM both (n = 21)	*p* Value
Axonal	10/250 (4.0)	9/64 (14.1)	0.006	8/42 (19.0)	0.001	6/40 (15.0)	0.013	3/24 (12.5)	n.s.	5/18 (27.8)	0.002
Equivocal	102/250 (40.8)	28/64 (43.8)	n.s.	21/42 (50.0)	n.s.	13/40 (32.5)	n.s.	15/24 (62.5)	0.040	6/18 (33.3)	n.s.
Inexcitable	6/250 (2.4)	8/64 (12.5)	0.002	7/42 (16.7)	<0.001	4/40 (10.0)	0.035	4/24 (16.7)	0.007	3/18 (16.7)	0.017

Abbreviations: CMV = cytomegalovirus; CNI = cranial nerve impairment; GBS-DS = GBS disability score; m = months; MRC = Medical Research Council; N.A. = not applicable; neg. = negative; pos. = positive; URTI = upper respiratory tract infection; w = weeks.
Associations of anti-GM1 IgG and IgM positivity with the clinical course and outcomes in GBS, compared with anti–GM1-negative patients. Data are presented as median (interquartile range) or number (percentage).

were present in pretreatment sera from 78 (20.7%) patients. Of these patients, 30 (38.5%) were tested positive for IgG only, 24 (30.8%) for IgM only, and 24 (30.8%) for both IgG and IgM. At entry, antibody titers were higher for the IgG isotype (median: 1,600, IQR: 800–12,800, range: 100–51,200) than for the IgM isotype (median: 200, IQR: 100–1,200, range: 100–25,600) ($p < 0.001$). Among patients with a high IgG antibody titer at entry (>1,600), 16/26 (61.5%) were also tested positive for IgM antibodies, compared with 8/28 (28.6%) in the group with a low IgG antibody titer at entry (≤800) ($p = 0.015$). Patients with anti-GM1 antibodies of both isotypes had higher IgG and IgM antibody titers than patients with a single isotype (IgG: $p = 0.006$, IgM: $p = 0.018$).

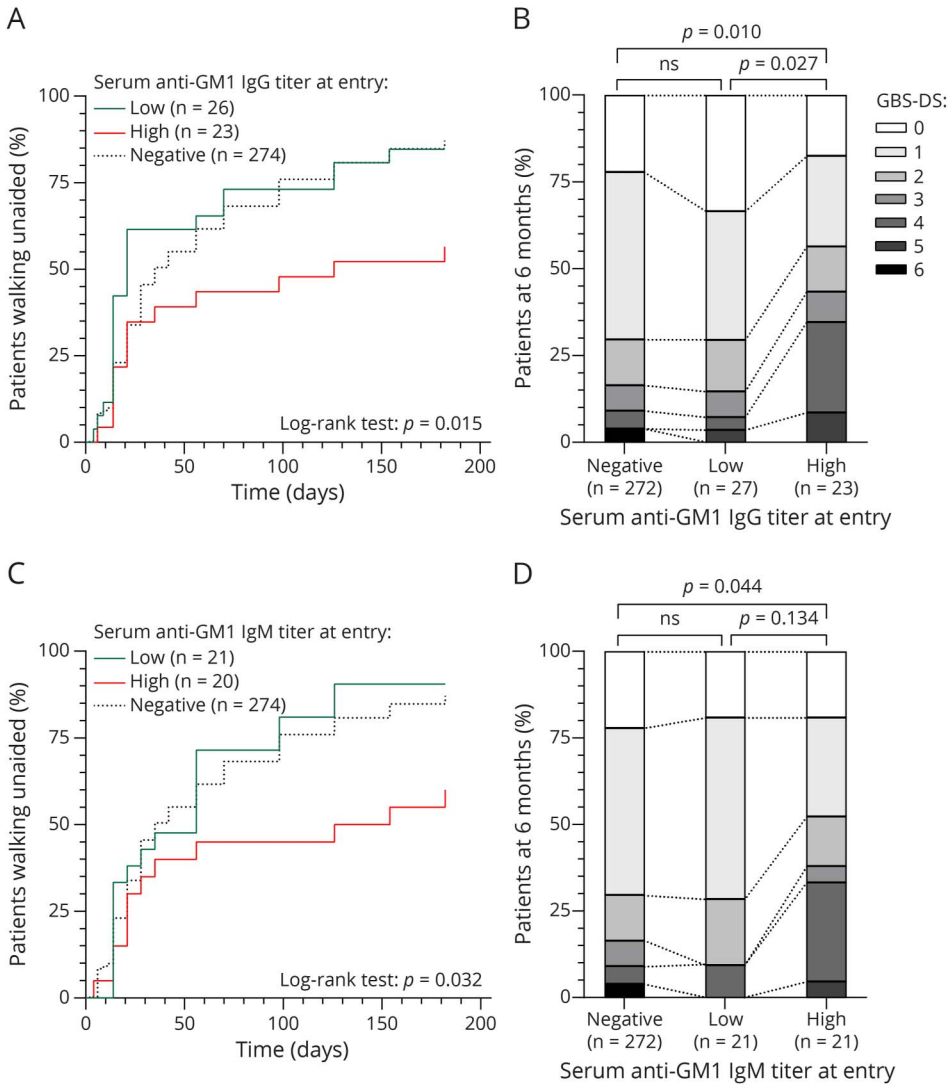
Highest antibody titers were found at entry in 74 of 78 (94.9%) patients. Although all antibody titers declined during follow-up, there was considerable variation in titer course between individual patients (Figure 1). Notably, a subgroup of patients with a high IgG antibody titer at entry had persistent antibody titers during follow-up. Anti-GM1 antibodies remained detectable in 27 patients (62.8% of 43 with available sera) at 3 months (IgG n = 21, median: 200, IQR: 100–1,600, range: 100–12,800; IgM n = 9, median: 200, IQR: 100–400, range: 100–800) and in 19 patients (46.3% of 41 with available sera) at 6 months (IgG n = 15, median: 400, IQR: 200–3,200, range: 100–12,800; IgM n = 8, median: 200, IQR: 150–300, range: 100–800). Among these patients, 4 (12.9%) of 31 had persistent high IgG antibody titers (>1,600) at 3 months and 5 (17.2%) of 29 at 6 months. IgM antibody titers were persistent high (>200) in 4 (17.4%) of 23 patients at 3 months and 2 (10.0%) of 20 at 6 months.

Ten patients positive for both IgG and IgM at entry remained anti-GM1 positive at 6 months (83.3% of 12 with available sera), compared with 5 in the group with IgG only (29.4% of 17 with available sera) and 4 in the group with IgM only (33.3% of 12 with available sera). IgG titers persisted longer than IgM titers in the group positive for both isotypes. Patients with a high IgG titer at entry more often had detectable IgG antibodies at 6 months than patients with a low titer at entry (11/14 [78.6%] vs 4/15 [26.7%], $p = 0.005$). None of the patients with IgM antibodies only at entry were tested positive for IgG during follow-up.

Anti-GM1 Antibody Titer Course in Relation to Clinical Course and Outcome in GBS

The presence of anti-GM1 antibodies was associated with preceding diarrhea, *C jejuni* infection, axonal polyneuropathy, inexcitable nerves, a lower MRC sum score at entry, nadir, and 3 months, and a higher GBS-DS at nadir (Table). In addition, anti–GM1-positive patients less often had a cytomegalovirus infection, demyelinating polyneuropathy, sensory deficits at entry, and cranial nerve impairment at entry. The majority of these associations were more pronounced for IgG compared with IgM and for IgG and IgM both compared with IgG only. Moreover, IgG positivity was associated with a lower MRC

Figure 2 Kaplan-Meier and Proportional Odds Analyses for the Ability to Walk Unaided and the GBS Disability Score at 6 Months in Patients Grouped by Anti-GM1 IgG and IgM Titer Height at Entry



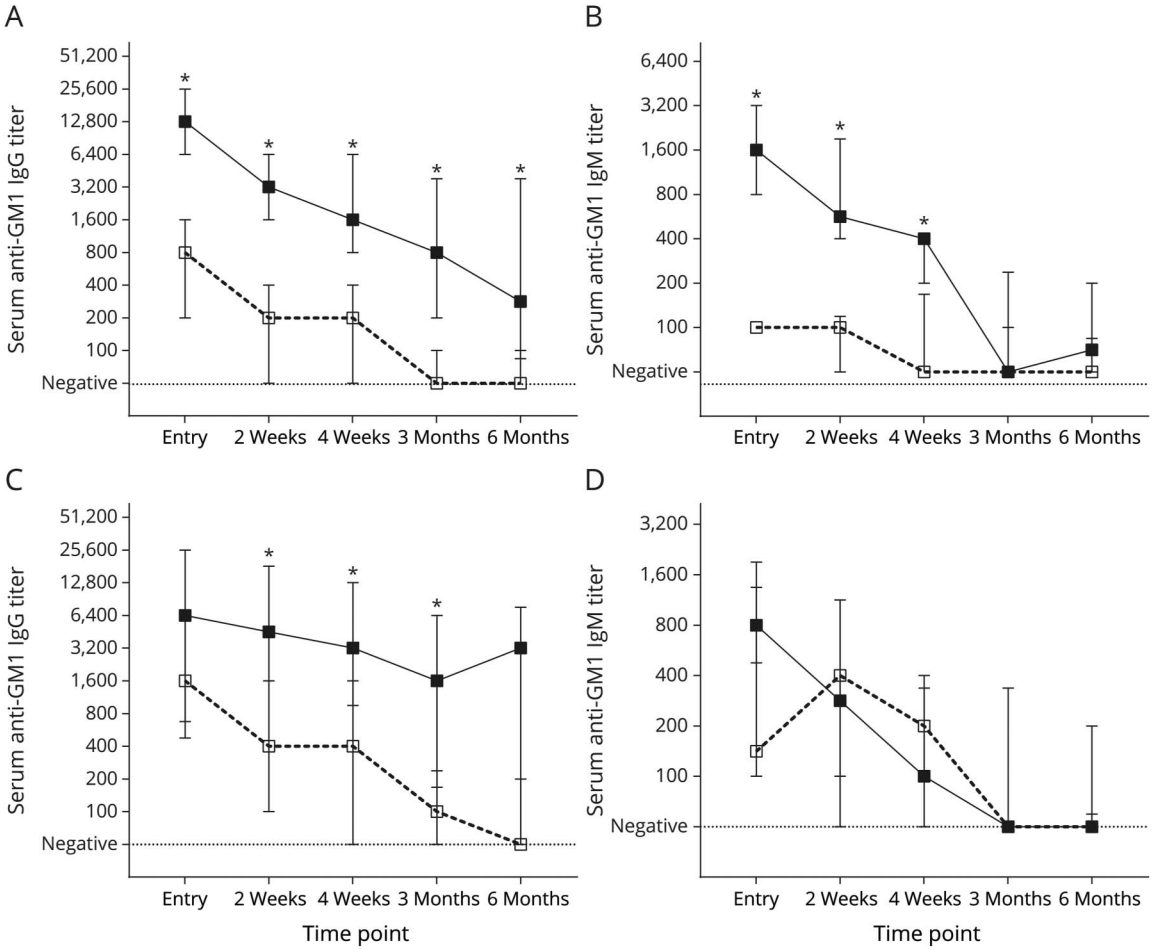
Kaplan-Meier curves for the ability to walk unaided at 6 months are shown in separate panels for serum anti-GM1 IgG (A) and IgM (C) antibody titers. Patients with a high anti-GM1 IgG and IgM titer at entry were compared with patients with a low titer of the corresponding isotype and anti-GM1-negative patients at entry. Corresponding proportional odds analyses for the distribution of the GBS disability score (0–6) at 6 months are also separately shown for the IgG (B) and IgM (D) isotypes. High titers at entry were defined as titers higher than the median titer at this time point, and low titers were defined as titers equal to or lower than the median titer. GBS-DS = GBS disability score; ns = not significant.

sum score at 6 months and patients less often requiring mechanical ventilation. These associations were also more pronounced for patients with IgG and IgM both.

The time to regain the ability to walk 10 m unaided differed between patients without anti-GM1 antibodies at entry, patients with a low IgG or IgM antibody titer at entry, and patients with a high IgG or IgM titer at entry (Figure 2). Following Bonferroni correction for pairwise comparisons, patients with a high IgG or IgM titer at entry (IgG: >1,600, IgM: >200) required more time to regain the ability to walk 10 m unaided and less often reached this end point compared with patients without anti-GM1 antibodies at entry (IgG: p = 0.015, IgM: p = 0.03). This difference did not remain when comparing patients with high vs low titers at entry (IgG: p = 0.06, IgM: p = 0.09). However, a high vs low IgG antibody titer at entry was independently associated with requiring more time to regain the ability to walk 10 m unaided after correcting for known prognostic factors, including age at onset, preceding diarrhea, and MRC sum score at entry (hazard ratio [95% CI]: 2.110 [1.015–4.388], p = 0.046). Furthermore, high anti-GM1 IgG and IgM antibody titers at entry were also associated with inexcitable nerves (IgG: p = 0.041, IgM: p = 0.033), and patients with a high IgM antibody titer at entry more often had preceding diarrhea (p = 0.022), a preceding upper respiratory tract infection (p = 0.026), and preceding *C jejuni* infection (p < 0.001) compared with patients with a low IgM antibody titer at entry.

Patients with poor outcome showed a consistently higher anti-GM1 IgG titer than patients with good outcome, with differences at 2 weeks (p < 0.001), 4 weeks (p = 0.013), and 3 months (p = 0.012) (Figure 3). There was no difference in anti-GM1 IgM titer between outcome groups at any time

Figure 3 Comparisons of Median Anti-GM1 IgG and IgM Antibody Titers at Each Time Point During Follow-up Based on Titer Height at Entry and Outcome



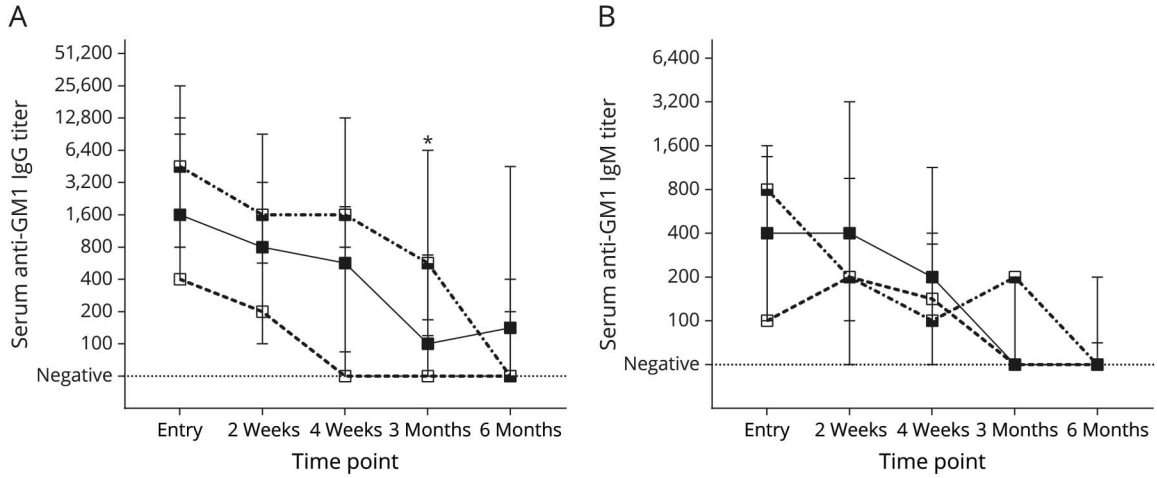
Median serum antibody titers with corresponding interquartile ranges are shown separately for the IgG (A and C) and IgM (B and D) isotypes. For comparisons related to the titer height at entry of each isotype (A and B), patients were grouped based on a high (solid line) or low (dashed line) titer at entry and for comparisons related to outcome (C and D) based on poor (solid line) vs good (dashed line) outcome. High titers at entry were defined as titers higher than the median titer at this time point, and low titers were defined as titers equal to or lower than the median titer.*p Value < 0.05.

point. High IgG antibody titers at 2 weeks (p = 0.022), 4 weeks (p = 0.006), and 6 months (p = 0.003) were associated with requiring more time to regain the ability to walk 10 m unaided and a lower frequency of reaching this end point compared with patients with a low titer at these time points. However, these associations did not remain after correcting for known prognostic factors. Among patients with a high anti-GM1 IgG titer at entry (n = 26/54 [48.1%]), a slow titer decline (observed in n = 14/19 [73.7%] at 4 weeks and n = 5/14 [35.7%] at 6 months) was associated with poor outcome at 4 weeks (p = 0.003) and 6 months (p = 0.032). Persistent anti-GM1 IgG and IgM antibodies at 3 and 6 months were not associated with the ability to walk unaided or the MRC sum score at 6 months. However, patients with persistent high IgG antibody titers at 3 and 6 months were less often able to walk 10 m unaided at 6 months (3 months: p = 0.022, 6 months: p = 0.004) and had a lower MRC sum score at 6 months (3 months: p = 0.018, 6 months: p = 0.002). This was not the case for patients with persistent high IgM antibody titers. The anti-GM1 IgG titer at entry did not differ between patients with a slow and a rapid titer decline at 4 weeks but was higher in the group with a slow decline at 6 months (p = 0.004). Also, patients with a persistent high IgG titer at 3 and 6 months had higher titers at entry compared with others (3 months: p = 0.002, 6 months: p < 0.001).

Anti-GM1 Antibody Responses in GBS Compared per Treatment Group

Patients treated with PE had the highest median anti-GM1 IgG antibody titers during follow-up, whereas patients treated with IVIg + MP had the lowest titers (Figure 4). The median anti-GM1 IgG antibody titer differed between patients treated with IVIg, IVIg + MP, or PE at 3 months (p = 0.027) (Figure 4). There was no association between anti-GM1 IgM antibody titers and treatment at any time point.

Figure 4 Comparisons of Median Anti-GM1 IgG and IgM Antibody Titers at Each Time Point During Follow-up Based on the Treatment Group



Median serum antibody titers with corresponding interquartile ranges are shown separately for the IgG (A) and IgM (B) isotypes. Treatment groups include IVIg only (solid line), IVIg and MP (dashed line), or PE (dash-dotted line).*$p < 0.05$.

Discussion

In this study, we determined the anti-GM1 antibody response in relation to clinical course and outcome in a well-defined cohort of patients with GBS who participated in previous therapeutic trials. Our results show that the anti-GM1 antibody response in GBS is highly variable, with titers ranging from 100 up to 51,200. In 46% of patients with anti-GM1 IgG antibodies at study entry, these antibodies remained present for at least 6 months, and this occurred more frequently in patients with a high titer at study entry. High anti-GM1 IgG and IgM antibody titers at entry and persistent high IgG antibody titers during follow-up were associated with poor outcome at 6 months. These findings indicate an ongoing production of anti-GM1 antibodies beyond the acute phase of the disease in a proportion of patients, which may predispose to poor outcome.

These results substantiate previous studies. One report described a correlation between high serum anti-GM1 IgG levels at disease onset and a high GBS disability score at discharge.[16] Similarly, another study reported a more severe disease course and worse outcomes among patients with high anti-GM1 IgA antibody titers, compared with patients with low titers.[17] In a third study with 34 patients, an association between disease severity at nadir and serum anti-GM1 IgG levels was found with a cell-based ELISA but not with ELISA.[18] Most of the studies on the anti-GM1 antibody titer course describe a gradually decreasing IgG and IgM antibody titer after disease onset, whereas some describe a subset of patients with prolonged antibody titers.[13-16,19-23] Some studies reported that the titer course was not associated with disease course or outcomes, whereas other studies did find an association with the disease course.[13,16,19,22] Two additional studies described antibody titer peaks during the (sub)acute phase of GBS, which were, respectively, associated with clinical exacerbations and a more severe acute phase.[14,15] In our study, we included a large and well-defined cohort of patients with GBS who had been prospectively included into previous therapeutic trials, providing more power for comparative analyses. Moreover, detailed clinical data provided the opportunity to correct for known prognostic factors, extending previous reports.

Several mechanisms may explain the association between high or persistent high anti-GM1 antibody titers and poor outcome in GBS. First, there may be a direct relation between antibody quantities and/or affinity of these anti-GM1 antibodies at disease onset and the extent of the nerve damage. Accordingly, we found that patients with a high IgG and IgM titer at entry more often had inexcitable nerves. Second, as patients with high anti-GM1 antibody titers at entry often also had elevated titers during follow-up, it is possible that these antibodies continue to damage neuronal membranes and/or myelin sheaths over time. Alternatively, the persistence of these antibodies in GBS may impair nerve recovery because antiganglioside antibodies are known to interfere with nerve regeneration in a mouse model for GBS.[9] The observed antibody titer persistency might have been caused by ongoing B-cell activation or prolonged antibody secretion by differentiation of B cells into long-lived plasma cells, but the mechanism of persistent antibody production in GBS requires further investigation.[32,33]

Considering the half-life of IgG antibodies (7–21 days), persistency of anti-GM1 IgG antibody titers observed in a subset of patients with GBS indicates ongoing antibody production long after the acute disease state in GBS. Moreover,

the associations of high anti-GM1 IgG and IgM antibody titers at entry and persistent high anti-GM1 IgG antibody titers with poor outcome in GBS provide further evidence for the pathogenicity of these antibodies and substantiate evidence for the pathogenicity acquired from previous studies.[8,34-37] In 2 animal studies, rabbits were immunized with GM1 or *C jejuni*–derived GM1-like LOS, which resulted in the development of anti-GM1 IgM and IgG antibodies and pathology resembling axonal GBS in humans.[34,35] In another study, passive transfer of an antiganglioside antibody–secreting hybridoma led to development of neuropathy in mice.[36] In humans, exogenous ganglioside injections have been shown to be immunogenic, causing production of anti-GM1 IgG antibodies with specificity for nodes of Ranvier and motor end plates and leading to axonal GBS.[22] Moreover, additional studies have shown that anti-GM1 IgG antibodies are able to induce complement-mediated disruption of voltage-gated sodium channels at the nodes of Ranvier and impair nerve repair.[8,37] It remains unclear whether anti-GM1 IgM antibodies have a pathogenic role in GBS. Low IgM antibody titers have been shown to occur in healthy human adults and can thus be considered to be part of natural immunity.[38] However, elevated titers of anti-GM1 IgM antibodies have been suggested to be pathogenic in neuropathies such as multifocal motor neuropathy.[39] Moreover, in our study, we found an association of high IgM titers at entry with poor outcome. Yet, this association may be the result of a correlation between IgG and IgM titer height at entry.

Our results may potentially have various implications for the treatment and care of patients with GBS. First, patients with GBS having persistent high anti-GM1 IgG antibody titers may benefit from additional or prolonged immune-modulatory treatment during the disease course. Monitoring of anti-GM1 antibodies could thereby potentially contribute toward personalized treatment strategies, although the effectiveness of immune-modulatory therapy after the acute stage of GBS has not been determined. Moreover, antibody persistency may be a new target for future treatment trials. Further research is needed on whether persistent antibodies are pathogenic and whether these patients may respond to treatments that interfere with these antibodies. Second, this study may contribute to improve the outcome prediction in GBS, as high anti-GM1 antibody titers at entry were independently associated with the inability to walk unaided at 6 months of follow-up when corrected for known prognostic factors. Notably, this would be a promising prognostic biomarker for GBS that can be determined at study entry and is potentially druggable.

Our study has several limitations. First, missing data may have led to selection bias and reduced power. Second, the population of trial patients included in the current study had a relatively severe form of GBS, which may partly limit extrapolation. Third, in patients treated with IVIg, anti-GM1 antibody titers may have been modified by the immunomodulatory effects of IVIg. Last, we have not assessed the IgG subclasses, fine specificity, and capacity to activate complement of the anti-GM1 antibodies, which may all influence their pathogenic effects. The 4 IgG subclasses (IgG1-4) are known to differ in their structure and function, affecting their pathogenicity and response to immunotherapy.[40,41] In GBS, IgG1 and IgG3 occur most frequently, and IgG1 has been associated with a more severe disease course and slower recovery.[40] The relative abundance of IgG1 and IgG3 in GBS may also explain the efficacy of IVIg in these patients.[41] The fine specificity of anti-GM1 antibodies has also been shown to affect clinical course and outcome in GBS, and formation of ganglioside complexes has been shown to enhance or attenuate immunopathogenic effects of individual antibodies.[6,18,40] Future studies should investigate these additional aspects, for which high-throughput antibody detection using glycoarrays would be an attractive method.[42]

In conclusion, high anti-GM1 IgG and IgM titers at entry and a persistent high anti-GM1 IgG antibody titer during follow-up are associated with poor clinical outcome in patients with GBS. Antibody persistency indicates ongoing antibody production long after the acute disease state in GBS. Monitoring anti-GM1 antibodies during the disease course may identify patients who require additional or prolonged treatment, but further research is required to determine whether persistent antibodies are pathogenic and whether antibody persistency may be a target for treatment.

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Disclosure

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High and Persistent Anti-GM1 Antibody Titers Are Associated With Poor Clinical Recovery in Guillain-Barré Syndrome

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