BRAIN
ORIGINAL ARTICLE

Large-scale profiling of antibody reactivity to glycolipids in patients with Guillain-Barré syndrome

Robin C. M. Thomma,[1,2,†] Susan K. Halstead,[3,†] Laura C. de Koning,[1] Eveline J. A. Wiegers,[1] Dawn S. Gourlay,[3] Anne P. Tio-Gillen,[1,2] Wouter van Rijs,[1,2] Henning Andersen,[4] Giovanni Antonini,[5] Samuel Arends,[1,6] Shahram Attarian,[7] Fabio A. Barroso,[8] Kathleen J. Bateman,[9] Luana Benedetti,[10] Peter Van den Bergh,[11] Jan Bürmann,[12] Mark Busby,[13] Carlos Casasnovas,[14] Efthimios Dardiotis,[15] Amy Davidson,[3] Thomas E. Feasby,[16] Janev Fehmi,[17] Giuliana Galassi,[18] Tania Garcia-Sobrino,[19] Volkan Granit,[20] Gerardo Gutiérrez-Gutiérrez,[21] Robert D. M. Hadden,[22] Thomas Harbo,[4] Hans-Peter Hartung,[23,24,25] Imran Hasan,[26] James K. L. Holt,[27] Zhahirul Islam,[26] Summer Karafiath,[28] Hans D. Katzberg,[29] Noah Kolb,[30] Susumu Kusunoki,[31] Satoshi Kuwabara,[32] Motoi Kuwahara,[31] Helmar C. Lehmann,[33] Sonja E. Leonhard,[34] Lorena Martín-Aguilar,[35] Soledad Monges,[36] Eduardo Nobile-Orazio,[37] Julio Pardo,[19] Yann Pereon,[38] Luis Querol,[35,39] Ricardo C. Reisin,[40] Simon Rinaldi,[17] Paolo Ripellino,[41,42] Rhys C. Roberts,[43] Olivier Scheidegger,[44] Nortina Shahrizaila,[45] Kazim A. Sheikh,[46] Nicholas J. Silvestri,[47] Soren H. Sindrup,[48] Beth Stein,[49] Cheng Y. Tan,[45] Hatice Tankisi,[50] Leo H. Visser,[51] Waqar Waheed,[30] Ruth Huizinga,[2] Bart C. Jacobs,[1,2] Hugh J. Willison[3] and the IGOS consortium

[†]These authors contributed equally to this work.

Guillain-Barré syndrome is an acute polyradiculoneuropathy in which preceding infections often elicit the production of antibodies that target peripheral nerve antigens, principally gangliosides. Anti-ganglioside antibodies are thought to play a key role in the clinical diversity of the disease and can be helpful in clinical practice. Extensive research into clinical associations of individual anti-ganglioside antibody specificities has been performed. Recent research has highlighted glycolipid complexes, glycolipid combinations that may alter antibody binding, as targets. In this study, we investigated antibody reactivity patterns to glycolipids and glycolipid complexes using combinatorial array, in relation to clinical features in Guillain-Barré syndrome.

In total, 1413 patients from the observational International Guillain-Barré syndrome Outcome Study (0–91 years, 60.3% male) and 1061 controls (healthy, family, infectious, vaccination, other neurological disease) were included. Acute-phase sera from patients were screened for IgM, IgG, and IgA reactivity against 15 glycolipids and one phospholipid and their heteromeric complexes, similarly to archived control sera. Antibody specificities and reactivity patterns were analysed in relation to clinical features.

Received November 08, 2024. Revised January 29, 2025. Accepted February 09, 2025. Advance access publication March 17, 2025

Of all patients, 1309 (92.6%) were positive for at least one anti-glycolipid (complex) antibody. Anti-GM1 and anti-GQ1b (complex) antibodies best distinguished motor Guillain-Barré syndrome and Miller Fisher syndrome from controls, with antibodies to glycolipid complexes outperforming antibodies to single glycolipids. Three models consisting of anti-glycolipid (complex) antibodies distinguished patients with Guillain-Barré syndrome, the motor variant, and Miller Fisher syndrome from controls with high sensitivity and specificity, performing better than antibodies to single glycolipids used in clinical practice. Seven patient clusters with particular antibody reactivity patterns were identified. These clusters were distinguished by geographical region, clinical variants, preceding *Campylobacter jejuni* infection, electrophysiological subtypes, the Medical Research Council sum score at study entry, and the ability to walk 10 m unaided at 26 weeks. Two patient clusters with distinct anti-GM1 (complex) reactivity (broad versus restricted) differed in frequency of the axonal subtype. In cumulative incidence analyses, 15 anti-glycolipid (complex) antibodies were associated with the time required to regain the ability to walk 10 m unaided. After adjustment for known prognostic factors, IgG anti-GQ1b:GM4, GQ1b:PS and GQ1b:Sulfatide remained associated with faster recovery. Addition of anti-glycolipid antibodies to clinical prognostic models slightly improved their discriminative capacity, though insufficiently to improve the models.

Measurement of anti-glycolipid antibodies by combinatorial array increases the diagnostic yield compared to assaying single glycolipids, identifies clinically relevant antibody reactivity patterns to glycolipids and glycolipid complexes, and may be useful in outcome prediction in Guillain-Barré syndrome.

1 Department of Neurology, Erasmus MC University Medical Center Rotterdam, Rotterdam 3015 GD, The Netherlands
2 Department of Immunology, Erasmus MC University Medical Center Rotterdam, Rotterdam 3015 GD, The Netherlands
3 School of Infection and Immunity, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow G12 8QQ, UK
4 Department of Neurology, Aarhus University Hospital, Aarhus 8200, Denmark
5 Department of Neurology, Mental Health and Sensory Organs (NESMOS), Faculty of Medicine and Psychology, University of Rome 'Sapienza', Sant'Andrea Hospital, Rome 00189, Italy
6 Department of Neurology, Haga Teaching Hospital, The Hague 2545 AA, The Netherlands
7 Department of Neurology, Reference Centre for NMD, CHU Timone, Marseille 13005, France
8 Department of Neurology, Instituto de Investigaciones Neurológicas Raúl Carrea, FLENI, Buenos Aires C1428, Argentina
9 Division of Neurology, Department of Medicine, Groote Schuur Hospital, University of Cape Town, Cape Town 7935, South Africa
10 Department of Neurology, IRCCS Ospedale Policlinico San Martino, Genova 16132, Italy
11 Department of Neurology, University Hospital St. Luc, University of Louvain, Brussels 1200, Belgium
12 Department of Neurology, MVZ Pfalzklinikum, Kusel 66869, Germany
13 Department of Neurology, Leeds Teaching Hospital, Leeds LS9 7TF, UK
14 Department of Neurology, Neuromuscular Unit, Bellvitge University Hospital-IDIBELL, CIBERER, Barcelona 08907, Spain
15 Department of Neurology, University of Thessaly, University Hospital of Larissa, Larissa 41110, Greece
16 Department of Clinical Neurosciences, University of Calgary, Alberta AB T2N 1N4, Canada
17 Nuffield Department of Clinical Neurosciences, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DU, UK
18 Department of Neurology, University Hospital of Modena, Modena 41125, Italy
19 Department of Neurology, Complejo Hospitalario Universitario de Santiago, Hospital Clínico, Santiago de Compostela 15706, Spain
20 Department of Neurology, Montefiore Medical Center, Bronx, NY 10065, USA
21 Department of Neurology, Faculty of Biomedical and Health Sciences, Hospital Universitario Infanta Sofia, Universidad Europea de Madrid, Madrid 28702, Spain
22 Department of Neurology, King's College Hospital, London SE5 9RS, UK
23 Department of Neurology, University of Düsseldorf, Düsseldorf 40225, Germany
24 Brain and Mind Centre, University of Sydney, Sydney NSW 2050, Australia
25 Department of Neurology, Medical University of Vienna, Vienna 1090, Austria
26 Infectious Diseases Division, Gut-Brain Axis Laboratory, Dhaka 1212, Bangladesh
27 Department of Neurology, The Walton Centre, Liverpool L9 7LJ, UK
28 Department of Neurology, Utah Valley University, Orem, UT 85048, USA
29 Division of Neurology, Department of Medicine, University Health Network, University of Toronto, Toronto ON M5S 1A1, Canada

30 Department of Neurology, University of Vermont Medical Centre, Burlington, VT 05401, USA
31 Department of Neurology, Faculty of Medicine, Kindai University, Osaka-Sayama, Osaka 589-8511, Japan
32 Department of Neurology, Graduate School of Medicine, Chiba University, Chiba 260-8670, Japan
33 Department of Neurology, University Hospital of Cologne, Cologne 50937, Germany
34 Department of Medical Microbiology and Infectious Diseases, Erasmus MC University Medical Center Rotterdam, Rotterdam 3015 GD, The Netherlands
35 Department of Neurology, Neuromuscular Diseases Unit, Hospital de la Santa Creu i Sant Pau, Universitat Autònoma de Barcelona, Barcelona 08025, Spain
36 Department of Neurology, Hospital de Pediatría J.P. Garrahan, Buenos Aires C1245, Argentina
37 Neuromuscular and Neuroimmunology Service, IRCCS Humanitas Research Hospital, Milan University, Milan 20089, Italy
38 CHU Nantes, Reference Centre for Neuromuscular Diseases AOC, Nantes 44093, France
39 Centro de Investigación Biomédica en Red de Enfermedades Raras, CIBERER, Instituto de Salud Carlos III Pabellón 11, Madrid 28029, Spain
40 Department of Neurology, Hospital Británico, Buenos Aires C1280, Argentina
41 Department of Neurology, Ospedale Regionale di Lugano, Lugano 6900, Switzerland
42 Faculty of Biomedical Sciences, Università della Svizzera Italiana, Lugano 6962, Switzerland
43 Department of Neurology, Box 165 Addenbrooke's Hospital, Cambridge University Hospitals NHS Foundation Trust, Cambridge CB2 0QQ, UK
44 Department of Neurology, Centre for Neuromuscular Diseases, Inselspital, Bern University Hospital, University of Bern, Bern 3010, Switzerland
45 Department of Medicine, University of Malaya, Kuala Lumpur 50603, Malaysia
46 Department of Neurology, The University of Texas Health Science Center at Houston, Houston, TX 77030, USA
47 Department of Neurology, University at Buffalo, Jacobs School of Medicine and Biomedical Sciences, Buffalo, NY 14203, USA
48 Department of Neurology, Odense University Hospital, Odense 5000, Denmark
49 Department of Neurology, ST Joseph Health, Paterson, NJ 07503, USA
50 Department of Clinical Neurophysiology, Aarhus University Hospital, Aarhus 8200, Denmark
51 Department of Neurology, St. Elisabeth-TweeSteden Hospital, Tilburg 5022 GC, The Netherlands

Correspondence to: Bart C. Jacobs
Departments of Neurology and Immunology, Erasmus MC University Medical Center Rotterdam
Dr. Molewaterplein 40, Rotterdam 3015 GD, The Netherlands
E-mail: b.jacobs@erasmusmc.nl

Keywords: peripheral neuropathy; autoantibody

Introduction

Guillain-Barré syndrome (GBS) is an acute immune-mediated polyradiculoneuropathy with an incidence of approximately 1–2 cases per 100 000 person-years.[1] Patients most typically present with rapidly progressive limb weakness accompanied by additional neurological symptoms including cranial nerve involvement, sensory deficits, autonomic dysfunction and respiratory insufficiency.[2] Disease severity may range from mild limb weakness to complete tetraparalysis with respiratory failure.[2] This heterogeneity in clinical presentation complicates early diagnosis and predictions of treatment response, clinical course and outcomes. Whilst the detailed pathophysiological and immunological factors underlying this clinical diversity remain largely unsolved, one major area of progress has been in the field of antibodies to glycolipids, principally gangliosides.[3]

In approximately two-thirds of patients with GBS, neurological symptoms are preceded by an infection.[4] Preceding infections, notably *Campylobacter jejuni*, elicit the production of antibodies that cross-react with peripheral nerve gangliosides as a result of structural identity, often termed molecular mimicry.[5] Gangliosides are sialylated glycolipids that are abundantly present in nerve cell membranes throughout the peripheral nervous system, with roles in nerve cell structure and physiology.[6] The binding of antibodies to these gangliosides in peripheral nerves leads to complement-mediated disruption of nerve membranes in axonal and Schwann cell membranes, notably at nodes of Ranvier.[3,6] Consequently, nerve damage ultimately results in neurophysiological changes and the development of clinical features.[3,6]

Clinical associations of antibodies to gangliosides and other glycolipids in GBS have been extensively investigated since their first discovery 40 years ago.[3,7] Most prominently, associations of anti-GM1 antibodies with motor-dominant GBS and anti-GQ1b antibodies with Miller Fisher syndrome (MFS) have been repeatedly described.[3] Antibodies to glycolipid complexes are a more recent important development.[3,8,9] Existing studies have generally covered a limited range of antibody specificities in small, geographically defined populations and especially antibodies to glycolipid heteromeric complexes remain less extensively studied.[3] Therefore, comprehensive clinical associations of antibody binding patterns to both single glycolipids and glycolipid complexes are lacking, limiting its current impact on the clinical evaluation of patients with GBS.

Combinatorial glyco-arrays, in which both single and heteromeric arrangements of glycolipids are spotted onto microarrays, are a relatively new and efficient method allowing for the testing of a vast repertoire of anti-glycolipid (complex) antibodies (AGAb) in a large number of samples.[10] Biophysical interactions between two glycolipids/gangliosides occur due to their clustering properties when spotted onto artificial membranes or surfaces. This ganglioside clustering is an important element of screening platform design and antibody discovery in this field. When two gangliosides interact to form a heteromeric cluster, this may alter the binding capacity of an antibody to either of the single gangliosides, causing enhancement or attenuation, or may not affect binding capacity (complex independence).[8,9] For example, an antibody to GM1, when presented as a single ganglioside, may fail to bind GM1 when clustered with GD1a; alternatively, antibodies can be detected that only bind a GM1:GD1a complex but bind neither ganglioside alone; lastly, an anti-GM1 antibody may bind GM1 irrespective of the presence or absence of GD1a.

In this study, we used the biobank and clinical database of the International Guillain-Barré syndrome Outcome Study (IGOS) to investigate on a large scale the presence of AGAb in glyco-arrays, in relation to clinical subtypes and characteristics, preceding infections, clinical course, and outcomes in patients with GBS. In addition to studying single glycolipids, the added diagnostic and categorical value of combinatorial array over single array was investigated.

Materials and methods

Study population

Clinical data and serum samples were acquired from patients included in IGOS, a prospective multicentre cohort study including patients with GBS irrespective of the clinical variant, electrophysiological subtype, and disease severity (Supplementary Fig. 1).[11,12] Patients with a final diagnosis other than GBS, insufficient clinical data, more than 17 days between onset of disease and inclusion, or protocol violations were excluded. Clinical data and serum samples were gathered at study entry and at standard time points during at least one year of follow-up. Only patients with a serum sample from study entry or Week 1 available were included in analyses ($n = 1413$; Supplementary Fig. 1 and Supplementary Table 1). Clinical data that were used for analyses included demographics, clinical variants and features, disease severity, and electrophysiological subtypes. Preceding infections associated with GBS were determined as described previously.[4] Electrophysiological subtypes were classified according to Hadden criteria and determined for the first 1500 patients included in IGOS.[13,14] The control population consisted of 1061 healthy (6.8%), family (27.3%), pre- and post-vaccination (16.8%), infectious (Zika virus, without neurological symptoms; 15.3%) and other neurological disease controls (multiple sclerosis and other inflammatory neurological diseases; 33.8%) from geographically diverse historical cohorts, including the UK, USA, Bangladesh and Colombia.[15,16] Control samples were used to determine AGAb positivity in patients with GBS and to identify AGAb that can distinguish patients from controls.

All patients provided written informed consent. IGOS was approved by the Institutional Review Board of the Erasmus MC University Medical Center Rotterdam (The Netherlands; MEC-2011-477) and by local review boards from each participating centre.

Antibody testing in glyco-array

Patient sera were tested for IgM, IgG and IgA against 15 individual glycolipids, including major gangliosides, and one phospholipid [GM1, GM2, phosphatidylserine (PS), GM4, GA1, GD1a, GD1b, GT1a, GT1b, GQ1b, GD3, sulfated glucuronyl paragloboside (SGPG), LM1, N-acetylgalactosaminyl GD1a (GalNAc-GD1a), galactocerebroside (GalC), and sulfatide] and all possible 1:1 (volume:volume) glycolipid complexes in glyco-arrays (136 targets, 408 antibodies), as described previously.[10] Sera from the control cohorts had been screened previously, some for only a subset of the glycolipid targets.[15,16] Of the 136 IgG targets included on the glyco-array panel for patients (Fig. 2), 55 (40.4% of total targets) had been tested in all controls, 65 (47.8% of total targets; GM2, GM4, GT1a, GT1b, and GD3 complexes) only in non-Bangladeshi controls ($n = 482$; 45.4% of total controls) and 16 (11.8% of total targets; GalNAc-GD1a complexes) only in UK controls ($n = 178$; 16.8% of total controls). IgM and IgA were screened against all 136 targets in UK controls only ($n = 98$; 9.2% of total controls).

In brief, array slides were printed in-house with each unique single or complex glycolipid target duplicated per array.[10] Slides were blocked with 2% bovine serum albumin (BSA) in PBS prior to application of individual serum samples diluted 1:50 in 1% BSA in PBS.[10] Following washing of unbound antibody, arrays were probed concomitantly with the following fluorescently conjugated, heavy chain specific, detection antibodies; anti-human IgG-Alexa Fluor 647 (Jackson Immuno Research Laboratories, 109-605-008; 3 μg/ml), anti-human IgM-TRITC (Southern Biotech, 2020-03; 3 μg/ml) and anti-human IgA-FITC (Southern Biotech, 2050-02; 3 μg/ml). Glyco-arrays were then washed and air dried. Fluorescent signals were sequentially detected with a GenePix 4300A microarray scanner (Molecular Devices) equipped with three lasers. For each antigen target on the array, the median fluorescent signal per immunoglobulin class was calculated, from which the local background signal was subtracted. As all unique targets were printed in duplicate, the mean of the two values was used for all subsequent analysis. Values ranged from 150 to 65 535 fluorescence intensity units.

Statistical analyses

Comparative analyses

Comparative analyses for AGAb fluorescence intensities and clinical features were performed with Chi-square, Fisher's exact, Mann–Whitney U- and Kruskal–Wallis tests, and univariable logistic regression analyses [for the latter, associations were described with odds ratios (OR) and their 95% confidence intervals (CI)]. Generally, an OR >1 indicates higher fluorescence intensities in the group of interest, whereas an OR <1 indicates lower fluorescence intensities. Multiple comparisons following Kruskal–Wallis tests were performed with *post hoc* Dunn's tests. Correlations were analysed with the Pearson correlation coefficient (r).

Assessment of the diagnostic value

The diagnostic value of AGAb in the diagnosis of GBS was assessed with logistic regression and receiver-operator characteristic (ROC) curve analyses. Discriminative performance was evaluated based on the area under the curve (AUC), for which a cut-off value of 0.75 was set to be classified as a clinically relevant test with high sensitivity and specificity.[17] In addition, bootstrapping was applied to acquire optimism-corrected C-statistics (C) and the goodness of

fit (R^2) of each model. The dataset was split into derivation and validation datasets for validation [derivation: 80% of patients ($n = 1134$) and controls ($n = 845$); validation: 20% of patients ($n = 279$) and controls ($n = 216$)]. Fluorescence intensities were log-transformed.

Univariable logistic regression analyses were employed to investigate associations of AGAb with GBS, motor GBS or MFS, and multivariable analyses were applied to explore whether combinations of AGAb could further improve the diagnostic value of AGAb. Final multivariable models were acquired through backwards variable selection of an initial model containing AGAb that remained after univariable analyses in the derivation dataset and were tested in all controls. Forward variable selection was applied to compare and validate models acquired from backwards variable selection. Principal components were created to adjust for multicollinearity across predictors in multivariable models. Generated models were validated in the validation and complete datasets and were compared to each other and to models based on antibodies currently used in clinical practice (GBS: IgG and IgM against GM1, GM2, GD1a, GD1b and GQ1b; motor GBS: IgG against GM1; MFS: IgG against GQ1b).[18] Since IgM and IgA were only tested in a relatively small subset of controls, we did not include these in model generation and model comparison as this would limit statistical power in complete-case analyses. Model comparison was performed through ANOVA for nested models and based on the Akaike information criterion (AIC) for non-nested models.

Clustering and heat map generation

Unsupervised hierarchical clustering was employed to explore whether AGAb reactivity patterns occur in patients with GBS. Ward's method (Ward D2) was applied to cluster both patients and AGAb based on min-max normalized (0–1) fluorescence intensities. Clusters were identified using dendrograms resulting from clustering. The optimal number of clusters was determined based on a combination of results from 26 distinct indices that each determine the optimal number of clusters, using the 'NbClust' package in RStudio, and clinical relevance.[19] For visualization in heat maps, fluorescence intensities were capped at a value of 0.2.

Associations of complex interactions with electrophysiological subtypes

Complex interactions between glycolipids were investigated in relation to electrophysiological subtypes according to Hadden criteria (normal, demyelinating, axonal, inexcitable or equivocal).[14] Complex enhancement was defined as an increased fluorescence intensity of anti-complex antibodies compared to the summed fluorescence intensities of antibodies to individual complex constituents (Eq. 1). Complex attenuation was defined as a decreased fluorescence intensity of anti-complex antibodies (Eq. 2). Anti-complex antibodies with unaltered fluorescence intensities were defined as complex independent.

$$\text{complex} > 2 \times (\text{constituent 1} + \text{constituent 2}) \quad (1)$$

$$\text{complex} < 0.5 \times (\text{constituent 1} + \text{constituent 2}) \quad (2)$$

Patients with fluorescence intensities below 500 U for antibodies against both complex constituents and the complex were excluded from these analyses. This threshold was chosen based on experience from previous studies, with the lower limit of reliable and valid detection (150 U) and assay variability taken into account.

Assessment of prognostic value

The time required to regain the ability to walk 10 m unaided was compared between patients classified as positive or negative for each AGAb using cumulative incidence analyses and log-rank tests. Correction for known prognostic factors [age, preceding diarrhoea and the Medical Research Council (MRC) sum score at entry] was performed for log-transformed fluorescence intensities in Cox regression.[20,21] Relative effects of variables in Cox regression were presented as hazard ratios (HR), along with their 95% CIs. HR values >1 indicate a higher probability to recover sooner, whereas values <1 indicate a higher probability to recover more slowly. In addition, we investigated the predictive performance of AGAb and their added value to existing clinical prognostic models for the prediction of regaining the ability to walk unaided by comparing the discriminative capacity [Erasmus GBS Outcome Score (EGOS) and modified EGOS (mEGOS)].[20-22] Validation was performed in the derivation and validation datasets.

Data processing and software

Cut-off values for antibody positivity were based on the 97.5th percentile of fluorescence intensities in controls. Two-sided *P*-values <0.05 were considered statistically significant. Bonferroni corrections were applied for multiple comparisons. Missing data were not imputed. The highest per cent missing data in clinical variables was 47.9% (MRC sum score at Week 13).

Statistical analyses were performed in RStudio version 2023.03.0 and GraphPad Prism version 9.5.1. Used RStudio packages include 'stats', 'FSA', 'rms', 'pROC', 'epiR', 'NbClust', 'ComplexHeatmap' and 'survival'.

Results

Anti-glycolipid (complex) antibodies discriminate patients from controls

Several AGAb reactivities were able to distinguish subgroups of patients from controls, for which antibodies to glycolipid complexes generally performed better than antibodies to single glycolipids. Of all patients, 1309 (92.6%) were positive for at least one of the 408 investigated IgG, IgM and IgA antibody reactivities. In univariable analyses on the complete dataset, higher fluorescence intensities of 125 AGAb (121 IgG and 4 IgA) and lower fluorescence intensities of 22 AGAb (2 IgG and 20 IgM) were associated with GBS (Table 1 and Supplementary Fig. 2). Several AGAb were able to discriminate specific subgroups of patients with GBS from controls with high sensitivity and specificity. Higher fluorescence intensities of 113 AGAb and lower intensities of 18 AGAb were associated with motor GBS and higher intensities of 118 AGAb and lower intensities of 16 AGAb with MFS ($n = 311$ and 153 patients, respectively; Table 1 and Supplementary Fig. 2). For patients with motor GBS and MFS, anti-GM1 and -GQ1b (complex) antibodies respectively best distinguished them from controls. Most antibodies to GM1 and GQ1b complexes performed better than antibodies to GM1 or GQ1b alone (Fig. 1A and B). Addition of sulfatide and GT1a to GM1 resulted in the highest performance increase for motor GBS, whereas addition of phosphatidylserine and sulfatide to GQ1b most improved the performance for MFS. Validation of these univariable analyses was performed using the derivation and validation datasets (Supplementary material, File 1).

Combinations of AGAb in multivariable models further improved the diagnostic value of AGAb. Backward selection of the

Table 1 Odds ratios with associated 95% confidence intervals for the top five anti-glycolipid antibodies in distinguishing Guillain-Barré syndrome, motor Guillain-Barré syndrome or Miller Fisher syndrome from controls

GBS versus controls		Motor GBS versus controls		MFS versus controls	
Top five AGAb	OR (95% CI)	Top five AGAb	OR (95% CI)	Top five AGAb	OR (95% CI)
Anti-GA1:Sulf	2.01 (1.85–2.20)	Anti–GA1:Sulf	2.34 (2.11–2.60)	Anti–GQ1b:SGPG	3.32 (2.79–4.00)
Anti-GA1:PS	1.83 (1.68–2.00)	Anti–GA1:PS	2.26 (2.05–2.52)	Anti–GQ1b:LM1	6.63 (5.01–9.04)
Anti-GA1:GalC	2.10 (1.89–2.35)	Anti–GM1:SGPG	2.94 (2.52–3.45)	Anti–GQ1b:Sulf	4.49 (3.58–5.79)
Anti-GM1:Sulf	3.90 (3.19–4.87)	Anti–GM1:Sulf	4.30 (3.47–5.45)	Anti–GQ1b:GalC	5.05 (3.93–6.78)
Anti-GD1b:Sulf	4.25 (3.41–5.45)	Anti–GA1:GalC	2.21 (1.96–2.52)	Anti–GQ1b:GD1b	7.19 (5.25–10.18)

All described anti-glycolipid antibodies are of the IgG isotype. Ranking of anti-glycolipid antibodies was based on *P* in univariable logistic regression analyses on the complete dataset. AGAb = anti-glycolipid antibodies; CI = confidence interval; GalC = galactocerebroside; GBS = Guillain-Barré syndrome; MFS = Miller Fisher syndrome; OR = odds ratio; PS = phosphatidylserine; SGPG = sulfated glucuronyl paragloboside; Sulf = sulfatide.

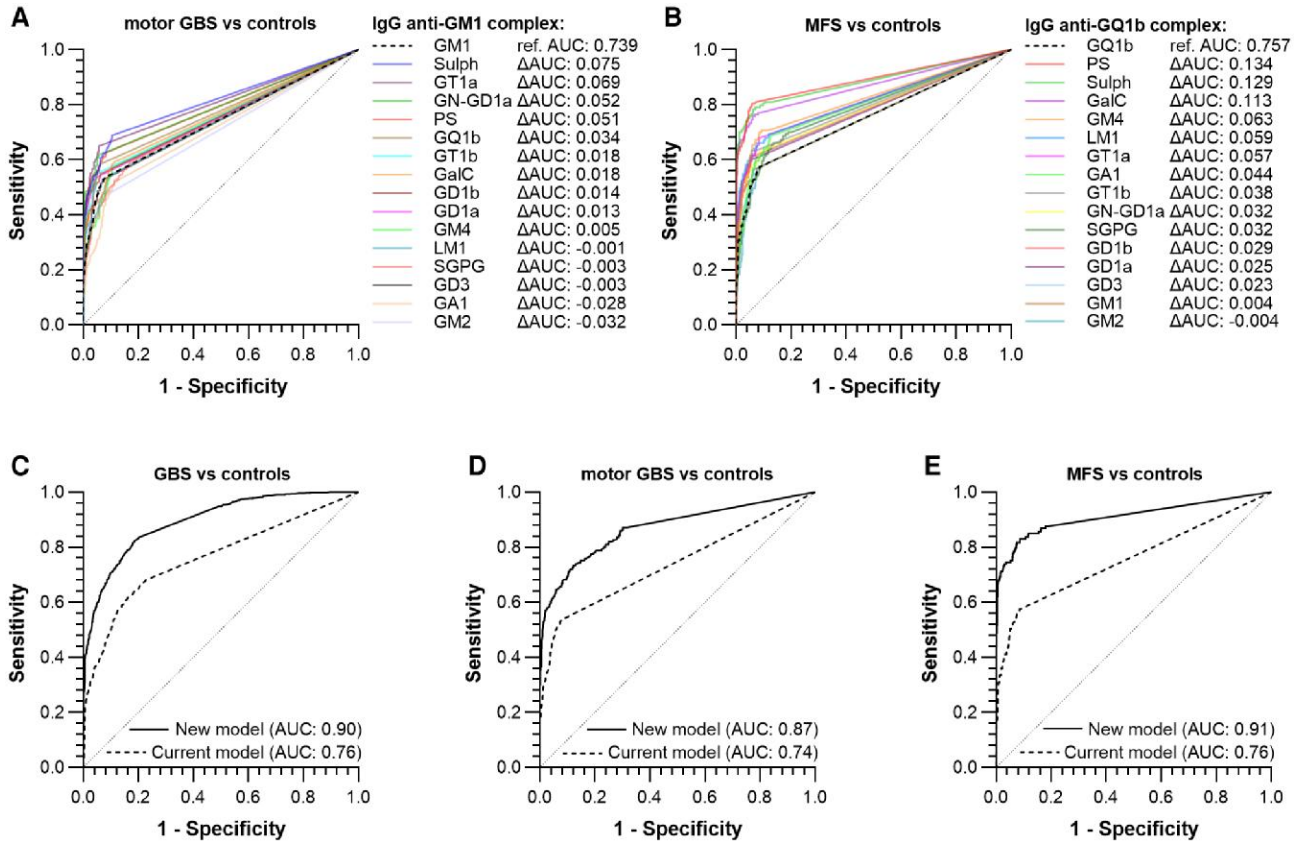

Figure 1 Comparison of receiver operating characteristic curves for models distinguishing patients with Guillain-Barré syndrome, motor Guillain-Barré syndrome or Miller Fisher syndrome from controls. Receiver operating characteristic curves are shown with associated values for the area under the receiver operating characteristic curve, for univariable models (**A** and **B**) and multivariable models (**C**–**E**). Using univariable models, the differentiating performance of IgG anti-GM1 complex antibodies and IgG anti-GQ1b complex antibodies were compared to IgG antibodies to GM1 or GQ1b alone for the distinction of motor Guillain-Barré syndrome (**A**) or Miller Fisher syndrome (**B**) from controls. Additionally, newly created multivariable models containing antibodies to both single gangliosides and ganglioside complexes were compared to currently used multivariable models based on antibodies to single gangliosides, for the distinction of Guillain-Barré syndrome (**C**), motor Guillain-Barré syndrome (**D**) or Miller Fisher syndrome (**E**) from controls. AUC = area under the receiver operator characteristic curve; GBS = Guillain-Barré syndrome; GalC = galactocerebroside; GN-GD1a = *N*-acetylgalactosaminyl GD1a; MFS = Miller Fisher syndrome; PS = phosphatidylserine; SGPG = sulfated glucuronyl paragloboside; Sulf = sulfatide.

AGAb that resulted from univariable analyses and were tested in all patients and controls resulted in three models to discriminate GBS, motor GBS, or MFS from controls in the derivation dataset (GBS: seven AGAb; motor GBS: two AGAb; MFS: two AGAb; Supplementary Table 2). The newly created models performed better than current models based on antibodies to single gangliosides (GBS: AIC = 2038 versus 2825; motor GBS: AIC = 892 versus 1175; MFS: AIC = 417 versus 714). At optimal thresholds, new models showed an increase in sensitivity compared to current models (GBS: from 62% to 83%; motor GBS: from 53% to 72%; MFS: from 58% to 83%) while maintaining high specificity (GBS: from 79% to 81%; motor GBS: from 93% to 89%; MFS: from 92% to 91%). Consequently, an additional 222/1413

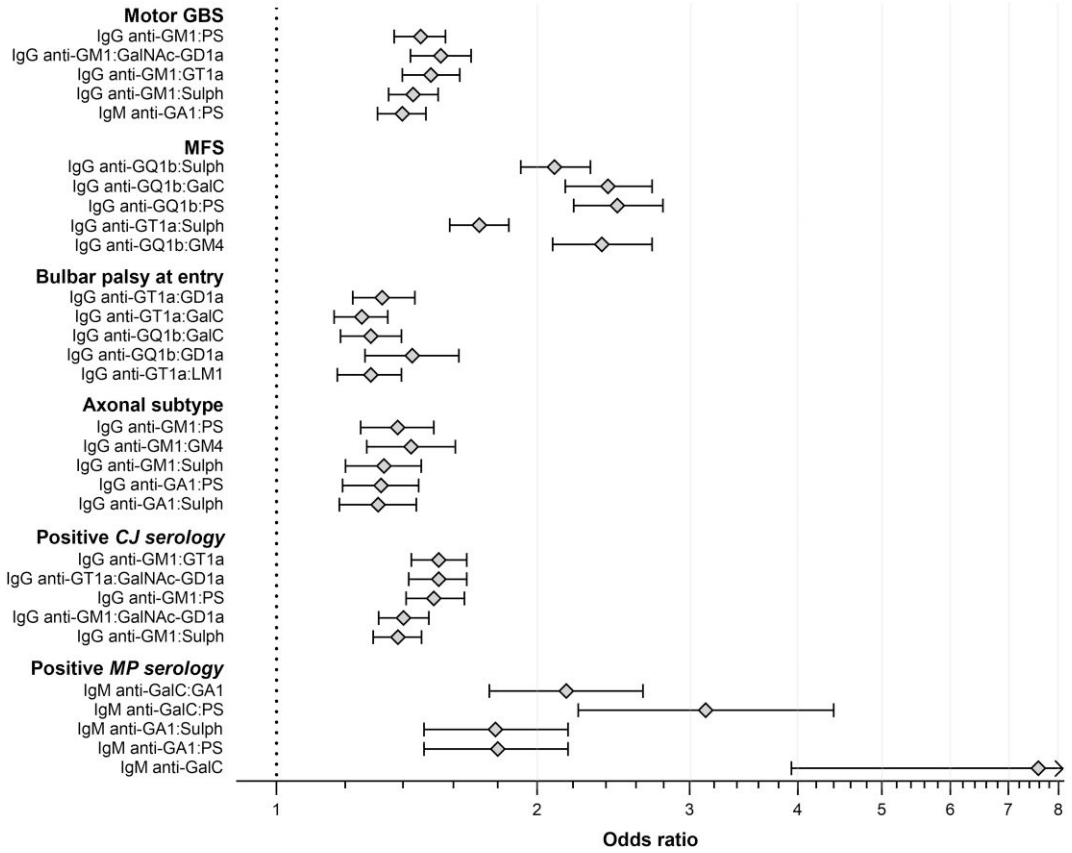

Figure 2 Forest plots depicting the top five anti-glycolipid (complex) antibodies associated with several clinical features in patients with Guillain-Barré syndrome. Associations of anti-glycolipid antibodies with motor Guillain-Barré syndrome, Miller Fisher syndrome, bulbar palsy at study entry, the axonal subtype, and preceding *Campylobacter jejuni* and *Mycoplasma pneumoniae* infections. Values indicate the odds ratio with their 95% confidence interval per anti-glycolipid antibody. Antibodies were ranked based on the *P*-value resulting from univariable logistic regression analyses. *CJ* = *Campylobacter jejuni*; GalC = galactocerebroside; GalNAc-GD1a = *N*-acetylgalactosaminyl GD1a; GBS = Guillain-Barré syndrome; MFS = Miller Fisher syndrome; *MP* = *Mycoplasma pneumoniae*; PS = phosphatidylserine; Sulf = sulfatide.

Table 2 Model performance statistics for models distinguishing Guillain-Barré syndrome, motor Guillain-Barré syndrome or Miller Fisher syndrome from controls

Group	Model	Constituent IgG anti-glycolipid antibodies	C-statistic	R^2
GBS	Current	GM1, GD1a, GD1b, GQ1b	0.76 (0.74–0.78)	0.27 (0.22–0.32)
	New	GD1b, GQ1b, SGPG, GM1:GD1a, GA1:LM1, GA1:Sulf, GalC:LM1	0.89 (0.88–0.91)	0.56 (0.52–0.61)
Motor GBS	Current	GM1	0.74 (0.71–0.78)	0.30 (0.23–0.37)
	New	GM1:GD1a, GA1:Sulf	0.87 (0.84–0.90)	0.52 (0.45–0.59)
MFS	Current	GQ1b	0.76 (0.70–0.80)	0.30 (0.21–0.39)
	New	GQ1b:GA1, GQ1b:GalC	0.91 (0.87–0.95)	0.64 (0.56–0.73)

Analyses were performed in the complete dataset. Data are presented as value (95% confidence interval). The C-statistic and R^2 were optimism-corrected.
GalC = galactocerebroside; GBS = Guillain-Barré syndrome; MFS = Miller Fisher syndrome; SGPG = sulfated glucuronyl paragloboside; Sulf = sulfatide.

(15.7%) patients with GBS, 58/311 (18.6%) patients with motor GBS, and 39/153 (25.5%) patients with MFS were diagnosed correctly using the newly created models when compared to the currently used models. When applying forwards instead of backwards selection to create the final models, similar models with comparable performance were acquired [GBS: three of seven AGAb differed (IgG against GD1b:SGPG, GD1a:Sulfatide and GA1:SGPG instead of GD1b, GM1:GD1a and GA1:LM1), AIC = 2077; motor GBS: one of two AGAb differed (IgG against GM1:Sulfatide instead of GM1:GD1a), AIC = 911; MFS: two of two AGAb differed (IgG against GQ1b:SGPG and GQ1b:Sulfatide instead of GQ1b:GA1 and GQ1b:GalC), AIC = 419]. Notably, each of the constituent AGAb of the models acquired from backwards selection was also among the most strongly associated AGAb for each step of the forwards selection. Application of all models acquired from backwards selection in the validation and complete (derivation and validation cohort together) datasets resulted in similar findings (Fig. 1C–E, Table 2 and Supplementary Table 2).

Associations of anti-glycolipid (complex) antibodies with preceding infections and clinical features

A subset of AGAb was associated with clinical features in GBS (Fig. 2 and Supplementary Figs 3–5). Several anti-GM1, -GalNAc-GD1a

and -GA1 (complex) antibodies were associated with preceding diarrhoea, preceding *C. jejuni* infection, motor GBS and the inability to walk 10 m unaided at 26 weeks. Patients with preceding *Mycoplasma pneumoniae* or cytomegalovirus infections had higher fluorescence intensities of several anti-GalC and IgM anti-GM2 (complex) antibodies, respectively. Anti-GQ1b and -GT1a (complex) antibodies were associated with preceding upper respiratory tract infections and MFS. Higher fluorescence intensities of several antibodies to GM1, GA1, GD1a, GD1b, GT1a, GT1b, GalNAc-GD1a and SGPG (complexes) correlated with lower MRC sum scores at each time point during follow-up (range of *r*: −0.28 to −0.10). Correlations were strongest for anti-GD1a and -GT1a (complex) antibodies. In contrast, higher levels of antibodies targeted to GQ1b:Sulfatide, GQ1b:PS and GQ1b:GalC correlated with higher MRC sum scores in the acute phase (range of *r*: 0.12–0.17). When excluding patients with MFS, the latter correlations were no longer present.

Cluster analysis of anti-glycolipid (complex) antibodies and clinical associations of clusters

Following clustering based on fluorescence intensities, seven patient clusters with particular IgG AGAb reactivity patterns were identified (A: broad-ranging GalNAc-GD1a reactivity; B: restricted GA1 and broad-ranging GM1 reactivity; C: restricted GalNAc-GD1a reactivity; D: restricted GA1, GD1b and GM1 reactivity; E: non-specific; F: restricted GQ1b and GT1a reactivity; and G: broad-ranging GT1a reactivity; Fig. 3). Broad-ranging clusters had reactivity against the majority of complexes containing a specific glycolipid, whereas restricted clusters had reactivity against specific glycolipids in the presence of sulfatide and PS. All patients that were negative for all investigated AGAb ($n = 104$, 7.4%) were included in the non-specific Cluster E. In the other clusters, all patients had antibody reactivity against at least 17 AGAb. Patient clusters were clinically distinct, differing in geographical regions, proportions of GBS forms and variants, preceding infection serology, cranial nerve involvement at study entry and the clinical course (Table 3). Cluster G consisted of a relatively high proportion of Argentinian patients that were included between 2013 and 2015 ($n = 11$, 28.2%), of which the majority had a preceding *C. jejuni* infection (10/11, 90.9%). Furthermore, patients in Cluster G were younger than patients in Clusters A, B, D, E and F (median age: 28 versus 48–54; range of $P = <0.001$–0.014) and patients in Cluster E had higher MRC sum scores at study entry than patients in Clusters B, D and G (median: 48 versus 32–37; range of $P = <0.001$–0.002) and patients in Cluster F had higher MRC sum scores at study entry than patients in all other clusters (median: 60 versus 32–48; $P < 0.001$). Notably, two clusters with particular anti-GM1 (complex) antibody reactivity patterns (Clusters B and D) were clinically distinct. Three clusters predominantly containing patients with motor GBS (Clusters A, C and D) also had distinct clinical features.

When further investigating patients from Cluster E, including patients without a particular AGAb reactivity pattern and with predominantly motor-sensory GBS, several subclusters were identified (E-a: non-specific, E-b: broad-ranging GalC reactivity, E-c: restricted GD1a and GT1a reactivity, E-d: restricted reactivity to GM1:GT1a and E-e: restricted reactivity to GM1 and GA1; Supplementary Fig. 6). All patients that were negative for all investigated AGAb were included in the non-specific Cluster E-a. Most notable among these subclusters was a cluster with AGAb reactivity against GalC complexes (Cluster E-b), which was associated with positive *M. pneumoniae* serology (Supplementary Table 3). When performing clustering analyses using antibodies to GD1b, GT1a, GT1b, GQ1b, GD3, and LM1 (complexes) only, still no patient clusters specific for any of these antibody reactivities could be found.

Distinction of electrophysiological subtypes based on anti-glycolipid (complex) antibodies

Electrophysiological subtypes were associated with various AGAb (Fig. 2 and Supplementary Figs 3–5). Patients with normal and equivocal nerve conduction studies had higher fluorescence intensities of some anti-GQ1b and -GT1a (complex) antibodies (IgG against GT1a:PS, GQ1b:PS, GQ1b:GM4, GQ1b:GT1a, GT1a:GalC, GT1a:Sulf, GQ1b:GT1b, GT1b:Sulf, GQ1b:GD3, GQ1b:SGPG, GQ1b:LM1, GQ1b:GalC and GQ1b:Sulf; IgA against GQ1b:GalC and GQ1b:Sulf), the demyelinating subtype was associated with lower intensities of these same AGAb groups (IgG against GQ1b:PS, GT1a:PS, GT1a:GM4, GQ1b:GM4, GT1a:GA1, GQ1b:GA1, GQ1b:GD1a, GQ1b:GD1b, GD1b:Sulf, GT1a:GalC, GT1a:Sulf, GQ1b:GT1b, GQ1b:GD3, GQ1b:LM1, GQ1b:GalC and GQ1b:Sulf), and the axonal and inexcitable subtypes were associated with the presence of anti-GM1, -GA1 and -GalNAc-GD1a (complex) antibodies (IgG against GM1, GA1, GM1:GM2, GM1:PS, GM1:GM4, GM1:GA1, GM1:GD1a, GM1:GD1b, GM1:GT1b, GM1:GQ1b, GM1:GD3, GM1:SGPG, GM1:GalNAc-GD1a, GM1:GalC, GM1:Sulf, GA1:PS, GA1:GM4, GA1:GalNAc-GD1a, GA1:Sulf, GalNAc-GD1a:GD1b, GalNAc-GD1a:GT1a and SGPG:Sulf; IgM against GM1:PS, GM1:GM4, GM1:Sulf, GA1:GM4 and GA1:GalNAc-GD1a; and IgA against GM1:PS and GM1:Sulf). Anti-GM1 (complex) antibody fluorescence intensities differed across electrophysiological subtypes (Fig. 4A). Notably, these antibodies occurred in each subtype with broad ranges of intensities. Moreover, proportions of electrophysiological subtypes differed across patient clusters based on antibody reactivity patterns and also across the two patient clusters with particular anti-GM1 (complex) reactivity (all clusters: $P < 0.001$; anti-GM1 clusters: $P = 0.008$; Fig. 4B and Table 3).

The complex interaction of GM1 with GD1a varied across patients with different electrophysiological subtypes (Fig. 4C). However, the proportions of these subtypes did not differ between groups based on complex interaction (Fig. 4D). Proportions of electrophysiological subtypes did differ for complex interactions of GM1 with PS (enhanced: 21.8% axonal; attenuated: 6.6% axonal; independent: 9.6% axonal; $P = 0.001$).

Prognostic value of anti-glycolipid (complex) antibodies

Positivity of 15 AGAb was associated with the time required to regain the ability to walk 10 m unaided. Patients positive for IgG antibodies against GM1:Sulfatide, GM1:SGPG, GM1:GD1b, GM1:GalC, GM1:GalNAc-GD1a, GalNAc-GD1a:GalC, GM1:GD3, GM1:GM4, GA1:PS, GM1:GT1b and GalNAc-GD1a:GD1b as well as IgA against GM1:Sulfatide required more time to regain this ability, whereas patients positive for IgG against GQ1b:GM4, GQ1b:PS and GQ1b:Sulfatide reached this end point more rapidly (range of $P = 0.045$ to < 0.001; Fig. 5A–C). Following adjustment of fluorescence intensities for known prognostic factors (age, preceding diarrhoea, MRC sum score at entry), associations remained for IgG antibodies against GQ1b:GM4, GQ1b:P and GQ1b:Sulfatide [HR (95% CI) = 1.56 (1.28–1.92), 1.50 (1.27–1.77) and 1.36 (1.15–1.60); P <0.001, <0.001 and 0.004]. When excluding patients with MFS, positivity of four AGAb was associated with requiring more time to regain the ability to walk 10 m unaided (IgG antibodies against GM1:Sulfatide, GM1:GD1b, GM1:SGPG and GM1:GalC; range of $P = 0.049$–0.011). Yet,

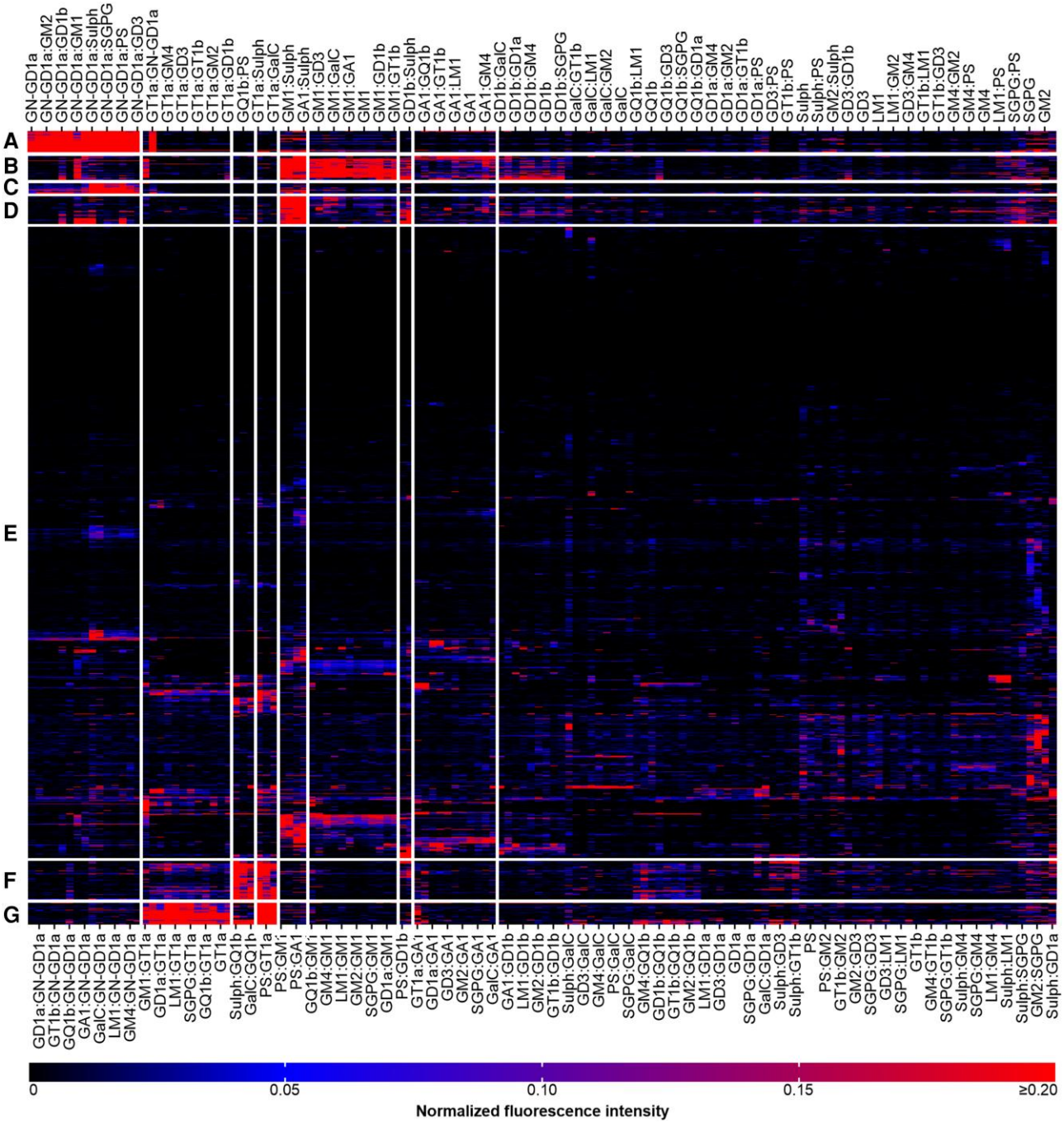

Figure 3 Heat map depicting patient clusters with particular IgG anti-glycolipid antibody reactivity patterns derived from unsupervised hierarchical clustering of anti-glycolipid antibodies in patients with Guillain-Barré syndrome. Patients were clustered on the *y*-axis (**A–G**) and anti-glycolipid antibodies were clustered on the *x*-axis. The clusters are separated by white lines. Each patient cluster is characterized by a distinct antibody reactivity pattern: (Cluster A) broad-ranging GalNAc-GD1a reactivity; (Cluster B) restricted GA1 and broad-ranging GM1 reactivity; (Cluster C) restricted GalNAc-GD1a reactivity; (Cluster D) restricted GA1, GD1b and GM1 reactivity; (Cluster E) non-specific; (Cluster F) restricted GQ1b and GT1a reactivity; and (Cluster G) broad-ranging GT1a reactivity. GalC = galactocerebroside; GN-GD1a = *N*-acetylgalactosaminyl GD1a; PS = phosphatidylserine; SGPG = sulfated glucuronyl paragloboside; Sulf = sulfatide.

none of these associations remained after adjusting for known prognostic factors, mainly due to the prognostic value of the MRC sum score at entry. Across patient clusters acquired from hierarchical clustering, the time required to regain the ability to walk 10 m unaided was different (Fig. 5D).

A subset of AGAb was associated with the inability to walk 10 m unaided at 4 and 26 weeks in the complete dataset [at 4 weeks ($n = 4$): IgG antibodies against GQ1b:PS, GQ1b:Sulfatide, GQ1b:GalC and GQ1b:GM4; at 26 weeks ($n = 17$, top four): IgG antibodies against GM1:GalC, GM1:Sulfatide, GM1 and GM1:SGPG]. Replication of these analyses in the derivation and validation datasets provided similar results in the derivation dataset but not in the validation dataset. Addition of 110 AGAb to the mEGOS at Week 1 predicting the inability to walk 10 m unaided at 26 weeks increased the AUC of the

Table 3 Clinical features of patient clusters derived from unsupervised hierarchical clustering of anti-glycolipid antibody fluorescence intensities in patients with Guillain-Barré syndrome

Variable	Cluster A ($n = 38$) GalNAc-GD1a (b)	Cluster B ($n = 43$) GM1 (b), GA1 (r)	Cluster C ($n = 18$) GalNAc-GD1a (r)	Cluster D ($n = 50$) GM1, GA1, GD1b (r)	Cluster E ($n = 1155$) Non-specific	Cluster F ($n = 70$) GQ1b, GT1a (r)	Cluster G ($n = 39$) GT1a (b)
Geographical region							
Europe	21 (55.3)	19 (44.2)	9 (50.0)	**33 (66.0)**[g]	**713 (61.7)**[g]	34 (48.6)	12 (30.8)
Americas	5 (13.2)	7 (16.3)	1 (5.6)	4 (8.0)	193 (16.7)	**21 (30.0)**[d,e]	**13 (33.3)**[d]
Africa	2 (5.3)	0 (0)	0 (0)	2 (4.0)	20 (1.7)	1 (1.4)	**5 (12.8)**[e]
Asia without Bangladesh	3 (7.9)	3 (7.0)	2 (11.1)	4 (8.0)	75 (6.5)	**14 (20.0)**[e]	3 (7.7)
Bangladesh	7 (18.4)	**14 (32.6)**[e]	6 (33.3)	7 (14.0)	154 (13.3)	0 (0)	6 (15.4)
Clinical variant							
Motorsensory	14 (36.8)	**22 (51.2)**[f]	2 (11.1)	19 (38.0)	**738 (63.9)**[a,c,d,f,g]	12 (17.1)	9 (23.1)
Motor	**24 (63.2)**[e]	**18 (41.9)**[e]	**15 (83.3)**[b,e]	**25 (50.0)**[e]	212 (18.4)	0 (0)	**17 (43.6)**[e]
Miller Fisher syndrome[h]	0 (0)	2 (4.7)	1 (5.6)	2 (4.0)	85 (7.4)	**53 (75.7)**[b,c,d,e,g]	**10 (25.6)**[e]
Preceding infection							
Campylobacter jejuni	**34/38 (89.5)**[b,c,d,e,f]	**26 (60.5)**[e,f]	**11 (61.1)**[e,f]	20 (40.0)	295/1153 (25.6)	18 (25.7)	**29 (74.4)**[d,e,f]
Mycoplasma pneumoniae	4/38 (10.5)	6 (14.0)	1 (5.6)	6 (12.0)	111/1153 (9.6)	5 (7.1)	8 (20.5)
Cytomegalovirus	0 (0)	0 (0)	0 (0)	0 (0)	53/1150 (4.6)	1 (1.4)	0 (0)
Hepatitis E virus	0 (0)	0 (0)	0 (0)	2 (4.0)	29/1153 (2.5)	0 (0)	1 (2.6)
Epstein-Barr virus	1/37 (2.6)	0 (0)	0 (0)	0 (0)	10/1151 (0.9)	0 (0)	1 (2.6)
Cranial nerve palsy at study entry							
Facial	2 (5.3)	4 (9.3)	5 (27.8)	10 (20.0)	**350/1143 (30.6)**[a,b]	19 (27.1)	8 (20.5)
Bulbar	2 (5.3)	6 (14.0)	3 (16.7)	7 (14.0)	275/1143 (24.1)	**28 (40.0)**[a,b,d,e]	**16 (41.0)**[a,d]
Oculomotor	0 (0)	5 (11.6)	1 (5.6)	6 (12.0)	115/1143 (10.1)	**55 (78.6)**[b,c,d,e,g]	**14 (35.9)**[e]
Electrophysiological subtype							
Normal	0 (0)	1/28 (3.6)	0 (0)	0 (0)	41/749 (5.5)	**16/53 (30.2)**[e]	1/20 (5.0)
Demyelinating	**17/32 (53.1)**[f]	8/28 (28.6)	6/17 (35.3)	**30/46 (65.2)**[b,f]	**452/749 (60.3)**[b,f]	10/53 (18.9)	**13/20 (65.0)**[f]
Axonal	**9/32 (28.1)**[e,f]	**11/28 (39.3)**[e,f]	3/17 (17.6)	7/46 (15.2)	59/749 (7.9)	2/53 (3.8)	1/20 (5.0)
Inexcitable	2/32 (6.3)	2/28 (7.1)	2/17 (11.8)	1/46 (2.2)	22/749 (2.9)	0 (0)	1/20 (5.0)
Equivocal	4/32 (12.5)	6/28 (21.4)	6/17 (35.3)	8/46 (17.4)	175/749 (23.4)	**25/53 (47.2)**[a,d,e]	4/20 (20.0)
Disease course							
Mechanical ventilation	1 (2.6)	6 (14.0)	4 (22.2)	9 (18.0)	210 (18.2)	9 (12.9)	7 (17.9)
Disability score ≥3 at nadir	29/37 (78.4)	33/38 (86.8)	15 (83.3)	39/49 (79.6)	871/1095 (79.5)	48/67 (71.6)	36/38 (94.7)
Disability score ≥3 at 26 weeks	**10/35 (28.6)**[f]	**12/34 (35.3)**[f]	3/16 (18.8)	10/40 (25.0)	161/894 (18.0)	2/52 (3.8)	6/33 (18.2)

Data are presented as count (%). Each cluster was compared to other clusters in logistic regression analyses. Significant differences between two clusters are indicated (in bold) for one of two clusters. Data to which logistic regression analyses were not applicable (due to all patients being in the same group) are presented in italics. (b) = broad-ranging reactivity; (r) = restricted reactivity; GalNAc-GD1a = *N*-acetylgalactosaminyl GD1a.

[a-g]Differs from Cluster A, B, C, D, E, F or G, respectively.

[h]Including overlap with Guillain-Barré syndrome.

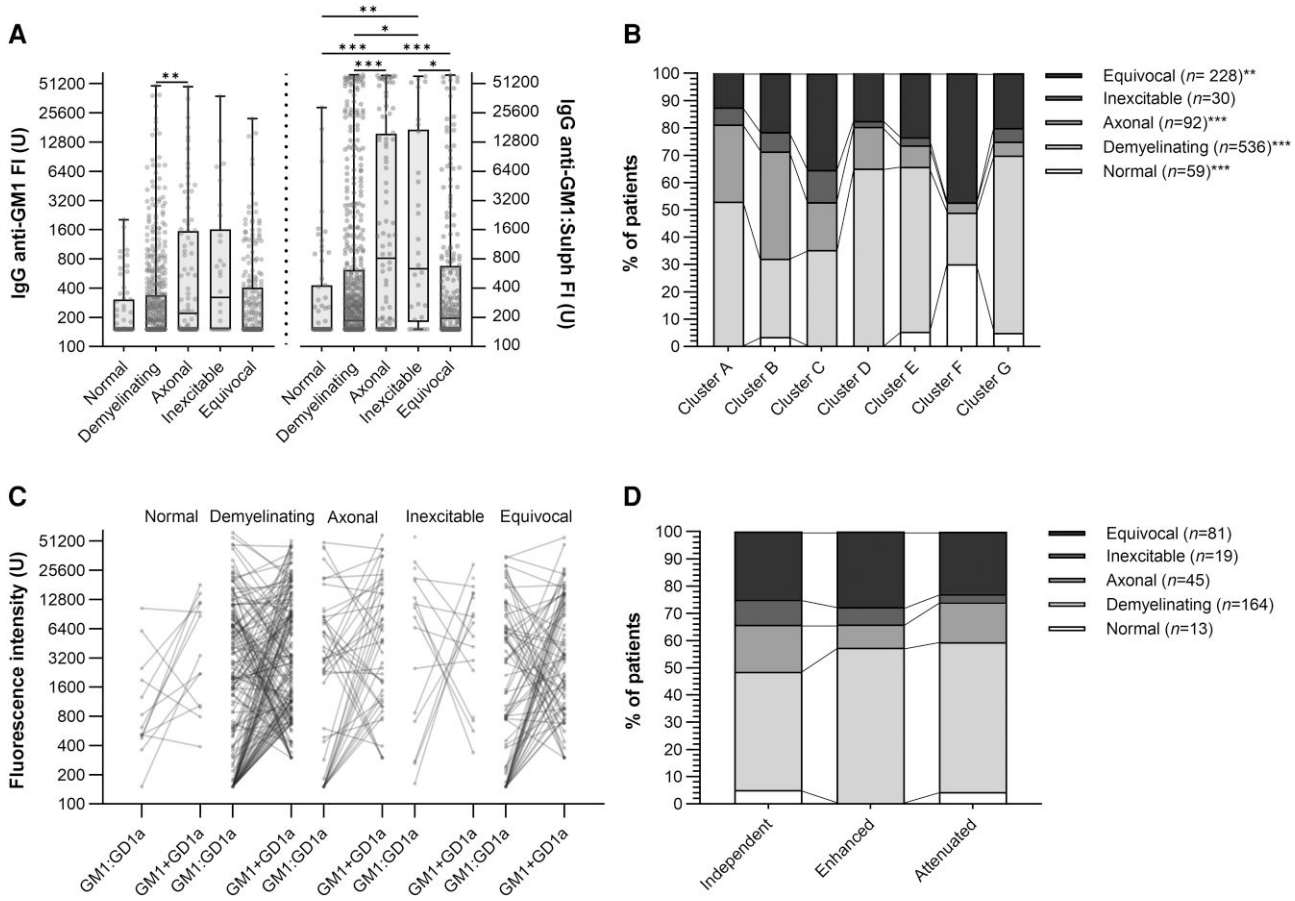

Figure 4 Dot plots and stacked bar plots illustrating the associations between anti-glycolipid antibodies (reactivity patterns) and electrophysiological subtypes in Guillain-Barré syndrome. (**A**) Box plots with individual anti-GM1 (*left*) and anti-GM1:Sulfatide (*right*) fluorescence intensities across electrophysiological subtypes. (**B**) Stacked bar plot depicting the distribution of electrophysiological subtypes across patient clusters based on anti-glycolipid antibody reactivity patterns. (**C**) Dot plot illustrating the interaction of GM1 with GD1a in patients, by comparing the sum of fluorescence intensities of anti-GM1 and anti-GD1a (anti-GM1 + anti-GD1a) with the fluorescence intensity of the anti-complex antibody anti-GM1:GD1a per individual patient. Each line connects the fluorescence intensity of anti-GM1 + anti-GD1a to the fluorescence intensity of anti-GM1:GD1a of one patient. Groups are based on electrophysiological subtypes. (**D**) Stacked bar plot showing the distribution of electrophysiological subtypes across three groups based on the interaction of GM1 with GD1a (complex independent, enhanced or attenuated). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. FI = fluorescence intensity; Sulf = sulfatide; U = units.

model in the derivation, validation and complete datasets [highest ΔAUC in the complete dataset = 0.01 (0.83–0.84; IgA anti-GM2:PS]. Similarly, addition of 199 AGAb to the mEGOS at study entry and 285 AGAb to the EGOS increased their AUC for the same outcome in all three datasets [mEGOS at study entry: highest ΔAUC in the complete dataset = 0.01 (0.78–0.79; IgG anti-GQ1b:PS), EGOS: highest ΔAUC in the complete dataset = 0.02 (0.87–0.89; IgG anti-GM1:GalC)]. However, these increases were insufficient to improve the predictive value.

Discussion

In this study, we determined an extensive repertoire of AGAb on glyco-array in a large prospective cohort of patients with GBS and related these to the diagnosis, clinical variants, electrophysiological subtypes, clinical course and outcome. We found that several antibodies against glycolipid complexes were able to distinguish motor GBS and MFS from controls more accurately than antibodies to single glycolipids. Moreover, combining multiple AGAb further improved their discriminative capacity, outperforming AGAb currently tested in clinical practice. Notably, we identified seven particular AGAb reactivity patterns with broad or restricted reactivities and distinct clinical phenotypes, of which two had specific anti-GM1 (complex) reactivity. Anti-GM1 (complex) antibodies were distributed amongst patients with all electrophysiological subtypes. Positivity of a subset of AGAb was associated with the clinical course and outcome, and the addition of several AGAb slightly improved the predictive value of current clinical prognostic models.

Several previous studies with comparable methodology have reported similar findings.[16,23,24] In these studies, glyco-arrays with varying AGAb panels, also including antibodies to glycolipid complexes, were employed to assess the occurrence of AGAb in patients with GBS. A subset of the tested AGAb was associated with GBS. Although the exact AGAb for which these associations were found differed between studies and from our study, the trend in specific glycolipids within glycolipid complexes was reproducible. For example, there was a consistently observed association between the type of preceding infection (with *C. jejuni*, *M. pneumoniae* and CMV) and AGAb (to GM1/GQ1b, GalC and GM2, respectively). Moreover, several clinical associations of AGAb subsets were

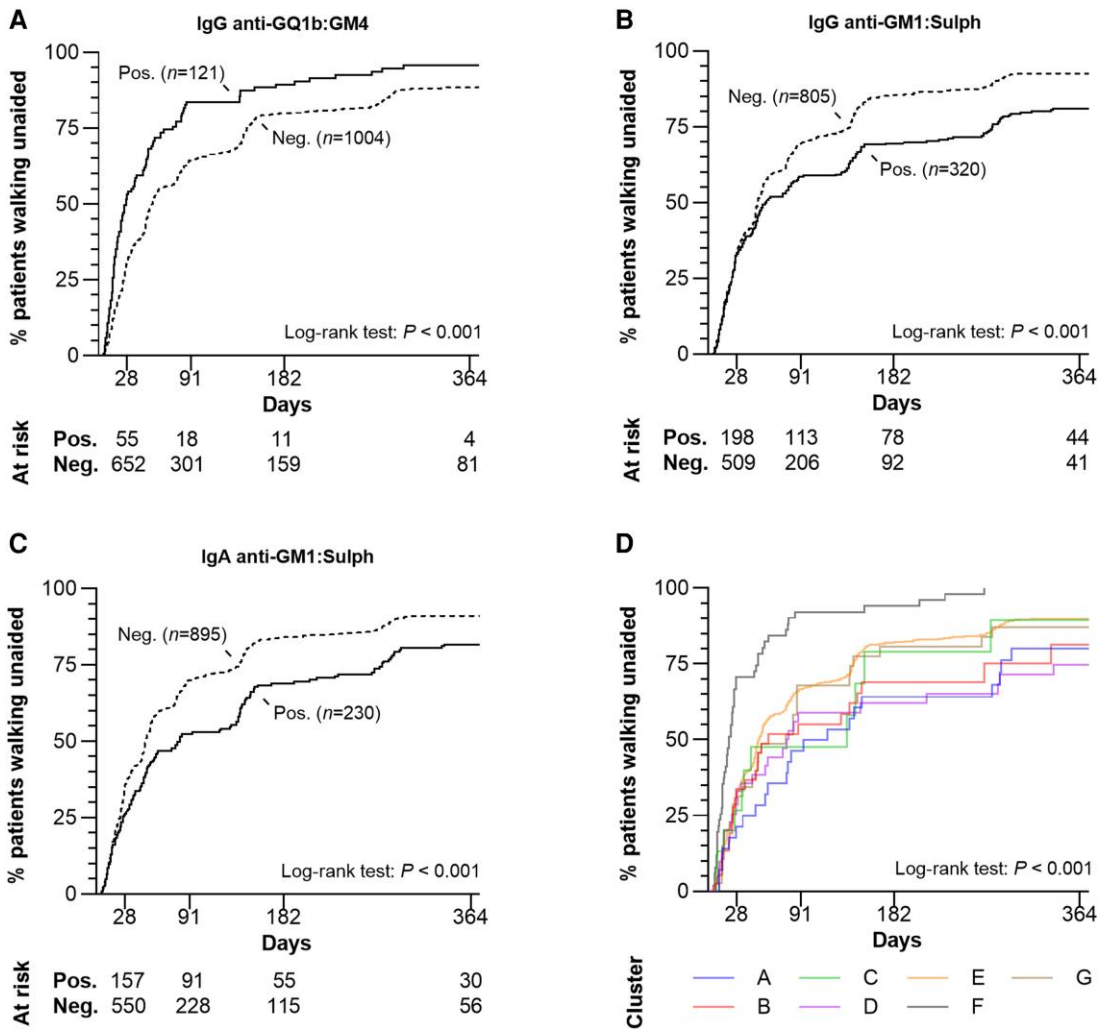

Figure 5 Cumulative incidence curves for the time to regain the ability to walk unaided in relation to anti-glycolipid antibody reactivity. Cumulative incidence curves are shown for IgG anti-GQ1b:GM4 (**A**), IgG anti-GM1:Sulfatide (**B**), IgA anti-GM1:Sulfatide (**C**) and patient clusters based on anti-glycolipid antibody reactivity patterns (**D**). Neg. = negative; Pos. = positive; Sulf = sulfatide.

described previously, which were similar to the current findings. In our study, we were able to test sera from a large, diverse, prospective, clinically well-defined cohort of patients for an AGAb panel including additional gangliosides and other glycolipids for IgG, IgM and IgA. As a result of this increased statistical power, previous findings could be confirmed and additional analyses could be performed.

Taken together, our study and previous studies provide evidence that not only one or a limited set of antibody specificities may play a role in the pathophysiology of GBS variants, but rather reactivity to a large number of antibody specificities together (including glycolipid complexes). Notably, antibodies to glycolipid complexes often had stronger clinical associations than antibodies to single glycolipids and may thus play an important role in the pathophysiology. Sulfatide and PS repeatedly appeared to enhance complex reactivity most potently, which may result from an inherently high ability to modify the accessibility or conformation of epitopes or from their anatomical distribution alongside gangliosides.[3,8,9]

Despite the identification of clear antibody reactivity patterns, some overlap in antibody reactivity was present across patient clusters. This could be a limitation of the applied clustering method. Since a limited number of antibody reactivity clusters were created, the algorithm may have preferentially clustered some AGAb over others. Though further clustering into a higher number of clusters may have provided even more specific clusters, this would have reduced statistical power and would have introduced clinically irrelevant clusters. Alternatively, the overlap in antibody reactivities could indicate that multiple clones of antibodies may be involved in the pathophysiology of GBS, but further research into this hypothesis is required.

The variety of AGAb specificities found in the current study may challenge their role in the pathogenesis of GBS, since they could reflect an epiphenomenon resulting from nerve damage or generally increased immune activity following a preceding infection.[3] An alternative viewpoint for the pathogenesis of GBS is that neuronal damage is a consequence of endoneurial ischemia resulting from inflammatory oedema in nerve trunks with epi-perineurium.[25] However, the numerous clinical associations of AGAb in GBS in the current and previous studies substantiate existing evidence for the pathogenicity of AGAb in GBS. Extensive studies into the pathogenicity of these antibodies have been performed in recent decades, using *in vitro*, *ex vivo* and *in vivo* animal models and human studies.[3] Several studies have looked into the pathogenicity of

anti-GM1 antibodies, showing that these antibodies cause GBS-like syndromes in rabbits and mice.[26-30] Other antibodies that have been shown to induce symptoms similar to GBS in animal models include anti-GD1a, anti-GalC, anti-GD1b and anti-GQ1b antibodies.[31-37] Although some studies have looked into anti-complex antibodies, the pathogenicity of these antibodies remains to be further studied in animal models.[3] Importantly, it should not be assumed that any or all of the described glycolipid complexes exist in vivo. Rather, the molecular shapes of glycolipids that allow for antibody binding can be manipulated in a wide variety of ways by cooperative lipids.[38,39] The biophysical basis for this phenomenon in living neural membranes has not been studied in detail. All evidence considered, at least for a subset of AGAb there is strong evidence that they are pathogenic.

Due to the focus of pathogenicity studies on IgG antibodies, it remains unclear whether IgM and IgA antibodies could be pathogenic in GBS. Interestingly, we found that patients with GBS had lower fluorescence intensities of multiple IgM antibodies than controls and that fluorescence intensities of several IgM and IgA antibodies were associated with clinical features in this study. The lower IgM fluorescence intensities in patients with GBS could be explained by the nature of included controls, since a natural occurrence of IgM AGAb has been described in healthy adults and IgM AGAb have been shown to be elevated in other neurological diseases such as multifocal motor neuropathy.[40-42] Alternatively, IgM could be downregulated in patients with GBS due to the relative upregulation of IgG or could be consumed or cleared from the circulation following antigen binding, but this remains to be further investigated. Although research on the role of IgA antibodies in GBS remains scarce, our study and several other studies provide evidence for a role of this isotype in the pathophysiology.[5,43,44] These IgA clinical associations may be specifically related to preceding (gastro-intestinal) infections.

Despite the presence of patient clusters with specific antibody reactivity patterns, the majority of patients in this study clustered in a cluster without characterizing antibody reactivity (Cluster E-a). Patients in this cluster were predominantly included from Europe, frequently had motorsensory and demyelinating GBS and had a low frequency of preceding infections. Relatively low frequencies of specific triggers or host factors for certain antibody reactivity patterns in some (European) regions may explain the absence of these patterns in this group of patients. Moreover, the pathophysiology of GBS in these patients may differ from patients with antibody reactivity patterns. Other anti-glycolipid antibodies or antibodies against other types of targets, that were not included in the antibody panel that we tested for this study may be involved. Alternatively, antibodies that may play a role in the pathophysiology of these patients could potentially be better detected with different ratios of complexes, e.g. 1:2 (volume:volume) or an increased number of glycolipids in the complex, e.g. three glycolipids. On the other hand, these patients may have a more T-cell driven pathophysiology instead of one driven by pathogenic antibodies.[45] Further studies are required to elucidate the pathophysiological mechanisms occurring in this group of patients with GBS.

Altogether, we describe several findings that could potentially improve diagnostics, prognostics and treatment strategies for patients with GBS. First, AGAb may be useful in patients with an atypical clinical presentation or differential diagnoses. Several antibodies to single gangliosides are already being tested in these cases.[18] However, their sensitivity and specificity are limited, and antibodies to glycolipid complexes could have a higher diagnostic value according to our findings. Second, our findings challenge the historical concept that anti-GM1 (complex) antibodies predominantly cause axonal GBS.[3] We found that these antibodies occur in all electrophysiological subtypes with a broad range of fluorescence intensities and that the proportion of patients with the axonal subtype differed across antibody reactivity patterns [including two anti-GM1 (complex) reactivity patterns]. These findings could be explained by the differential anatomical distribution of different GM1 complexes on the axon and myelin or by differences in disease severity across electrophysiological subtypes.[3,8,9] Third, AGAb may potentially be useful in improving outcome prediction in patients with GBS, alongside or in combination with current clinical prognostic models.[20,21] In our study, AGAb only slightly increased the AUC of current prognostic models ((m)EGOS), which may be related to their associations with incorporated clinical features. Their predictive potential could be further explored using other methods (such as machine learning), by addition of multiple AGAb or by combining AGAb with other clinical features. Fourth, AGAb reactivity patterns may reflect endemics of microbes that are able to elicit the production of cross-reactive antibodies and subsequently cause GBS, as we described for the relatively high proportion of Argentinian patients with a preceding *C. jejuni* infection in patient Cluster G (broad GT1a reactivity). Lastly, determining AGAb reactivity patterns could potentially help identify patients who may benefit from additional or alternative treatments.

Future implementation of antibody testing for the AGAb that we found to be clinically relevant into clinical practice could be feasible, though additional studies are required. For diagnostic purposes, a set of antibodies could be confined to nine AGAb to distinguish GBS, motor GBS and MFS from controls (GD1b, GQ1b, SGPG, GM1:GD1a, GA1:LM1, GA1:Sulf, GalC:LM1, GQ1b:GA1 and GQ1b:GalC). Alternatively, addition of sulfatide or PS to gangliosides currently used in clinical practice, such as GM1, could already improve their diagnostic value. In addition, some of these nine and several other AGAb, as well as AGAb reactivity patterns, could potentially be used for the other described purposes, such as improving prognostics and treatment strategies. Testing these antibodies on glyco-array could be feasible, although antibody detection by ELISA may be more accessible for clinical practice, as the vast majority of laboratories are conversant with this method. Generally, results from both methods correlate, although in a small number of cases a different result could be obtained. Validation of our findings with ELISA would therefore be required prior to implementation into clinical routines.

Our study had several limitations. Firstly, missing clinical data and serum samples in subsets of patients and controls may have led to some selection bias and to limited statistical power. Likewise, the use of Week 1 samples if study entry samples were not available may also have introduced some bias. However, in preliminary subgroup analyses, AGAb fluorescence intensities did not differ between the sets of samples from study entry versus Week 1 and were only higher for IgG anti-GM2 and IgG anti-Sulfatide in posttreatment versus pretreatment samples. Secondly, the applicability of our control cohorts in diagnostics was limited. Patients with diseases that specifically mimic GBS variants would be preferred controls for diagnostic analyses over healthy, family, vaccination and other neurological disease controls. Moreover, including IgM and IgA AGAb from control cohorts with sufficient statistical power could provide further possibilities to improve diagnostic models. Thirdly, a high proportion of patients with GBS, in particular those with viral preceding infections, have no detectable AGAb. These patients may have an alternative immunological mechanism.[45] Likewise, functional characteristics of antibodies that could affect the found associations, such as affinity, subclass and the ability to elicit complement activation, were not studied.

In conclusion, combinatorial array has added value over single array in diagnostics, enabled the identification of AGAb reactivity patterns with distinct clinical phenotypes and may have added value in prognostics. Importantly, anti-GM1 (complex) antibodies occur in patients with any electrophysiological subtype, despite their particular association with axonal pathology. Further studies are required to validate these finding externally.

Data availability

Data of patients included in IGOS will be used for future studies and may be made available on reasonable request after consulting the IGOS Steering Committee. Raw AGAb fluorescence intensity unit data may be made available on reasonable request through the IGOS website (https://www.igosresearch.com/).

Acknowledgements

We thank all members of the IGOS consortium for their support and work in the inclusion and follow-up of patients with GBS. Several authors of this publication are members of the Netherlands Neuromuscular Center (NL-NMD) and the European Reference Network for rare neuromuscular diseases (EURO-NMD).

Funding

IGOS is financially supported by the GBS/CIDP Foundation International, GAIN, Erasmus Medical Center, Rotterdam; University of Glasgow, CSL Behring, Grifols, Annexon and Hansa Biopharma. H.J.W. was supported by Wellcome Trust (092805 and 202789), GAIN and the Chandra Mehta Foundation.

Competing interests

V.G. is currently an employee of Biohaven Pharmaceuticals. RDMH received honoraria from Takeda, CSL Behring, ArgenX, and Dianthus Therapeutics. J.K.L.H. has served on advisory boards and received support to attend conferences from CSL Behring and Takeda outside the submitted work. Su.Ku. received honoraria from CSL Behring, Japan Blood Product Organization, Takeda Pharmaceuticals, and KMBiologics; served on the data and safety monitoring board for ArgenX. Sa.Ku. received honoraria from CSL Behring, ArgenX, and Takeda Pharmaceuticals outside the submitted work. M.K. received speaker honoraria from CSL Behring, Japan Blood Product Organization, and Takeda Pharmaceuticals. L.Q. received speaker or expert testimony honoraria from CSL Behring, Novartis, Sanofi-Genzyme, Merck, Annexon, Alnylam, Janssen, ArgenX, UCB, Dianthus Therapeutics, LFB, Avilar Therapeutics, Nuvig Therapeutics, Takeda, and Roche; was supported by Instituto de Salud Carlos III—Ministry of Economy and Innovation (Spain), CIBERER, Fundació La Marató, GBS-CIDP Foundation International, UCB, ArgenX, and Grifols; serves at Clinical Trial Steering Committees for Sanofi Genzyme, Takeda, and ArgenX and was Principal Investigator for UCB's CIDP01 trial. P.R. served on advisory boards for UCB, ArgenX, Biogen, Alexion, and Roche outside the submitted work. KAS was supported by Grifols (Grifols Investigator-Sponsored Research, 31 August 2015-30 August 2017). R.H. was supported by GBS-CIDP Foundation International and the T2B collaboration project funded by PPP Allowance made available by Top Sector Life Sciences & Health to Samenwerkende Gezondheidsfondsen (SGF) under project number LSHM18055-SGF to stimulate public-private partnerships and co-financing by health foundations that are part of the SGF.Health~Holland. The remaining authors report no competing interests.

Supplementary material

Supplementary material is available at *Brain* online.

Appendix 1

Members of the IGOS consortium

J. M. Addington, S. Ajroud-Driss, H. Andersen, G. Antonini, S. Arends, S. Attarian, U. A. Badrising, C. Balducci, F. A. Barroso, K. Bateman, I. R. Bella, L. Benedetti, B. van den Berg, P. Y. K. van den Bergh, T. E. Bertorini, R. Bhavaraju-Sanka, F. M. Bozzano, T. H. Brannagan, C. Briani, J. Bürmann, M. Busby, S. Butterworth, G. Capodivento, C. Casasnovas, G. Cavaletti, C. C. Chao, S. Chen, E. Cisneros, K. G. Claeys, M. E. Conti, D. R. Cornblath, J. S. Cosgrove, M. C. Dalakas, P. van Damme, E. Dardiotis, A. Davidson, G. W. van Dijk, M. M. Dimachkie, A. Y. Doets, P. A. van Doorn, A. Echaniz-Laguna, F. Eftimov, C. G. Faber, R. Fazio, T. E. Feasby, J. Fehmi, J. Fernández-Travieso, C. Fokke, T. Fujioka, E. A. Fulgenzi, G. Galassi, T. Garcia-Sobrino, M. P. J. Garssen, C. Giannotta, C. J. Gijsbers, J. M. Gilchrist, H. J. Gilhuis, J. M. Goldstein, K. C. Gorson, N. A. Goyal, V. Granit, A. M. Grapperon, G. Guttiérrez-Guttiérrez, L. Gutman, R. D. M. Hadden, T. Harbo, H. P. Hartung, S. Hayat, R. A. Hendriks, Jakob V. Holbech, J. K. L. Holt, S. T. Hsieh, M. Htut, R. A. C. Hughes, R. Huizinga, A. M. Humm, T. Hundsberger, B. Islam, Z. Islam, B. C. Jacobs, I. Jahan, K. Jellema, I. Jericó Pascual, K. Kaida, S. Karafiath, H. D. Katzberg, H. Kerkhoff, M. A. Khoshnoodi, L. Kiers, N. Kokubun, N. A. Kolb, L. C. de Koning, R. van Koningsveld, A. J. van der Kooi, J. C. H. M. Kramers, K. Kuitwaard, T. Kuntzer, S. Kusunoki, S. Kuwabara, J. Y. Kwan, S. S. Ladha, L. Landschoff Lassen, A. M. Lascano, V. Lawson, H. C. Lehmann, S. E. Leonhard, C. Lleixa-Rodriguez, L. W. G. Luijten, M. P. T. Lunn, A. Magot, H. Manji, C. Marchesoni, G. A. Marfia, C. Márquez Infante, L. Martín-Aguilar, E. Martinez Hernandez, G. Mataluni, M. Mattiazi, C. J. McDermott, G. D. Meekins, J. A. L. Miller, Q. D. Mohammad, M. S. Monges, M. Morales de la Prida, G. Morís de la Tassa, P. Nair, C. Nascimbene, L. Nobbio, E. Nobile-Orazio, R. J. Nowak, M. Osei-Bonsu, J. Pardo, F. Pelouto, Y. Péréon, M. T. Pulley, L. Querol, S. W. Reddel, T. van der Ree, R. C. Reisin, S. Rinaldi, P. Ripellino, R. C. Roberts, I. Rojas-Marcos, J. Roodbol, S. A. Rudnicki, G. M. Sachs, J. P. A. Samijn, L. Santoro, A. Savransky, O. Scheidegger, A. Schenone, L. Schwindling, M. J. Sedano Tous, N. Shahrizaila, K. A. Sheikh, N. J. Silvestri, S. H. Sindrup, V. Siokas, C. L. Sommer, B. Stein, A. M. Stino, T. Suichi, H. Tankisi, R. C. M. Thomma, P. Tsouni, P. Twydell, J. D. Varrato, J. C. Verboon, C. Verhamme, F. H. Vermeij, J. Verschuuren, L. H. Visser, M. V. Vytopil, W. Waheed, C. Walgaard, Y. Z. Wang, E. J. A. Wiegers, H. J. Willison, P. W. Wirtz, M. van Woerkom, Y. Yamagishi, K. Yoshikawa, L. L. Zhang, L. Zhou and S. A. Zivkovic.

References

1. Shahrizaila N, Lehmann HC, Kuwabara S. Guillain-Barré syndrome. *Lancet*. 2021;397:1214-1228.

2. Leonhard SE, Mandarakas MR, Gondim FAA, et al. Diagnosis and management of Guillain-Barré syndrome in ten steps. *Nat Rev Neurol*. 2019;15:671-683.
3. Kusunoki S, Willison HJ, Jacobs BC. Antiglycolipid antibodies in Guillain-Barré and Fisher syndromes: Discovery, current status and future perspective. *J Neurol Neurosurg Psychiatry*. 2021;92: 311-318.
4. Leonhard SE, van der Eijk AA, Andersen H, et al. An international perspective on preceding infections in Guillain-Barré syndrome: The IGOS-1000 cohort. *Neurology*. 2022;99:e1299-e1313.
5. Laman JD, Huizinga R, Boons GJ, Jacobs BC. Guillain-Barré syndrome: Expanding the concept of molecular mimicry. *Trends Immunol*. 2022;43:296-308.
6. Cutillo G, Saariaho AH, Meri S. Physiology of gangliosides and the role of antiganglioside antibodies in human diseases. *Cell Mol Immunol*. 2020;17:313-322.
7. Ilyas AA, Willison HJ, Quarles RH, et al. Serum antibodies to gangliosides in Guillain-Barré syndrome. *Ann Neurol*. 1988;23: 440-447.
8. Kaida K, Morita D, Kanzaki M, et al. Ganglioside complexes as new target antigens in Guillain-Barré syndrome. *Ann Neurol*. 2004;56:567-571.
9. Kusunoki S, Kaida K, Ueda M. Antibodies against gangliosides and ganglioside complexes in Guillain-Barré syndrome: New aspects of research. *Biochim Biophys Acta*. 2008;1780:441-444.
10. Halstead SK, Gourlay D, Willison HJ. Detection of autoantibodies using combinatorial glycolipid microarrays. *Methods Mol Biol*. 2022;2460:183-191.
11. Doets AY, Verboon C, van den Berg B, et al. Regional variation of Guillain-Barré syndrome. *Brain*. 2018;141:2866-2877.
12. Jacobs BC, van den Berg B, Verboon C, et al. International Guillain-Barré syndrome outcome study: Protocol of a prospective observational cohort study on clinical and biological predictors of disease course and outcome in Guillain-Barré syndrome. *J Peripher Nerv Syst*. 2017;22:68-76.
13. Arends S, Drenthen J, van den Bergh P, et al. Electrodiagnosis of Guillain-Barré syndrome in the international GBS outcome study: Differences in methods and reference values. *Clin Neurophysiol*. 2022;138:231-240.
14. Hadden RD, Cornblath DR, Hughes RA, et al. Electrophysiological classification of Guillain-Barré syndrome: Clinical associations and outcome. Plasma exchange/sandoglobulin Guillain-Barré syndrome trial group. *Ann Neurol*. 1998;44: 780-788.
15. Davies AJ, Lleixà C, Siles AM, et al. Guillain-Barré syndrome following Zika virus infection is associated with a diverse spectrum of peripheral nerve reactive antibodies. *Neurol Neuroimmunol Neuroinflamm*. 2022;10:e200047.
16. Halstead SK, Kalna G, Islam MB, et al. Microarray screening of Guillain-Barré syndrome sera for antibodies to glycolipid complexes. *Neurol Neuroimmunol Neuroinflamm*. 2016;3:e284.
17. Jones CM, Athanasiou T. Summary receiver operating characteristic curve analysis techniques in the evaluation of diagnostic tests. *Ann Thorac Surg*. 2005;79:16-20.
18. van Doorn PA, Van den Bergh PYK, Hadden RDM, et al. European academy of neurology/peripheral nerve society guideline on diagnosis and treatment of Guillain-Barré syndrome. *Eur J Neurol*. 2023;30:3646-3674.
19. Charrad M, Ghazzali N, Boiteau V, Niknafs A. Nbclust: An R package for determining the relevant number of clusters in a data set. *J Stat Softw*. 2014;61:1-36.
20. van Koningsveld R, Steyerberg EW, Hughes RAC, Swan AV, van Doorn PA, Jacobs BC. A clinical prognostic scoring system for Guillain-Barré syndrome. *Lancet Neurol*. 2007;6:589-594.
21. Walgaard C, Lingsma HF, Ruts L, van Doorn PA, Steyerberg EW, Jacobs BC. Early recognition of poor prognosis in Guillain-Barré syndrome. *Neurology*. 2011;76:968-975.
22. Doets AY, Lingsma HF, Walgaard C, et al. Predicting outcome in Guillain-Barré syndrome: International validation of the modified erasmus GBS outcome score. *Neurology*. 2022;98: e518-e532.
23. Morikawa M, Kuwahara M, Ueno R, Samukawa M, Hamada Y, Kusunoki S. Serological study using glycoarray for detecting antibodies to glycolipids and glycolipid complexes in immune-mediated neuropathies. *J Neuroimmunol*. 2016;301:35-40.
24. Rinaldi S, Brennan KM, Kalna G, et al. Antibodies to heteromeric glycolipid complexes in Guillain-Barré syndrome. *PLoS One*. 2013;8:e82337.
25. Berciano J. The pathophysiological role of endoneurial inflammatory edema in early classical Guillain-Barré syndrome. *Clin Neurol Neurosurg*. 2024;237:108131.
26. Lopez PH, Zhang G, Zhang J, et al. Passive transfer of IgG anti-GM1 antibodies impairs peripheral nerve repair. *J Neurosci*. 2010;30:9533-9541.
27. Yuki N, Susuki K, Koga M, et al. Carbohydrate mimicry between human ganglioside GM1 and Campylobacter jejuni lipooligosaccharide causes Guillain-Barré syndrome. *Proc Natl Acad Sci U S A*. 2004;101:11404-11409.
28. Yuki N, Yamada M, Koga M, et al. Animal model of axonal Guillain-Barré syndrome induced by sensitization with GM1 ganglioside. *Ann Neurol*. 2001;49:712-720.
29. Sheikh KA, Zhang G, Gong Y, Schnaar RL, Griffin JW. An anti-ganglioside antibody-secreting hybridoma induces neuropathy in mice. *Ann Neurol*. 2004;56:228-239.
30. Susuki K, Rasband MN, Tohyama K, et al. Anti-GM1 antibodies cause complement-mediated disruption of sodium channel clusters in peripheral motor nerve fibers. *J Neurosci*. 2007;27: 3956-3967.
31. Kusunoki S, Chiba A, Hitoshi S, Takizawa H, Kanazawa I. Anti-Gal-C antibody in autoimmune neuropathies subsequent to mycoplasma infection. *Muscle Nerve*. 1995;18:409-413.
32. Nagai Y, Momoi T, Saito M, Mitsuzawa E, Ohtani S. Ganglioside syndrome, a new autoimmune neurologic disorder, experimentally induced with brain gangliosides. *Neurosci Lett*. 1976;2: 107-111.
33. Saida T, Saida K, Dorfman SH, et al. Experimental allergic neuritis induced by sensitization with galactocerebroside. *Science*. 1979;204:1103-1106.
34. Kusunoki S, Shimizu J, Chiba A, Ugawa Y, Hitoshi S, Kanazawa I. Experimental sensory neuropathy induced by sensitization with ganglioside GD1b. *Ann Neurol*. 1996;39:424-431.
35. Plomp JJ, Molenaar PC, O'Hanlon GM, et al. Miller Fisher anti-GQ1b antibodies: Alpha-latrotoxin-like effects on motor end plates. *Ann Neurol*. 1999;45:189-199.
36. Halstead SK, Zitman FMP, Humphreys PD, et al. Eculizumab prevents anti-ganglioside antibody-mediated neuropathy in a murine model. *Brain*. 2008;131(Pt 5):1197-1208.
37. Takada K, Shimizu J, Kusunoki S. Apoptosis of primary sensory neurons in GD1b-induced sensory ataxic neuropathy. *Exp Neurol*. 2008;209:279-283.
38. Greenshields KN, Halstead SK, Zitman FMP, et al. The neuropathic potential of anti-GM1 autoantibodies is regulated by the local glycolipid environment in mice. *J Clin Invest*. 2009; 119:595-610.
39. Zitman FMP, Greenshields KN, Kuijf ML, et al. Neuropathophysiological potential of Guillain-Barré syndrome anti-ganglioside-complex antibodies at mouse motor nerve terminals. *Clin Exp Neuroimmunol*. 2011;2:59-67.

40. Mizutamari RK, Wiegandt H, Nores GA. Characterization of anti-ganglioside antibodies present in normal human plasma. *J Neuroimmunol*. 1994;50:215-220.
41. Willison HJ, Yuki N. Peripheral neuropathies and anti-glycolipid antibodies. *Brain*. 2002;125(Pt 12):2591-2625.
42. Budding K, Bos JW, Dijkxhoorn K, et al. IgM anti-GM2 antibodies in patients with multifocal motor neuropathy target Schwann cells and are associated with early onset. *J Neuroinflammation*. 2024;21:100.
43. van Sorge NM, Yuki N, Koga M, et al. Ganglioside-specific IgG and IgA recruit leukocyte effector functions in Guillain-Barré syndrome. *J Neuroimmunol*. 2007;182:177-184.
44. Ilyas AA, Mithen FA, Chen ZW, Cook SD. Anti-GM1 IgA antibodies in Guillain-Barré syndrome. *J Neuroimmunol*. 1992;36:69-76.
45. Súkeníková L, Mallone A, Schreiner B, et al. Autoreactive T cells target peripheral nerves in Guillain-Barré syndrome. *Nature*. 2024;626:160-168.