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CLINICAL IMPACT AND RELEVANCE OF ANTIGANGLIOSIDE ANTIBODIES TEST RESULTS

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Autoantibodies most commonly found in association with neuropathies are those against the ganglioside family antigens — GM1, GQ1b, asialo-GM1, GM2, GD1a, and GD1b. The major diagnostic role is set for two of antibodies — anti-GM1 and anti-GQ1b. This retrospective study was designed to evaluate the status of antiganglioside antibodies in patients with possible autoimmune neuropathy. The study included 85 patients tested for antiganglioside antibodies during their hospitalization. Clinical information such as demographic data and antecedent illness was collected for all patients, and paraclinical studies including results from cerebrospinal fluid and neuroelectrophysiological examination were analyzed. In our study, a total of 27 patients (32%) were found positive for at least one antiganglioside antibody. The most commonly found antibodies were against asialoGM1 (n=13) and GM1 (n=10) gangliosides. Eight patients were diagnosed with a disease where antiganglioside antibodies are used as a diagnostic marker: five patients — Guillain-Barré syndrome (GBS), 1 patient - Miller-Fisher syndrome (MFS), two patients — multifocal motor neuropathy (MMN). Three out of five patients diagnosed with GBS and one of two patients diagnosed with MMN were seronegative. The acute course of disease, positive antiganglioside antibodies and cytoalbuminologic dissociation in cerebrospinal fluid-induced patients is preference for a specific immune therapy. The results of our study support the previously described immunological association between antiganglioside antibodies and GBS, MFS, and MMN.

Key words: *gangliosides, Guillain-Barré syndrome, Miller-Fisher syndrome, multifocal motor neuropathy, autoimmune neuropathy, cytoalbuminologic dissociation.*

INTRODUCTION

Gangliosides are a family of glycosphingolipids that are broadly distributed on plasma membrane components of the nervous system. Gangliosides reside in membrane microdomains referred to as lipid rafts or detergent-resistant membranes, together with other sphingolipids, cholesterol and glycosylphosphatidylinositol (GPI)-anchored proteins. These microdomains form platforms and facilitate a variety of membrane-mediated functions, including signal transduction (Simons *et al*., 2002; Kusonoki *et al.*, 2008). Ganglioside nomenclature is represented by the following scheme: G refers to ganglio; M, D, T, and Q refer to the number of sialic acid residues (mono, di, tri, and quad, respectively); numbers and lower case letters refer to the sequence of migration as measured by thin layer chromatography (TLC).

Antibodies against these gangliosides are typically found in patients presenting with inflammatory peripheral neuropathies. The most commonly found antibodies are against GM1, GQ1b, asialo-GM1, GM2, GD1a and GD1b gangliosides (Table 1). The major diagnostic role is set for two of them — anti-GM1 and anti-GQ1b. Antiganglioside antibodies are used as differential diagnostic markers for mainly Guillain-Barré syndrome (GBS), multifocal motor neuropathy (MMN) and Miller-Fisher syndrome (MFS) in case of a clinically presented peripheral neuropathy (Rojas-Garcia *et al.*, 2012). Anti-GM1 IgM antibody is associated with multifocal motor neuropathy (MMN), anti-GM1 and anti-GD1a IgG antibodies are found in association with acute motor axonal neuropathy (AMAN) form of GBS, anti-GQ1b IgG antibodies — Miller-Fisher syndrome (Tatsumoto *et al*., 2006; Yu *et al*., 2006; Kaida, 2013; Bourque *et al*., 2015).

Antiganglioside antibodies are not a unique marker for autoimmune neuropathies, suggesting the need for a development of specific criteria for a rational use of antiganglio-

Table 1

SUMMARY OF THE MAJOR ANTIBODIES AND THE ASSOCIATED CLINICAL NEUROPATHY

Antibody	Clinical features	Antigen localization in human peripheral nervous system
Anti-GM1	AMAN, pure motor GBS, MMN	Not determined
Anti-AsialoGM1	ALS or GBS	Not determined
Anti-GD1a	AMAN	Not determined
Anti-GD1b	Ataxia in GBS	Large neurons in DRG, paranodal myelin
Anti-GM2	GBS	Not determined
Anti-GQ1b	MFS, GBS with ophthalmoplegia	Paranodal myelin of oculomotor, trochlear, and abducens nerves. A part of DRG neurons. Nerve terminals inside muscle spindles and near intrafusal fibers
Anti-GT1a	Bulbar palsy in GBS, PCB-GBS	Not determined
Anti-GD3	AIDP, CIDP	Not determined
Anti-GM1b	Pure motor GBS	Not determined

AMAN, acute motor axonal neuropathy; GBS, Guillain-Barrè syndrome; CIDP, chronic inflamatory demyelinating polyneuropathy; AIDP, acute inflammatory demyelinating polyradiculoneuropathy; MFS, Miller Fisher syndrome; PCB, pharyngeal-cervical-brachial; DRG, dorsal root ganglion; ALS, amyotrophic lateral sclerosis.

side antibodies in differential diagnostics. Determining specific clinical features, acquiring laboratory data and a serological pattern against gangliosides in autoimmune neuropathies may assist in recognising the existence of a clinical-immunological association. The establishment of an algorithm for cases where patients present with one of the above-mentioned diseases is crucial. Our aim was to study the clinical importance of antiganglioside antibodies: impact on patient's diagnosis and specific therapy, as well as their relevance as a diagnostic marker together with other clinical and laboratory findings.

MATERIALS AND METHODS

A retrospective study included 85 patients who were tested for antiganglioside antibodies during their hospitalisation in the time frame from January 2013 to December 2014, they were selected from the database of Clinical Immunology Centre of Pauls Stradiņš Clinical University Hospital.

Antiganglioside antibody test results were obtained from the database of Clinical Immunology Centre. Bühlmann laboratories GanglioCombi® ELISA (Enzyme-Linked Immunosorbent Assay) kit was used for quantitative antiganglioside antibody detection. GanglioCombi™ includes gangliosides such as GM1, asialo-GM1, GM2, GD1a, GD1b, and GQ1b. Patients were analysed for each of these gangliosides to obtain an immunoglobulin G profile per patient. The measured absorbance is proportional to the titer of antiganglioside-antibodies present in a given sample, meaning higher absorbance for patients with higher antibodies titer. Results are expressed as a percentage ratio of the absorbance of samples and the (averaged) absorbance of the calibrator: less than 30% are negative; 30–50% — grey zone (borderline); 50–100% — positive, more than 100% — strongly positive.

All patients were also subjected to full history and data analysis including age, sex, antecedent illness, history of smoking and/or alcohol use, complete clinical and neurological examination, including motor and sensory systems, neurophysiological studies. Furthermore, lumbar puncture results, cytoalbuminologic dissociation in cerebrospinal fluid (< 10 cells/mm^3, protein > 0.45 g/L), electrophoresis and plasma levels of various autoantibodies, duration and course of illness were evaluated. Therapy with corticosteroids, intravenous immunoglobulin or use of plasmapheresis was recorded. This study was approved by a research Ethics Committee. Data analysis was performed using IBM SPSS Statistics 20 and Microsoft Office Excel softwares.

RESULTS

Overall, 85 patients aged 23–78 years were enrolled and retrospectively analyzed in our study. 27 patients (32%) were positive for at least one antiganglioside antibody (Fig. 1). Fourteen patients had a positive reaction against a single ganglioside, 8 patients — against two gangliosides, 4 patients — against three gangliosides, 1 patient — against four gangliosides. Fifty-eight patients (68%) were found negative for all of the antibodies. Anti-asialoGM1 (13 cases) and anti-GM1 (10 cases) were the most frequently found antibodies (Fig. 2). Anti-GM2 antibodies had low seropositivity, it was found positive only for one patient (Fig. 2).

Of paraclinical studies, cerebrospinal fluid (CSF) examination was done in 47 patients; cytoalbuminologic dissociation was present in 28 out of 47. As a distinct group, 13 patients were chosen who had both, positive antiganglioside antibodies test and a cytoalbuminologic dissociation in CSF (Fig. 3). Of 27 antibody-positive patients only 10 patients had a clinically and neurographically approved polyneuropathy (2 — GBS, 1 — MFS, 6 — motor sensory axonal demyelinating neuropathy, 1 — pure sensory polyneuropathy), and the rest of the group had cerebrovascular diseases,

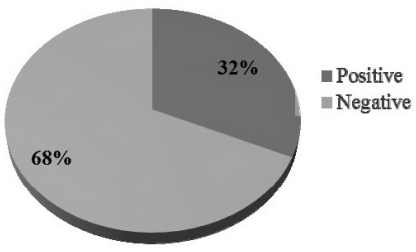
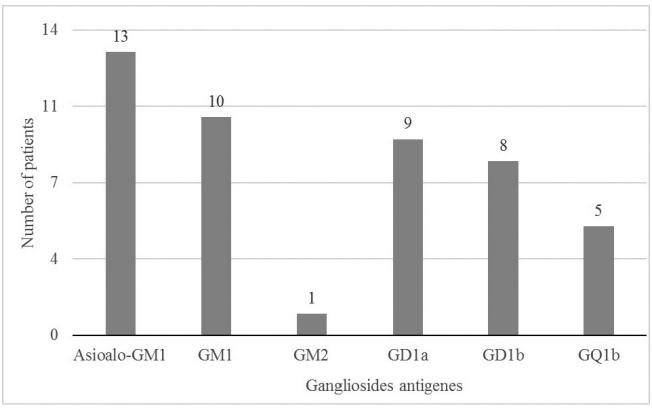


Fig. 1. Total positivity of antiganglioside antibody test.



Antiganglioside antibody	Asioalo-GM1	GM1	GM2	GD1a	GD1b	GQ1b
Positive	13	10	1	9	8	5
Negative/borderline	72	75	84	76	77	80

Fig. 2. Seroprevalence of IgG autoantibodies.

neuroinfections or radiculopathies. Specific treatment was given to seven antibody-positive acutely presenting patients. Three patients received intravenous methylprednisolone, 2 patients — intravenous immunoglobulin (IVIG), 2 — combination of IVIG and plasmapheresis therapy. Complete description of patient profiles is shown in Table 2.

DISCUSSION

The current guidelines in case of autoimmune neuropathies released by the Latvian Society of Neuroimmunologists in Latvia are discussing and advising the detection of antiganglioside antibodies in cases of Guillain-Barrè syndrome, Miller-Fisher syndrome and multifocal motor neuropathy (Logina *et al*., 2014). Similarly, as currently indicated in other scientific papers, the strongest biomarker associations are those linking Miller-Fisher syndrome with anti- GQ1b and multifocal motor neuropathy with anti-GM1 (Kaida, 2013; Bourque *et al*., 2015). The general testing of antiganglioside antibodies proved to have a 32% efficiency in detecting at least one positive antiganglioside antibody (27 out of 85 patients), but the impact on the diagnosis was relatively low in our study — only 4 out of these 27 patients were diagnosed with one of the corresponding diseases (GBS, MFS, MMN) (Table 2). This low efficiency of the test diagnosing GBS, MFS or MMN (even when antiganglioside antibodies are positive) could possibly be explained by borderline increase of antibody titers against ganglioside epitopes also in patients with motor neuron disease or motor neuropathy, overlaps with other diseases, for example, amyotrophic lateral sclerosis (Vernino *et al*., 2007).

Most studies have shown the peak incidence rise for GBS in two age groups: 15–35 years and 50–75 years (Hughes *et al.,* 2005; Sevjar *et al*., 2011). Supporting data were found in our study: four out of five patients who were diagnosed

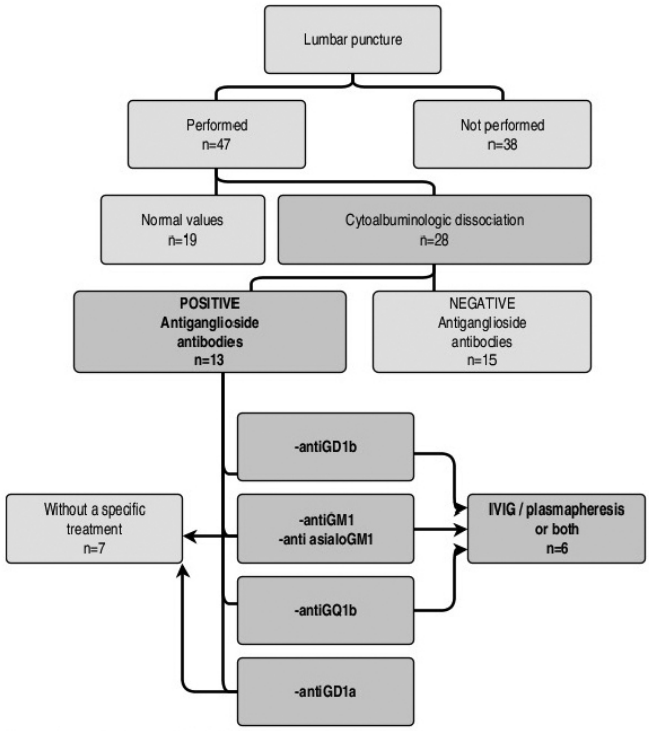


Fig. 3. Relation between cytoalbuminologic dissociation in CSF, seropositivity and preference of treatment.

with GBS fitted in the mentioned age-specific peaks (patients age in a row: 54, 62, 63, 72 years) (Table 2).

We found a noteworthy connection between cytoalbuminologic dissociation in CSF, treatment preference and antiganglioside seropositivity, proving it a potential criterion for more studies in the future (Fig. 3). Antiganglioside seropositive patients (n = 6) who were treated with either IVIG, plasmapheresis or intravenous glucocorticoids, all had cytoalbuminologic dissociation in cerebrospinal fluid, even more highlighting the autoimmune nature of the neuropathy and a possible relevance of these two diagnostic units used in cooperation. This indicates that antiganglioside antibodies decisively should be tested together with CSF examination to prefer treatment — all six patients received IVIG or plasmapheresis (Fig. 3) Analyzing those data simultaneously it could also be possible to exclude other causes of the clinical picture (infections etc.) Preference of IVIG or plasmapheresis treatment was not determined by type of antiganglioside antibody.

As earlier suggested, the gangliosides GM1 and GQ1b have proven to be the most useful markers for our patients (Figs. 2 and 3), these two were the only ones from the whole group having any clinical impact on the patient's diagnosis and therapy. In this study, a clear relevance is observed for the detection of anti-GQ1b in the case of MFS.

In the future perspective, we should discuss whether it is effective to detect all panel of antiganglioside antibodies for one patient, for example anti-GM2 antibody. It was found positive only for one patient (Fig. 2) and did not have any

Table 2

PROFILES OF ALL SEROPOSITIVE PATIENTS

Patients Nr.	Gender	Age (y)	Antiganglioside antibody test results (%)						Cytoalbuminologic dissociation (cells in mm3/ protein g/L)	Areflexia	Neurophysiology-data about PNP	Specific Therapy	GBS, MFS or MMN
			Asialo GM1	GM1	GM2	GD1a	GD1b	GQ1b					
Patient 1	female	54	60.8	31.1	11.3	9.2	8.8	6.9	not performed	-	-	none	-
Patient 2	male	48	172	85.5	17.8	24.5	13.5	17.8	5/5.36	+	+	IVIG	MMN
Patient 3	male	69	37.7	10.7	5.6	191.1	6.1	4.4	1/0.57	-	-	none	-
Patient 4	female	33	92.2	44	21.3	116.7	57.4	31	not performed	-	+	none	-
Patient 5	female	57	13.3	4.9	5	210.6	7.1	4.7	5/0.48	-	-	none	-
Patient 6	male	59	82.6	56.4	16.7	45	47.3	34.5	1/1.0	-	-	none	-
Patient 7	male	69	82.2	47.8	62.4	42.8	57.6	49.9	not performed	+	-	none	-
Patient 8	female	77	27.3	23.8	13	55.7	8	8.1	1/0.75	-	-	none	-
Patient 9	female	64	23.7	33.9	27.7	55	48.3	33.7	normal values	-	+	i/v GC	-
Patient 10	male	58	31.2	6.4	7.9	99.3	55.7	121.8	not performed	-	-	none	-
Patient 11	female	57	30.6	19	19	43.5	51.3	27.3	not performed	+	+	none	-
Patient 12	male	74	37.2	14.3	32.2	86.7	26	14.6	traumatic	-	+	none	-
Patient 13	male	38	45.3	68.9	32.3	39.5	54.1	10.9	4/0.86	+	+	none	-
Patient 14	female	55	32.6	41.3	20.5	22.2	64.1	27.3	not performed	-	-	i/v GC	-
Patient 15	female	72	67.4	413,8	18.8	48.6	409.6	14.9	2/0.68	+	+	IVIG	GBS
Patient 16	female	78	43.1	65.2	23.2	11.8	11.8	10.5	normal values	+	+	none	-
Patient 17	female	36	10.3	6.6	12.9	80.2	8	5.3	normal values	-	-	i/v GC	-
Patient 18	female	59	109.6	28.5	18.4	219.3	10.6	51.8	normal values	-	-	none	-
Patient 19	male	63	26	97.3	15.4	13.2	80.1	8.7	normal values	-	-	none	-
Patient 20	male	54	19.3	53.7	12.9	9.7	14.1	54	6/1.0	+	-	plasmapheresis IVIG	MFS
Patient 21	male	29	52.2	52.6	30.3	28.6	33.6	20	0/0.53	-	-	none	-
Patient 22	female	37	59.5	8.5	17.5	9.5	11.9	7.5	normal values	-	-	none	-
Patient 23	male	77	35.3	12.7	22.6	11.2	13	73.2	16/1.35	-	-	none	-
Patient 24	male	60	52.2	18.5	20.9	10.7	13.8	22.1	not performed	-	-	none	-
Patient 25	male	54	100.3	27.9	30.2	27.9	22.4	8.2	0/0.84	+	-	none	-
Patient 26	female	71	149.4	100.1	14.9	23.5	30.9	16.9	not performed	+	+	none	-
Patient 27	female	39	53	85	18.7	33.1	40	17	2/1.23	+	+	plasmapharesis	GBS

GBS, Guillain-Barrè syndrome; MFS, Miller Fisher syndrome; MMN, Multifocal motor neuropathy; IVIG, intravenous immunoglobulin; i/v GC, intravenous glucocorticoids; PNP, polyneuropathy.

impact on diagnosis or treatment. The same could be asked about antiGD1a antibodies detection. Furthermore, in our study, three out of five GBS patients were seronegative for anti-GM1, and one of two MMN patients was seronegative for both anti-GM1 and anti-GD1a, raising up the questions of a possible seronegative disease and appropriate timing for a reliable use of the test.

As in the retrospective study, conclusions of the study can be conditioned by limited availability and accuracy of the clinical data. No firm conclusions can be performed, as statistical data were not obtained for such a small group of patients.

In conclusion, antiganglioside antibodies as an isolated diagnostic unit are not practical enough. Together with such findings as positive cytoalbuminologic dissociation and neurography data suggesting polyneuropathy, they can be a valid marker for the diagnosis of MFS, GBS and MMN. As we are still lacking certain guidelines in case of an autoimmune neuropathy, the establishment of a cost-effective diagnostic algorithm is crucial, where the goal is to select a patient group who could actually benefit from the antiganglioside antibody test.

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ANTIGANGLIOZĪDU ANTIVIELU TESTA REZULTĀTU KLĪNISKĀ IETEKME UN NOZĪME PACIENTAM

Visbiežāk atrastās autoantivielas asociācijā ar perifērām neiropātijām ir pret gangliozīdiem GM1, GQ1b, asialo-GM1, GM2, GD1a un GD1b. Atzīta diagnostiska vērtība ir divām no tām — anti-GM1 un anti-GQ1b. Tika veikts retrospektīvs pētījums ar mērķi izvērtēt antigangliozīdu antivielu noteikšanas nozīmi pacientiem ar iespējamu autoimūnu neiropātiju. Pētījumā tika iekļauti 85 pacienti, kuri bija stacionēti Paula Stradiņa Klīniskajā universitātes slimnīcā laika posmā no 2013. gada janvāra līdz 2014. gada decembrim un kuru perifērajās asinīs tika noteiktas antigangliozīdu antivielas. Tika reģistrēti un analizēti arī pacientu demogrāfiskie dati, iepriekšējas un esošas saslimšanas, paraklīnisko izmeklējumu dati, t.sk. cerebrospinālā šķidruma atradne un elektrofizioloģiskās izmeklēšanas rezultāti. Mūsu pētījumā 27 pacienti (32%) bija seropozitīvi attiecībā vismaz uz vienu no antigangliozīdu grupas antivielām. Visbiežāk atrastās antivielas bija pret asialo-GM1 (n = 13) un GM1 (n = 10) gangliozīdiem. Astoņu pacientu diagnozes sakrita ar slimībām, kur antigangliozīdu antivielām ir atzīta diagnostiska marķiera vērtība: pieciem pacientiem — Gijēna-Barē sindroms (GBS), vienam pacientam — Millera-Fišera sindroms (MFS), diviem pacientiem — multifokāla motora neiropātija (MMN). Trīs no pieciem pacientiem, kuriem bija diagnosticēts GBS, un viens no diviem pacientiem, kuriem bija diagnosticēta MMN, bija seronegatīvi. Akūta slimības gaita, pozitīvas antigangliozīdu antivielas un citoalbumināra disasociācija cerebrospinālajā šķidrumā bija faktori, kas noteica pacientam nepieciešamu specifisku imūnterapiju. Mūsu pētījuma rezultāti atbilst iepriekš aprakstītām novērotām asociācijam starp antigangliozīdu grupas antivielām un GBS, MFS un MMN.