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Laboratory Studies

Anti-glycolipid antibodies in patients with neuropathy: A diagnostic assessment

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ABSTRACT

Anti-glycolipid antibodies are associated with immune-mediated neuropathies and screening is often performed as part of the diagnostic assessment for patients presenting with peripheral neuropathy. We report our experience in testing for immunoglobulin (Ig) G and IgM anti-glycolipid (GM1, GM2, GM3, GM4, GD1a, GD1b, GD2, GD3, GT1a, GT1b, GQ1b, sulfatides) antibodies in 290 consecutive patients presenting with neuropathy. Anti-glycolipid antibodies were detected significantly more often (43%) in patients who were diagnosed with definite immune-mediated neuropathy than in patients without a final diagnosis of immune-mediated neuropathy (control group) (23%). With positive and negative predictive values of 22% and 90%, respectively, anti-glycolipid antibodies are not a very reliable diagnostic tool in early patient contact. Certain antibodies (IgM to GM2, GT1a and IgG to GM3, GD3 and GT1b) were equally or more prevalent in the control group; clinicians should be aware of this distribution when receiving positive screening results for these antibodies. Concomitant IgG and IgM reactivities were found for GM1, GM2, GD1b and sulfatides, and were detected more frequently in patients with definite immune-mediated neuropathies.

1. Introduction

Peripheral neuropathy is a common clinical entity in neurology and anti-glycolipid antibody screening is reported to be a supportive diagnostic tool in various stages of diagnostic assessment by numerous authors. Indeed, anti-glycolipid screening has been included in the guidelines of the German Society of Neurology [1–5]. Anti-glycolipid antibodies are associated with various acute and chronic autoimmune-mediated peripheral neuropathies and are thought to possibly have pathogenic roles in these conditions [6–10]. The clinical manifestations of immune-mediated neuropathies are remarkably diverse. Similarly, there is high variability in the antibody profiles of these disorders.

The closest association between antibody and disorder has been observed between immunoglobulin (Ig) G antibodies against ganglioside GQ1b/GT1a and Miller-Fisher syndrome (MFS) [6,11]. The most common antibody in Guillain–Barré syndrome (GBS) is IgG anti-GM1, but antibodies against GD1a, GD1b and GQ1b have also been detected [6,8]. Antibodies in chronic immune-mediated neuropathies are mostly of the IgM isotype and are found to be directed towards GM1, and less frequently towards GM2 in multifocal motor neuropathy (MMN). Patients with chronic inflammatory demyelinating polyneuropathy (CIDP) have been reported to test positive for GM2, GD1a and GD1b antibodies [9]. In neuropathies associated with monoclonal gammopathy, myelin-associated glycoprotein, sulfatides and various gangliosides have been recognised as target antigens in a significant proportion of these patients [12]. In addition to these immune-mediated peripheral neuropathies, anti-glycolipid antibodies have also been found in individuals with other neurological diseases and non-neurological diseases, as well as healthy controls [13–17]. The evaluation of the diagnostic usefulness of anti-glycolipid antibody screening is further complicated by the use of various testing methods and different target antigen panels that are included in commercial assays. We therefore report our laboratory experience in anti-glycolipid antibody screening in a consecutive series of neurological patients presenting with neuropathy to a German university hospital.

2. Materials and methods

2.1. Materials

Over a 3 year period from 2009 to 2011, 301 consecutive serum samples of patients with neuropathy or related clinical syndromes were screened for IgG- and IgM-glycolipid antibodies as requested by the referring neurologist per the clinical standard of care. We retrospectively evaluated the screening results considering the final primary neurological diagnosis included in the hospital discharge letter. The guidelines of the German Society for Neurology

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served as the foundation of the diagnostic approach applied to the patients in this study. Eleven patients were excluded because of incomplete clinical information. The remaining 290 patients were grouped accordingly into three categories: (1) definite immune-mediated neuropathy (37 patients including 21 with GBS, three with MFS, eight with CIDP and five with monoclonal gammopathy-associated immune-mediated neuropathy); (2) possible immune-mediated peripheral neuropathy (12 patients); and (3) non-immune-mediated peripheral neuropathy or related disease (241 patients; control group). The most common diagnoses in the control group were polyneuropathy without underlying cause, diabetic and toxic polyneuropathy and motor neuron disease.

2.2. Methods

Testing for serum anti-glycolipid antibodies was conducted using a commercially available multiparametric immunodot assay coated with the antigens ganglioside GM1, GM2, GM3, GM4, GD1a, GD1b, GD2, GD3, GT1a, GT1b, GQ1b and sulfatides (Generic Assays GmbH, Dahlewitz, Germany). Prior to testing, the sera were stored at −20 °C for up to a maximum of 7 days. According to the manufacturer's instructions, samples were diluted to 1:100 and incubated at 4 °C for 2 hours. After a single washing, the bound antibodies reacted with anti-IgG or anti-IgM conjugated to horseradish peroxidase during a second incubation for 60 minutes at 4 °C. A second wash step was performed and substrate was added to visualise the immune reaction. A sample was considered positive for a given antibody if the coloration of the test line was of greater intensity than the band on the identification template.

Four patient categories were established based on antibody status and discharge diagnosis and arranged in a contingency table. Statistical measures, including sensitivity, specificity, predictive values and odds ratios (OR) were calculated. All data were categorical and all expected frequencies were >5; to test for differences between patient groups, the chi-squared (χ^2) test was applied. p values <0.01 indicated significant results at the 0.01 alpha level, which implied highly significant results. To present statistical data in Table 1 more clearly (and only for this purpose), patient groups were defined as Group A: patients with definite immune-mediated peripheral neuropathies; and Group B: patients with definite or possible immune-mediated peripheral neuropathies. Controls were patients without immune-mediated peripheral neuropathies.

3. Results

The statistical analysis yielded comparable results for Group A and Group B: low sensitivity rates (43% Group A, 49% Group B) and low positive predictive values (22% Group A, 30% Group B), and moderate specificity rates (77%) and therefore acceptable to good negative predictive values (90% Group A *versus* 88% Group B). Antibodies were present with significantly greater frequency in both immune-mediated neuropathy groups (Group A: $\chi^2 = 6.69$, $p < 0.01$, degrees of freedom [df] = 1; Group B: $\chi^2 = 13{,}509$, $p < 0.01$, df = 1) compared to the control group. Furthermore, the OR were significantly greater than 1 (Group A: OR = 2.52, 95% confidence interval [CI] 1.23–5.15; Group B: OR = 3.17, 95% CI 1.68–5.98) (Table 1).

Table 1
Frequency distribution of diagnosis and glycolipid-antibody status

	Group A	Group B	Controls
Antibody positive	16 (43%)*	24 (49%)*	56 (23%)
Antibody negative	21 (57%)	25 (51%)	185 (77%)
Total	37	49	241

* $p < 0.01$ compared to control group.

In the group of patients with definite immune-mediated neuropathy, the most common IgG antibody was to GM1; that of the control group, however, was to GM3 and to sulfatides. The IgG antibodies that were exclusively detected in the control group were to GM3, GD3 and GT1b. All of the other IgG anti-glycolipid antibodies that were detected in controls and in patients with definite immune mediated peripheral neuropathy were found more frequently in the latter of the two. An IgG GD2 antibody was not found in any of the 290 patients. The most frequently detected antibody across all of the groups was IgM to GM1. IgM antibodies to GM2, GM3, GD1a and GT1a were found more frequently in the control group than in the group of patients with definite immune mediated peripheral neuropathies. IgM antibodies against GQ1b were found exclusively in the group of patients with definite immune-mediated peripheral neuropathy. Concomitant IgG and IgM reactivities were found for GM1, GM2, GD1b and sulfatides; all of these antibodies were detected more frequently in the group of patients with definite immune-mediated peripheral neuropathy than in the control group. Because of the small number of samples in most of the subgroups, further statistical analysis was not performed (Table 2). The antibody profiles in patients with immune-mediated peripheral neuropathies are shown in Table 3.

4. Discussion

The overall prevalence of peripheral neuropathy is approximated 2.4%, with higher numbers in the elderly; it is therefore a common clinical entity presenting to neurology departments [18]. An extensive diagnostic workup is usually performed and testing for anti-glycolipid antibodies is often recommended as a diagnostic tool. Of the 290 patients evaluated in the present study, 37 were discharged with the diagnosis of an immune-mediated peripheral neuropathy (13%). This number is fairly consistent with the data presented in the German national guidelines, which state that the sum of GBS, CIDP and paraproteinemic neuropathies amounted to 11.5% of a large collective of patients with polyneuropathy [5].

To save time related to processing laboratory reports and to avoid additional patient venipunctures, many neurologists in our hospital request antibody testing at an early stage of patient contact in which differentiated clinical and neurophysiological evaluations are yet to be conducted. In this setting, the diagnostic sensitivity of anti-glycolipid antibodies in patients with a definite immune-mediated peripheral neuropathy (Group A) was 43%, and the diagnostic specificity was 77%. Positive and negative predictive values of 22% and 90%, respectively, demonstrated that anti-glycolipid antibody testing at an early stage of the diagnostic procedure in our institution may, at the utmost, be considered a supportive tool in ruling out definite immune-mediated neuropathies and identifying possible differential diagnoses. The OR represented a good approximation of the relative risk in the present study and demonstrated a 2.52-fold chance of patients with positive anti-glycolipid antibody results of actually having definite immune-mediated peripheral neuropathy. Comparable results were obtained for Group B (patients with definite or possible immune-mediated peripheral neuropathies) and are therefore not further elaborated.

Regarding single antibody reactivities, a high specificity of over 95% was achieved for all antibodies except for IgM-GM1 antibodies (specificity of 91%). However, this must be viewed in the context of accordingly low sensitivity, with rates $\geqslant$10% for only IgG-GM1 (12%), IgM-GM1 (24%) and IgM-GD1b (10%) in the complete collective of definite immune-mediated peripheral neuropathies. Higher sensitivity rates were achieved in respect to defined peripheral immune-mediated neuropathies. The corresponding antibody profiles