Serum anti-ganglioside antibodies in patients with autoimmune limbic encephalitis

ABSTRACT

Background/aim: Ganglioside antibodies are identified not only in patients with inflammatory neuropathies but also several central nervous system disorders and paraneoplastic neuropathies. Our aim was to investigate whether ganglioside antibodies are found in autoimmune encephalitis patients and may function as a diagnostic and prognostic biomarker.

Materials and methods: Sera and cerebrospinal fluid (CSF) samples of 33 patients fulfilling the criteria for probable autoimmune encephalitis were collected within the first week of clinical manifestation. None of the patients had evident symptoms and findings of peripheral polyneuropathy. Well-characterized anti-neuronal and paraneoplastic antibodies were investigated in sera and CSF and anti-ganglioside (anti-GM1, GM2, GM3, GD1a, GD1b, GT1b and GQ1b) IgG and IgM antibodies were measured in sera using commercial immunoblots.

Results: Twenty-eight of 33 autoimmune encephalitis patients displayed antibodies against neuronal surface or onco-neural antigens with N-methyl-D-aspartate receptor (NMDAR), glutamic acid decarboxylase (GAD) and Hu antibodies being the most prevalent. While no anti-ganglioside IgG antibodies were found, 4 patients (2 anti-NMDAR+, 1 anti-GAD+ and 1 antibody negative) with autoimmune limbic encephalitis displayed anti-GM1, anti-GM2, anti-GM3 or anti-GQ1b IgM antibodies. There was no apparent association between anti-ganglioside positivity and clinical and demographic features.

Conclusion: Serum ganglioside IgM antibodies may infrequently emerge during the clinical course of autoimmune limbic encephalitis without evident polyneuropathy.
Absence of the IgG response suggests that these antibodies might have developed as a hyperacute immune response to neuro-axonal destruction. Nevertheless, potential impact of ganglioside antibodies on axonal degeneration and neuronal loss in limbic encephalitis pends to be further investigated.

**Key words:** Ganglioside, antibody, autoimmune encephalitis, paraneoplastic, autoimmunity
1. Introduction

Ganglioside antibodies are typically found in patients with acute or chronic inflammatory demyelinating polyneuropathies, which include variants of Guillain-Barré syndrome, chronic inflammatory demyelinating polyradiculoneuropathy and multifocal motor neuropathy [1]. Nevertheless, gangliosides are abundantly expressed by central nervous system (CNS) neurons and are involved in cell signaling, nerve transduction and neuroplasticity [2]. Thus, anti-ganglioside antibodies might conceivably interact with their target antigens on the axonal membrane and subsequently lead to impairment in nerve conduction and axonal degeneration.

Ganglioside antibodies have been implicated in several CNS disorders such as Rasmussen encephalitis, amyotrophic lateral sclerosis, multiple sclerosis and neurodegenerative diseases [1,3,4]. Notably, GQ1b ganglioside antibodies are found in patients with Bickerstaff brainstem encephalitis, which, in addition to external ophthalmoplegia, manifests with ataxia, disturbance of consciousness and hyperreflexia [5]. Some Bickerstaff encephalitis patients may develop acute-onset seizures and psychosis, suggesting that autoimmune encephalitis and ganglioside autoimmunity may coexist [6]. Moreover, serum IgG of ganglioside antibody positive Miller-Fisher syndrome cases may show specific interaction with cerebellar antigens and may encompass voltage-gated potassium channel complex antibodies [7,8].

Cancer cells express gangliosides and several cancer-associated polyneuropathy cases with ganglioside antibodies have been reported, suggesting that gangliosides may act as putative onco-neural antigens [9]. In this study, we aimed to identify the prevalence and clinical relevance of a broad panel of ganglioside antibodies in autoimmune limbic encephalitis with or without an underlying malignancy.
2. Materials and methods

2.1. Patients

A total of 33 consecutive patients fulfilling the criteria for probable autoimmune encephalitis and 30 age/gender-matched healthy individuals were included [10]. All patients had a disease duration of less than 3 months, at least 2 of the symptoms of altered mental status, short-term memory loss, psychosis and seizures, magnetic resonance imaging (MRI) features suggestive of autoimmune encephalitis and CSF pleocytosis with or without CSF-specific oligoclonal bands (OCB). Alternative causes were excluded by total blood count, blood biochemistry, thyroid function tests, thyroid antibodies, antibody panel for vasculitic and rheumatological disorders, a screen panel for viral causes of encephalitis and cranial MRI. None of the patients reported a concomitant neurological or systemic disease or a previous clinical episode suggestive of autoimmune encephalitis. Also, none of the patients showed subjective symptoms and neurological examination findings of polyneuropathy or ophthalmoplegia. Underlying malignancies were identified by whole-body computed tomography (CT) scan, pelvic ultrasound, mammography, endoscopic procedures and pathological examination of biopsy or surgery material, as required. All patients received the standard treatment of pulse iv methylprednisolone and intravenous immunoglobulin within the first month and treatment resistant patients received rituximab and/or cyclophosphamide, thereafter. Treatment responsiveness was defined as a reduction of at least 2 points in modified Rankin score (mRS) at the end of the follow-up period. The patients gave informed consent for inclusion in the study, which was approved by the local medical research ethics committee.

2.2. Anti-neuronal antibody tests
Sera and CSF were collected within the first week of the clinical episode prior to administration of immunosuppressive treatment. All samples were stored at −80°C prior to investigation. Serum ganglioside (anti-GM1, GM2, GM3, GD1a, GD1b, GT1b and GQ1b) IgM and IgG antibodies and serum/CSF onco-neural (anti-Hu, Yo, CV2, Ma2, Ri and amphiphysin) IgG antibodies were investigated by commercial immunoblots (Euroimmun, Lübeck, Germany) using standard protocols (serum dilution 1:50) [11]. Serum/CSF IgG antibodies against neuronal surface antigens [anti-N-methyl-D-aspartate receptor (NMDAR), contactin-associated protein-like 2 (CASPR2), leucine-rich, glioma inactivated 1 (LGII), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR), gamma-aminobutyric acid B receptor (GABA_B)] were investigated by a commercial kit using a cell-based assay (serum dilution 1:20) (Euroimmun). Glutamic acid decarboxylase (GAD) antibodies were measured by Enzyme-linked immunosorbent assay (ELISA), as per instructions of the manufacturer (Euroimmun).

3. RESULTS

3.1. Clinical features

Eighteen of 33 autoimmune encephalitis (18 men, 15 women; 46±18-year-old; age range, 11-76) patients presented with definite autoimmune limbic encephalitis, as per relevant criteria [10]. In the remaining patients, autoimmune encephalitis findings were coupled with brainstem [7], cerebellar [3], stiff-person syndrome (SPS) [2] or peripheral nerve hyperexcitability (PNH) [2] symptoms and 1 patient had both cerebellar and SPS findings. In a follow-up period of 22.2±17.9 months, treatment resistance was observed in 13 patients, 5 of whom eventually died. An accompanying malignancy was detected in 12 patients [4 small-cell lung cancer (SCLC), 2 ovarian teratoma, 2 testis cancer, 2
thymic cancer, 1 breast cancer and 1 rectum cancer]. CSF-specific OCB were detected in 10 patients.

3.2. Antibody profile

Anti-neuronal antibodies were found in sera and/or CSF of 28 patients (anti-NMDAR [11], anti-GAD [5], anti-Hu [4], anti-LGI1 [2], anti-Ma2 [2], anti-Ri [2], anti-GABA\_B\_R [1], anti-amphiphysin [1]). Five patients who did not display any of the well-characterized anti-neuronal antibodies displayed at least two of short-term memory loss, psychosis and seizures, bilateral temporal lobe hyperintensity and CSF pleocytosis and thus fulfilled the criteria for autoantibody-negative limbic encephalitis [10]. IgM antibodies against GM1, GM2, GM3 and GQ1b were found in four patients, only (Table). None of the patients showed anti-ganglioside IgG antibodies. Anti-ganglioside IgG/IgM antibodies were not found in sera of healthy individuals.

3.3. Clinical features of ganglioside antibody positive patients

Anti-ganglioside IgM antibodies were found in 4 patients (11-57 year-old, 3 men). All patients had limbic encephalitis as the clinical syndrome. One of the patients displayed additional symptoms of SPS and cerebellar syndrome, as well. Two of these patients had NMDAR antibody, one had GAD antibody and one patient did not display any of the investigated anti-neuronal antibodies. The autoantibody negative patient showed SCLC, whereas no malignancies could be found in other patients. CSF-specific OCB was only detected in the GAD-antibody positive patient. Two patients were treatment resistant, whereas the other 2 patients showed good response to immunosuppressive treatment. A detailed study of nerve conduction and needle electromyography was done only for the patient with SPS findings. This study did not reveal any findings of
neuropathy, nerve conduction block or myopathy. Other 3 patients did not report symptoms suggestive of peripheral nerve involvement.

4. Discussion

Our study has shown that ganglioside IgM antibodies are found only in a small fraction of patients with autoimmune limbic encephalitis. Moreover, presence of these antibodies is not strictly associated with age, any peculiar clinical features, response to immunosuppressive treatment or presence of an underlying malignancy. Only one of five antibody-negative autoimmune encephalitis patients were positive for ganglioside antibodies arguing against their potential use in diagnosis of antibody-negative autoimmune encephalitis. Therefore, the prognostic and diagnostic significance of ganglioside antibodies is somewhat limited and a routine screening of these antibodies appears to be unnecessary.

Viral infection, which is a well-known initiator of ganglioside autoimmunity, may also be encountered shortly before the autoimmune encephalitis episode [12,13]. Thus, whether ganglioside antibodies identified in our study were induced by a concomitant subclinical viral infection needs to be further studied. Similarly, whether post-infectious autoimmune encephalitis may generate clinically significant ganglioside antibodies pends to be characterized.

The absolute lack of the IgG response against gangliosides might be due to the absence of the microenvironment for affinity maturation and class switching in the lymphatic tissue of the patients. An alternative reason could be collection of blood samples in the very early stage of antibody production, at a time point when the predominant antibody isotype was IgM [14,15]. Clinically significant anti-neuronal antibodies associated with autoimmune encephalitis are of the IgG isotype [16]. Immunization experiments
performed in rodents and patients have shown that production of measurable levels of serum IgG might take around 7-10 days following the encounter of the lymphatic cells with antigens including gangliosides [17, 18]. Congruently, anti-ganglioside IgG antibodies may not be detected in the first week of ganglioside immunization [18]. Therefore, anti-ganglioside IgG antibodies might plausibly be detected in samples obtained in the more advanced stages of encephalitis.

Hippocampi, major CNS target of autoimmune encephalitis, are known to abundantly express gangliosides including GM1, GM2, GM3 and GQ1b, which exhibit diverse neuronal functions such as learning and memory [2]. Particularly, GQ1b is involved in the facilitation of the NMDA receptor signaling pathway in the hippocampus [19,20]. Due to close proximity of membrane glycolipids and cell surface targets of anti-neuronal antibodies, gangliosides might putatively be dispersed and exposed to the immune cells infiltrating the brain tissue of autoimmune encephalitis patients ultimately leading to ganglioside antibody formation.

By virtue of our inclusion criteria, all patients had classical features of autoimmune limbic encephalitis and, as a result, around 84% of our patients displayed anti-neuronal antibodies [21]. Therefore, it is not surprising that anti-ganglioside antibodies were mostly found in association with well-characterized anti-neuronal antibodies, further implying that anti-ganglioside antibodies in encephalitis patients occur as a bystander effect in response to neuronal damage induced by anti-neuronal antibodies. Similar antibody co-occurrences have been described in anti-voltage-gated potassium channel (VGKC)-complex, anti-NMDAR and anti-GAD antibody positive patients [3, 8, 22,23]. Regardless of emerging by primary or bystander (secondary to neuro-axonal destruction) mechanisms, anti-ganglioside or anti-glycolipid IgM antibodies are detected in several inflammatory disorders (e.g. multifocal motor neuropathy; chronic
ataxic neuropathy and encephalomyeloradiculoneuropathy) and serve as useful diagnostic biomarkers [24]. Moreover, there is substantial evidence suggesting that these IgM antibodies are involved in neuronal damage particularly through complement activation [24]. None of the ganglioside antibody positive patients in our cohort presented with findings congruent with peripheral nervous system involvement. A detailed peripheral nerve assessment done for the 19-year-old woman with SPS proved unremarkable, thus suggesting that detected ganglioside antibodies did not have a pathogenic action on peripheral nerves. However, as reported previously, ganglioside antibodies may be associated with pure CNS findings without profoundly affecting peripheral nerve functions [25,26]. All ganglioside antibody positive patients in our cohort had relatively high maximum mRS. Whether ganglioside antibodies contribute to neuronal destruction and increased clinical severity in autoimmune encephalitis needs to be further studied. Also, autoimmune encephalitis patients with peripheral nerve involvement should be investigated for ganglioside antibodies.

One of the ganglioside antibody (anti-GM2) positive patients in our cohort was a man with SCLC, limbic encephalitis and no anti-neuronal antibodies. Although SCLC patients with neurological syndromes often present with well-characterized anti-neuronal antibodies such as anti-Hu, antibody-negative SCLC cases with limbic encephalitis have also been reported [27]. Notably, GM2 is abundantly expressed by SCLC and other tumor types [9,28]. Gangliosides have been implicated to serve as onco-neural antigens triggering the immune response and our case lends further support to this notion [9].

A limitation of our study is the absence of patients with limbic system and peripheral nerve involvement in our cohort, except for 2 patients with PNH and no ganglioside antibodies. Albeit rare, this clinical combination might prove to be a better target for...
identification of encephalitis-related ganglioside antibodies. Another limitation was absence of ganglioside antibody measurements in CSF samples. Ganglioside antibodies are generally investigated in sera of patients and thus procurement of positive CSF samples as an assay control is extremely difficult. Since validation of our assay system for the CSF anti-ganglioside measurements was not possible without these positive controls, no attempt was done for CSF identification of ganglioside antibodies. Finally, serial measurement of ganglioside antibodies in samples obtained at different time points of disease course might provide seminal information about transiency/permanency and isotype switching (from IgM to IgG) characteristics of ganglioside antibodies in autoimmune encephalitis.

5. Conclusion

In summary, we identified anti-ganglioside IgM antibodies in a small group of autoimmune limbic encephalitis cases with or without accompanying malignancy. The clinical and pathogenic significance of this finding is presently uncertain. However, previous research has shown ganglioside antibodies in patients presenting with isolated CNS findings and pathogenicity of ganglioside antibodies has been demonstrated. Thus, despite their rarity in autoimmune encephalitis, potential pathogenic contribution of these antibodies pends to be further characterized.

Acknowledgments/disclaimers/conflict of interest

No potential conflict of interest was reported by the authors.

Informed consent

This study was approved by the local medical research ethics committee. Informed consent forms were signed by all patients.
REFERENCES


### Table. Clinical and demographic features of patients with anti-ganglioside antibodies.

<table>
<thead>
<tr>
<th>Age</th>
<th>Gender</th>
<th>Clinical syndrome</th>
<th>Follow-up (months)</th>
<th>mRS max/final</th>
<th>Anti-neuronal Ab</th>
<th>Cancer</th>
<th>Ganglioside Ab</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>M</td>
<td>Limbic encephalitis</td>
<td>20</td>
<td>5/4</td>
<td>Anti-NMDAR</td>
<td>-</td>
<td>anti-GM1 IgM</td>
</tr>
<tr>
<td>19</td>
<td>F</td>
<td>Limbic encephalitis stiff-person, cerebellar syndrome</td>
<td>22</td>
<td>4/3</td>
<td>Anti-GAD</td>
<td>-</td>
<td>anti-GQ1b IgM</td>
</tr>
<tr>
<td>24</td>
<td>M</td>
<td>Limbic encephalitis</td>
<td>12</td>
<td>4/0</td>
<td>Anti-NMDAR</td>
<td>-</td>
<td>anti-GM3 IgM</td>
</tr>
<tr>
<td>57</td>
<td>M</td>
<td>Limbic encephalitis</td>
<td>18</td>
<td>4/1</td>
<td>None</td>
<td>Small-cell lung Ca</td>
<td>anti-GM2 IgM</td>
</tr>
</tbody>
</table>

M, male; F, female; mRS, modified Rankin score; max, maximum; NMDAR, N-methyl-D-aspartate receptor; GAD, glutamic acid decarboxylase; Ca, cancer; MRI, magnetic resonance imaging; R, right; BL, bilateral; HI, hyperintensity; CSF, cerebrospinal fluid; OCB, oligoclonal band; Ab, antibody.