How Much GAD65 Do You Have? High Levels of GAD65 Antibodies in Autoimmune Encephalitis

Neurologic Syndromes Related to Anti-GAD65 Clinical and Serologic Response to Treatment

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Objective: Antibodies against glutamic acid decarboxylase 65 (anti-GAD65) are associated with a number of neurologic syndromes. However, their pathogenic role is controversial. Our objective was to describe clinical and paraclinical characteristics of anti-GAD65 patients and analyze their response to immunotherapy. Methods: Retrospectively, we studied patients (n = 56) with positive anti-GAD65 and any neurologic symptom. We tested serum and cerebrospinal fluid with enzyme-linked immunosorbent assay (ELISA), immunohistochemistry, and cell-based assay. Accordingly, we set a cutoff value of 10 000 IU/mL in serum by ELISA to group patients into high-concentration (n = 36) and low-concentration (n = 20) groups. We compared clinical and immunologic features and analyzed response to immunotherapy. Results: Classical anti–GAD65-associated syndromes were seen in 34 of 36 patients with high concentration (94%): stiff-person syndrome (7), cerebellar ataxia (3), chronic epilepsy (9), limbic encephalitis (9), or an overlap of 2 or more of the former (6). Patients with low concentrations had a broad, heterogeneous symptom spectrum. Immunotherapy was effective in 19 of 27 treated patients (70%), although none of them completely recovered. Antibody concentration reduction occurred in 15 of 17 patients with available pre- and posttreatment samples (median reduction 69%; range 27%-99%), of which 14 improved clinically. The 2 patients with unchanged concentrations showed no clinical improvement. No differences in treatment responses were observed between specific syndromes. Conclusion: Most patients with high anti-GAD65 concentrations (>10 000 IU/mL) showed some improvement after immunotherapy, unfortunately without complete recovery. Serum antibody concentrations’ course might be useful to monitor response. In patients with low anti-GAD65 concentrations, especially in those without typical clinical phenotypes, diagnostic alternatives are more likely.

Commentary

Autoimmune encephalitis (AE) is a well-recognized etiology of seizures and epilepsy. There is an ever growing list of antibodies associated with AE, which can be directed against intracellular antigens or neuronal surface antigens. While classical intracellular antigens are located in the cell nucleus and are not involved in neuronal function, a few others including glutamic acid decarboxylase 65 kDa (GAD65) are expressed in the presynaptic terminals of neurons. GAD65 is found throughout the central nervous system, including the cerebral cortex, hippocampus and cerebellum, and in pancreatic cells. GAD65 antibodies have been associated with a variety of autoimmune neurological syndromes, from stiff-person syndrome and cerebellar ataxia to limbic encephalitis (LE) and epilepsy. However, the pathogenic role of the antibodies has often been questioned due to variability of the clinical presentation and the high prevalence of GAD65 antibodies in patients without any neurological manifestation. They are found in up to 80% patients with type 1 diabetes mellitus. Also, additional antibodies with a clear pathogenic effect, such as γ-aminobutyric acid (GABA) B or GABAA receptor, can be found in association with GAD65 antibodies in patients with autoimmune neurological manifestations. Finally, response to immunotherapy is often disappointing, further questioning their pathogenic significance. A first clue to reconcile these discrepancies is that patients with clear autoimmune neurological syndromes and GAD65 antibodies seem to have very high serum titers (>2000 IU/mL with radioimmunoassay [RIA]) or intrathecal synthesis of the antibodies, as opposed to patients without neurological manifestations. Table 1 summarizes the key features of well-characterized cases of patients with epilepsy or LE and high titers of GAD65 antibodies reported in the literature.
| Authors          | Case presentation | Age (range) | F:M ratio | Autoimmune diseases and Ab | Associated neoplasm | Treatment | Treatment response | MRI | CSF               |
|------------------|-------------------|-------------|-----------|-----------------------------|---------------------|-----------|--------------------|-----|-------------------|
| Peltola et al9   | TLE (2)           | 46-50       | 1:1       | Thyroid (2)                 | None                | Not attempted | NA                 | NA  | Elevated protein (1) |
| Peltola et al9   | TLE (2)           | 46(13-70)   | 5:1       | DM1: 5 Thyroid: 4 SLE (1)   | SCLC (1) with LE   | IV steroids + IVIG (1) | Seizure freedom? | Encephalitis (2) | Normal (1) |
| Malter et al7    | LE (8)            | 23 (17-66)  | 7:2       | DM1                          | None                | IV steroids ± oral steroids (8) | Seizure free (0) | LE (9) | MTLS (3)          |
| Liimatainen et al5 | TLE (7)          | 48 (31-71)  | 5:2       | Thyroid (5)                 | None                | NA        | NA                 | MTLS (3) | NA               |
| Borovsz et al11  | LE (3)            | 64 (57-70)  | 1:2       | GABABR (3)                  | SCLC (2)            | Steroids (3) | Recovery (1)      | Normal (3) | Pleocytosis (1)   |
| Borovsz et al11  | TLE (4)           | 35 (20-44)  | 6:0       | DM1: 1 Coeliac disease: 2    | SCLC 4 (all had LE and with GABAAR or GABABR antibodies) | NA        | NA                 | NA  | NA                |
| Borovsz et al11  | TLE (12)          | 24 (8-47)   | 11:2      | DM1 (2)                      | None                | IV ± oral steroids (10) | Seizure free (1) | LE (13) | MTLS (1)          |
| Li et al10       | LE (4)            | 35 (27-49)  | 5:1       | Thyroid (1)                 | Thymoma (1)         | IV ± oral steroids (5) | Seizure free (2) | LE (4) | MTLS (1)          |
| Li et al10       | LE (15)           | 30 (2-63)   | 31:4      | DM1 (8) Thyroid (9)          | NA                  | IVIG (28) | Seizure free (7) | LE (15) | Pleocytosis (6) |
| Muiñoz-Lopetegi et al14 | LE (9)     | NA         | NA        | NA                           | NA                  | NA        | NA                 | NA  | NA                |

Abbreviations: Ab, antibodies; CA, cerebellar ataxia; CPA, cyclophosphamide; CSF, cerebrospinal fluid; DM1, type 1 diabetes mellitus; DN, downbeat nystagmus; F, female; GABA, y-aminobutyric acid; IV, intravenous; IVIG, intravenous immunoglobulin; LE, limbic encephalitis; M, male; MG, myasthenia gravis; MMF, mycophenolate mofetil; MRI, magnetic resonance imaging; MTLS, mesial temporal lobe sclerosis; MTX, methotrexate; NA, not available; OCB, oligoclonal bands; ON, optic neuritis; PLEX, plasma exchange; RTX, rituximab; SLE, systemic lupus erythematosus; SPS, stiff-person syndrome; TLE, temporal lobe epilepsy; MS, multiple sclerosis; RA, rheumatoid arthritis; SCLC, small cell lung carcinoma.
To further clarify this relationship between antibodies level and clinical presentation, the authors conducted a retrospective review of patients with neurological symptoms whose serum or cerebrospinal fluid (CSF) tested positive for GAD65 antibody at their institution. They identified 71 patients, of whom 56 were available and agreed to participate.

Different methods exist to detect and characterize antibodies, each with their pros and cons. One of the strengths of the study is that the authors used a combination of these methods. Enzyme-linked immunosorbent assay (ELISA) allows for the direct quantification of antibody levels, replacing the RIA used in prior studies. Immunohistochemistry on hippocampal tissue only provides semiquantitative titers and is less specific, as any antibody present in the sample can bind to the slice, although the latter issue can be circumvented by the identification of specific staining patterns. Finally, cell-based assay (CBA), while also semiquantitative, is the most sensitive method and provides indisputable evidence of the specificity of the antibodies for the target antigen.

The authors found a high concordance between methods, with high ELISA levels (cutoff: >10,000 IU/mL in serum and >100 IU/mL in CSF) associated with intracerebral hemorrhage (ICH) and CBA detection in all CSF samples and in nearly all serum samples. Other antibodies were identified in a minority of patients: 2 patients had GABAB receptor antibodies (1 with low and 1 with high GAD65 antibody levels) and 1 had glycine receptor antibodies (and low GAD65 antibody level).

Thirty-six patients had a high serum (>10,000 IU/mL) or CSF (>100 IU/mL) level. They all presented typical syndromes, including LE (N = 9), epilepsy (N = 9), stiff-person syndrome (N = 7), and cerebellar ataxia (N = 3). Almost a third of them had overlapping manifestations of 2 or more of the different syndromes, either concomitantly or developing at an interval of several years. One patient with LE had coexisting GABAB receptor antibodies. Most patients had normal magnetic resonance imaging and normal CSF cytology and protein levels but oligoclonal bands were often present in the CSF.

In contrast, patients in the low-level group (N = 20) had more variable neurological manifestations, including nonspecific gait disorders, poorly characterized stiffness, and peripheral nerve disorders. Twelve had an obvious alternative etiology than AE. Ten had seizures or were diagnosed with epilepsy, of which 1 was attributed to glioblastoma and 3 were due to LE (1 with GABAB receptor antibody and 2 seronegative). Six thus had seizures or epilepsy from unknown etiology. Some of them had quite high serum and CSF antibody levels (up to 6580 IU/mL and 81 UI/mL, respectively). Thus, while their levels were below the cutoff values, I wonder if some of these patients could still have autoimmune GAD65-related encephalitis. No patient in the low-level group had overlapping syndromes, suggesting that this might be a specific feature of pathogenic GAD65 antibodies.

Four patients in the high-level group and 1 in the low-level group were considered paraneoplastic. The associated tumors were not the typical lung carcinoma but included lymphoma, testicular, breast, pancreatic, and prostate cancers.

Twenty-seven patients in the high-titer group received immunotherapy. Most (26/27) received intravenous immunoglobulins (IVIG). Eight patients received intravenous (IV) steroids, 3 received plasma exchange, 3 received rituximab, and 1 received cyclophosphamide. Chronic immunotherapy consisted of azathioprine (n = 9), oral steroids (n = 8), mycophenolate mofetil (n = 5), and cyclosporine (n = 1). Clinical improvement was observed in 1 of 27 patients. However, only 2 of 17 patients with LE or epilepsy achieved seizure freedom, and relapses were frequent. So, while some patients responded, most did not fully recover, suggesting either a chronic autoimmune process or postinflammatory irreversible structural injury. In line with the latter, some patients with GAD65 antibodies develop hippocampal sclerosis (Table 1), and histological studies have demonstrated the presence of inflammatory cytotoxic T cells in brain tissue of patients with GAD65 antibodies, similar to patients with onconeural antibodies.

A reduction in serum antibody titer was observed in 15 of 17 patients in which pre-and posttreatment sera were available. Of those, 14 of 15 improved, while the 2 patients in whom titers remained stable did not. There was a temporal relation between clinical improvement—including a decrease in seizure frequency—and antibody titers. This lends further credence to the pathogenic role of the antibodies and suggests that serum levels might be used to follow response to treatment.

Nine patients in the low-level group also received immunotherapy, mostly IVIG and IV steroids. While detailed outcome data were not available, most patients were reported to improve and a few completely recovered. This suggests that, although the pathogenic role of GAD65 antibodies is questionable, these patients still had an autoimmune disorder. My view is that, in patients with suggestive neurological syndromes, the presence of GAD65 antibodies could be seen as a nonspecific marker of autoimmunity and should motivate the search for additional, possibly still unknown, pathogenic antibodies and a trial of immunotherapy. In the presence of nonspecific neurological manifestations, I would simply regard them as a spurious finding.

Altogether, this study lends further support to the clinical significance of high serum levels or presence in the CSF of GAD65 antibodies in patients with autoimmune neurological disorders, including LE and autoimmune epilepsy. In the latter group, the latency between the onset of seizures and diagnosis sometimes exceeds 2 decades in published series, and hippocampal sclerosis may occur. It is thus likely that we all follow at least 1 patient with longstanding drug-resistant temporal lobe epilepsy, which in fact has autoimmune epilepsy with GAD65 antibodies. The identification of GAD65 antibodies should not deter the clinician from looking for additional antibodies. In particular, GABAA or GABAB receptor antibodies might coexist, and they have clinical implications, as they seem to increase the risk of an underlying neoplasm.

By Nicolas Gaspard.
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