Paraneoplastic Neurological Syndromes and Glutamic Acid Decarboxylase Antibodies

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IMPORTANCE Little is known of glutamic acid decarboxylase antibodies (GAD-abs) in the paraneoplastic context. Clinical recognition of such cases will lead to prompt tumor diagnosis and appropriate treatment.

OBJECTIVE To report the clinical and immunological features of patients with paraneoplastic neurological syndromes (PNS) and GAD-abs.

DESIGN, SETTING, AND PARTICIPANTS Retrospective case series study and immunological investigations conducted in February 2014 in a center for autoimmune neurological disorders. Fifteen cases with GAD65-abs evaluated between 1995 and 2013 who fulfilled criteria of definite or possible PNS without concomitant onconeural antibodies were included in this study.

MAIN OUTCOMES AND MEASURES Analysis of the clinical records of 15 patients and review of 19 previously reported cases. Indirect immunofluorescence with rat hippocampal neuronal cultures and cell-based assays with known neuronal cell-surface antigens were used. One hundred six patients with GAD65-abs and no cancer served as control individuals.

RESULTS Eight of the 15 patients with cancer presented as classic paraneoplastic syndromes (5 limbic encephalitis, 1 paraneoplastic encephalomyelitis, 1 paraneoplastic cerebellar degeneration, and 1 opsoclonus-myoclonus syndrome). When compared with the 106 non-PNS cases, those with PNS were older (median age, 60 years vs 48 years; \( P = .03 \)), more frequently male (60% vs 13%; \( P < .001 \)), and had more often coexisting neuronal cell-surface antibodies, mainly against \( \gamma \)-aminobutyric acid receptors (53% vs 11%; \( P < .001 \)). The tumors more frequently involved were lung (n = 6) and thymic neoplasms (n = 4). The risk for an underlying tumor was higher if the presentation was a classic PNS, if it was different from stiff-person syndrome or cerebellar ataxia (odds ratio, 10.5; 95% CI, 3.2-34.5), or if the patient had coexisting neuronal cell-surface antibodies (odds ratio, 6.8; 95% CI, 1.1-40.5). Compared with the current series, the 19 previously reported cases had more frequent stiff-person syndrome (74% vs 13%; \( P = .001 \)) and better responses to treatment (79% vs 27%; \( P = .005 \)). Predictors of improvement in the 34 patients (current and previously reported) included presentation with stiff-person syndrome and the presence of a thymic tumor.

CONCLUSIONS AND RELEVANCE Patients with GAD-abs must be screened for an underlying cancer if they have clinical presentations different from those typically associated with this autoimmunity or develop classic PNS. The risk for cancer increases with age, male sex, and the presence of coexisting neuronal cell-surface antibodies.
High serum levels of antibodies to the synaptic enzyme glutamic acid decarboxylase (GAD-abs) is a very sensitive biomarker of stiff-person syndrome (SPS) and have also been described in subgroups of patients with limbic encephalitis (LE),1 cerebellar ataxia,2 epilepsy, and isolated cases of palatal tremor, as well as downbeat or periodic alternating nystagmus.3 Patients with neurological syndromes associated with GAD-abs are not considered at risk for cancer and extensive search for a tumor is not indicated unless they harbor additional onconeural antibodies. However, there are case reports of patients with GAD-abs whose cancer was identified by the time of the neurological diagnosis, suggesting a paraneoplastic mechanism.4,5 Whether these cases represent a casual association or a true GAD-ab–associated paraneoplastic neurological syndrome (PNS) is unclear.

The discovery of antibodies against neuronal cell-surface receptors and synaptic antigens in patients with encephalitis adds complexity to the study of GAD-ab–associated neurological syndromes. Patients with LE may have coexistent GAD-abs and antibodies against the γ-aminobutyric acid (GABA) b receptor, and this association seems more frequent in patients with cancer.6 A systematic determination of neuronal cell-surface antibodies has not been done in patients with GAD-abs and suspected PNS.

In this study, we retrospectively examined a cohort of patients with clinical criteria of definite or possible PNS but without onconeural antibodies in whom GAD-abs were identified during investigations for a paraneoplastic etiology. In addition, we performed a systematic review of previously reported cases of GAD-ab–associated PNS. The aims of this study were to describe the PNS and tumor types associated with GAD-abs, the occurrence of additional neuronal cell-surface antibodies, and the neurological response to cancer treatment and immunotherapy, as well as to provide the more frequent GAD-abs clinical settings in which a tumor screening is warranted.

Methods

Patients

In February 2014, we retrospectively identified patients examined between 1995 and 2013 with definite or possible diagnosis of PNS according to the PNS Euronetwork criteria,7 whose serum samples were sent to our laboratory for the determination of onconeural antibodies but routine immunohistochemistry on paraformaldehyde-perfused brain tissue revealed GAD-ab reactivity (a positive brain tissue serum reactivity indicates high GAD-ab levels, usually >2000 U/mL when determined by radioimmunooassay).3 In all samples with evidence of GAD-ab reactivity, the presence of GAD-abs was subsequently confirmed by radioimmunooassay. All patients were seen by at least 1 of the authors. Basic clinical information was obtained from medical records and additional information was collected through a structured questionnaire focused on symptom presentation, type of tumor, and response to immunotherapy and tumor treatment. Neurological disability was measured by the modified Rankin Scale8; a change of 1 point was required to define improvement or symptom progression.

To compare the findings of our patients with those of previously reported cases, we performed a comprehensive PubMed search using the terms GAD antibodies AND cancer and identified all cases until January 1, 2015. Only cases published in English that included clinical information were included. Articles were also identified by searches of the authors’ files.

To define the frequency of a paraneoplastic etiology among different GAD-ab–associated neurological syndromes, we searched in our database for all cases diagnosed as having GAD-ab–associated neurological syndromes without cancer during the same period as the PNS cases were diagnosed. In total, 106 patients were identified, including 39 with cerebellar ataxia, 32 with SPS, 18 with isolated epilepsy, and 17 with LE (eFigure in the Supplement).

Patients’ serum and cerebrospinal fluid (CSF) samples are deposited in the collection of biological samples named Neuroinmunología registered in the Biobank of Institut d’Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS). Written informed consent for the storage and use of the samples for research purposes was obtained from all patients. The study was approved by the ethics committee of the Hospital Clinic of Barcelona, Barcelona, Spain.

Autoantibody Assays

Levels of GAD65-abs were detected by enzyme-linked immunosorbent assay (RSR Limited) using a commercial kit following the manufacturer’s specifications. Because GAD65 titers in neurologic syndromes are high, serum and CSF samples were titrated to determine the optimal dilution factor. Briefly, enzyme-linked immunosorbent assay wells were seeded for 1 hour with patients’ serum samples diluted 1:10 000 or CSF diluted 1:200, followed by 1-hour incubation with GAD65 biotinylated protein, and 20-minute incubation with streptavidin peroxidase. In addition, serum and CSF samples were tested for antibodies to intracellular and neuronal cell-surface antigens using brain immunohistochemistry, as previously reported.9,10 Onconeural antibodies to Hu, Yo, Ri, CV2, amphiphysin, and Ma1/2 were determined with immunoblot assays and GAD67, gephyrin (cotransfected 1:1 with collybistin), and neuronal cell-surface antibodies were investigated using in-house cell-based assays, including leucine-rich, glioma-inactivated 1, contactin-associated protein-like 2, GluN1/2B subunits of the N-methyl-D-aspartate receptor, GluR1 and 2 subunits of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate, B1 and B2 subunits of the GABAβR, α1 and β1 subunits of the GABAαR, and the α1 subunit of the glycine receptor (GlyR), as reported.11 All DNA sequences were purchased from Origene except those of GlyR, gephyrin, and collybistin (a gift of R. J. Harvey, PhD, Department of Pharmacology, University College London School of Pharmacy, London, England).

To demonstrate the expression of GAD65 and GAD67 in the tumor, paraffin sections were deparaffinized and the antigen retrieved, as reported.4 After inhibition of endogenous peroxidase with 0.3% hydrogen peroxide in phosphate-buffered saline for 15 minutes, sections were incubated with GAD65 (HybriMed Bank) or GAD67 (Abcam) monoclonal antibodies (diluted 1:1000) overnight at 4°C, and developed with the avidin-biotin peroxidase technique (Vector Laboratories).
The diagnosis of a tumor relapse after 10 years of remission in 10 patients and led to a neurological syndrome in 3 patients with GABA receptor antibodies (2 LE with SCLC and 1 thymic carcinoma), 2 patients with antinuclear antibodies, and 1 paraneoplastic syndrome. The neurological syndrome and cancer was 2.7 months (interquartile range, 1.2-4.5 months).

All patients received immunotherapy: 11 high-dose corticosteroids, associated with intravenous immunoglobulins in 6; 2 with isolated intravenous immunoglobulins; 1 with intravenous immunoglobulins combined with rituximab; and 1 with intravenous immunoglobulins followed by cyclophosphamide. In 3 patients, paraneoplastic cerebellar degeneration; PEM, paraneoplastic encephalomyelitis; PNS, paraneoplastic neurological syndrome; SCLC, small cell lung cancer. Table 1. Clinical and Immunological Features of Patients With and Without PNS With GAD Antibodies

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>PNS (n = 15)</th>
<th>Non-PNS (n = 106)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (range), y</td>
<td>60 (29-80)</td>
<td>48 (5-79)</td>
<td>.03</td>
</tr>
<tr>
<td>Female, No. (%)</td>
<td>6 (40)</td>
<td>88/102 (87)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Diabetes mellitus at onset, No./total No. (%)</td>
<td>2/13 (15)</td>
<td>31/74 (42)</td>
<td>.12</td>
</tr>
<tr>
<td>Other organ-specific autoimmune disorder, No./total No. (%)</td>
<td>4/13 (31)</td>
<td>43/75 (57)</td>
<td>.13</td>
</tr>
<tr>
<td>Clinical syndrome</td>
<td>Encephalitis (LE, n = 5)</td>
<td>6 (26)</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Cerebellar ataxia (PCD, n = 1)</td>
<td>4 (9)</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>Stiff-person syndrome</td>
<td>2 (6)</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Opsoclonus-myoclonus syndrome</td>
<td>1 (100)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>PEM*</td>
<td>1 (100)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Otherc</td>
<td>1 (100)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Isolated epilepsy</td>
<td>0 (0)</td>
<td>18</td>
</tr>
<tr>
<td>Serum, ×10⁵ U/mL</td>
<td>10.5 (1.2-31.9)</td>
<td>5.9 (2.9-13.2)</td>
<td>.79</td>
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<tr>
<td>CSF, ×10³ U/mL</td>
<td>3.5 (1.2-57.1)</td>
<td>7.5 (1.7-17.2)</td>
<td>.86</td>
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<tr>
<td>GAD67-ab, No./total No. (%)</td>
<td>8/13 (62)</td>
<td>93/106 (88)</td>
<td>.03</td>
</tr>
<tr>
<td>GAD67-ab, No./total No. (%)</td>
<td>4/7 (57)</td>
<td>61/100 (100)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Neuronal cell-surface antibodies, No./total No. (%)</td>
<td>8/15 (53)</td>
<td>12/106 (11)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Abbreviations: CSF, cerebrospinal fluid; GAD, glutamic acid decarboxylase; IQR, interquartile range; LE, limbic encephalitis; PCD, paraneoplastic cerebellar degeneration; PEM, paraneoplastic encephalomyelitis; PNS, paraneoplastic neurological syndrome.

Statistical Analysis
The Fisher exact test was used to assess proportions when the expected frequencies were small (<5). For multivariate analysis of the probability of paraneoplastic origin in the present series, a logistic regression, including factors with a P value of .10 or less in the univariate analysis, was used. P ≤ .05 was considered statistically significant. The software used was Stata version 13.1 (StataCorp).

Results
Fifteen patients with PNS and GAD-abs were identified. None of them had onconeural antibodies (Table 1). Eight of them fulfilled the criteria of definite PNS and 7 of possible PNS. The mean age was 60 years (range, 29-80 years) and 6 (40%) were women. The most frequent clinical syndromes included encephalitis (6 patients; 5 of them had typical LE) and cerebellar ataxia (4 patients; 1 of them had been previously reported). One patient fulfilled criteria of paraneoplastic cerebellar degeneration, whereas the remaining 3 had a slowly progressive course of the disease more suggestive of degenerative ataxia. Two additional patients developed SPS, 1 opsoclonus-myoclonus syndrome, 1 paraneoplastic encephalomyelitis (previously reported), and 1 syndrome that included vertigo, ataxia, axial rigidity, and dysautonomia (eTable 1 in the Supplement).

Six patients had lung cancer (4 of them small-cell lung cancer [SCLC]), 4 had neuroendocrine tumors (2 pancreas and 2 thymic carcinoids), 2 had thymoma, 2 had breast cancer, and 1 had non-Hodgkin lymphoma. The neurological syndrome antedated the diagnosis of the cancer in 10 patients and led to the diagnosis of a tumor relapse after 10 years of remission in another patient. The median delay between the diagnosis of another patient. The median delay between the diagnosis of the neurological syndrome and cancer was 2.7 months (interquartile range, 1.2-4.5 months).

All patients received immunotherapy: 11 high-dose corticosteroids, associated with intravenous immunoglobulins in 6; 2 with isolated intravenous immunoglobulins; 1 with intravenous immunoglobulins combined with rituximab; and 1 with intravenous immunoglobulins followed by cyclophosphamide. In addition, 10 patients (71%) had oncological treatment (surgery, chemotherapy, and radiotherapy alone or combined). Clinical follow-up was available for all 15 patients: 8 (53%) had clinically progressed (5 had died owing to PNS); 4 were stable; and 3 had neurological improvement (all 3 with neoplasms of the thymus; 2 thymomas and 1 thymic carcinoma) (eTable 1 in the Supplement).

Immunological Studies
Serum and CSF GAD65-ab levels were similar in patients with or without PNS. The frequency of GAD67-obs was significantly lower in patients with PNS, particularly in CSF (Table 1). Gephyrin antibodies were not detected. Neuronal cell-surface antibodies were detected in 8 of 15 patients (53%). The antibodies, type of associated syndrome, and tumor were as follows: 3 patients with GABAAbR antibodies (2 LE with SCLC and 1 paraneoplastic cerebellar degeneration with thymic carcinoma), 2 patients with GABAAbR antibodies (2 LE with SCLC and thymoma), 1 patient with GlyR antibodies (cerebellar ataxia and thymic carcinoma), and 2 patients with antibodies against unknown neuronal cell-surface antigens (2 epidermoid lung cancer with opsoclonus-myoclonus syndrome and ataxia and myoclonus).

Tumor expression of GAD was assessed in 3 patients, 2 of them were previously reported. The 3 cases had a neuroendocrine tumor (2 pancreatic and 1 thymic carcinoma) that were
found to express GAD65. Only one tumor was tested for expression of GAD67 and it was found to be positive (Figure 1).

Comparison With Patients With GAD-abs Without Cancer
Compared with the 106 patients with nonparaneoplastic GAD-ab–associated disorders, patients with PNS were older (median age, 60 years vs 48 years; \( P = .03 \)), were more frequently male (60% vs 13%; \( P < .001 \)), had more often coexistent neuronal cell-surface autoantibodies (53% vs 11%; \( P < .001 \)), and presented a different clinical profile (more classic PNS than SPS or cerebellar ataxia) (Table 1). Taking into account the clinical presentation, patients presenting with a syndrome different from SPS, cerebellar ataxia, or isolated epilepsy had a 10-fold increased risk for being paraneoplastic (odds ratio, 10.5; 95% CI, 3.2-34.5). Similarly, the detection of neuronal cell-surface antibodies carried a 7-fold increased risk for the presence of an underlying tumor (odds ratio, 6.8; 95% CI, 1.1-40.5). In a multivariate analysis that included age, sex, and the presence of neuronal cell-surface antibodies, the clinical presentation remained the most robust predictor of a paraneoplastic origin (odds ratio, 33.2; 95% CI, 5.0-220.2).

Comparison With Previously Reported GAD-ab–Associated PNS
A literature search identified 23 patients with PNS and isolated GAD-abs. Three patients were excluded from analysis because the period between the development of PNS and tumor diagnosis was unknown or longer than 2 years.7 An-
other patient was excluded because the neurological syndrome (SPS) occurred after autologous bone marrow transplantation for multiple myeloma, raising the possibility of an abnormal immunoreconstitution rather than PNS as the cause of SPS.15 The clinical information of the remaining 19 patients is summarized in eTable 2 in the Supplement.16-34

Among these 19 patients, 53% were female. Stiff-person syndrome was the most common neurological syndrome (74%), with a remarkable frequency of focal forms (36%). By contrast, none of the patients developed LE, which was the most prevalent syndrome in the present series. The distribution of associated tumors was also different. Thymic tumors were the most frequent neoplasm (6 patients), followed by lung cancer (4 patients) and breast cancer (4 patients). Compared with the present series, the previously reported cases appeared to have better outcomes (79% vs 27%; P = .005; Table 2).

When considering together the 15 current PNS cases and the 19 previously reported, the probability of clinical improvement was greater in patients with thymic tumors, either benign or malignant (100% vs 38% other tumors; P = .001) and in those with SPS (75% vs 39% other syndromes; P = .03). Survival curves are shown in Figure 2.

Discussion

In some neurological syndromes with antibodies different from those strongly associated with cancer (onconeural antibodies), the immune response can be occasionally triggered by an underlying tumor and the syndrome is considered paraneoplastic. An example is LE, which, when it is associated with GABAbR-abs, may be idiopathic (autoimmune) or caused by an immune response against a tumor, usually SCLC, that expresses GABAbR.5,11 Patients with SPS, cerebellar ataxia, or other neurological syndromes that typically associate with GAD-abs rarely have cancer and therefore an aggressive or repeated tumor search is not indicated. However, our study showed that when GAD-abs occur in patients with LE or other classic PNS (paraneoplastic cerebellar degeneration, opsoclonus-myoclonus syndrome, or paraneoplastic encephalomyelitis)7 the risk for cancer is 10-fold higher than that in patients with SPS or cerebellar ataxia and the workup for a tumor is mandatory. The role of the tumor as a trigger of the immune response is supported by the demonstration of GAD65 in the tumors of these patients.4,5 The higher frequency of classic PNS in our series compared with the predominance of SPS in previously published cases probably reflects the fact that our laboratory receives samples from patients with a wide spectrum of neurological syndromes, not only from those typically related to GAD-abs.

A second important observation of our study was that the probability of an underlying cancer was 7 times higher in patients with GAD-abs and coexisting antibodies against neuronal cell-surface antigens. Therefore, the determination of these antibodies in patients with GAD-abs is indicated particularly in cases of LE or cerebellar ataxia. The neuronal cell-surface antibodies predominantly identified were against GABA re-
ceptors. γ-Aminobutyric acid bR antibodies usually occur in patients with LE and 58% of patients with these antibodies associate with cancer, mainly SCLC. γ-Aminobutyric acid aR and GlyR antibodies have been reported in a few patients with thymoma. However, it is important to keep in mind that with the exception of GABAbR-absthatareoftendetectedinGAD-ab patients with cancer, the antibodies against GABAaR or GlyR are more frequently found in nonparaneoplastic patients with GAD-abs.

The analysis of our series and the cases previously reported identified 3 subgroups of patients in whom GAD-ab–associated syndromes can be paraneoplastic. The first group included patients with classic PNS or neurological syndromes not usually associated with GAD-abs (38%; 13 of 34 patients). These patients had additional neuronal cell-surface antibodies (46%) and lung cancer was the most frequent tumor (46%), whereas thymomas were rare (15%); only 42% of patients in this group improved with treatment. Limbic encephalitis was the most frequent PNS. It is usually considered that LE associated with GAD-abs often occurs in young women with a predominant or isolated epileptic syndrome and it is not paraneoplastic. However, our study indicated that patients with LE and GAD-abs may have a tumor, usually an SCLC, and that this possibility is higher if LE occurs in older men.

The second group included patients with SPS and represented 47% (16 of 34) of all cases. Unlike patients of the first group, thymomas and breast cancer accounted for 53% of the tumors and 75% responded to therapy. Although patients with classic PNS and GAD-abs are more likely to be men, this is not the case in the subgroup of paraneoplastic SPS, where 69% of patients were women. The association of SPS with thymoma probably reflects the propensity of this tumor to induce a variety of autoimmune disorders and circulating autoantibodies. There is no evidence that thymoma cells express GAD65 or GAD67; therefore, the possible pathogenic mechanisms remain unclear. Patients with SPS and breast cancer usually harbor amphiphysin antibodies rather than GAD-abs. Some of the patients with SPS reported with GAD-abs and breast can-

Figure 2. Kaplan-Meyer Survival Curves of 34 Patients With Paraneoplastic Glutamic Acid Decarboxylase Syndromes by Tumor and Neurological Syndrome
cer also had type 1 diabetes mellitus and other organ-specific autoimmunities commonly seen in idiopathic SPS, therefore, the diagnosis of the breast cancer, a very frequent tumor, could be a coincidence. On the other hand, GAD65 is expressed in breast cancer cells and the GABABR pathway is implicated in breast cancer cell invasion and migration.\(^4\) This observation supports the possibility that GAD may act as a tumor antigen and thus trigger, in some patients, an immune response leading to the development of SPS.

The third group included patients with cerebellar ataxia or progressive encephalomyelitis with rigidity and myoclonus that is usually associated with GAD-abs without cancer (15%; 5 of 34 patients). The most frequent tumors were lung and breast cancer, and 2 of the 5 patients improved. These patients, as those with classic PNS, were more frequently men (80%).

A previous study on patients who underwent extensive paraneoplastic screening, including also GAD autoimmunity, identified 62 patients with GAD-abs, none of them with cancer.\(^4\) Our experience also indicated that paraneoplastic GAD autoimmunity is infrequent\(^2,3\) but this possibility cannot be overlooked.

**Conclusions**

Patients with high levels of GAD-abs (in our setting >2000 U/mL by radioimmunoassay) and classic PNS or neurological syndromes not typically associated with GAD-abs should be screened for an underlying cancer. Considering the tumors identified in the current series and previous reported cases, the tumor workup should include mammogram and chest computed tomography or computed tomography–positron emission tomographic scan, depending on the clinical setting. The cancer risk increases with age, male sex, and presence of concomitant antibodies against neuronal cell-surface antigens.

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