Spectrum of neurological syndromes associated with glutamic acid decarboxylase antibodies: diagnostic clues for this association

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The association of high levels of autoantibodies to glutamic acid decarboxylase (GAD-ab) and stiff-person syndrome (SPS) is well known. However, the full spectrum of neurological syndromes associated with GAD-ab is not well established. In addition, these patients usually present type I diabetes mellitus (DMI) that could justify the presence of high GAD-ab levels. To clarify these issues, we reviewed the clinical and immunological features of patients in whom high GAD-ab levels were detected in a reference centre for DMI and for the detection of antineuronal antibodies in suspected paraneoplastic neurological syndromes (PNS). High GAD-ab levels were defined as values ≥2000 U/ml by radioimmunoassay. Intrathecal synthesis (IS) of GAD-ab was calculated in paired serum/CSF samples. Values higher than the IgG index were considered indicators for positive GAD-ab-specific IS. High GAD-ab levels were identified in 61 patients, 22 (36%) had SPS, 17 (28%) cerebellar ataxia, 11 (18%) other neurological disorders (epilepsy—four, PNS—four; idiopathic limbic encephalitis—two; myasthenia gravis—one), and 11 (18%) isolated DMI. Patients with SPS and cerebellar ataxia had the same frequency of female gender (86% vs 94%), DMI (59% vs 53%), CSF oligoclonal bands (35% vs 69%). Three of the four PNS patients, with paraneoplastic encephalomyelitis, a predominant gait cerebellar ataxia, and limbic encephalitis, had neuroendocrine carcinomas. GAD expression was confirmed in the two tumours in which the study was done. The fourth patient presented with paraneoplastic cerebellar degeneration antedating a lung adenocarcinoma. The frequency of increased IS of GAD-ab was 85% in SPS, 100% in cerebellar ataxia, and 86% in other neurological disorders. In conclusion, our study emphasizes that high GAD-ab levels associate with other neurological disorders besides SPS. Cerebellar ataxia, the second most common syndrome associated with high GAD-ab levels, shares with SPS the same demographic, clinical and immunological features. The demonstration of an increased IS of GAD-ab is important to confirm that the GAD autoimmunity is related to the neurological syndrome particularly when there is a concomitant DMI that could justify the presence of high GAD-ab levels. Lastly, in patients who develop neurological syndromes that suggest a PNS, the finding of GAD-ab does not rule out this possibility and appropriate studies should be done to confirm an underlying cancer.

Keywords: glutamic acid decarboxylase; stiff-person syndrome; cerebellar ataxia; paraneoplastic neurological syndromes; diabetes mellitus

Abbreviations: DMI = Type I diabetes mellitus; ELISA = enzyme-linked immunosorbent assay; GAD = Glutamic acid decarboxylase; GAD-ab = Autoantibodies to GAD; IS = Intrathecal synthesis; IVIg = Intravenous immunoglobulins; LE = Limbic encephalitis; PNS = Paraneoplastic neurological syndromes; RIA = Radioimmunoassay; SCLC = Small cell lung cancer; SPS = Stiff person syndrome

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Introduction

Glutamic acid decarboxylase (GAD) is the rate-limiting enzyme for the synthesis of γ-aminobutyric acid (GABA), the major inhibitory neurotransmitter in the CNS. The enzyme is selectively expressed in GABA-ergic neurones and in pancreatic β-cells. GAD is a major autoantigen in type 1

diabetes mellitus (DM1), and autoantibodies to GAD (GAD-ab) are detected in about 80% of newly diagnosed DM1 patients (Baekkeskov *et al.*, 1990; Solimena and De Camilli, 1991). High GAD-ab levels, usually more than 100-fold higher that those found in DM1 (Baekkeskov *et al.*, 1990; Kim *et al.*, 1994), are present in up to 80% of patients with stiff person syndrome (SPS) (Walikonis *et al.*, 1998; Meinck *et al.*, 2001), a subgroup of patients with late onset isolated cerebellar ataxia usually associated with DM1 or polyendocrine autoimmunity (Honnorat *et al.*, 2001), and a few patients with epilepsy (Peltola *et al.*, 2000), nystagmus (Antonini *et al.*, 2003), palatal tremor (Nemni *et al.*, 1994) and brainstem dysfunction (Pittock *et al.*, 2006).

SPS is a rare CNS disorder characterized by progressive muscular rigidity, predominantly of the trunk muscles, with superimposed spasms (Lorish and Thorsteinsson, 1989). The syndrome is frequently associated with other autoimmune diseases, mainly DM1, and with a high incidence of organ-specific autoantibodies (Solimena and De Camilli, 1991). The diagnosis of SPS is based on established clinical and neurophysiological features that show a continuous co-contraction of agonist and antagonist muscles caused by involuntary firing of motor units at rest (Lorish and Thorsteinsson, 1989; Thompson, 1994). GAD-ab are not a requirement for the diagnosis. By contrast, in other neurological syndromes, particularly cerebellar ataxia, the presence of GAD-ab is used to confirm a possible autoimmune pathogenesis of the syndrome (Saiz *et al.*, 1997; Honnorat *et al.*, 2001). However, in many instances the neurological syndrome occurs in the setting of DM1 or polyendocrine autoimmunity that by itself may associate with high GAD-ab levels (Costa *et al.*, 2002).

GAD-ab are detected in many laboratories by radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA). The main target of these techniques are DM1 patients or subjects at risk for DM1 and the assays can detect very low levels of GAD-ab (Schmidli *et al.*, 1995; Walikonis and Lennon, 1998). The diagnostic value of low titres of GAD-ab in a patient with a neurological syndrome is unknown. Another way to identify GAD-ab is to screen sera by immunohistochemistry and confirm the positive cases by RIA. This approach only identifies sera with high GAD-ab levels that are rarely present in patients with DM1 or autoimmune polyendocrine syndrome without neurological disease (Solimena *et al.*, 1990; Saiz *et al.*, 1997). Previous studies suggested that GAD-ab of DM1 patients can be distinguished from those of SPS patients because the later recognize linear epitopes that can be identified by immunoblot (Baekkeskov *et al.*, 1990; Kim *et al.*, 1994; Daw *et al.*, 1996). However, GAD-ab were identified by immunoblot in patients with DM1 or with polyendocrine autoimmunity without associated neurological disease (Baekkeskov *et al.*, 1990; Solimena *et al.*, 1990; Honnorat *et al.*, 2001; Vianello *et al.*, 2005). Presently, there are no assays that unambiguously distinguish GAD-ab of patients with neurological syndromes.

We undertook the present study with the following aims: (i) to review the clinical features of patients with high GAD-ab levels detected in our hospital that is a reference centre for detection of GAD-ab in diabetic patients and antineuronal antibodies in patients with suspected PNS, and (ii) to provide guidelines on the value of high GAD-ab levels in patients with neurological syndromes different from SPS.

Methods

Patients

We selected 47 patients whose serum or CSF was sent to the Hospital Clinic's Neurology Laboratory for detection of antineuronal antibodies between January 1994 and December 2007 and presented GAD-ab reactivity by immunohistochemistry. The clinical information was obtained from written questionnaires by the referring neurologist, telephone interviews and review of the clinical records. Twelve (24%) patients were seen personally by at least one of the authors. Clinical and/or serological evidence of organ-specific autoimmunity and the relation with the neurological syndrome was also recorded. On the basis of the information received, patients were classified in one of the following groups: SPS, cerebellar ataxia and other neurological disorders. The diagnosis of SPS was based on clinical and neurophysiological criteria (Lorish and Thorsteinsson, 1989). Patients who fulfilled the criteria except for the distribution of the affected muscles that were restricted to one limb (focal form of SPS) (Saiz *et al.*, 1998, 1999) were also included. Criteria for GAD-associated cerebellar ataxia included a predominant or exclusive cerebellar dysfunction at presentation, and absence of another diagnosis that could explain the cerebellar disorder.

To know the frequency among DM1 patients of high GAD-ab levels, in the range observed in patients with SPS or other neurological syndromes, we reviewed the patients in whom the detection of GAD-ab by RIA was requested to the Hospital Clinic's Hormonal Laboratory because the diagnosis of diabetes. Between January 2003 and December 2006, 20 out of 2447 samples (0.8%) from 14 patients had high GAD-ab levels defined by a RIA value ≥2000 U/ml (see below). We reviewed the medical records of the 14 patients to identify associated neurological disorders. The study was approved by the Ethic Committee of the Hospital Clinic.

Detection of GAD-ab

GAD-ab were analysed in serum and CSF, when available, by immunohistochemistry and the positive cases confirmed by RIA. In patients with isolated diabetes or polyendocrine syndromes, GAD-ab were directly analysed by RIA.

Immunohistochemistry. GAD immunoreactivity was analysed (serum screening dilution 1:500; CSF undiluted) using an avidin-biotin technique on paraformaldehyde-fixed frozen rat tissues as described previously (Saiz *et al.*, 1997).

RIA

GAD-ab were measured by RIA using a commercial kit (CIS Biointernational, France) following the manufacturer's instructions (Saiz *et al.*, 1997). Briefly, 20 µl of standards (SPS serum containing GAD-ab at different dilutions expressed in arbitrary units, U/ml), and serum samples, were incubated with 50 ml of ^{125}I-labelled human recombinant GAD65 for 2 h at room temperature.

Then, 50 µml of protein A-sepharose was added, and the mixture incubated for 1 h at room temperature. After centrifugation at 1500g for 30 min at 4°C, the precipitates were counted for ^{125}I with a gamma scintillation counter. The results were interpolated in the standard curve constructed using the dilutions of the positive control serum. For each serum, conditions were established in that appropriate dilutions of the serum produced results that were in the straight line portion of the standard curve. All samples were tested in duplicate. This assay has a sensitivity and specificity of 84% and 92%, respectively, for the diagnosis of diabetes mellitus, considering as positive result >0.5 U/ml in the Diabetes Autoantibodies Standarization Program of 2005.

Definition of high GAD-ab level

We arbitrarily defined high GAD-ab levels as those that show a GAD pattern on immunohistochemistry. To analyse the correlation between GAD-ab RIA values and the presence of GAD labelling in the immunohistochemistry, serum samples from 11 patients with SPS or cerebellar ataxia and 17 with DM1, were analysed by immunohistochemistry by two investigators (A.S. and F.G.) without knowledge of the GAD-ab RIA values. Only serum samples with GAD-ab RIA values ≥2000 U/ml produced the characteristic GAD-ab pattern by immunohistochemistry. Therefore, a high GAD-ab level was defined by a RIA value ≥2000 U/ml.

IS of GAD-ab

Paired serum and CSF samples, when available, were used to analyse the total albumin and IgG values, IgG index and presence of oligoclonal IgG bands by isoelectrofocusing. The index for IS of GAD-ab was calculated using the values obtained by immunohistochemistry, based on the following formula:

$$\frac{\text{CSF GAD} - \text{ab titre/Serum GAD} - \text{ab titre}}{\text{CSF albumin (mg/l)/Serum albumin (mg/l)}}$$

Values higher than the IgG index, particularly higher than 1, are strong indicators for IS of antibody-specific IgG (Dalakas et al., 2001). To know whether the amount of GAD-ab-specific IS in patients with SPS and in patients with cerebellar ataxia was different, the median CSF/Serum GAD-ab titre ratio to the median CSF/Serum IgG ratio expressed as x-fold of GAD-ab IS was calculated as previously reported (Rakocevic et al., 2006).

Results

High GAD-ab levels (≥2000 U/ml by RIA) were identified in 61 patients, 47 of them from the Neurology Laboratory and 14 from the Hormonal Laboratory. Fifty (82%) patients had a neurological disorder (SPS 22, cerebellar ataxia 17, other neurological disorders 11), and 11 (18%) isolated DM1. In 47 of the 50 patients with neurological syndromes, GAD-ab were initially detected by immunohistochemistry and confirmed by RIA. In the other three patients, high GAD-ab levels were found when requested for the recent diagnosis of DM1 and the review of the clinical records showed a previous history of epilepsy.

Eleven patients with high GAD-ab levels (mean RIA value 16 147 U/ml; range 2050–44 487 U/ml) had DM1 without any neurological disorder (median time since DM1 diagnosis

5 years; range 2–26 years). The median age at diagnosis was 38 years (range 8–72 years). Ten patients were female and seven (64%) had other autoimmune endocrine syndromes. The observation that patients with high GAD-ab levels may not develop a neurological disorder was further confirmed during the follow-up (median 14 years; range 9–28 years) of nine patients with DM1 and high GAD-ab levels included in our previous study (Honnorat et al., 2001). CSF could not be obtained in these patients to analyse the presence of oligoclonal IgG bands or IS of GAD-ab.

SPS

The clinical and immunological features of the 22 SPS patients are summarized in Table 1. The mean age at diagnosis was 56 years (range 14–77 years) and 19 (86%) patients were female. Four (18%) patients had a focal form of SPS (the detailed description of two patients was reported previously; Saiz et al., 1998), three of them limited to one leg for more than 8 years and the fourth limited to one arm during the 4 years of follow-up. CSF oligoclonal IgG bands were present in 35% of the patients and IS of GAD-ab was detected in 85% of the patients analysed.

Cerebellar ataxia

Gad-ab-associated cerebellar ataxia was diagnosed in 17 patients, and 94% were female. Six patients were included in a previous European series of cerebellar ataxia and GAD-ab (Honnorate et al., 2001) (Table 1). The mean age at diagnosis was 59 years (range 39–77 years). Seven (41%) of the 17 patients presented a cerebellar ataxia with a sub-acute evolution in a few weeks to less than 6 months. The cerebellar syndrome progressed more slowly, between 1 and 6 years, suggesting a degenerative disease, in 10 patients. The main cerebellar sign, which was present in all patients, was gait ataxia that was scored, as previously described (Honnorate et al., 2001), as moderate or severe in 11 and mild in one patient. Limb ataxia was present in 10 patients, but it was moderate or severe in only two. Dysarthria and nystagmus was observed in 11 patients. Associated neurological symptoms at presentation were rigidity in the legs (two patients) and myasthenia gravis (two patients). During the follow-up (median 8 years; range 0.5–21 years), one patient developed stiffness in both legs that required treatment with intrathecal baclofen. Two patients had breast cancer 1–4 years before the onset of the cerebellar syndrome but none of them developed the classical picture of paraneoplastic cerebellar degeneration (Graus et al., 2004). Both patients were in remission at the time of the neurological diagnosis.

Autoimmune endocrine associations

The clinical spectrum of autoimmune disorders was not different from that seen in the 22 patients with SPS (Table 1). Nine (53%) patients had DM1 that was diagnosed after the age of 30 years in eight of them.

Table I Clinical and immunological findings of patients with cerebellar ataxia and SPS

	Cerebellar ataxia n = 17 (%)	SPS n = 22 (%)
Female	16 (94)	19 (86)
Age (range)	59 (39–77)	56 (14–77)
Organ-specific autoimmune associations		
Type I diabetes mellitus (DMI)	9 (53)	13 (59)
Other[a]	7 (41)	6 (27)
None	5 (29)	7 (32)
Epilepsy	2 (11)	1 (4)
Temporal relation of the neurological syndrome		
Before DMI, n (%); median, in years (range)	1 (11); 5	6 (46); 3.5 (1–12)
After DMI, n (%); median, in years (range)	8 (89); 7 (0.1–38)	7 (54); 5 (0.1–15)
GAD-ab titre by immunohistochemistry, median (range)	6000 (1000–80 000)	8000 (500–40 000)
GAD-ab level by RIA (U/ml), median (range)	16 737 (2000–99 000)	17 500 (3450–144 000)
CSF oligoclonal IgG bands	9/13 (69)	5/14 (35)
Intrathecal synthesis of GAD-ab[b]	12/12 (100)	11/13 (85)
No. of folds of GAD-ab intrathecal production[c]	8×	4×
Duration of the syndrome median, in years (range)	7 (1–17)	8 (0.5–21)

[a]Other clinical organ-specific autoimmune association: SPS (four patients thyroiditis and two vitiligo); cerebellar ataxia (seven patients thyroiditis, two of them with pernicious anaemia and one with vitiligo).
[b]In one patient with cerebellar ataxia and three with SPS the intrathecal synthesis of GAD-ab was lower than 1 but higher than the IgG index.
[c]Median CSF/Serum GAD-ab titre ratio to median CSF/Serum IgG ratio. No significant differences were found between cerebellar ataxia and SPS patients.

The diagnosis of DM1 preceded the onset of the ataxia (median 8 years; range 0.5–38 years) in eight (89%) patients. DM1 did not appear in eight (47%) patients after a follow-up of 6 years (range 1–10 years), but three of them had other associated organ-specific autoimmune diseases. The follow-up of the five patients without clinical evidence of polyendocrine autoimmunity was shorter (2 years; range 1–7 years) than those who had other autoimmune endocrine diseases (9 years; range 7–10 years).

Immunological features

Paired serum and CSF samples were available in 13 of the 17 patients. CSF oligoclonal IgG bands were present in nine (69%) patients. IS of GAD-ab was demonstrated in all of the 12 patients tested. In one patient, the paired samples were only available to analyse the presence of oligoclonal IgG bands. These data were not different from that seen in patients with SPS (Table 1). Similarly, no differences in GAD-ab levels were noted between patients with cerebellar ataxia and SPS. However, the IS of GAD-ab was 2-fold higher in the cerebellar ataxia group (Table 1).

Other neurological disorders

There were 11 patients who presented different neurological syndromes and high GAD-ab levels (Table 2). Patient 1 was a female with previous history of late-onset DM1 in whom high GAD-ab levels were found when she developed diplopia, dysphagia and dysarthria at the time a colon cancer was diagnosed. Further work-up confirmed the diagnosis of myasthenia gravis with positive acetylcholine receptor antibodies.

Epilepsy

Four patients (Table 2, Patients 2–5) had epilepsy. In three of them, GAD-ab were requested at the time of diagnosis of DM1 of late-onset (at age 49, 47 and 32 years, respectively), 10, 19 and 26 years after the onset of the epilepsy. Patients 2 and 3 had drug-resistant temporal lobe epilepsy associated with hippocampal sclerosis, and clinical or serological evidence of other organ-specific autoimmune diseases (Table 2). In one of them, CSF analysis disclosed a positive IS of GAD-ab. Patient 4 had epilepsy since the age of 13 related to cortical heterotopias. This patient has been followed for 5 years with good control of the seizures and absence of other neurological problems. Patient 5 had epilepsy since the age of 20 associated with hippocampal sclerosis. High GAD-ab levels were found at the age of 50 years when she presented with a 5-month history of oscillopsia with normal serum levels of antiepileptics. Neurological examination disclosed a left lateral and down-gaze nystagmus. She did not have DM1 and the study of organ-specific autoimmunity was negative. She had positive CSF oligoclonal IgG bands and IS of GAD-ab. The nystagmus did not improve despite a change of the antiepileptic medication and a trial of high dose of steroids and two courses of intravenous immunoglobulins (IVIg).

Non-paraneoplastic limbic encephalitis

Two patients (Table 2, Patients 6, 7) developed a clinical syndrome compatible with idiopathic limbic encephalitis (LE) (Graus *et al.*, 2004). Patient 6 was a 49-year-old female who presented with a 1-week history of short-term

Table 2 Clinical and immunological characteristics of patients with other neurological syndromes and high GAD-ab levels

Patient No	Clinical features	Age/sex	Diabetes, age at onset	Other autoimmune associations	Cancer	GAD-ab titre (U/ml)	Oligoclonal IgG bands	GAD-ab IS
1	Myasthenia	71/F	Yes, 60	No	Colon	3500	No	N.D.
2	Epilepsy	39/F	Yes, 49	TPO	No	5305	No	Yes
3	Epilepsy	21/F	Yes, 47	Grave's disease, SLE	No	9372	N.D.	N.D.
4	Epilepsy	13/F	Yes, 32	Grave's disease	No	11 000	N.D.	N.D.
5	Down-beat nystgamus	50/F	No	No	No	56 000	Yes	Yes
6	Limbic encephalitis	49/F	Yes, 49	Hypothyroidism	No	23 000	N.D.	N.D.
7	Limbic encephalitis	47/F	Yes, 43	No	No	58 000	Yes	Yes
8	Paraneoplastic limbic encephalitis	70/M	No	TPO, TGB	SCLC	5733	No	Yes
9[a]	Paraneoplastic encephalomyelitis	67/M	No	No	Pancreas[b]	146 000	No	Yes
10	PCD	79/M	No	No	Non-SCLC	6771	No	No
11	PCD	55/M	No	TPO, TGB	Thymic carcinoma[b]	21 200	No	Yes

IS = intrathecal synthesis; PCD = paraneoplastic cerebellar degeneration; N.D. = not done; SCLC = small cell lung cancer; SLE = systemic lupus erythematosus; TPO = thyroid microsomal antibodies; TGB = thyroglobulin antibodies.
[a]Previously reported (Hernandez-Echebarria et al., 2006).
[b]Tumor cells showed GAD expression.

memory loss, incoherent language and partial seizures with dysautonomic symptoms coincident with the diagnosis of DM1. There was a past history of autoimmune hypothyroidism. The neurological examination disclosed severe short-term memory impairment. The EEG showed a left temporal epileptic focus, and the MRI a high FLAIR signal involving both medial temporal lobes (Fig. 1). Whole body CT and mammograms were normal. She was treated with insulin and valproic acid with clinical and EEG improvement of the seizures. A progressive improvement of the memory function was observed after discharge. Four years later neuropsychological tests only disclosed a mild recall deficit. The follow-up MRI lesions were compatible with an evolution to mesial sclerosis.

Patient 7 (Table 2) was a 47-year-old female who developed subacute short-term memory loss and language difficulties that impaired her job as a teacher. DM1 had been diagnosed 4 years earlier. Neuropsychological testing revealed memory impairment and a dysexecutive syndrome with difficulties in planning, decision-making, temporal sequencing problems, poor attention and impaired abstraction. EEG showed background slowing over the left posterior temporal region. Brain MRI was normal. CSF analysis revealed oligoclonal IgG bands and a positive IS of GAD-ab. After nine monthly 5-day courses of IVIg, the neuropsychological examination confirmed an improvement of the memory and stabilization of the executive dysfunction that remained stable over the ensuing 8 years. Five years later, she developed epilepsy with good response to valproic acid.

Fig. 1 Axial fluid-attenuated inversion recovery (FLAIR) MRI shows asymmetric, left greater than right, mesial temporal high signal abnormalities.

Paraneoplastic neurological syndromes

Four patients had a PNS (Graus et al., 2004) (Table 2, Patients 8–11). Patient 8 was a 70-year-old male who presented with a classical syndrome of LE characterized
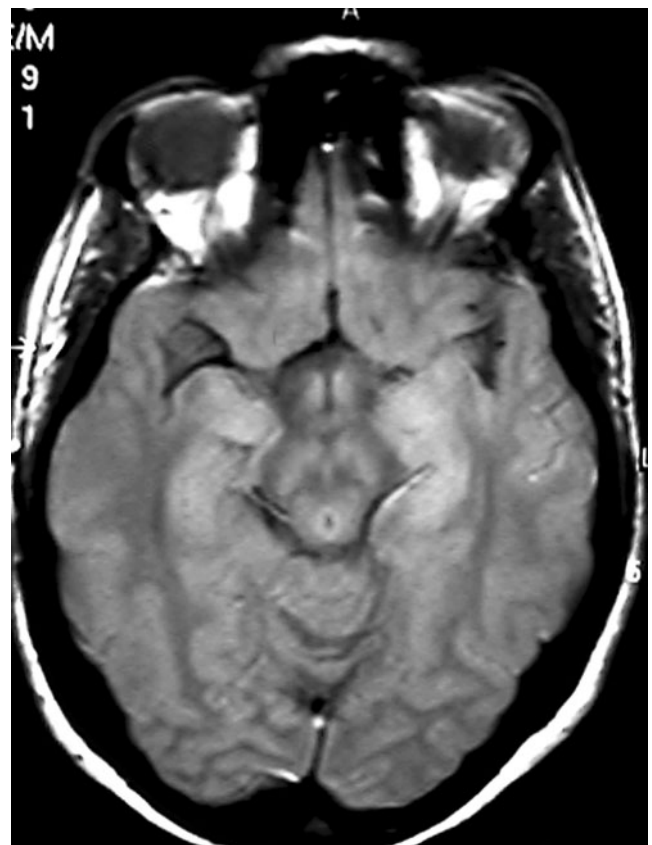
by seizures, confusion, short-term memory loss and hallucinations. Brain MRI was normal. Onconeuronal antibodies were negative, but GAD-ab were detected in serum and CSF with a positive IS of GAD-ab. There was no

medical history of organ-specific autoimmunity but positive thyroglobulin and thyroid microsomal antibodies were detected. Chest CT scan revealed a mediastinal tumour that was confirmed as small cell lung cancer (SCLC). The LE did not improve after a course of high-dose steroids and IVIg and the patient died 1 month after the onset of the LE. Patient 9 (Table 2), reported previously (Hernandez-Echebarria *et al.*, 2006), was a 67-year-old male who presented with encephalomyelitis associated with a pancreatic endocrine tumour. The analysis of the tumour demonstrated expression of GAD.

The other two patients presented a cerebellar ataxia that was considered paraneoplastic. Patient 10 developed a classic paraneoplastic cerebellar degeneration (Graus *et al.*, 2004) associated with non-SCLC. Unlike idiopathic GAD-ab cerebellar ataxia, there was no clinical or serological evidence of organ-specific autoimmunity. Patient 11 was a 55-year-old male who presented with a 6-month course of gait ataxia followed by mild dysarthria and left arm ataxia. He had no history of autoimmune endocrine diseases, but positive thyroglobulin and thyroid microsomal antibodies were detected. CSF was normal, without oligoclonal IgG bands, but positive for IS of GAD-ab. CT scan revealed the presence of a mass in the anterior mediastinum. The histological study revealed a thymic neuroendocrine carcinoma that showed positive immunoreactivity for GAD. After treatment with local radiotherapy and chemotherapy, he has remained stable for 6 months.

Discussion

We report the clinical and immunological features of 61 patients with high GAD-ab levels who were identified in a reference centre for the detection of GAD-ab in patients with diabetes and antineuronal antibodies in patients with suspected PNS. The study shows that the majority of patients (82%) had an associated neurological syndrome: SPS (36%) or cerebellar ataxia (28%), and confirms the previous impression (Saiz *et al.*, 1997; Honnorat *et al.*, 2001) that both syndromes share the same demographic, clinical and immunological features. Other neurological disorders were found in low numbers; some of them previously described such as epilepsy (Giometto *et al.*, 1998; Peltola *et al.*, 2000; Yoshimoto *et al.*, 2005) or isolated myasthenia gravis (Vernino and Lennon, 2004). However, the association of GAD-ab with PNS and tumours different from thymoma had not been emphasized in previous studies. We realize that the distribution of neurological syndromes associated with GAD-ab in our series may be biased because the main reason to send the samples to our laboratory was to detect antineuronal antibodies to diagnose PNS. In this setting, the frequency of SPS may be underestimated.

The prevalence of GAD-ab in neurological syndromes different from SPS was reported in a previous study that included patients with low GAD-ab levels. However, no description of the neurological syndromes was provided (Meinck *et al.*, 2001). The authors identified GAD-ab in 14 (5%) of 279 patients with a wide variety of neurological disorders but only three, with cerebellar ataxia, epilepsy and basal ganglia disease, had high GAD-ab levels (Meinck *et al.*, 2001).

In a recent series of 62 patients in whom GAD-ab were detected during paraneoplastic autoantibody screening, neurological manifestations were multifocal in 41 (66%) patients and included cerebellar ataxia (63%), brainstem involvement (29%), seizures (27%), stiff-man phenomenon (26%), extrapiramidal signs (16%) and myelopathy (8%). One-third of the patients had DM1 (Pittock *et al.*, 2005). The study emphasized that 20 (32%) of the patients were African American, and 55% of them had multifocal involvement characterized by a predominant brainstem dysfunction (Pittock *et al.*, 2005). The combination of brainstem involvement, ataxia, rigidity and seizures resembles the pattern described in patients with progressive encephalomyelitis with rigidity and myoclonus and GAD-ab (Meinck and Thompson, 2002). The absence of similar patients in our series is unclear and we cannot exclude a bias in the pattern of referral.

The clinical and immunological profile of the 22 SPS patients of our study is in agreement with that described in previous series except for a higher, but not significant, frequency of female (86%) and DM1 (59%) (Table 3). The temporal relation between the diagnosis of SPS and the onset of DM1 was not previously described (Solimena *et al.*, 1990; Dalakas *et al.*, 2000; Dalakas *et al.*, 2001; Meinck and Thompson, 2002; Rakocevic *et al.*, 2004). In our series, 46% of the patients developed DM1 after the onset of the SPS and it occurred as late as 12 years (median 3.5 years). The rest of the patients (54%) had been diabetics for months to 15 years (median 5 years) and all but one had late-onset DM1. Whether patients, particularly female, with late-onset DM1 and high GAD-ab levels are at high risk to develop SPS or cerebellar ataxia (see below) is unclear. However, other variables, besides high GAD-ab levels, are probably necessary for the development of the neurological dysfunction because no evidence of SPS or other neurological syndromes were observed in the nine patients with DM1 and high GAD-ab levels that we have been following for many years (median 14 years).

The clinical characteristics of the 17 patients with cerebellar ataxia in this study, confirms the information we previously provided on 14 patients collected from different European centres (Honnorat *et al.*, 2001), and they are similar to those described in case reports published after that study (Table 4). Taken together, these data suggest that the possibility of GAD-ab-associated cerebellar ataxia should be considered in patients, mostly female, with DM1 or polyendocrine autoimmunity who develop cerebellar gait ataxia, but at the same time indicates that a minority of patients may develop the cerebellar syndrome in absence of clinical evidence of polyendocrine autoimmunity.

Table 3 Comparison of clinical and immunological features in different series of SPS associated with GAD-ab

	Present series	Solimena et al. (1990)	Dalakas et al. (2000)	Dalakas et al. (2001)	Meinck and Thompson (2002)[a]	Rakocevic et al. (2004)
Number of patients	22	20	20	18	39	16
Females (%)	86	66	70	N.A.	74	62
Mean age (range)	56 (14–77)	N.A.	41.2 (N.A.)	N.A.	N.A. (17–72)	52 (37–62)
Diabetes (%)	59	30	40	N.A.	30	44
Epilepsy (%)	4	20	20	N.A.	0	31
Onset of the neurological syndrome before/after DMI, n (%)	6 (46)/7 (54)	N.A.	N.A.	N.A.	N.A.	N.A.
Focal SPS (%)	18	N.A.	17	N.A.	15	N.A.
Oligoclonal IgG bands (%)	5/14 (35)	3/11 (27)	N.A.	10/15 (67)	19/32 (59)	N.A.
IS of GAD-ab (%)	11/13 (85)	N.A.	N.A.	11/13 (85)	N.A.	N.A.
Mean follow-up in years (range)	8.1 (0.5–21)	N.A.	N.A.	N.A.	N.A.	11 (5–30)

IS = intrathecal synthesis; N.A. = not available.
[a]Twenty-eight of the 39 patients were GAD-ab positive.

Table 4 Summary of reported cases of cerebellar ataxia associated with GAD-ab published since our 2001 study (Honnorat et al., 2001)

Reference	Age/sex	Clinical features	Diabetes, age at onset	Other autoimmune associations	GAD-ab serum/CSF	Oligoclonal IgG bands	GAD-ab IS	Follow-up
Ishida et al. (1999) Ishida et al. (2007)	66/F	Subacute cerebellar ataxia	Yes, 67	No	+/+	No	N.A.	SPS at 70 years. Dead at 71 years. Autopsy: loss of Purkinje cells
Vulliemoz et al. (2007)	58/F	Focal epilepsy at 40, subacute cerebellar ataxia	No	PA;TGB, TPO	+/+	Yes	28.8	8 months
Chang et al. (2007)	56/F	Subacute cerebellar ataxia	No	No	+/+	N.A.	N.A.	15 months
Kim et al. (2006)	40/M	Subacute cerebellar ataxia and SPS	Yes, 37	Thyroiditis	+/+	No	4.8	4 months
Birand et al. (2006)	38/F	Chronic cerebellar ataxia	No	Graves disease	+/N.A.	N.A.	N.A.	3 years
McFarland et al. (2006)	70/M	Cerebellar ataxia	Yes, N.A.	No	+/+	N.A.	4.67	1 year
Zivotofsky et al. (2006)	46/F	Chronic SPS and cerebellar ataxia with DBN	No	PA	+/N.A.	N.A.	N.A.	18 months
Tilikete et al. (2005)	76/F	Chronic cerebellar ataxia with PAN, and cognitive dysfunction	No	No	+/+	Yes	N.A.	3 weeks
Ances et al. (2005a)	55/F	Subacute cerebellar ataxia with DBN and SPS	Yes, years before	Thyroiditis	+/+	N.A.	N.A.	N.A.
Lauria et al. (2003)	66/F	Subacute cerebellar ataxia and leg spasms	No	Thyroiditis, PA	+/+	Yes	N.A.	18months
Matsumoto et al. (2002)	63/F	Subacute brainstem and cerebellar ataxia	No	TPO, TGB	+/+	No	10.7	3 weeks
Rüegg et al. (2002)	62/F	Chronic cerebellar ataxia with DBN	No	TGB, TPO, GPC	+/+	No	N.A.	N.A.
Kono et al. (2001)	56/F	Chronic cerebellar ataxia and SPS	Yes, 58	GPC	+/+	N.A.	N.A.	N.A.

Subacute: <6months; Chronic: >6 months.
IS = intrathecal synthesis; DBN = down-beat nystagmus; SPS = stiff-person syndrome; PAN = periodic alternating nystagmus; PA = pernicious anemia; TPO = thyroid microsomal antibodies; TGB = thyroglobulin antibodies; GPC = gastric parietal cell antibodies; N.A. = not available.

Table 5 Summary of reported cases of isolated epilepsy associated with GAD-ab

Reference	Age/sex	Clinical features	Diabetes, age at onset	Other autoimmune associations	GAD-ab serum/CSF	Oligoclonal IgG bands	GAD-ab IS	Follow-up
Giometto et al. (1998)	19/M	Subacute complex partial seizures	No	No	+/+	Yes	5.7	N.A.
Peltola et al. (2001)	49/M	Resistant temporal-lobe epilepsy for 33 years	No	TPO, antigliadin, anticardiolipin antibodies	+/+	No	12.6	N.A.
Peltola et al. (2001)	50/F	Resistant temporal-lobe epilepsy for 34 years	No	Hypothyroidism oligomenorrhea	+/+	No	0.8	N.A.
McKnight et al. (2005)	31/F	Drug-resistant complex partial seizures for 17 years	No	No	+/N.A.	N.A.	N.A.	N.A.
McKnight et al. (2005)	28/F	Drug-resistant complex partial seizures for 15 years	Yes, N.A.	Hypothyroidism	+/N.A.	N.A.	N.A.	N.A.
McKnight et al. (2005)	35/F	Drug-resistant complex partial seizures for 31 years	No	No	+/N.A.	N.A.	N.A.	N.A.
Olson et al. (2002)	6.5/M	Epilepsia partialis continua for days	Yes, 6 years	No	+/+	N.A.	N.A.	Resolution after high-dose strenoids, plasmapheresis and immunoglobulins
Yoshimoto et al. (2005)	55/M	Complex partial seizures for 8 months	Yes, 50 years	TPO, TGB	+/+	N.A.	1.62	6 months

IS = intrathecal synthesis; TPO = thyroid microsomal antibodies; TGB = thyroglobulin antibodies; N.A. = not available.

Therefore, GAD-ab analysis should be included in the routine work-up of sporadic adult-onset cerebellar ataxias when more common causes are ruled-out even in patients without DM1.

We did not find significant differences between patients with cerebellar ataxia and SPS in the number of associated autoimmune disorders, frequency of oligoclonal IgG bands or IS of GAD-ab (Table 1). Three patients presented symptoms of SPS associated with the predominant cerebellar ataxia. The coexistence of SPS and cerebellar ataxia has been described in a few case reports (Kono et al., 2001; Ances et al., 2005a; Kim et al., 2006). A recent study showed that patients with SPS and cerebellar ataxia had a higher IS of GAD-ab than patients with isolated SPS (Rakocevic et al., 2006). In line with this observation, we also found a higher IS of GAD-ab in patients with cerebellar ataxia compared to that seen in SPS patients (Table 1). The significance of this difference is presently unclear but further supports the evidence that GAD autoimmunity is implicated in the pathophysiology of the cerebellar damage (Ishida et al., 1999; Manto et al., 2007).

The immunological findings in this series of cerebellar ataxia provide useful information on the diagnostic value of the GAD-ab in a patient with a given neurological disorder. Although the sole presence of high GAD-ab levels probably suggests that the underlying neurological syndrome may be immunomediated, our observation that a specific IS of GAD-ab is found in all patients with cerebellar ataxia strongly suggest that these studies must be done in every patient with a neurological syndrome and GAD-ab to confirm the syndrome is related to GAD autoimmunity. The evaluation of

IS of GAD-ab should be specifically indicated in those patients with concomitant DM1 or other autoimmune endocrine syndromes where high GAD-ab levels could just reflect the presence of the associated endocrine disorders.

The frequency of high GAD-ab levels in patients with epilepsy is low and ranges from 0% to 4% (Kwan et al., 2000; Peltola et al., 2000; Verrotti et al., 2003; Sokol et al., 2004; McKnight et al., 2005; Majoie et al., 2006). GAD-ab-positive patients are more likely to have drug-resistant epilepsy of long duration (Peltola et al., 2000). Four (7%) patients of this series had epilepsy. In one of them, GAD-ab were requested because of the recent onset of an isolated downbeat nystagmus. Downbeat nystagmus, the most frequently eye movement abnormality associated with GAD-ab (Tilikete et al., 2005; Zivotofsky et al., 2006), has been reported as isolated manifestation (Antonini et al., 2003) but mostly in the context of cerebellar ataxia (Rüegg et al., 2002; Ances et al., 2005a; Zivotofsky et al., 2006). In the other three patients, GAD-ab were detected at the time of diagnosis of late-onset DM1 many years after the onset of the epilepsy. The temporal profile of epilepsy antedating the diagnosis of DM1 for many years is different from what is described in previous patients who either had no diabetes or the epilepsy appeared years after the diagnosis of the DM1 (Giometto et al., 1998; Peltola et al., 2000; Olson et al., 2002; McKnight et al., 2005; Yoshimoto et al., 2005) (Table 5). The possible pathogenic role of GAD autoimmunity in patients with epilepsy and Gad-ab is unclear. The presence of a positive IS of GAD-ab in all five patients (Tables 2 and 5) in whom this analysis was done

further emphasizes the possible autoimmune origin of the epilepsy in these patients.

A new observation of our study is that GAD-ab may occur in patients with classical PNS (Graus *et al.*, 2004). In addition to the well-known association of paraneoplastic SPS with amphiphysin-ab (Rosin *et al.*, 1998; Pittock *et al.*, 2005), there have been reports of paraneoplastic SPS patients who presented GAD-ab. The associated tumours included thymoma and different solid tumours (McHugh *et al.*, 2007). High GAD-ab levels were described in five (13%) of 37 patients with thymoma and several types of neurological disorders. However, no clinical information was provided on the neurological syndrome of these patients (Vernino and Lennon, 2004). Two of our patients, with paraneoplastic encephalomyelitis and cerebellar ataxia, presented IS of GAD-ab, the tumours showed neuroendocrine features, and expressed GAD. These data suggest that the immunity raised against the neoexpression of GAD by the tumour may cause a PNS as occurs in patients with classical onconeural antibodies (Darnell and Posner, 2003). This observation emphasizes that the possibility of a paraneoplastic origin of the neurological syndrome should be considered in patients with high levels of GAD-ab particularly when there is no evidence of DM1 or other autoimmune endocrine disorders.

Three patients of our series presented with clinical features compatible with LE (Gultekin *et al.*, 2000). In two of them, the brain MRI was normal, a feature that does not rule out this diagnosis (Gultekin *et al.*, 2000), and both presented positive IS of GAD-ab. The third patient showed typical features of LE in the brain MRI. However, the clinical and MRI improvement with the control of the seizures in absence of any immunotherapy does not rule out that the MRI features suggestive of LE could be secondary to brain edema induced by the epilepsy (Kim *et al.*, 2001). LE associated with GAD-ab may be paraneoplastic or idiopathic. LE was reported in two patients of a series of 62 with high GAD-ab levels and no evidence of cancer (Pittock *et al.*, 2006). Paraneoplastic LE with GAD-ab has been described in a few patients with thymoma (Vernino and Lennon, 2004; Ances *et al.*, 2005*b*; Knudsen *et al.*, 2007;). In one of them, antibodies against the neuronal surface were identified concomitant with GAD-ab suggesting that the former could be responsible for the LE whereas the GAD-ab were associated with the SPS syndrome that the patient also presented (Ances *et al.*, 2005*b*). One patient of this study had GAD-ab-positive LE antedating the diagnosis of a SCLC an association not previously reported. Although we could not analyse the tumour for the expression of GAD, this possibility would justify the relation of LE with GAD autoimmunity (Hernandez-Echebarria *et al.*, 2006).

In conclusion, our study emphasizes several important issues that neurologists and other specialists must keep in mind when they evaluate a patient with high GAD-ab levels and a neurological syndrome. First, high GAD-ab levels associate with different neurological syndromes besides SPS. Second, GAD-ab may just reflect the presence of concomitant DM1 or other endocrine autoimmune disorders. In this setting, the demonstration of a positive IS of GAD-ab is important to confirm that the GAD autoimmunity is related to the neurological syndrome and the patient merits the possibility of treatment with immunotherapy. Third, cerebellar ataxia, the second most common syndrome associated with high GAD-ab levels, shares with SPS the same demographic, clinical and immunological features. Fourth, in patients with no previous history of DM1 who develop neurological syndromes that suggest a paraneoplastic origin, the finding of GAD-ab does not rule out this possibility and appropriate studies should be done to confirm an underlying cancer.

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