GLUTAMIC ACID DECARBOXYLASE ANTIBODIES IN NEUROLOGICAL DISEASES: CLINICAL RETROSPECTIVE EVALUATION OF DIAGNOSIS PERFORMED BY ENZYME-LINKED IMMUNOSORBENT ASSAY

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1 BACKGROUND

GAD antibodies are markers of type 1 diabetes mellitus and autoimmune neurological syndromes. The main neurological syndromes linked to GAD abs are stiff-person syndrome, cerebellar ataxia, limbic encephalitis and epilepsy. Titers of GAD abs in neurological patients are much higher than in diabetes. The meaning of low titres in neurological syndromes is still uncertain and also the cut off for the relevance of GAD abs is still debated. Moreover, commercial kits for detection of GAD abs are validated only in patients with T1DM.

2 OBJECTIVE

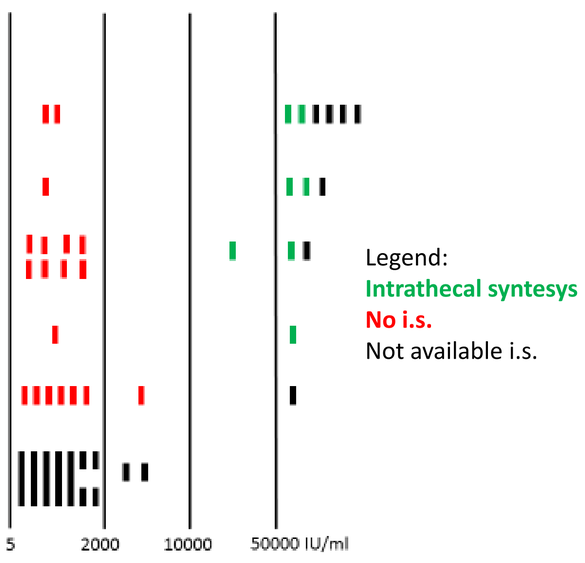
- To validate a commercial ELISA assay for GAD abs detection in serum and CSF of patients with suspected neurological autoimmune diseases
- To set up a clinically relevant cut-off for GAD abs
- To explore the meaning of low-titre GAD abs
- To investigate the presence of coexisting antineuronal antibodies

3 METHODS

We tested 328 patients with suspected autoimmune neurological syndromes for GAD abs by a commercial ELISA kit (RSR Limited). Sera and CSF were titrated by multiple dilutions, using a mathematical model. When paired sera and CSF were available, intrathecal synthesis was calculated. Specimens were also tested by immunofluorescence on monkey cerebellum (Euroimmun) and cell-based assay (Euroimmun) for other antineuronal abs. We arbitrarily selected a cut-off of relevance on the basis of positivity of GAD abs intrathecal synthesis in our series. We assigned to each patient a clinical score on the basis of probable autoimmune features. Then the positive patients were divided into high- and low-titre groups. 53 controls and 21 T1DM patients were tested, too.

4 RESULTS: titres and intrathecal synthesis in neurological and T1DM pts

CLINICAL SYNDROME	NUMBER	GAD ABS RANGE (IU/ml)
SPS	8	(169 – 2019681)
ATAXIA	4	(39 – 643289)
ENCEPHALITIS	11	(5,4 – 1373559)
EPILEPSY	2	(11 – 1165654)
OTHER	8	(14 – 187202)
DIABETICS	21	(6 – 4627)



Control group: 53 patients (8 healthy subjects, 25 degenerative diseases, 14 multiple sclerosis, 6 neuropathy). All resulted negative (< 5 IU/ml)

SELECTED CUT-OFF = 10000 IU/ml

5 RESULTS: high-titre and low-titre groups

HIGH TITRE

14 patients

1 M : **13 F**

27-80 years (median 56)

6 stiff-person syndrome
3 limbic encephalitis
3 cerebellar ataxia
1 epilepsy
1 other

LOW TITRE

19 patients

12 M : 7 F

15-82 years (median 54)

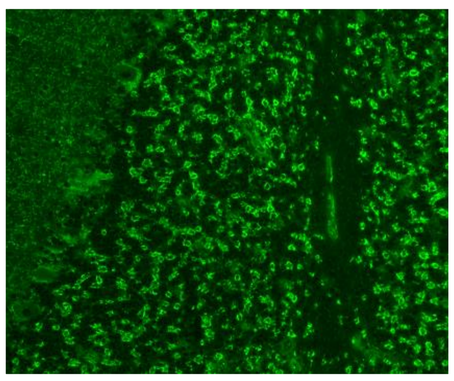
8 limbic encephalitis
2 stiff-person syndrome
1 cerebellar ataxia
1 epilepy
7 other

6 RESULTS: high titre vs low titre

	HIGH TITRE (n. 14)	LOW TITRE (n. 19)
Female	**13**	**7**
Autoimmune comorbidity	**12**	**2**
Typical autoimmune features	**13**	**5**
Anti-neuronal abs*	1	3 (+ anti-GlyR, + anti-neuronal filament)
Neoplasm	2	4
Response to therapy	13/13	12/14

* Other anti-neuronal abs: 1 anti-amphyphisin (HT), 3 anti-LG1 (LT), 1 anti-GlyR (LT), anti-neuronal filament (LT)

7 RESULTS: indirect immunofluorescence



IF on monkey cerebellum (serum 1:10, IgAGM)

- 5/13 specimen with HT GAD abs were positive on IIF;
- All LT pts had negative IIF;
-IF was positive only over 100000 IU/ml (serum)

→ Commercial immunofluorescence is less sensitive than ELISA.

8 CONCLUSION

The study validates this ELISA kit in routine detection of GAD abs in neurological patients. On the basis of intrathecal synthesis, we identified the threshold of 10000 IU/ml as a discriminant cut-off for probable autoimmune neurological diseases associated to GAD abs. We found some significant differences between HT and LT group, which confirm a probable irrelevance of low-titer GAD abs. Search for GAD abs on cerebrospinal fluid and calculation of intrathecal synthesis in patients with suspected autoimmune neurological diseases are warranted to establish clinical relevance of serum positivity.