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Short Communication

# Can serum GAD65 antibody levels predict neurological disease or cancer?

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ABSTRACT

The clinical relevance of antibodies that bind to glutamic acid decarboxylase 65 (GAD65) is controversial regarding diagnostic utility in screening for neurological disease or cancer.

We did a retrospective study of 3152 GAD65 antibody-positive patients to examine whether analysis of the antibody levels could predict neurological disease or cancer. Serum GAD65 antibody levels were not associated with any of the following groups: neurological disease, neurological disease and diabetes, diabetes only, no neurological diagnosis and no diabetes mellitus, or cancer. Analysis of serum GAD65 antibody levels had no prognostic value in neurological disease or cancer. GAD65 antibodies should therefore be measured in selective cases of autoimmune neurological diseases.

## 1. Introduction

Glutamate is the most abundant excitatory neurotransmitter. It is the precursor for synthesis of inhibitory gamma-aminobutyric acid (GABA) in GABA-ergic neurons. This reaction is catalyzed by glutamate decarboxylase (GAD), which is abundant in the cerebellum and pancreas (Erlander and Tobin, 1991).

GAD exists in two isoforms: GAD65 and GAD67. In patients with neurological disorders, antibodies can be detected against GAD65, which is the standard biomarker, but also less frequently, against GAD67 (Gresa-Arribas et al., 2015). GAD65 antibodies are associated with various neurological diseases such as stiff-person syndrome, cerebellar ataxia, limbic encephalitis, and epilepsy (Honnorat et al., 2001; Malter et al., 2010; Peltola et al., 2000). Such antibodies are frequently found in patients with diabetes mellitus 1, as well as in patients with poly-endocrine syndromes (Lampasona and Liberati, 2016). Antibodies to GAD65 and GAD67 are also detected in 1 to 2% of healthy people (Brooking et al., 2003).

GAD65 antibodies have been detected in cancer patients with possible or confirmed paraneoplastic neurological syndrome who do not express classical onconeural antibodies (Ariño et al., 2015). Although the utility of GAD65 antibodies as a paraneoplastic marker has not been established, GAD65 antibodies are included in commercially available paraneoplastic line-blots. In the present study, we examined whether analysis of serum GAD65 antibody levels is associated with neurological disease, diabetes, and/or cancer in a large population of patients.

## 2. Methods

### 2.1. Patients

Data on 3168 patients with GAD65 antibodies treated from 1999 until 2013 at Haukeland University Hospital, Bergen, Norway were collected. Clinical information was not available for 16 patients, and therefore 3152 patients (41.8% males and 58.2% females) were included in the study. ICD-10 diagnoses were obtained from hospital records and the Cancer Registry of Norway from 1995 to 2015. The study was approved by the regional ethics committee (REK Vest # 2011/2433).

The patients were grouped into the following categories: those with a neurological diagnosis only (G00-G99), those with a neurological diagnosis and additionally diabetes type 1 or 2 (E10-E14), those with only diabetes type 1 or 2 (E10-E14), and those with no neurological diagnosis and no diabetes diagnosis. Presence of cancer diagnoses (C00-C97) was compared between the four groups. We have no information on the rationale for ordering a test for GAD65 antibodies in these patients.

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### 2.2. Antibodies

Levels of GAD65 antibodies in serum were determined by radioimmunoprecipitation at the Laboratory of Clinical Biochemistry, Haukeland University Hospital, Bergen, Norway. In patients with more than one GAD65 measurement, we used the peak level in our study. GAD65 antibody levels were classified as low (< 50 U/ml), medium (50–100 U/ml), or high (> 100 U/ml). GAD65 antibody levels were not measured in cerebrospinal fluid (CSF).

### 2.3. Statistics

The association between antibody level and the diagnostic classification was tested using Pearson's chi-square test. For multiple regression analysis GAD65 antibody levels were natural log-transformed (adding 1 to avoid log of zeros). Multiple logistic regression analysis was used to relate risk of cancer to ln(peak GAD65 antibody level) and diagnostic classification, adjusting for age and sex.

## 3. Results

In our cohort of 3152 patients, 2585 (82.0%) had GAD65 antibody levels in the low range (< 50 U/ml), 450 (14.3%) in the mid-range (50–100 U/ml), and 117 (3.7%) in the high range (> 100 U/ml). None of the patients had GAD65 levels > 300 U/ml.

The 3152 patients were characterized into the following diagnostic groups: 41 (1.3%) had only a neurological diagnosis, 166 (5.3%) had a neurological diagnosis and diabetes, 1274 (40.4%) had only diabetes, and 1078 (34.2%) had no neurological diagnosis and no diabetes. As expected, type 1 diabetes was more prevalent than type 2 diabetes among the GAD65 antibody-positive patients. Associations between the diagnostic groups and GAD65 antibody levels are shown in Table 1 and Fig. 1. Epilepsy (G40X) was the most common diagnosis in patients with GAD65 antibodies (16.1%), followed by other disorders of central nervous system (G96X) (12.3%), upper extremity mononeuropathy (G56X) (9.9%), and polyneuropathy (G62.X) (7.9%). None of the ICD10 subgroups of neurological diseases were significantly associated with GAD65 antibody levels ($p$ = .125).

According to the Cancer Registry of Norway, 145 (4.6%) of the GAD65 antibody-positive patients had a cancer diagnosis in the period of 1995–2015. Among the cancer patients, 5 (3.4%) had a neurological diagnosis, 20 (13.8%) had a neurological diagnosis and diabetes, 100 (60.0%) had only diabetes, and 20 (13.8%) had no neurological diagnosis and no diabetes (13.8%). There was no association between GAD65 antibody and diagnostic class grouping ($p$ = .182). Among the 145 cancer patients, 62 were diagnosed with cancer within 3 years of quantification of GAD65 antibody. For these cancer patients, the distribution into the four diagnostic groups did not differ by low, medium, or high levels of GAD65 antibody ($p$ = .440). Prostate cancer (C61) was the most common diagnosis in patients with GAD65 antibodies (18.6%), followed by cecum cancer (C18) (9.7%), breast cancer (C50) (6.9%), and urinary cancer (C66–68) (6.2%). None of the ICD10 subgroups of cancer were significantly associated with the presence of GAD65 antibodies ($p$ = .908).

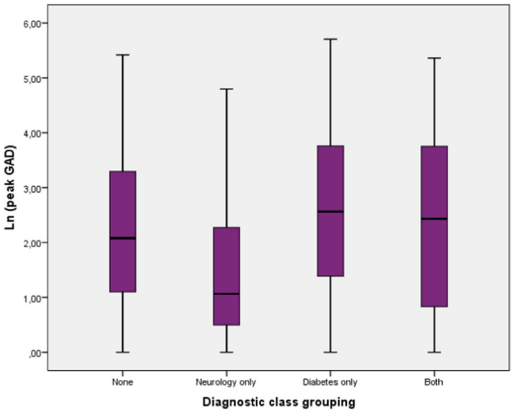


**Fig. 1.** Boxplot of ln(peak GAD65 antibody level) for each diagnostic class. The median is indicated by a line inside each box. The length of the box is the interquartile range (IQR) computed from Tukey's hinges.

In multiple logistic regression analyses, adjusted for age and sex, we did not find higher odds for cancer in any of the groups compared to the group with no neurological diagnosis and no diabetes (Table 2, $p$ = .510). Furthermore, there was no increase in the likelihood of cancer diagnosis in patients with GAD65 antibodies ($p$ = .463) and there was no difference between males and females ($p$ = .100). Age at detection of GAD65 antibody (grouped in 10-year increments) was, as expected, highly significant, as in older subjects the odds of cancer diagnosis were higher than in younger subjects ($p$ < .001).

## 4. Discussion

Analysis of GAD65 antibody levels were not associated with any of the following groups: neurological disease, neurological disease and diabetes, diabetes only, no neurological diagnosis and no diabetes mellitus or with cancer. Furthermore, GAD65 antibody levels were not associated with ICD10 subgroups of neurological diagnoses or cancer

**Table 1**
Number of patients at indicated peak GAD65 antibody levels by diagnostic class grouping.

| Diagnostic grouping | GAD65 antibody level | | | Total | p value[a] |
|---|---|---|---|---|---|
| | Low | Medium | High | | |
| | < 50 | 50–100 | > 100 | | |
| | n (%) | n (%) | n (%) | N (%) | |
| Neurology only | 36 (1.4) | 4 (0.9) | 1 (0.9) | 41 (1.3) | |
| Neurology and diabetes 1 | 133 (5.1) | 27 (6.0) | 6 (5.2) | 166 (5.3) | |
| Neurology and diabetes 2 | 69 (2.7) | 13 (2.9) | 9 (7.8) | 91 (2.9) | |
| Diabetes 1 only | 1014 (39.2) | 202 (44.9) | 58 (50.0) | 1274 (40.4) | |
| Diabetes 2 only | 387 (15.0) | 87 (19.3) | 28 (24.1) | 502 (15.9) | |
| None of the above | 947 (36.6) | 117 (26.0) | 14 (12.1) | 1078 (34.2) | |
| Total | 2586 (100) | 450 (100) | 116 (100) | 3152 (100.0) | < 0.001 |

[a] Pearson's chi-square test.

**Table 2**
Multiple logistic regression analysis of risk for cancer ($n$ = 62) in patients diagnosed within 3 years of peak GAD65 antibody quantification.

| Variables | Odds ratio | 95% CI[a] | p value[b] |
|---|---|---|---|
| Age at anti-GAD65 analysis[c] | 1.99 | (1.71, 2.33) | < 0.001 |
| Male vs. female | 1.56 | (0.92, 2.65) | 0.100 |
| Diagnosis | | | 0.510[b)] |
| None | 1.00 | Reference | |
| Neurology only | 0.92 | (0.18, 4.62) | |
| Diabetes I only | 0.58 | (0.25, 1.31) | |
| Diabetes II only | 1.14 | (0.55, 2.36) | |
| Diabetes I and neurology | 0.75 | (0.20, 2.77) | |
| Diabetes II and neurology | 0.71 | (0.21, 2.38) | |
| ln(GAD65 level) | 0.94 | (0.79, 1.11) | 0.463 |

[a] CI: confidence interval; $p$-value from likelihood ratio test.
[b] Likelihood ratio test.
[c] Odds ratio is per 10 years increase in age.

diagnoses. We found that epilepsy was the most common diagnosis associated with GAD65 antibodies; however, there were no significant associations with neurological ICD10 subgroups and GAD65 antibody levels. We did not have access to CSF and therefore could not determine whether there was an association between GAD65 antibodies in CSF and neurological disease.

We did not find any differences in diagnostic groups among low, medium, and high GAD65 antibody levels. However, in a previous retrospective study of 61 patients with high GAD65 antibody levels (radioimmunoassay values ≥2000 U/ml), 36% had stiff person syndrome, 28% cerebellar ataxia, 18% other neurological disorders, and 18% isolated diabetes mellitus (Saiz et al., 2008). We also found these diagnoses among the patients with high, medium, and low GAD65 antibody levels, but there was no significant association between neurological disease and any level of GAD65 antibody. However, there were no patients in our cohort with GAD65 levels > 300 U/ml using a radioimmunoprecipitation assay.

GAD65 antibodies have been detected in patients with small-cell lung cancer without paraneoplastic neurological syndromes (Gozzard et al., 2015). In our study, prostate cancer was most prevalent of the cancer subtypes; there were no patients diagnosed with small cell lung cancer. We did not find any significant associations with any ICD10 cancer subgroups and GAD65 antibodies. We have previously found a significant association of Hu antibodies with small cell lung cancer without paraneoplastic neurological syndromes (Monstad et al., 2004), but such antibodies could not be used as prognostic markers for the development of lung cancer in patients without paraneoplastic neurological syndromes (Qvale et al., 2014).

We did not find any increase in cancer risk for patients within 3 years of detection of GAD65 antibodies when adjusting for age, sex, and neurological disease and/or diabetes. Results were similar when we expanded the cancer interval from 3 to 5 years (data not shown). Therefore, screening for GAD65 antibodies cannot be used as a paraneoplastic marker. On the other hand, patients with classic paraneoplastic neurological syndromes must be screened for an underlying cancer (Titulaer et al., 2011; Ariño et al., 2015).

Our findings show that analysis of serum GAD65 antibody levels is not prognostic of neurological diseases or cancer. However, as previously reported measuring GAD65 antibodies can be of importance in clinical decisions of certain neurological diagnoses, such as stiff person syndrome, cerebellar ataxia, limbic encephalitis, and epilepsy (McKeon and Tracy, 2017). Recently, GAD65-reactive B cells were found in in blood of patients with stiff person syndrome, cerebellar ataxia and limbic encephalitis (Thaler et al., 2019). An increased intrathecal antibody response against GAD65 supports the hypothesis that these antibodies have pathogenic relevance (Saiz et al., 2008). Therefore, the potential pathogenic relevance of serum GAD65 antibodies should be confirmed by measuring these antibodies also in the CSF. Since there is epitope-dependent pathogenic actions of GAD65 antibodies, future tests should make it possible to differentiate between GAD65 antibodies implicated to have a role in neurological disease or diabetes (Manto et al., 2019).

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## Declaration of Competing Interest

The authors have no competing interests.

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