2025 Pathology Update

GAD-ting the Picture: Developing A Strategy For GAD Antibody Testing In CSF

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BACKGROUND: Glutamic acid decarboxylase antibodies (GAD Ab), seen in type I diabetes mellitus (T1DM), are also associated with neurological conditions, including Stiff Person Syndrome and cerebellar ataxia[1]. Assays for GAD Ab are primarily validated for the diagnosis of T1DM, as such the optimal assessment of GAD Ab in neurological disease remains uncertain. GAD Ab levels in the serum of neurological patients are significantly higher than levels seen in T1DM patients.[1-3] The presence of GAD Ab in CSF suggests intrathecal antibody production, which is supportive in diagnosing GAD Ab-associated neurological disease[1-3]. GAD Ab can be visualised by indirect immunofluorescence (IIF) with characteristic patchy granular layer staining on primate cerebellum tissue (Figures 1 and 2). As part of our workflow, GAD Ab requests on CSF samples are tested by IIF.

AIM: 1. To determine the utility of incorporating CSF GAD Ab testing for detecting neurological conditions **2.** Evaluate the correlation between CSF GAD Ab detected by IIF and serum GAD Ab level by ELISA.

METHOD: An audit was conducted over 20 months from March 2023 to October 2024, reviewing CSF samples tested by IIF on cerebellum substrate with paired serum GAD Ab by ELISA and immunoblot, to review the correlation between CSF IIF and serum GAD Ab levels. Serum samples included in the study were collected within three months of the CSF sample. GAD Ab levels in serum were measured using the RSR GAD Ab ELISA kit (Cardiff, UK). IIF patterns were reported as "neuronal antibody negative" in absence of any staining pattern or "not excluded" when staining was observed on monkey cerebellum NOVA Lite substrate (Nova Lite® CA USA). The presence of GAD Abs were further confirmed with immunoblot analysis on serum samples using the Ravo PNS II Line Assay (Freiburg, FRG).

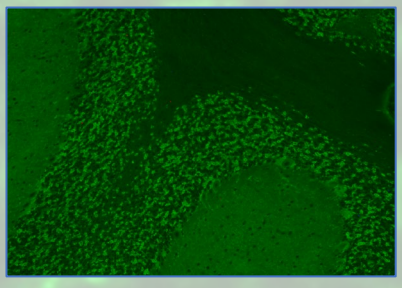

Figure 1. Granular layer staining on patient serum on IIF.

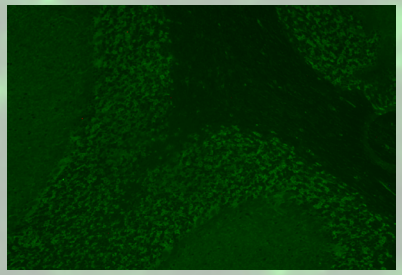

Figure 2. Granular layer staining on patient CSF on IIF.

RESULTS: 28 CSF samples were reviewed, 85% of the samples were negative by IIF. Of the remaining 4 samples, 3 had an IIF pattern consistent with GAD Ab on CSF, high titre serum GAD Ab by ELISA (31,934-200,000 u/mL) and were positive on detection by immunoblot on serum. All 3 patients were female and diagnosed with GAD Ab-associated neurological disease - Stiff person syndrome (n=2), autoimmune encephalitis (n=1). 1 other CSF sample showed molecular layer staining not consistent with GAD Ab - like staining and was from a patient diagnosed with LGI1 autoimmune encephalitis. Out of 4 samples that were positive for GAD Ab on serum by ELISA (9-6008 u/mL) and negative on IIF for CSF, 2 were positive for GAD Ab on immunoblot; clinical diagnoses included paraneoplastic encephalitis, psychosis and lithium toxicity, 3 of 4 of these samples were negative on IIF for serum.

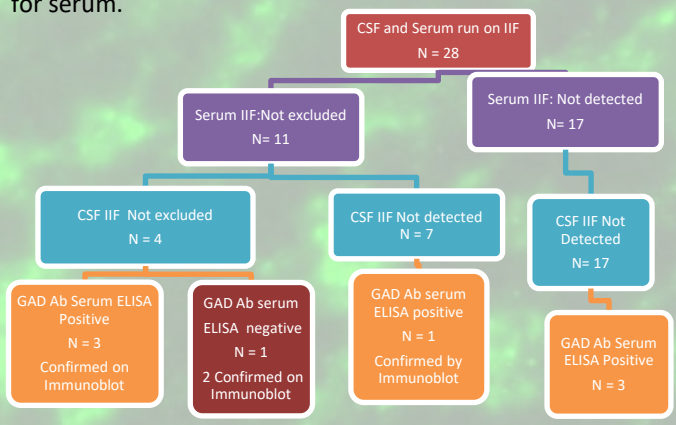

Figure 4. Flow chart showing the number of patients with positive GAD Ab results for IIF on CSF, ELISA and/or immunoblot on serum.

DISCUSSION: This audit shows good correlation between the GAD Ab CSF IIF pattern, high titre GAD Ab on serum and detection by immunoblot on serum in patients with clinical features of anti-GAD antibody associated neurological disease. Patients with GAD Ab neurological disease had serum titres >10,000IU/mL on ELISA, consistent with what is reported in the literature[2-3]. A testing algorithm that includes correlation of GAD Ab testing for CSF and GAD Ab titres on serum may aid in identifying intrathecal GAD Ab production to separate clinically associated neurological syndromes from incidental GAD antibodies, however a larger sample size is required to confirm this.

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