

## Supplementary Online Content

Korff CM, Parvex P, Cimasoni L, et al. Encephalitis associated with glutamic acid decarboxylase autoantibodies in a child: a treatable condition? *Arch Neurol*. 2011;68(8):1065-1068.

**eAppendix.** Laboratory methods.

This supplementary material has been provided by the authors to give readers additional information about their work.

## **eAppendix: Laboratory Methods**

Plasma exchanges were performed by plasma filtration with the Hospal Prisma TPE dialyzer (Prisma TPE 2000 ET; Hospal Renal Intensive Care, Lyon, France). Exchanges of 1.5-times the expected plasma volume were applied, using individual units of fresh frozen plasma.

Autoantibodies to GAD65 and GAD67 were determined by antibody radioligand binding assay (RBA). Briefly, recombinant [<sup>35</sup>S]-AD (human GAD65 and rat GAD67) were produced in an in vitro coupled transcription and translation system with SP6 RNA polymerase and nuclease-treated rabbit reticulocyte lysate (Promega, Madison, Wisconsin). Sera were incubated with [<sup>35</sup>S]-GAD (25 000 of TCA precipitable radioactivity). Anti-GAD bound [<sup>35</sup>S]-GAD was precipitated with Protein A Sepharose (PAS) (Invitrogen) and counted on a Wallac Microbeta Liquid Scintillation Counter (Perkin Elmer Life and Analytical Sciences, Inc, Boston, Massachusetts). The World Health Organization standard for GAD65Ab, negative samples, and a buffer blank were included in each assay to correct for interassay variation and to express GAD65Ab as a relative Unit. In the Diabetes Antibody Standardization Program (DASP) workshop 2005, the GAD65Ab analysis ranked at 80% sensitivity and 91% specificity. The capacity of the recombinant Fab (rFab) to inhibit GAD65 binding by human serum GAD65Ab was tested in a competitive epitope-specific radioligand binding assay (ESRBA), as described. Samples at their half-maximal binding concentration were incubated with radiolabeled GAD65 in the presence or absence of rFab b96.11 or b78. Noncompeted GAD65 binding was established by no addition of rFab. Binding of anti-GAD to GAD65 in the presence of rFab was expressed as anti-GAD (U/mL) in the presence of rFab/anti-GAD (U/mL) in the absence of rFab. All samples were analyzed in triplicate determination. Human monoclonal antibodies b96.11 and b78 specific to GAD65 were similarly derived from a patient with APS-1, and recognize epitopes located at amino acid residues 308-365 and 451-585, respectively. GAD65 enzyme activity was measured by the <sup>14</sup>CO<sub>2</sub>-trapping method described elsewhere. Briefly, recombinant human GAD65 was incubated with 0.018 μCi <sup>14</sup>C29 glutamate (Amersham Life Science Inc, Arlington Heights, Illinois) in the presence or absence of serum/CSF. Released <sup>14</sup>CO<sub>2</sub> was captured and determined in a Beckman scintillation counter. Inhibition (%) is reported as: 100 – (cpm in the presence of serum/CSF/cpm in the absence of serum/CSF × 100). Injecting the serum containing the abnormal antibodies in a healthy animal, to reproduce the disease, was not performed.