

# Anti-Glutamic Acid Decarboxylase Antibodies in the Serum and Cerebrospinal Fluid of Patients With Stiff-Person Syndrome

## Correlation With Clinical Severity

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**Background:** Stiff-person syndrome (SPS) is an immune-mediated central nervous system disorder characterized by fluctuating muscle stiffness, disabling spasms, and heightened sensitivity to external stimuli. Up to 80% of patients with SPS have anti-glutamic acid decarboxylase (GAD) antibodies in the serum or cerebral spinal fluid (CSF). Whether these antibodies are clinically relevant and correlate with disease severity is unknown.

**Objective:** To correlate anti-GAD antibody titers in the serum and CSF of patients with SPS with the degree of clinical severity.

**Design:** Patients studied the last 6 years.

**Setting:** The Clinical Center of the National Institutes of Health, Bethesda, Md.

**Patients:** Sixteen patients with typical SPS and elevated serum anti-GAD antibody titers.

**Interventions:** Antibody titers in serum and CSF were measured by radioimmunoassay, and the intrathecal anti-GAD-specific IgG production was calculated.

**Main Outcome Measures:** Comparison of antibody titers with stiffness index and heightened sensitivity scores based on scales that reliably measure disease severity.

**Results:** The mean disease duration was 11 years (range, 5-30 years). The mean anti-GAD antibody titer in the serum was 51 500 U/mL (range, 24 000-200 000 U/mL); and in the CSF, 181 U/mL (range, 30-400 U/mL). A 10-fold increased intrathecal production of GAD-specific IgG antibodies was noted. No correlation was found between antibody titers in serum or CSF with disease severity. In 4 patients, the anti-GAD antibody titers measured serially during a 2-year period did not correlate with clinical fluctuations.

**Conclusions:** In patients with SPS, the anti-GAD antibody titers in serum and CSF do not correlate with disease severity or duration. Anti-GAD antibodies are an excellent marker for SPS, but monitoring their titers during the course of the disease may not be of practical value.

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**S**TIFF-PERSON SYNDROME (SPS) is an immune-mediated central nervous system disorder characterized by rigidity of the axial and proximal limb muscles, intermittent superimposed spasms, and heightened sensitivity to external stimuli.<sup>1-3</sup> Continuous cocontraction of agonist and antagonist muscles caused by involuntary firing of motor units at rest are the clinical and electrophysiologic hallmarks of the disease.<sup>1-4</sup>

Stiff-person syndrome is an autoimmune disorder that responds to immunotherapies<sup>5,6</sup> and is frequently associated with other autoimmune diseases, autoantibodies, and certain HLA haplotypes.<sup>1-3</sup> A characteristic autoimmune marker for SPS seen in more than 80% of the patients is the presence of antibodies against glu-

tamic acid decarboxylase (GAD65), the rate-limiting enzyme for the synthesis of  $\gamma$ -aminobutyric acid (GABA), the brain's main inhibitory neurotransmitter.<sup>1-3</sup> These antibodies are produced intrathecally and may impair the synthesis of GABA, resulting in low GABA levels in the brain and cerebrospinal fluid (CSF).<sup>2,3</sup> To determine the clinical relevance of anti-GAD antibodies in patients with SPS, we correlated their intrathecal production and serum titers with the severity of symptoms, using validated clinical scales.

## METHODS

We examined serum and CSF from 16 patients with SPS, 6 men and 10 women, aged 37 to 62 years (mean, 52 years), studied under institutional review board-approved protocols at

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the Neuromuscular Diseases Section, National Institutes of Health, Bethesda, Md, from 1997 to 2003. All patients had elevated titers of serum anti-GAD antibodies and fulfilled the established diagnostic criteria for SPS.<sup>1,3</sup> The serum and CSF were examined for total immunoglobulins, immunofixation electrophoresis, IgG index, and intrathecal synthesis of IgG-specific anti-GAD antibodies. The integrity of the blood-brain barrier was assessed by the ratio of serum albumin to CSF albumin (normal, >130). The CSF IgG index was calculated according to the following formula:

$$(\text{CSF IgG/Serum IgG})/(\text{CSF Albumin/Serum Albumin}),$$

in which CSF IgG and CSF albumin are measured in nanograms per milliliter and serum IgG and serum albumin are measured in milligrams per milliliter.

The CSF IgG index is considered normal when it is below 0.67.<sup>2,7</sup> For detection and quantification of anti-GAD antibodies in serum and CSF, we used a radioimmunoassay kit (Kronus, Boise, Idaho). The results are reported in manufacturer's units per milliliter (normal, <1 U/mL). For the calculation of IgG GAD-specific index, the following formula was used in paired serum and CSF specimens:

$$(\text{CSF IgG Anti-GAD Titer/Serum IgG Anti-GAD Titer})/(\text{CSF Albumin/Serum Albumin}),$$

in which the CSF IgG and serum IgG anti-GAD titers are measured in units per milliliter, CSF albumin is measured in nanograms per milliliter, and serum albumin is measured in milligrams per milliliter.

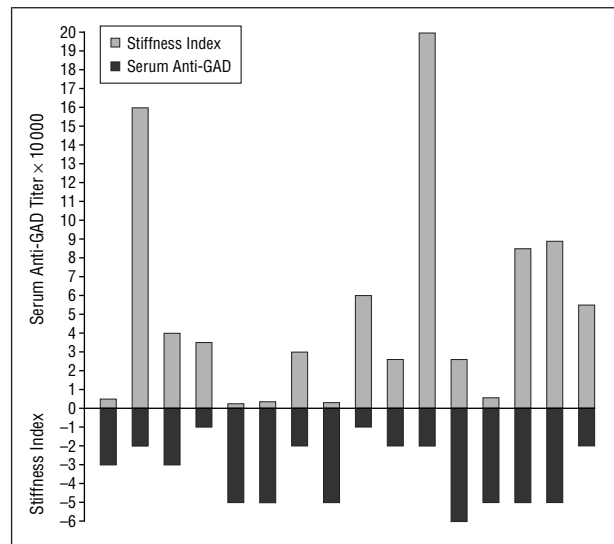
The final concentration of antibodies was obtained after normalization of the different dilutions to the same standard curve. Antibody titers were blindly analyzed and compared with the stiffness index and heightened sensitivity scores, determined for each patient at the time of antibody draw.

The following scales that measure disease severity were used, as previously described.<sup>1,2,6</sup> For *heightened sensitivity*, the following were rated as present or absent: noise-induced stiffness and cramps, visual stimuli-induced stiffness and cramps, somatosensory-induced stiffness and cramps, voluntary activity-induced spasms, emotional upset and "stress"-induced spasms, awakenings due to nocturnal spasms, and untriggered cramps and spasms. The presence of each item added 1 point (maximum score, 7). For *distribution of stiffness and cramps*, the presence of each of the following was given 1 point: stiffness in the face, stiffness in the arms, stiffness in the upper trunk, stiffness in the abdomen, stiffness in the lower trunk, and stiffness in the legs (maximum score, 6).

## RESULTS

### CLINICAL DATA

The duration of disease ranged from 5 to 30 years (mean, 11 years); the time from onset of symptoms to diagnosis ranged from 1 to 13 years (mean, 4 years). The most prevalent initial symptom was lower back muscle stiffness (8 patients), followed by proximal leg stiffness (7 patients). Single-limb stiffness was the initial symptom in 1 patient, and stiffness confined to respiratory and abdominal muscles in another. The median score on the stiffness index was 3.3 (range, 1-6) (**Figure 1**). The median score for heightened sensitivity was 3.8 (range, 2-7). At the time of GAD antibody determination, all studied patients were symptomatic or incapacitated, despite receiving GABA-enhancing drugs such as diazepam, clonazepam, or baclofen. None of the study patients were taking



**Figure 1.** Relationship between serum anti-glutamic acid decarboxylase (GAD) antibodies and stiffness index. Each black bar represents the degree of stiffness for each patient rated on a scale from 0 to 6 (shown as 0 to -6), while the corresponding gray bar shows the patient's serum anti-GAD antibody titer ( $\times 10,000$ ). No consistent correlation was found between serum anti-GAD antibody titers and disease severity.

immunomodulating therapies, but 1 patient had received intravenous immunoglobulin 4 months before determination of GAD antibodies. Seven patients also had insulin-dependent diabetes mellitus, 6 had immune-mediated thyroiditis, and 2 had pernicious anemia. Five patients had strongly positive antinuclear antibodies, 3 had thyroid microsomal antibodies, and 2 others had anti-islet or anti-Jo antibodies.

Neuropsychiatric symptoms were ubiquitous, with anxiety being most prevalent in 9 patients and depressed mood in 3. Five patients had seizures.

### ANTIBODY TITERS AND CORRELATION WITH CLINICAL SEVERITY

No consistent correlation was found between the serum anti-GAD antibody titers and disease severity; the titers were high in some patients with mild disease and low in some others with severe disease (Figure 1).

The mean anti-GAD antibody titer in the CSF was 181 U/mL (range, 30-400 U/mL) and the mean serum titer was 51,500 U/mL (range, 24,000-200,000) (normal, <1 U/mL). The mean ratio of the CSF to serum anti-GAD antibody titer was 0.02 (range, 0.001-0.166), compared with the mean ratio of 0.002 for the CSF to serum IgG (range, 0.001-0.004), reflecting a 10-fold increased production of GAD-specific IgG antibodies in the CSF.

The CSF to serum albumin ratio was normal in all patients, indicating intact blood-brain barrier. No correlation was found between the CSF anti-GAD antibody titers and their intrathecal production with severity of clinical symptoms (**Figure 2**). Although 6 patients with high intrathecal anti-GAD antibody production had clinically severe disease, others with equally severe disease had low GAD-specific CSF IgG index.

The level of anti-GAD antibodies in serum or CSF did not correlate with the presence of other autoantibodies or

