

In Vivo Assessment of Clinically Relevant Autoantibodies in Intravenous Immunoglobulin Preparations

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The present study was designed to determine if intravenous immunoglobulin (IVIG) infusions result in detectable levels of clinically relevant autoantibodies in the serum of IVIG infusion recipients. Serum levels of anti-cyclic citrullinated peptide antibody, antinuclear antibody, cytoplasmic staining antineutrophil cytoplasmic antibody, perinuclear staining antineutrophil cytoplasmic antibody, and thyroid peroxidase antibody (anti-TPO) were measured in patients who receive regular IVIG infusions. Thirty-one subjects enrolled in the study. Anti-TPO levels exceeded the reference range in 21 of 31 (68%) subjects immediately after IVIG infusion. We conclude that patients who receive IVIG at standard replacement doses may have clinically relevant levels of apparent anti-TPO antibodies, as measured with routine diagnostic assays, and this finding is directly attributable to receiving IVIG.

PRIMARY ANTIBODY DEFICIENCY syndromes often predispose patients to the development of autoimmune diseases.^{1,2} Antibody-deficient patients also receive immunoglobulin replacement therapy with intravenous immunoglobulin (IVIG) preparations that contain autoantibodies.³⁻⁷ While it is unlikely that IVIG therapy would transfer a sufficient amount of disease-causing autoantibodies, the presence in IVIG of autoantibodies relevant to the investigation of autoimmune disease may create diagnostic problems for patients who receive IVIG. The present study addressed the specific question of whether IVIG infusions may result in false-positive tests for clinically relevant autoantibodies.

Patients >1 year of age receiving standard IVIG replacement therapy for the diagnosis of X-linked agammaglobulinemia (XLA), common variable immunodeficiency (CVID), or other humoral immunodeficiencies were enrolled sequentially at the time of infusion appointments. The study was approved by the local human subjects review board, and written informed consent was obtained from all patients or parents/legal guardians. Subjects were excluded if they were known to have or suspected of having an autoimmune disease or if they were known to have autoantibodies from prior serum analysis.

Blood samples for serum separation were collected immediately before IVIG infusions and immediately after the infusion of IVIG. For each subject, one set of preinfusion and postinfusion serum samples was collected. Postinfusion samples were analyzed for the presence of anti-cyclic citrullinated peptide antibody (anti-CCP), cytoplasmic staining antineutrophil cytoplasmic antibody (c-ANCA),

perinuclear staining antineutrophil cytoplasmic antibody (p-ANCA), or thyroid peroxidase antibody (anti-TPO) with standard immunoassays (Quest Diagnostics™, San Juan Capistrano, CA). Antinuclear antibody (ANA) was determined with a standard immunofluorescent assay (Immuno Concepts™, HEp-2000 ANA-Ro Test System, Sacramento, CA). Preinfusion samples were sent for analysis only if antibody levels detected in the postinfusion samples were greater than the laboratory reported upper limit of normal. Confidence intervals were calculated for the total number of positive tests in each antibody category. Results were deemed significant if the historically expected number of positive results was outside the confidence interval range.

A total of 31 subjects enrolled in the study. Thirteen of 31 (42%) subjects were female. Age ranged from 9 months to 71 years. Diagnoses included CVID (12 subjects), XLA (8 patients), immunodeficiency not otherwise specified (5 subjects), hyper IgM Syndrome (1 patient), protein losing enteropathy (2 patients), combined immunodeficiency (2 subjects), and X-linked lymphoproliferative disorder (1 subject). Duration of IVIG therapy ranged from <1 to 20 years.

Post-IVIG infusion, anti-TPO levels exceeded the reference range in 21 of 31 (68%) subjects. In 3 patients, insufficient blood was obtained for postinfusion analysis. Of 28 subjects for whom adequate samples were obtained, 21 (75%) were positive, and of those, 20 (95%) converted from negative to positive after their standard IVIG infusion. The confidence interval was 59% to 91%. This range exceeded the value of 5% that is the expected overall presence of TPO

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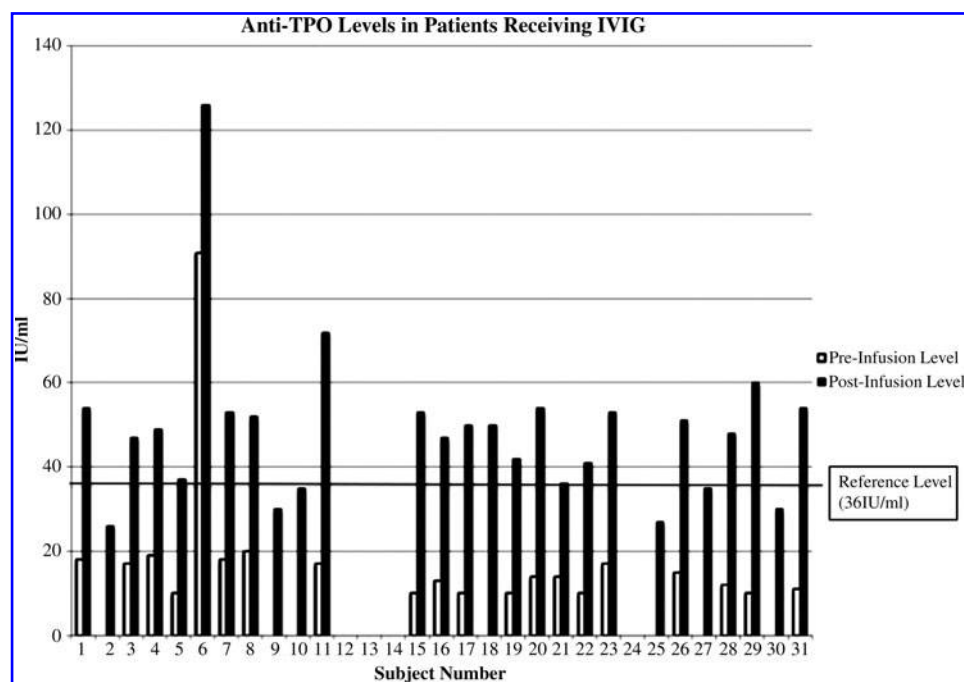


FIG. 1. Anti-TPO levels in postinfusion serum were elevated compared to preinfusion serum. For subjects whose post-infusion anti-TPO level was lower than the reference level, preinfusion serum was not analyzed. For subjects 12, 13, and 14, the volume of serum obtained was insufficient for analysis. Subject 24 refused blood draw. Anti-TPO, thyroid peroxidase antibody.

antibody in this population.⁸ Of the TPO-positive subjects, one subject had a positive value immediately before infusion; the others were negative for anti-TPO (see Fig. 1). The range of IVIG dose for TPO-positive patients was 374–1,030 mg/kg (mean = 550 mg/kg; standard deviation [SD] = 133) and for TPO-negative patients was 240–610 mg/kg (mean = 449 mg/kg; SD = 139). The difference between these means was not statistically significant ($P = 0.12$). The range of durations of IVIG therapy for TPO-positive patients was first dose to 248 months and for TPO-negative patients was 10 to 102 months. The difference between these means was not statistically significant ($P = 0.32$).

ANAs were detected in 5 of 31 patients (16%) when a titer of 1:80 was used as the cut-off.⁹ The confidence interval at this cut-off included the amount expected to be positive in historical populations of normal controls.⁹ No patient converted from negative to the more clinically relevant threshold titer of 1:160. Antibody assays for c-ANCA, p-ANCA, or anti-CCP were negative in the postinfusion samples from all 31 subjects.

The present study demonstrates that patients who receive IVIG at standard replacement doses may have clinically relevant levels of apparent anti-TPOs as measured with routine diagnostic assays, and that this finding is directly attributable to receiving IVIG. This reactivity may reflect true anti-TPO or an epiphenomenon of IVIG. We did not compare our population with subjects who receive IVIG for other reasons, so we do not know if this phenomenon is seen in nonimmunodeficient patients. It is not possible to comment on the pathogenicity of these antibodies. On the basis of extensive historical experience with IVIG infusions, we believe that IVIG infusions do not cause thyroid disease.

The impact of IVIG infusions on autoantibody tests other than anti-TPO was very limited. ANA titers were elevated, but only at a threshold generally not considered clinically significant. It is possible that other IVIG products or doses of IVIG used for immune modulation that are 2 to 5 times as high as the doses used in this study may cause false-positive autoantibody assays. Results of serologic testing for autoimmune disease in patients receiving IVIG must be interpreted with care. Comparing autoantibody levels obtained immediately before and after an IVIG infusion may assist in this regard.

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Author Disclosure Statement

Joseph Church declares that he has no conflict of interest. Jordan Abbott declares that he has no conflict of interest.

References

1. Lopes-da-Silva S, Rizzo LV. Autoimmunity in common variable immunodeficiency. *J Clin Immunol* 2008; 28:S46–S55.
2. Cunningham-Rundles C. Autoimmune manifestations in common variable immunodeficiency. *J Clin Immunol* 2008; 28:S42–S45.
3. Saenko VA, Kabakov AE, Poverenny AM. Hidden high-avidity anti-DNA antibodies occur in normal human gammaglobulin preparations. *Immunol Lett* 1992; 34:1–5.
4. Stratta P, Canavese C, Schinco, Montaruli B, Giorda L, Bessone P, et al. Intravenous immunoglobulin contains detectable amounts of antiphospholipid antibodies. *Br J Haematol* 1997; 96:872–873.
5. Krause I, Blank M, Shoenfeld Y. Anti-DNA and antiphospholipid antibodies in IVIG preparations: *in vivo* study in naive mice. *J Clin Immunol* 1998; 18:52–60.

6. Jolles S, Deacock S, Turnbull W, Silvestrini R, Bunn C, White P, et al. Atypical c-ANCA following high dose intravenous immunoglobulin. *J Clin Pathol* 1999; 52:177–180.
7. Hao W, Davis C, Daniels T, Hampe CS, Lernmark A. Epitope-specific glutamic acid decarboxylase-65 autoantibodies in intravenous immunoglobulin preparations. *Transfus Med* 1999; 9: 307–310.
8. Baloch Z, Carayon P, Conte-Devolx B, Demers LM, Feldt-Rasmussen U, Henry JF, et al. Laboratory medicine practice guidelines. Laboratory support for the diagnosis and monitoring of thyroid disease. *Thyroid* 2003; 13:3–126.
9. Tan EM, Feltkamp TE, Smolen JS, Butcher B, Dawkins R, Fritzler MJ, et al. Range of antinuclear antibodies in “healthy” individuals. *Arthritis Rheum* 1997; 40:1601–1611.

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