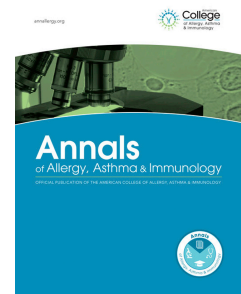


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Deepti Vellaichamy Manian, MD, Chelsee Jensen, Pharm.D., R.Ph., Elitza S. Theel, Ph.D, John R. Mills, Ph.D, Avni Joshi, MD., MS.



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Title page:

Non-neutralizing antibodies and limitations of serological testing for SARS-CoV-2 in patients receiving replacement immunoglobulin products

Authors

Deepti Vellaichamy Manian, MD¹, Chelsea Jensen Pharm.D², R.Ph. Elitza S. Theel, Ph.D³, John R. Mills, Ph.D³, and Avni Joshi, MD¹, MS.

¹Division of Allergic Diseases, Mayo Clinic, Rochester, MN 55905

²Pharmaceutical Formulary Manager, Mayo Clinic, Rochester, MN 55905

³Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN 55905

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Corresponding author:

Avni Joshi, M.D., M.S. Division of Allergic Diseases, Mayo Clinic

200 First St SW Rochester MN 55905

507-284-2511 Fax-507-284-0727

Joshi.avni@mayo.edu

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With the growing surges in coronavirus disease 2019 (COVID-19) cases caused by the Severe Acute Respiratory Syndrome coronavirus-2 (SARS-CoV-2) across the globe, there has been growing concern of its implications in patients with primary immune deficiency disorders (PIDs). Common variable immunodeficiency (CVID) is a common PID characterized by a failure in B-cell differentiation with defective immunoglobulin production, making the patients uniquely susceptible to recurrent infections.¹ While current evidence suggests higher risk for more severe disease in patients with both primary and secondary immune deficiency states, the exact burden of COVID-19 in these disease states is unknown.^{2,3}

There are multiple diagnostic strategies to identify COVID-19 infection. RT-PCR or antigen based testing is used to detect an active infection, while serologic tests are useful to detect past infections. A positive antibody test is suggestive of an infection at some time in the past, but whether these antibodies are protective against SARS-CoV-2 reinfection is unclear. A negative antibody result may indicate remote or no prior infection with SARS-CoV-2, however individuals may be negative if samples are collected too soon following infection or if the individual is incapable of mounting a humoral immune response, particularly in PIDs or secondary immunosuppressed states. Immunity against SARS-CoV-2 will likely be multifaceted and not solely dependent on the antibody response, with some studies demonstrating that innate and cell mediated immunity may also play a significant role in recovery and prevention of re-infection with SARS-CoV-2.^{4,5} Neutralizing antibodies are those that inhibit the virus from infecting other cells and are generally thought to play a direct role in protective immunity. However not all antibodies are neutralizing and currently FDA Emergency Use Authorization

has yet to be granted to assays that are capable of distinguishing between neutralizing and non-neutralizing antibodies.

Here, we report a case of a 59-year-old female, weighing 71 kg with COVID who presented for a yearly follow up visit. She has been maintained on supplemental IgG therapy with 10% Gamunex-C at a dose of 35 grams every 3 weeks. As per institutional guidelines, on May 20th, 2020, she underwent SARS-CoV-2 testing as a prerequisite for pulmonary function testing.. The patient tested negative for SARS-CoV-2 by RT-PCR, but was positive for IgG-class antibodies against the SARS-COV-2 spike protein. She used to work in the local school system but had been mostly homebound over the past 2 months due to the state-wide “shelter in place” recommendations. Her potential exposure to SARS-CoV-2 might have been through her adult son, who was a health care worker, and had tested positive for SARS-CoV-2 via RT-PCR in March 2020. She reported very limited interactions with him and adherence to physical distancing and universal masking recommendations. It was unclear if the SARS-CoV-2 antibodies were de novo production or exogenous from her IVIG infusions. Therefore, additional SARS-CoV-2 antibody levels were tested around her IVIG infusion to assess for a trend. We tested her again prior to her next IVIG infusion on June 5th, 2020 and post IVIG infusion on June 8th, 2020.

Her serial SARS-CoV-2 antibody levels showed an initial positive results at an index value of 1.13 (5/20/2020, positive is ≥ 1.1 index) and then intermediate results (index values between 0.8 and 1.0) on 6/5/2020 and 6/8/2020; with no changes in her titers post IVIG infusion on

6/5/2020 (Figure 1), likely indicating the natural trajectory of waning immunity after natural infection or variability of the lots for SARS-CoV-2 antibody concentrations. To further assess, whether her seropositivity was a result of the IVIG infusions she received, we tested 6 samples from different commercially available IgG products (*Privigen*, *Hizentra*, *Gamunex-C*, and *Gammagard Liquid*) for SARS-CoV-2 IgG antibodies. All six samples were positive when tested undiluted using the Euroimmun SARS-CoV-2 IgG ELISA. At a 1:101 dilution, the recommended dilution by the manufacturer for testing serum, four commercial IgG preparations were still positive, two were negative and one indeterminate. We further sought to assess if these antibodies were neutralizing and found that none of the IVIG products were positive for neutralizing antibodies (Table 1).

The IVIG solutions are pooled from 3000 to 10,000 donors, which contributes to a wide range of antibody specificities against various infectious agents. These include but are not limited to bacteria and, viral pathogens including those causing respiratory coronaviruses infections that are ubiquitous, reflecting the cumulative exposure of the donor population to the environment.⁶ The lead time for IVIG preparation is typically 6-9 months after donor collection pooling. Recently, antibodies against several antigens of human common *betacoronaviruses* in IVIG preparations have shown to have positive cross-reactivity with SARS-CoV-2 antigens.⁷

Cohorts representing the general population have shown low sero-prevalence even in COVID-19 hotspots, suggesting that these antibodies may not be long lasting or individuals have not yet seroconverted. A recent cohort study of 175 patients with clinically mild COVID-19 infection reported robust induction of SARS-CoV-2-specific neutralizing antibodies in 94% of patients

within 2 weeks of symptom onset. Of note, 10 of these patients recovered without developing detectable neutralizing antibodies, suggesting that other components of the serological response and cellular immune response may play a role in convalescence.⁸

The positive SARS-CoV-2 IgG antibody results with negative neutralizing antibodies found in commercial IgG preparations may signify cross reacting antibodies or false reactivity due to the presence of these antibodies in the IVIG or SCIG samples or differences in analytical sensitivity between assays.

. Future studies should seek to explore if patients receiving commercial IVIG therapy have a lower incidence of SARS-CoV-2 infections or milder disease courses when infected.

Additionally, the potential to use commercial IVIG in patients as a prophylactic or as a therapeutic strategy are been explored on a clinical trial basis⁹.

In summary, the utility of SARS-CoV-2 antibody testing in patients with humoral immunodeficiency on IgG replacement therapy is unclear. Our case highlighting the caveats of serologic testing for COVID-19. Our survey of several commercially available IgG products for SARS-CoV-2 antibodies showed the presence of cross reacting antibodies which may be non-neutralizing in nature.

Table 1: Commercial IgG products and SARS-CoV-2 Antibodies

Sample ID	Index Value (1:1	SARS-CoV-2 IgG antibody result*	Index Value (1:101	SARS-CoV-2 IgG antibody result	Neutralization antibody results

	dilution)		dilution)		
PRIVIGEN 6709	7.33	Positive	1.00	Indeterminate	Negative
PRIVIGEN 0054	7.81	Positive	1.06	Indeterminate	Negative
HIZENTRA 8411	3.07	Positive	1.62	Positive	Negative
HIZENTRA 0624	1.62	Positive	0.38	Negative	Negative
GAMUNEX 0013	7.22	Positive	1.38	Positive	Negative
GAMMAGARD 39AB	8.48	Positive	1.68	Positive	Negative
GAMUNEX 0362	N/A	N/A	N/A	N/A	Negative

87

88 * Positive > 1.10. ; Indeterminate: 0.8-1.1; Negative: <0.8

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