

Repeated Negative Serological Testing in Otherwise Healthy Patients With Coronavirus Disease 2019

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TO THE EDITOR—We read with great interest the study by Zhang et al [1] on longitudinal changes of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibodies in coronavirus disease 2019 (COVID-19). The authors suggest that serological testing is useful for early COVID-19 diagnosis. However, the short- and long-term utility of serological testing remains unclear, because the rate of seroconversion after documented SARS-CoV-2 on molecular testing ranges from 30% to 71% [2]. Therefore, we aimed to prospectively study the rates of seroconversion in our patients.

We prospectively enrolled consecutive patients with signs and/or symptoms of COVID-19. Molecular testing was performed with a reverse-transcription polymerase chain reaction (PCR) kit (VIASURE SARS-CoV-2 Real-Time PCR Detection Kit, CE-IVD; CerTest BIOTEC). Detected genes include the *ORF1ab* and *N* and an internal control to monitor extraction efficiency and PCR inhibition. Serological testing was performed 10–19 days after the syndrome's initiation. The antibody level was evaluated by 2 methods: the 2019-nCoV, immunoglobulin (Ig)G, and IgM chemiluminescence immunoassay (CLIA) assay (Maglumi) and the SARS-CoV-2 IgG chemiluminescent microparticle immunoassay (CMIA) (Abbott). Maglumi's IgG antibodies method reports sensitivity and specificity of 95.6% and 96% and Abbott's IgG antibodies 99% and 100%, respectively, according to the manufacturers. The IgM and IgG seronegative patients were also tested by the rapid 2019 nCoV IgG/IgM combo test card (Xiamen Boson Biotech). Complement activation

products were measured in these seronegative patients using the complement C3/C4 reagent kit (Abbott). Immunoglobulin levels (IgA, IgG, IgM) were also measured in these patients (Abbott). The study was performed according to the Helsinki Declaration. Written informed consent was obtained from all patients.

Median age of patients was 22 years, ranging from 22 to 62, and the majority of them were male (61%). Among 26 patients, IgG antibodies were positive in 24 patients. Mean IgG levels were 1.129 (index) with a standard deviation of 6.879. Regarding IgM antibodies, mean IgM levels were 0.489 (index) with a standard deviation of 0.843. Only 4 patients were positive for IgM antibodies.

Detailed history was recorded in the 2 seronegative patients (both IgM and IgG). They were otherwise healthy individuals, a male aged 26 years and a female aged 43 years. Although both had a positive molecular test, the clinical course of infection was mild. Symptoms included anosmia and fatigue in both patients and grade 1 fever in one. The serological testing was repeated 10 days after the first negative results, within 20–29 days after the molecular testing. Immunoglobulin M and IgG remained negative by all methods. Complement testing was negative. Immunoglobulin levels were within normal limits. No signs or symptoms of an underlying disease were documented during the presentation or the 2-month follow-up of the patients.

It is interesting to note that there are only a few reports of persistently seronegative patients after COVID-19 [1]. Zhang et al [1] have documented seronegative patients at different time points, but it remains unclear whether there were persistently seronegative patients in their cohort. Furthermore, COVID-19 diagnosis was not confirmed with molecular testing [1]. Solodky et al [2] included patients with molecular testing. Among positive patients, 4 of 14 otherwise healthy

individuals remained negative 15 days after the infection. However, this testing was performed only with 1 method and not repeated over time [2]. In addition, no data were reported on the severity of infection.

Furthermore, a recent study has reported an excellent specificity and sensitivity of the Abbott's antibody testing [3]. Optimal measurement was defined within the time period used in our study. Given that seronegative patients were also tested with additional methods in our study, these patients are considered true negative. A potential explanation of seronegative results in our patients could be their mild course of infection. Given that severe COVID-19 is life-threatening [4, 5], linking the severity of infection with seropositivity would be useful for guiding management of COVID-19.

In conclusion, our prospective study suggests that serological testing is useful for the majority of patients. Nevertheless, a small percentage of otherwise healthy individuals remained seronegative despite documented infection. Further prospective studies are needed to highlight the clinical usefulness of serological testing.

Notes

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