Clinical Validity of Serum Antibodies to SARS-CoV-2: A Case-Control Study

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Objective

Determine the clinical validity and utility of SARS-CoV-2 antibodies.

Methods

First month of COVID-19 nasopharyngeal nucleic acid amplification test (NAAT) testing at the Johns Hopkins Hospital, Baltimore, Maryland, performed in 11,066 subjects. 115 of 11,066 (1%) were adult, hospitalized patients investigated for COVID-19. 614 were laboratory controls not tested by NAAT: 160 healthy laboratory employees, 101 EBV capsid, 215 thyroperoxidase, 101 immunodeficient, and 37 rheumatoid factor antibody positive subjects. The 729 participants contributed 977 sera. Clinical record review to classify 115 patients into COVID-19 cases (No. = 60) or non-COVID-19 controls (No. = 55).

All sera were tested for the presence of IgG and IgA antibodies against the S1 domain of the SARS-CoV-2 spike protein using a manual commercial ELISA (enzyme-linked immune-sorbent assay, from Euroimmun, Lübeck, Germany), which received an Emergency Use Authorization by the Food and Drug Administration on May 4, 2020. The assay yields a unit-less ratio calculated by dividing the absorbance (optical density) of antibody reactivity in patient serum by that of an assay calibrator (a proprietary humanized monoclonal antibody to the SARS-CoV-2 spike protein). The assay was performed according to the manufacturer's recommendations, testing the sera at a 1:101 dilution and respecting specified incubation temperatures, with two main modifications. We extensively honed the assay to select an in-house threshold value of the ratio that best distinguished COVID-19 cases from controls. We also serially diluted (1:1000 and 1:2000) samples that violated the Beer-Lambert law, which describes the linear relationship between absorbance and concentration, to obtain a quantitative value instead of a "greater than" estimate for samples with high antibody reactivity.

Results

Sensitivity and specificity of SARS-CoV-2 IgG assay were 0.976 (0.928 – 0.995) and 0.988 (0.974–0.995), respectively, when performed 14 days or later after symptom onset, but sensitivity decreased at earlier time points. IgG developed rapidly and sustained high levels throughout follow-up (up to 58 days). SARS-CoV-2 antibodies predicted odds of developing acute respiratory distress syndrome, which increased by 62% (95% CI: 48%-81%, P<0.001) for every two-fold increase in IgG. Of 11,066 NAAT-tested subjects, 457 were repeatedly NAAT-negative, and sera were obtained for 18 such patients: 6 COVID-19 cases and 12 non-COVID-19 controls. Antibodies were present in 6 of 6 cases and none of the 12 controls (P=0.001).

Conclusions

SARS-CoV-2 antibodies demonstrate infection when measured at least 14 days after symptom onset, associate with clinical severity, and provide valuable diagnostic support in patients who test negative by NAAT but remain clinically suspicious for COVID-19.

Figure 1

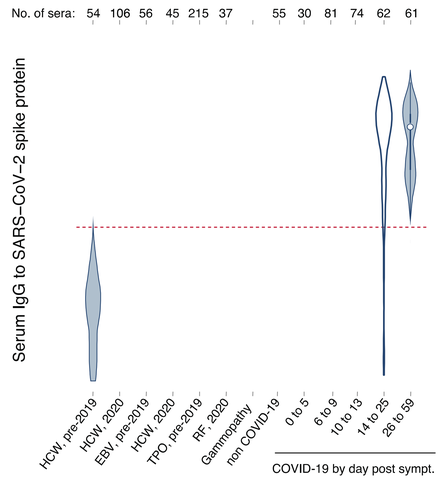

Figure 1. interquartile. Violin plot showing the distribution of IgG levels, indicating median and range. The horizontal dashed line represents the cutoff (1.1 units).

Figure 2

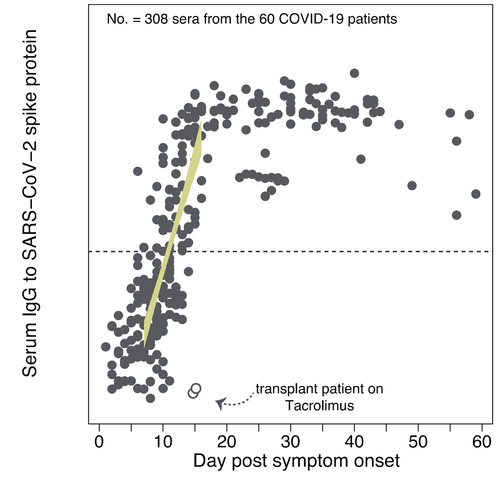

Figure 2. Overall relationship between IgG levels and day post symptom onset. The white circles represent a hypogammaglobulinemic patient receiving immunosuppressive therapy due to kidney transplant.

Figure 3

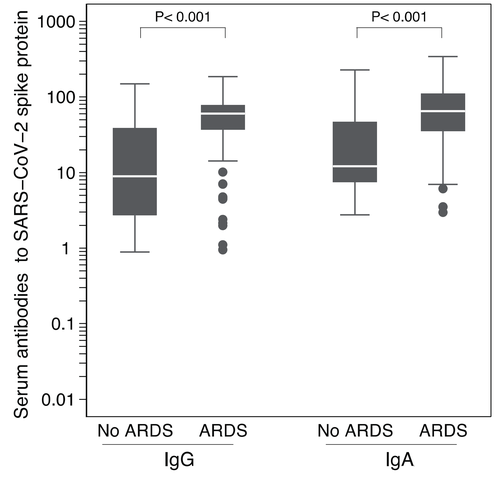

Figure 3. Serum IgG and IgA antibody levels, stratified by the presence or absence of ARDS.

Figure 4

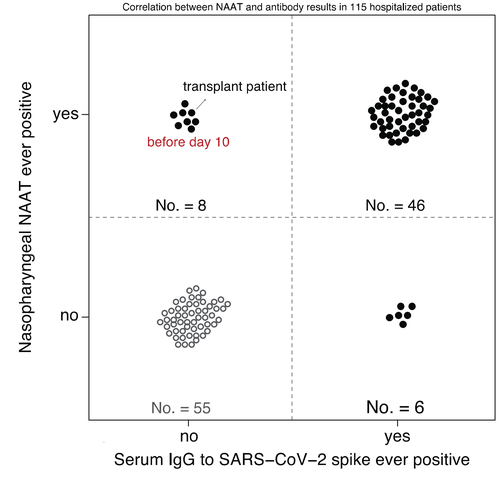

Figure 4. Integration of clinical data and molecular testing with serum antibodies to SARS-CoV-2 spike protein.