False-Positive Rates in Pediatric SARS-CoV-2 Serology Testing

Biological, Clinical, and Technical Considerations

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TO THE EDITOR

We read with interest the report by Geisler et al1 and commend the authors for their efforts to address the important need to evaluate the clinical performance of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) diagnostic assays in pediatric populations, especially with respect to serology, given the noted differences in immune responses between children and adults.2 We do feel, however, that the title of this paper may be misleading. First, given the timing of sample collection during the pandemic (including shortly after a peak phase), positive results are certainly not implausible. In addition, only a fraction of the positive samples that were positive by EUROIMMUN Anti–SARS-CoV-2 IgG ELISA (73/85 [86%]) were retested with 2 different immunoassays, although it would have been preferable to concomitantly investigate all 2,238 samples with all methods to achieve an unbiased set of data and thus compare the cumulative positivity rate in this pediatric cohort. Moreover, no demographic or clinical information has been provided about these patients, which significantly limits our interpretations.

As the authors have noted, discordant results among assays could in part be caused by differences in the target antigen, determined immunoglobulin (Ig) subclasses and analytical technique among the 3 assays. Therefore, a certain degree of discrepancy in test results cannot be excluded, but they could be substantially improved with inter assay harmonization.3 The receptor-binding domain (RBD) is the antigenic domain used in the Beckman and Siemens immunoassays, whereas the full-length S1 protein subunit, which includes the RBD, is used in the EUROIMMUN IgG assay. Thus, compared with the RBD moiety only, the EUROIMMUN S1 antigen contains additional epitopes to which antibodies could bind. S1 antibodies have been shown to be more sensitive and specific than those that target antigens within the RBD because they would be capable of capturing both non-RBD and RBD-binding coronavirus disease 2019 antibodies.4 Indeed, although RBD-binding antibodies are commonly referred to as “neutralizing” antibodies, perfect equivalence between RBD-binding antibodies’ neutralizing antibodies and the actual neutralizing potential of serum or plasma does not exist. For example, a recent study found that the concordance among 7 anti–SARS-CoV-2 immunoassays and virus-neutralization tests varied widely between a minimum of 0.24 and a maximum of 0.72.1 It may also be that anti–SARS-CoV-2 antibodies may differ in that decay of more specific anti-RBD antibodies may precede decay of generic anti-S1 (or S1/2) antibodies. This behavior would make it challenging to define whether anti-RBD antibodies are false negatives or if anti-S1 antibodies are false positives. These considerations are important when performing serology studies, with anti-S1/S2 serology tests preferably used to assess true seroprevalence; anti-RBD-specific serology immunoassays may be better suited to reflecting the presence of neutralizing antibodies.

Finally, it is noteworthy that the EUROIMMUN Anti–SARS-CoV-2 IgG assay has been well validated in the current scientific literature; it was the first serology assay to be independently validated by the Frederick National Laboratory for Cancer Research. That said, we encourage all diagnostic companies to provide pediatric-specific diagnostic performance data and support the independent validation and publication of their assay performance in pediatric populations by academic medical centers.

Ultimately, more studies are required to determine the false positivity rate in children using SARS-CoV-2 serologic assays. Clinically characterized samples are critical to such conclusions, while differences in the technology, antigen used, signal detection (ie, enzyme-linked immunosorbent assay [ELISA] vs chemiluminescence), and detected immunoglobulin classes are essential when interpreting test results.

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REFERENCES

1. Geisler D, Freeman MC, Rapsinski GJ, et al. Unexpected false-positive rates in pediatric SARS-CoV-2 serology using the EUROIMMUN Anti–SARS-CoV-2 IgG assay. Am J Clin Pathol. 2021;155:773–775.

2. Weisberg SP, Connors TJ, Zhu Y, et al. Distinct antibody responses to SARS-CoV-2 in children and adults across the COVID-19 clinical spectrum. Nat Immunol. 2021;22:25–31.

3. Plebani M, Padoan A, Negriini D, et al. Diagnostic performances and thresholds: the key to harmonization in serological SARS-CoV-2 assays? Clin Chim Acta. 2020;509:1–7.

4. Tian Y, Lian C, Chen Y, et al. Sensitivity and specificity of SARS-CoV-2 S1 subunit in COVID-19 serology assays. Cell Discov. 2020;6:75.
TO THE EDITOR

We at EUROIMMUN have read the article published by Geisler et al1 and would like to provide our response to it. Despite the interesting study, including a cohort of pediatric patients, we believe that the authors have misleadingly settled on the title “Unexpected False-Positive Rates in Pediatric SARS-CoV-2 Serology Using the EUROIMMUN Anti–SARS-CoV-2 ELISA IgG Assay.” After reviewing the publication, we disagree with the authors’ result interpretations and would like to address and highlight the various reasons for it.

First, it is important to consider that the samples investigated in this study were collected during the coronavirus disease 2019 (COVID-19) pandemic. It has been estimated that 16% to 50% of the pediatric infections are asymptomatic.2 Therefore, it is possible that one could expect few positive results in the cohort tested. Additionally, only samples that were positive by EUROIMMUN assay were retested using the other commercial assays. The evaluation of all the samples with all assays would have been preferred to avoid underestimating results and to be able to compare the overall positivity rates across manufacturers.

As the authors reported, it is important to note that there are differences among the 4 assays in terms of the antigen target, measured immunoglobulin classes, and detection technology implemented. Therefore, one would expect to find inconsistencies in the results. The EUROIMMUN assay incorporates the full-length S1 protein subunit, including the receptor-binding domain (RBD), while the other assays incorporate only the RBD. Compared with the RBD moiety, more epitopes are available on the full-length S1 subunit.

The results of the study showed a positive rate of 3.64% (95% CI, 2.91%-4.48%) with the EUROIMMUN assay. This rate translates into a specificity of 96.36%, which is greater than the 95% criterion that the US Food and Drug Administration accepted. This high specificity rate is not proportionate to the high false positivity rate (60.82%) that the authors reported. The performance of our enzyme-linked immunosorbent assay (ELISA), including its excellent specificity, has been confirmed by several external studies conducted to date.3 Additionally, our assay was the first serology assay independently validated by federally funded research (the National Cancer Institute). The results reported excellent sensitivity and specificity for the EUROIMMUN assay.4 Recently, another external research institute validated our assay, which resulted in inclusion of our assay in the list of tests for use in the manufacture of COVID-19 convalescent plasma.5

During CE validation of EUROIMMUN assay, we also tested a set of 100 pediatric samples of children aged 3 to 10 years (samples collected before 2020). Only 1 sample tested weak positive, which corresponds to an overall specificity of 99% (unpublished data).

In conclusion, the study by Geisler et al does not accurately represent the true specificity of the EUROIMMUN Anti–SARS-CoV-2 IgG ELISA. We ask the editors to reevaluate this publication for appropriate interpretation of the results and to address additional limitations of this study as highlighted above. We agree on the need for more studies with pediatric samples to improve the accuracy of serology tests, but it is important to include clinically characterized samples for these studies to draw appropriate conclusions.

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REFERENCES

1. Geisler D, Freeman MC, Rapsinski GJ, et al. Unexpected false-positive rates in pediatric SARS-CoV-2 serology using the EUROIMMUN Anti–SARS-CoV-2 ELISA IgG assay. Am J Clin Pathol. 2021;155:773-775.
2. Information for pediatric healthcare providers. Centers for Disease Control and Prevention Web site. https://www.cdc.gov/coronavirus/2019-ncov/hcp/pediatric-hcp.html#:~:text=A%20recent%20systematic%20review%20estimated,pediatric%20infections%20may%20be%20asymptomatic. Updated December 30, 2020. Accessed June 18, 2021.
3. Beavis KG, Matushek SM, Abeleda APF, et al. Evaluation of the EUROIMMUN Anti–SARS-CoV-2 ELISA assay for detection of IgA and IgG antibodies. J Clin Virol. 2020;129:104468.
4. Coronavirus (COVID-19) update: daily roundup May 4, 2020 [press release]. Silver Spring, MD: US Food and Drug Administration; May 4, 2020. https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-daily-roundup-may-4-2020. Accessed June 18, 2021.
5. Letter to Nikki Bratcher-Bowman regarding tests acceptable for use in the manufacture of high titer COVID-19 convalescent plasma [departmental letter]. Silver Spring, MD: US Food and Drug Administration; March 9, 2021. https://www.fda.gov/media/141477/download. Accessed May 3, 2021.

THE AUTHORS’ REPLY

We thank the authors for their interest in our work and appreciate the further discussion of our data and study limitations. For severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), it has been and will be critical to determine whether children are exposed to the virus to inform public health decisions, particularly as many children remain ineligible for vaccination. Because of a lack of published data on test performance in pediatric patients, assessment of serologic testing using multiple approaches is critical for proper test use and clinical interpretation. As the authors indicated, larger and more comprehensive studies are needed. Pretest sensitivity and specificity of assays are critical performance metrics that compare a cohort of known positive with known negative patients. All assays used in this work have independently tested sensitivity and specificity of more than 98% in our own and other adult studies. These assays have demonstrated excellent concordance when compared with the assay consensus of 6 clinical tests using receptor-binding domain or S1 protein antigens.1 In our study, we had sufficient remnant specimen for...
follow-up testing for 73 of the 85 initially positive samples from a total cohort of 2,338 specimens, which limits our analysis. Limited sample volume is common with residual clinical samples, however, particularly in pediatrics.  

Serology plays a key role in seroprevalence studies, the case definition for multisystem inflammatory syndrome in children, a reportable disease, and in determining prior SARS-CoV-2 infection. In clinical settings, we are interested in the posttest probabilities calculated with the validation sensitivity/specificity and disease prevalence. Typical performance characteristics assessed are the false-positive rate (FPR) and the positive predictive value (PPV) or their negative complements, false-negative rate, and negative predictive value. In vaccinated populations, seroprevalence resulting from vaccination is quickly rising, but in pediatric cohorts, prevalence is often still quite low. At the time of this study, the estimated pediatric prevalence based on adult seroprevalence was approximately 1%. At a prevalence of 1% in a serosurvey of 2,338 children and an assay with 99% sensitivity and specificity, 50% of our positive results are calculated to be false positives. When performing orthogonal testing in our study, the first test screens a low-prevalence population (1%). The second test, with identical sensitivity and specificity, will be screening a population with an expected 50% prevalence, based on the anticipated true-positive rate of the first test. Therefore, on this principle, one would calculate no false positives or false negatives by the second test because of the enriched prevalence. Any minor change in specificity (because of population differences), however, will lead to large changes in the FPR. At a 1% prevalence, with a sample size of 2,338 and a sensitivity of 99%, we calculate that specificities of 99%, 98%, 97%, 96%, and 95% would result in FPRs of 50%, 67%, 75%, 80%, and 83%, respectively. These data are not a criticism of the tests themselves, but they remind us of the importance of orthogonal testing, particularly in a low-prevalence population. Small changes in specificity caused by lack of assay characterization in a population can also alter FPR and PPV. Arguably, the most straightforward way to manage this alteration is to incorporate a second test of all positive samples, which increases the prevalence for the second test, and our findings support this recommendation. As we all note, more work is needed to fully understand assay performance and immune response to SARS-CoV-2 in children.

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REFERENCES  

1. Zilla M, Wheeler BJ, Keetch C, et al. Variable performance in 6 commercial SARS-CoV-2 antibody assays may affect convalescent plasma and seroprevalence screening. Am J Clin Pathol. 2021;155:343-353.  
2. Geisler D, Freeman MC, Rapsinski GJ, et al. Unexpected false-positive rates in pediatric SARS-CoV-2 serology using the EUROIMMUN Anti–SARS-CoV-2 ELISA IgG assay. Am J Clin Pathol. 2021;155:773-775.  
3. Brownstein NC, Chen YA. Predictive values, uncertainty, and interpretation of serology tests for the novel coronavirus. Sci Rep. 2021;11:5491.  
4. Interim guidelines for (COVID-19) antibody testing. US Centers for Disease Control and Prevention Web site. https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antibody-tests-guidelines.html. Updated March 17, 2021. Accessed October 6, 2020.  
5. Diagnostic test calculator. Alan Schwartz’s Web site. http://araw.med.uic.edu/cgi-bin/testcalc.pl. Accessed May 7, 2021.