Manufacturer Signal-to-Cutoff Threshold Underestimates Cumulative Incidence of SARS-CoV-2 Infection: Evidence from the Los Angeles Firefighters Study

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Background: The objective of this analysis was to compare the performance sensitivity and specificity of manufacturer-recommended signal-to-cutoff (S/Co) thresholds with modified S/Co values to estimate the prevalence of SARS-CoV-2-specific antibodies in a cohort of firefighters with a known infection history.

Methods: Plasma venipuncture samples were used for serologic analysis of firefighters in Los Angeles, CA, USA, in October 2020. Seropositivity was assessed using the manufacturer's recommended S/Co ($\geq$1.4 IgG) and modified S/Co thresholds based on measured antibody levels in 178 negative control patients who had blood drawn prior to the emergence of COVID-19. Optimal S/Co threshold was determined by receiver operating characteristic (ROC) curve analysis.

Results: Of 585 firefighters included in the study, 52 (8.9%) reported having a PCR-positive test history prior to antibody testing. Thirty-five (67.3%) firefighters with a previous PCR-positive test were seropositive based on the manufacturer S/Co thresholds, consistent with an estimated 67.3% sensitivity and 100% specificity. After evaluating multiple modified S/Co thresholds based on pre-pandemic negative samples, a modified S/Co of 0.36 was found to yield optimal sensitivity (88.5%) and specificity (99.4%) by ROC curve analysis. This modified threshold improved serostatus classification accuracy by 21.2%.

Conclusions: S/Co thresholds based on known negative samples significantly increase seropositivity and more accurately estimate cumulative incidence of disease compared to manufacturer-based thresholds.

INTRODUCTION

Designing an appropriate public health response to the pandemic requires an accurate estimate of the cumulative incidence of SARS-CoV-2 infection. While serologic tests for antibodies against SARS-CoV-2 have served as the primary method for modeling the cumulative

IMPACT STATEMENT

While serology tests are a critical tool for evaluating the cumulative incidence of SARS-CoV-2 infection, the exact signal-to-cutoff (S/Co) thresholds used for defining seropositivity are unclear. Manufacturer thresholds were established with serum collected from hospitalized patients, and the performance characteristics of these thresholds in those with milder disease is unknown. We demonstrate that modified S/Co thresholds using pre-pandemic negative samples more accurately estimate cumulative incidence of disease compared to manufacturer-based thresholds. These findings have important implications for correctly classifying serostatus and understanding the cumulative incidence of SARS-CoV-2, which will benefit epidemiologists and public health researchers studying COVID-19.

incidence of disease, factors including test kit sensitivity, waning antibody levels, and disease severity can influence seropositivity estimates ([1–3]). Another important consideration when determining serostatus is the signal-to-cutoff (S/Co) threshold used in antibody assays to define seropositive cases. For many commonly used platforms, manufacturer-recommended S/Co thresholds were established by testing hospitalized COVID-19 patients, or those with severe illness. Although these thresholds have performed well in populations of similar disease severity, their performance when applied to the general population of those infected with SARS-CoV-2 remains unclear.

We conducted serologic testing to assess the prevalence of SARS-CoV-2-specific antibodies in a cohort of firefighters with a known infection history. We compared the performance sensitivity and specificity of manufacturer-recommended S/Co values with modified S/Co values as determined by testing pre-pandemic negative control samples. We hypothesized that alternate S/Co thresholds would improve performance characteristics and more accurately estimate the cumulative incidence of disease in this cohort of firefighters with mild sickness representative of the majority of SARS-CoV-2-infected individuals.

MATERIALS AND METHODS

This is a prospective, cohort study of firefighters employed by the Los Angeles Fire Department. Firefighters were invited to respond to a questionnaire and received PCR and antibody tests (see online Supplemental Table 1). The Los Angeles County Department of Public Health Institutional Review Board approved this study. We obtained written informed consent from all study participants. We collected data on participant demographics and PCR-confirmed SARS-CoV-2 infection history through electronic surveys.

An estimated 3000 firefighters actively employed by the Los Angeles Fire Department were eligible for study participation. Participants were recruited through an employee intranet portal from July 2020 to October 2020. Participant onboarding was performed through a proprietary web- and mobile-based application developed by Gauss Surgical (Menlo Park, CA, USA). We collected EDTA plasma venipuncture samples from participants who did not report symptoms on the sample collection day. Serology testing was conducted at Cedars-Sinai Medical Center's CLIA-certified laboratory with US Food and Drug Administration (FDA) Emergency Use Authorization (EUA) approvals, using the Abbott Architect SARS-CoV-2
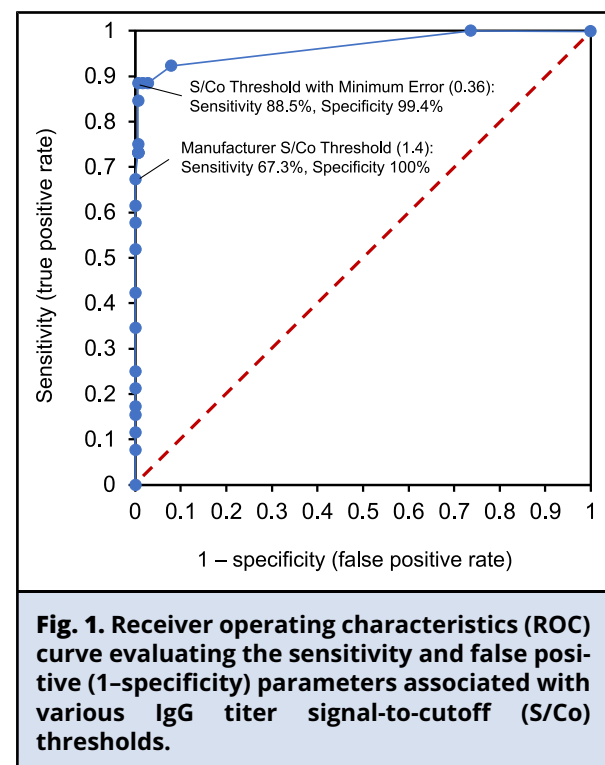
assays for IgM and IgG antibodies against spike and nucleocapsid proteins (Abbott Laboratories, Chicago, IL). We classified participants as being seropositive based on different thresholds: the manufacturer's recommended S/Co threshold (≥1.4 for IgG) and modified S/Co thresholds based on measured antibody levels in 178 negative control specimens collected prior to the emergence of COVID-19 and stored at −80°C in the hospital's biobank until they were selected for use for this study. Frozen samples were thawed at room temperature and assayed for antibodies within 6 h after thawing, thus undergoing one freeze–thaw cycle. All validation study specimens were assayed in the same CLIA-certified lab on the same analyzer with the same assays and methods as research study specimens. Descriptive statistics were summarized for participant and negative control cohorts. We calculated the sensitivity and specificity for manufacturer and modified S/Co thresholds and used receiver operating characteristic (ROC) curve to identify the optimal S/Co threshold based on these samples.

RESULTS

Overall, 585 firefighters that received an antibody test had a previous PCR test for SARS-CoV-2. Of these, 52 (8.9%) reported having a PCR-positive test history at a median 3.3 months (interquartile range [IQR], 1.2 to 4.2 months) prior to antibody testing. Online Supplemental Table 2 presents demographic characteristics for individuals with a known history of infection. Most firefighters were male (90.4%) and between 31 and 59 years of age (88.5%). The most commonly identified racial/ethnic categories in this cohort included white (48.1%), followed by Hispanic (26.9%), Asian (11.5%), black or African American (7.7%), and other (5.8%). There were no firefighters that reported being previously hospitalized for the treatment of COVID-19, indicating that this cohort

primarily exhibited mild illness following SARS-CoV-2 infection. For the negative control sample, we measured SARS-CoV-2 antibodies in blood specimens drawn from 178 individuals prior to the emergence of COVID-19. Most individuals in the negative control sample were male (56.2%), white (71.3%), and over 60 years of age (70.2%). The most commonly associated comorbidities described in this cohort included solid tumor cancer (69.1%), hypertension (57.9%), metastatic cancer (32.0%), hypothyroidism (30.3%), and cardiac arrhythmias (28.1%).

Thirty-five (67.3%) firefighters with a previous PCR-positive test were found to be seropositive for SARS-CoV-2 IgG antibodies based on the manufacturer-recommended S/Co thresholds, corresponding to a sensitivity of 67.3% and specificity of 100%. We then tested the performance of several modified S/Co thresholds based on the index titer values measured in the negative control sample by ROC curve analysis (Fig. 1). As expected,

Fig. 1. Receiver operating characteristics (ROC) curve evaluating the sensitivity and false positive (1–specificity) parameters associated with various IgG titer signal-to-cutoff (S/Co) thresholds.
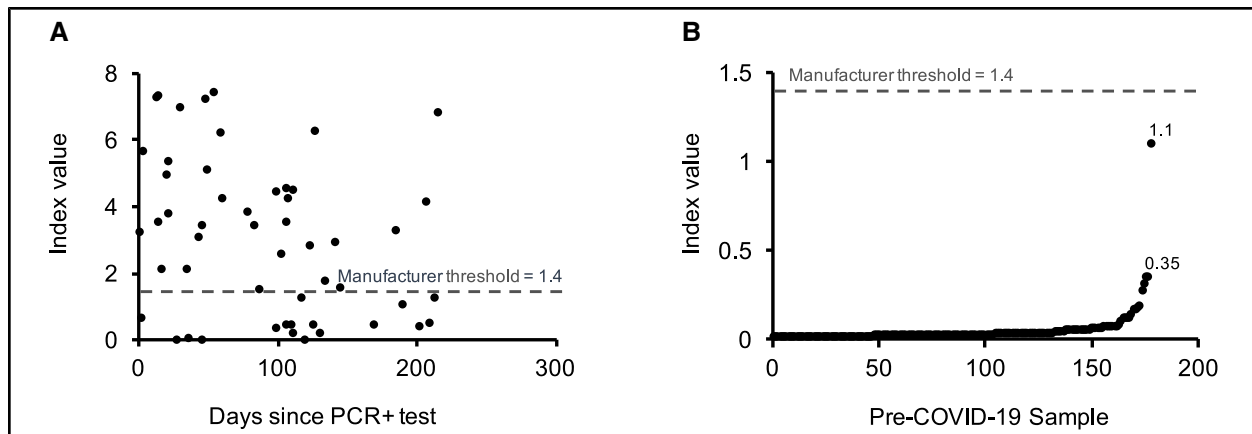
Fig. 2. Index values of SARS-CoV-2 antibody titers measured in: (A), serum collected from firefighters shown by days since PCR test positivity; (B), negative control serum collected from hospitalized individuals prior to COVID-19. The gray line denotes the manufacturer recommended signal-to-cutoff (S/Co) threshold.

we observed a general improvement in test sensitivity on lowering S/Co thresholds at the expense of declining specificity. We found that a modified S/Co of 0.36 yields optimal sensitivity (88.5%) and specificity (99.4%) performance in this cohort, with minimal error in serostatus classification (Fig. 1 and 2). When applying this modified threshold to estimate overall serostatus in firefighters, 46 (88.5%) individuals with a previous PCR-positive test result were determined to be seropositive.

DISCUSSION

Serologic tests can be used to identify active and resolved SARS-CoV-2 infections, which is critical for the epidemiological tracking of COVID-19 in the population. Manufacturer-recommended S/Co thresholds for serologic tests are typically based on samples collected from symptomatic patients within a few weeks of infection. Review of 6 major serology testing assays listed on the FDA EUA website (Abbott Architect, Abbott Alinity AdviseDx, BioRad Laboratories Bioplex 2200, Euroimmun Anti SARS-CoV-2 ELISA, Roche Elecsys, Siemens

Healthcare Diagnostics ADVIA Centaur) demonstrates that only 2 such platforms validated performance characteristics in samples that included non-hospitalized individuals (4). While manufacturer recommended cutoffs have been shown to perform well in hospitalized cohorts, manufacturer instructions for use specifically caution that these thresholds may underestimate cumulative incidence when applied to individuals with milder disease severity or as antibodies wane over time (2–4). Previous analysis of the same Abbott Architect instrument utilized in this study showed that manufacturer-based S/Co thresholds were highly sensitive when applied to samples collected from hospitalized patients assayed within 17 days of PCR positivity (5). However, owing to the highly selective characteristics of the sensitivity cohort evaluated in their study, the authors speculated that modified S/Co thresholds may be considered for diagnostic serology in different target populations (5).

To this end, we evaluated the performance of the Abbott Architect using manufacturer-recommended S/Co thresholds in a cohort of firefighters with a known history of SARS-CoV-2 infection. In contrast to previous studies evaluating the performance

characteristics of this instrument, none of the firefighters included in the present analysis were hospitalized for the treatment of COVID-19. Our data demonstrate that the sensitivity of manufacturer S/Co thresholds significantly underperforms in ambulatory patients, particularly with increasing time since the initial PCR positive test. Other groups have shown similar results with other serology platforms, describing comparatively lower antibody levels after seroconversion in those with milder illness ([6], [7]). As a result, we pursued alternate S/Co thresholds based on IgG-antibody titer index values measured in known negative samples collected from pre-COVID-19 serum. Analysis of the ROC curve according to multiple alternate S/Co thresholds determined the optimal S/Co threshold to be 0.36. The sensitivity and specificity of this threshold was 88.5% and 99.4%, respectively. When applied to the firefighter cohort, this threshold improved the overall seroprevalence estimate by 21.2% compared to the manufacturer-recommended S/Co threshold. Given that approximately 85% of individuals infected by SARS-CoV-2 are not expected to be hospitalized for the management of their disease, our data suggest that lowering S/Co thresholds from manufacturer-recommended levels may be more optimal for antibody assessments in the general population ([8]).

Overall, our data demonstrate that the use of a modified S/Co based on known negative samples significantly increases the percent seropositive and more accurately estimates cumulative incidence of infection in a cohort with a disease severity largely representative of the general COVID-19 population. These results are consistent with evidence from studies from the US and Belgium that find detectable antibodies several months after infection ([9], [10]). In summary, our findings suggest that estimation of cumulative incidence of COVID-19 using serology-based assays must apply diagnostic thresholds that account for weaker antibody response exhibited by those with mild disease ([11]).

The findings of this study should be viewed in light of its limitations. First, seroprevalence was assessed based on blood specimens drawn at a single, cross-sectional time point, resulting in varying times since initial PCR positivity. Future serology studies evaluating S/Co thresholds may benefit from repeated measurements to longitudinally track antibody kinetics over time. In addition, PCR positivity was determined by participant survey, thus we cannot rule out false-positive PCR test histories. Finally, it is unclear whether factors such as age, race/ethnicity, concomitant comorbidities, and cross-reactivity with other known coronaviruses influenced SARS-CoV-2 antibody measurements in the negative control cohort utilized in this study. Additional studies evaluating SARS-CoV-2 antibody levels in larger and more representative negative control specimens are needed.

SUPPLEMENTAL MATERIAL

Supplemental material is available at *The Journal of Applied Laboratory Medicine* online.

REFERENCES

1. Takahashi S, Greenhouse B, Rodríguez-Barraquer I. Are seroprevalence estimates for severe acute respiratory syndrome coronavirus 2 biased? J Infect Dis 2020;222: 1772–5.

2. Ibarrondo FJ, Fulcher JA, Goodman-Meza D, Elliot J, Hofmann C, Hausner MA, et al. Rapid decay of anti–SARS-CoV-2 antibodies in persons with mild COVID-19. N Engl J Med 2020;383:1085–7.

3. Seow J, Graham C, Merrick B, Acors S, Pickering S, Steel KJA, et al. Longitudinal observation and decline of neutralizing antibody responses in the three months following SARS-CoV-2 infection in humans. Nat Microbiol 2020;5:1598–607.

4. US Food and Drug Administration. EUA Authorized Serology Test Performance (December 3, 2021). https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/eua-authorized-serology-test-performance (Accessed December 2021).

5. Bryan A, Pepper G, Wener MH, Fink SL, Morishima C, Chaudhary A, et al. Performance characteristics of the Abbott Architect SARS-CoV-2 IgG assay and seroprevalence in Boise, Idaho. J Clin Microbiol 2020;58:e00941-20.

6. Trabaud MA, Icard V, Milon MP, Bal A, Lina B, Escuret V. Comparison of eight commercial, high-throughput, automated or ELISA assays detecting SARS-CoV-2 IgG or total antibody. J Clin Virol 2020;132:104613.

7. Wellinghausen N, Voss M, Ivanova R, Deininger S. Evaluation of the SARS-CoV-2-IgG response in outpatients by five commercial immunoassays. GMS Infect Dis 2020;8: Doc22.

8. Nakamichi K, Shen JZ, Lee CS, Lee A, Roberta EA, Simonson PD, et al. Hospitalization and mortality associated with SARS-CoV-2 viral clades in COVID-19. Sci Rep 2021;11:4802.

9. Duysburgh E, Mortgat L, Barbezange C, Dierick K, Fischer N, Heyndrickx L, et al. Persistence of IgG response to SARS-CoV-2. Lancet Infect Dis 2021;21: 163–4.

10. Wajnberg A, Amanat F, Firpo A, Altman DR, Bailey MJ, Mansour M, et al. Robust neutralizing antibodies to SARS-CoV-2 infection persist for months. Science 2020; 370:1227–30.

11. Toubat O, Berg AH, Sobhani K, Mulligan K, Hori AM, Bhattacharya J, Sood N. Manufacturer signal-to-cutoff threshold underestimates cumulative incidence of SARS-CoV-2 infection: evidence from the Los Angeles Firefighter Study. Preprint at https://www.medrxiv.org/content/10.1101/2021.04.20.21255829 (2021).