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Progression and Predictors of SARS-CoV-2 Antibody Seroreactivity In US Blood Donors

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\textbf{A B S T R A C T}

The second largest US blood center began testing for antibodies to SARS-CoV-2, the etiologic agent of Coronavirus Disease-2019 (COVID-19) to identify potential COVID-19 Convalescent Plasma (CCP) donors and encourage blood donation. We report the non-vaccine seroprevalence of total immunoglobulin directed against the S1 spike protein of SARS-CoV-2 in our donors. Unique non-CCP donor sera from June 01 to December 31, 2020 were tested with the Ortho VITROS Anti-SARS-CoV-2 total immunoglobulin assay (reactive: signal-to-cutoff (S/C) ≥ 1). Multivariate regressions including age, sex, race-ethnicity, ABO, RhD, highest education level, donor experience, regional collection center and drive type factors were conducted to identify demographics associated with the presence of antibodies and with S/C values. Unique donors (n = 523,068) showed an overall seroprevalence of 6.12% over 7 months, with the highest prevalence in December 2020 around Lubbock, TX (24.3%). In a subset of donors with demographic information (n = 394,470), lower odds of antibody reactivity were associated with female sex, non-Hispanic White or Asian race/ethnicity, age ≥ 65, graduate education, blood group O, and history of blood donation. In reactive donors (n = 24,028), antibody signal was associated with male sex, race/ethnicity other than non-Hispanic White, low educational attainment, age 16–17 years and geographic location. Seroprevalence continues to grow in US blood donors but varies significantly by region. Temporal trends in reactivity may be useful to estimate effectiveness of public health measures. Before generalizing these data from healthy donors to the general population, rates must be corrected for false-positive test results and adjusted to match the wider US demography.

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\textbf{Introduction}

The Coronavirus Disease 2019 (COVID-19) pandemic challenged blood centers to supply a new component, COVID-19 Convalescent Plasma (CCP), while struggling to maintain the blood supply by rescheduling and redirecting donations of traditional blood and platelet components. Blood drives were declared critical infrastructure, allowing donors to travel despite shelter in place orders and the need for CCP was publicized by governmental agencies, blood banking organizations, and hospitals. An initial decrease in hospital demand due to cancellation of elective procedures to accommodate surging COVID-19 admissions was followed by blood shortages due to re-scheduled elective procedures and stem cell transplants [1]. A program to screen blood donors by serological methods to identify individuals with SARS-CoV-2 was developed as a mechanism to identify and recruit CCP donors and as a potential incentive for blood donation. This program also provided a mechanism for serosurveillance.

Surveillance studies are known to identify significantly higher numbers of infections than those defined by reported cases [2]. This reflects individuals with asymptomatic illness, those with milder symptoms who did not seek medical care, and those who could not undergo testing. Because of the reduced positive predictive value of antibody testing in what were originally low prevalence groups, orthogonal testing and statistical adjustments are needed to assist in interpretation and generalize data to larger populations [2]. Unadjusted serial sampling for SARS-CoV-2 antibodies can, however, assess community viral spread in relation to containment measures, or their relaxation. Antibody studies can
also determine demographic risk factors for infection, allow assessment of the durability of the immune response, and potentially detect reinfection when signal strength intensifies. Also important, high-throughput semi-quantitative binding antibody assays correlating with defined titers of neutralizing antibodies may identify and qualify donors for CCP donation, while also identifying units with higher antibody titers. We describe 7 months’ results of serologic testing of healthy allogeneic blood donors prior to the widespread availability of SARS-CoV-2 vaccines using a single total binding antibody assay conducted by the second largest US blood center and its affiliate.

Despite the best performance in a study of four high-throughput tests then-available in the US, the signal-to-cutoff (S/C) ratio in the Ortho VITROS Immunodiagnostic Products Anti-SARS-CoV-2 Total Ig test (αCoV2Tlg), which we used to screen donors, does not precisely correlate with neutralization titers [3,4]. S/C ratios on this platform do however, correlate with the presence of binding antibody across the test’s high dynamic range. This assay is also uniquely suited for serorevérence estimation as results are quite stable over time, despite declining neutralizing antibody activity [5]. IgG and IgM signals have been reported to be higher in those recovering from more-severe COVID-19 [6,7]. We thus also sought to characterize the signal strength of reactive samples.

Methods

Donor Testing

Vitalant (Scottsdale, AZ) began testing all allogeneic whole blood and apheresis donors for SARS-CoV-2 antibodies on June 01, 2020. LifeStream (San Bernardino, CA), a Vitalant affiliate, commenced testing on June 8, 2020. Donors were asked to refrain from donating within 28 days of COVID-19 symptoms or diagnosis. They were informed about antibody testing and gave consent for the research use of anonymized information, blood and blood samples. Test results are reported to donors using a secure portal for information and recruitment purposes. FDA does not require donor deferral for reactive antibody test results [8].

Serum was tested using sample tubes collected with successful donations and routinely sent to Creative Testing Solutions (CTS - Bedford, TX; St. Louis, MO; Tampa, FL; Tempe, AZ). SARS-CoV-2 antibody testing used the VITROS Immunodiagnostic Products Anti-SARS-CoV-2 Total Reagent Pack (αCoV2Tlg, Ortho Clinical Diagnostics, Rochester, NY) on VITROS XT 7600 and 3600 instruments. The test was FDA-authorized under an Emergency Use Authorization as an aid in identifying individuals with an adaptive immune response to the virus. αCoV2Tlg is a chemiluminescent immunometric test qualitatively measuring total antibody (IgG, IgM and IgA) to SARS-CoV-2 S1 spike antigens. Results with signal-to-cutoff (S/C) ratios ≥1 are reported as reactive.

CTS transmitted test results (excluding CCP donations and therapeutic phlebotomies) to centers’ blood establishment computer systems (BECs). S/C ratios were obtained by donation ID numbers from test instruments and relayed electronically in batches. Data warehouse extracts were performed for all donations from June 01 to December 31, 2020 in our 6 unique BECs which included anonymized, encoded donation numbers and donor identifiers along with test results. Unique donor results were used to characterize the rate of COVID-19 antibody reactivity within geographic collection regions. Anonymized donor demographics were available for the largest BECs (eProgressa, MAK-SYSTEM, Paris, France) comprising ~75% of tested collections. This subset database included:

- Donor demographics: age, sex, race/ethnicity, ABO, RhD, highest education level, and experience (first-time, active [prior presentation within 2 years], or reengaged [prior presentation >2 years ago])
- Donation information: date, Vitalant regional collection center, drive type (fixed site or mobile), and antibody test S/C result.

Unique donors were counted based on having one or more donations within a calendar month for the temporal analysis or all 7 months for the demographic analysis. Donors with at least one reactive test within any month were counted as antibody reactive. For the signal strength analysis, the donation with the highest S/C was selected if the donor had more than 1 reactive donation.

Donors reporting SARS-CoV-2 vaccination after the September 19, 2020 introduction of questioning for CCP eligibility determination were excluded from these analyses (n = 267).

Statistics

SARS-CoV-2 antibody reactivity was calculated as the proportion of unique-donor reactivity to the total number of donors tested in each analysis period. The effects of donor demographics on the presence of detectable antibody were explored in a logistic regression model for donations with retrievable demographic information. An unadjusted logistic regression was initially performed to assess the crude association of each predictor with a reactive antibody test. Multivariate logistic analysis was then performed to calculate the adjusted odds ratios (OR) and 95% confidence intervals (CIs) (Stata 15.1, Stata Corp., LLC, College Station, TX, USA). The final model included nine donor and donation characteristics as predictors.

To further explore the potential effects of donor characteristics on antibody levels, reactive S/Cs were transformed for analysis to meet model assumption requirements using the Box-Cox method, where Transformed S/C = [(S/C)^2 - 1]/λ, λ = 0.25 (Proc Transreg, SAS v9.4, SAS Institute, Cary, NC). The transformed S/C from donors with the highest reactive αCoV2Tlg test results were regressed against seven donor demographic characteristics and blood type in a linear mixed effects model (Proc Mixed, SAS), initially with collection region as a random effect. Based on Akaike information criteria, the random effect was removed and effects with P < .3 were retained in the final model. Pearson marginal residuals were examined to evaluate the assumptions and final model fit and were found to support the key assumptions of independence and normality. Exploratory hypotheses were tested using the model. P values were not adjusted for multiple comparisons. The least-squared means (with 95% confidence intervals) of fixed effects were determined from the model. Estimates were converted to the linear S/C scale.

Results

From June 01 through December 31, 2020, 523,068 unique donors completed a whole blood or non-CCP apheresis donation and were tested for SARS-CoV-2 antibodies (Table 1). Figure 1 shows the changing rate of antibody reactivity across the geographies in which Vitalant and its affiliate collect blood. There was a demonstrable increase in overall serorevérence from 1.4% in June to 11.2% in December across all unique donors each month and

| Donations Tested for Ab | All | Demographics subset |
|------------------------|-----|---------------------|
| Donations Tested for Ab | 620,940 | 625,067 |
| Unique Donors | 523,068 | 394,470 |
| All Donations αCoV2Tlg-negative | 491,044 | 370,315 |
| At Least 1 Donation αCoV2Tlg-reactive | 32,024 | 24,155 |
from 2.3% in June to 17.5% in December among first-time donors (latter data not shown). Of the 180,775 individuals donating more than once during this time period, 7,606 donors (4.2%) seroconverted between their first and a later donation any time within the entire 7-month time period.

Bivariate comparisons within the large subset of donors for which expanded demographic information was available show antibody reactivity by sex, race/ethnicity, age, education, blood group, donor experience, collection site type and region (Table 2). Significant differences are observed within all categories except collection site type. Results of the multivariate analysis for factors associated with reactive antibody test results by donor and donation characteristics are presented in Figure 2. Significant differences were observed for all categories. Compared with non-Hispanic White donors, those who are non-Hispanic Native Americans/Alaskans, non-Hispanic Blacks, or Hispanic donors were significantly more likely to have a reactive test result; non-Hispanic Asian/Pacific Islanders were less likely to have a reactive result. Individuals aged 16–17 had the highest odds of a reactive result, but those under 64 were also significantly more likely than those over 64 to be reactive. Individuals with no high-school experience were much more likely to be reactive compared with donors with graduate degrees who were the least likely overall to have a reactive test result. First-time donors also had a higher risk for reactive results. Donors at the reduced number of mobile drives had a lower risk for antibody positivity. Figure 2B shows geographic differences in seroprevalence, adjusted for all donor and donation characteristics.
A small but statistically significant increase in reactivity was seen in all non-Group O individuals and donors who are RhD-positive. To explore the role of potential false-positive test results, donors with αCoV2Tlg S/C values < 10 were excluded from the analysis (Table 2). REDS-IV-P data demonstrate that only 51% of donor sera with these low S/C values confirmed reactive by anti-nucleocapsid immunoglobulins or had neutralizing antibodies, but for S/C values at or above 10, > 99% confirmed reactive [2]. Restricting the bivariate analysis to higher S/C values did not change the ABO/RhD findings. However, when the analysis was limited to first-time donors who are not differentially recruited by blood type, Group B individuals and RhD-positive donors were no longer statistically more likely to be reactive than Group O or RhD-negative donors.
Of note, the extended bivariate analysis limited to S/C values ≥ 10 (eliminating ~3,000 donors; data not shown) did not substantially change any results of the larger analysis.

The distribution of reactive donor S/C values (maximum S/C observed per donor in the 7-month period) is shown in Figure 3. From June 01 through December 31, 2020, 24,028 reactive donors (127 had missing S/C or ABO) had a aCoV2TIg S/C ≥ 1 (median 79.6, IQR 27.1–180.0, maximum 1080). Log10 transformation demonstrates a left-skewed distribution (Figure 3B) with greater numbers of donors with lower amounts of binding antibody and/or a significant number of low-signal false positives. The Box-Cox transformation resolves the left-skew as shown in Figure 3B inset.

We next examined the association of donor and donation site characteristics with S/C in aCoV2TIg-reactive donors. While not claimed as a quantitative assay outcome, the S/C is likely indicative of the degree of antibody response (quantity and avidity). We did not observe a significant effect on S/C for ABO and RhD and these were removed from the initial model (Table 3, Supplemental Tables 1 and 2), while the ABO⁺RhD interaction was significant and was retained in the final model. Male S/Cs were found to be significantly higher than female values (P < .0001) for reactive donors perhaps indicating a stronger antibody response (Supplemental Tables 1 and 2, Figure 4). All identified race/ethnicity groups had significantly higher S/Cs than non-Hispanic Whites. There was a wide range of S/C values across regional collection locations. Eleven of 18 sites had S/Cs significantly lower than Phoenix, and 6 were not different from the reference. Age ranges 30–49 years had lower S/Cs compared to the 65– to 74-year-old group (P < .0001), while 16– to 17-year-olds had higher S/Cs compared to the reference 65– to 74-year-old group. Donors with an education at/below 8th grade had significantly higher S/Cs than those with 9th grade education or higher (P < .0001). Group A-positive donors had higher S/Cs than all others (P = .0006). Donors present at mobile blood drives also had S/Cs higher than donors at fixed site collections (P = .0496).

**Discussion**

We present seroprevalence data from a large cohort of geographically diverse healthy blood donors over the first seven months of SARS-CoV-2 antibody testing on the aCoV2TIg platform. This cohort is composed of almost exclusively unvaccinated individuals. The overall seroreactive rate was 6.12% amongst 523,068 unique donors tested over 7 months. An increasing reactivity trend was shown from June to December as expected based upon progression of the pandemic within Vitalant collection regions. We
Fig. 3. Distribution of S/C Ratios for Reactive Samples (S/C ≥ 1.0). A: Linear frequency with cumulative percentages; B: Log10-transformed S/C distribution with cumulative percentages. Inset shows Box-Cox S/C transformation.

Fig. 4. Reactive donor S/C multivariate model estimates – least-squares estimates of fixed effects from the mixed effects linear model. S/C Estimates (Supplemental Table 2) are transformed to linear scale. □: reference category [95% CI]; ○: estimate [95% CI] statistically-significantly different from reference [P < .05]; ♦: estimate [95% CI] NOT significantly different from reference.
observed higher reactivity rates associated with middle school or lesser education (unadjusted point estimate, 33.5% reactive), age (highest result [11.7%] in those under 18 years old), race/ethnicity (highest point estimate in Hispanics [8.3%]), and first-time donors (7.0% compared with 6.0% in active donors). There was a small, but statistically significant increase in reactivity seen in non-blood Group O donors (limited to Groups A and AB in first-time donors). Significant regional differences were observed, with June to December unadjusted reactivity rates highest at 12.3% in the McAllen, TX area and the lowest rate (1.9%) in San Francisco, CA.

SARS-CoV-2 serosurveillance studies of North American blood donors are beginning to appear in the literature [2,9-13]. As data are compared, it is important to consider the characteristics of the population studied, geographic location, dates of testing, and screening laboratory methods. As noted in a global systematic review and meta-analysis of serosurveillance studies, most are methodologically flawed, without correction for demographics or test performance, and employ non-representative sampling [9].

The growing number of larger, US-based studies have however, surveilled blood donor populations within minimally-overlapping geographies, which in toto paint a more complete picture of the national burden of COVID-19 [2,10-12].

Demographic associations of test reactivity in North American blood donors have been reported [2,10-12]. Consistently, race/ethnicity other than non-Hispanic White and younger age appear to confer greater risk for seroreactivity. This has been potentially attributed to disparities in the ability (racial/ethnic minorities at home or the workplace) or willingness (younger individuals) to social distance [2]. Greater fractions of antibody reactivity in new donors in this study and others suggests some effect from the announcement of antibody testing [2,10]. This may have contributed to a disproportionate temporal increase over observed geographic progression of COVID-19 as donors with exposures or untested minor symptoms sought testing or came forward to determine eligibility to donate CCP.

Blood group results appeared to be in concert with the conclusions of other studies, all subject to biases of various types, that non-Group O individuals may be more likely to contract COVID, possibly of greater severity [14]. Because Group O and RhD-negative blood donors are heavily recruited for RBC donation, they may respond differently to calls for additional blood donation than less-recruited individuals, thus introducing bias in our analysis. This may also explain the seeming increase in reactivity among RhD-negative individuals. Increased reactivity in Group B and RhD-positive donors disappeared when analysis was restricted to first-time donors whose blood type is unknown. While other demographic characteristics of these new donors may be non-random, their blood type is and thus, the role of Group A glycans in viral entry or anti-A immunoglobulins in viral neutralization remains a potential explanation for findings in first-time donors. The higher S/C observed in Group A-positive donors may thus reflect a group-specific propensity, enhanced by the effects of differential response to requests for donations, and curiosity about antibody status. In a reanalysis of the S/C data among first-time donors (data not shown), the ABO-RhD interaction was no longer significant and dropped out of the model, casting doubt on the role of blood type in disease severity.

SARS-CoV-2 binding Total Ig antibodies appear to be stable over months for individuals with previous infection and are thus well-suited for surveillance studies [2,5]. While ideal for surveillance, these results do not correlate as closely with neutralizing antibody titer, which may be an important direct or proxy measure of immunity and CCP potency. Other assays in which antibody reactivity wanes over time are less suited to detect previously infected individuals but tend to correlate more closely with neutralizing antibody titer [5]. In our study, higher S/C values for spike Total Ig were associated with male sex, declared race/ethnicity other than non-Hispanic White, and low educational attainment. Whether this correlates with the severity of infection or indicates a more vigorous immunologic response remains to be determined. First-time donors and individuals in geographies with a higher prevalence of antibody reactivity also had higher S/C values.

This study has several limitations. First, this is a report on the first 7 months of screening blood donors for antibodies to SARS-CoV-2, primarily in the western half of the country; continued reporting is necessary to define trends as public health measures are enacted and relaxed. The EUA of several vaccines beginning in December 2020 will represent a confounder in future analyses [15]. This will require orthogonal testing (eg, reliably persistent nucleocapsid antibody) to identify vaccine-only reactivity. We did eliminate a very small number (267) individuals who had been vaccinated after we initiated questioning in mid-September and believe the pre September number is even smaller, therefore not appreciably affecting the present results. Our experience should also be viewed through the lens of the geography represented in Figure 1. Second, there is a lack of donor clinical information (symptomatology and laboratory confirmation of infection by PCR) and orthogonal supplemental testing to weed out false-positive test results was not reported in this growing-prevalence population. The left tail observed in Figure 3B suggests a substantial fraction of low-S/C results (ie, < 10), which in at least 1 report, is correlated with a lower rate of confirmatory antibody reactivity [2]. These limit the ability to correlate antibody reactivity and S/C strength with the presence or severity of symptoms. Last, blood donors are a significantly healthier population than the general public, with substantially different demography than the general population. Reactivity rates were clearly affected by prior appeals to high-demand blood groups, the offer of SARS-CoV-2 antibody testing, and inducement of donors with known COVID to present for CCP as well as non-CCP donations. Generalization of data from this group of tested individuals to the population at large must be corrected for potential false-positive test results and adjusted to reflect the demography of the general population before estimates of pathogen exposure are valid. Trends over time however, are valuable in the evaluation of imposition or relaxation of efforts to limit spread of the disease. While longitudinal studies are under way to better characterize pathogen exposure, data like ours also provide a rapid estimate of regional differences in the number of infected individuals as we progress through substantial morbidity and mortality toward herd immunity. Our report complements the growing number of surveillance studies in North America.

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Conflict of Interest

Drs. Bravo, Dumont, Hazegh, Kamel and Vassallo have declared no relevant potential conflicts of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.trmrv.2021.07.003.

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