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Characteristics of US Blood Donors Testing Reactive for Antibodies to SARS-CoV-2 Prior to the Availability of Authorized Vaccines

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ABSTRACT

In the United States, many blood collection organizations initiated programs to test all blood donors for antibodies to SARS-CoV-2, as a measure to increase donations and to assist in the identification of potential donors of COVID-19 convalescent plasma (CCP). As a result, it was possible to investigate the characteristics of healthy blood donors who had previously been infected with SARS-CoV-2. We report the findings from all blood donations collected by the American Red Cross, representing 40% of the national blood supply covering 44 States, in order to characterize the seroepidemiology of SARS-CoV-2 infection among blood donors in the United States, prior to authorized vaccine availability. We performed an observational cohort study from June 15th to November 30th, 2020 on a population of 1.531 million blood donors tested for antibodies to the S1 spike antigen of SARS-CoV-2 by person, place, time, ABO group and dynamics of test reactivity, with additional information from a survey of a subset of those with reactive test results. The overall seroreactivity was 4.22% increasing from 1.18% to 9.67% (June 2020 - November 2020); estimated incidence was 11.6 per hundred person-years, 1.86-times higher than that based upon reported cases in the general population over the same period. In multivariable analyses, seroreactivity was highest in the Midwest (5.21%), followed by the South (4.43%), West (3.43%) and Northeast (2.90%). Seroreactivity was highest among donors aged 18-24 (Odds Ratio 3.02 [95% Confidence Interval 2.80-3.26] vs age > 55), African-Americans and Hispanics (1.50 [1.24-1.80] and 2.12 [1.89-2.36], respectively, vs Caucasian). Group O frequency was 51.5% among nonreactive, but 46.1% among seroreactive donors (P < .0001). Of surveyed donors, 45% reported no COVID-19-related symptoms, but 73% among those unaware of testing. Signal levels of antibody tests were stable over 120 days or more and there was little evidence of reincorporation. Evaluation of a large population of healthy, voluntary blood donors provided evidence of widespread and increasing SARS-CoV-2 seroprevalence and demonstrated that at least 45% of those previously infected were asymptomatic. Epidemiologic findings were similar to those among clinically reported cases.

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Introduction

Despite the extent of the COVID-19 pandemic in the United States, there has been limited information about the overall seroprevalence and the frequency of recognized asymptomatic infection. Although blood donors are not fully representative of the US population, they provide a healthy subset with more than 10 million samples per year [1]. Large-scale testing for antibodies to SARS-CoV-2 has been implemented by many US blood collection organizations, offering an opportunity to examine these issues. An early analysis indicated that the demographics and regional distribution of seroreactivity were similar to national data on COVID-19 [2]. Also, evaluation of data from retained samples from donors suggested the presence of SARS-CoV-2 earlier than the generally recognized date of first appearance of the virus in the US [3].

We report on the frequency of reactivity using a specific spike antibody test for SARS-CoV-2, validated by using an alternate test directed to a different viral target, by person, place, time and blood type within the American Red Cross (ARC) donor population. We report the frequency of asymptomatic infections, infection incidence relative to clinical case findings, the dynamics of antibody levels post-infection, self-reported symptoms, and test awareness. Combined data for these attributes in a large segment the US population have not been reported previously in a single defined study.

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Methods

Test Population

The ARC collects about 40% of the US blood supply from 44 states and has tested every donor for antibodies to SARS-CoV-2 since June 15, 2020 using the Ortho VITROS anti-SARS-CoV-2 S1 Total Ig assay (identified as the Ortho test) (Raritan NJ) [2,4]. Descriptive studies were conducted for 1,531,221 donors who made 2,191,731 donations of whole blood, double red-cells or single-donor platelets between June 15th and November 30th, 2020, before the general availability of SARS-CoV-2 vaccines in the US; donors of COVID-19 convalescent plasma were excluded. On average, each donor gave 1.43 donations. Donors with any donation having a reactive test result (signal-to-cutoff [S/CO] ratio ≥ 1.00) were defined as seroreactive. Information from the donation record included donor status (first-time or repeat), sex, age, self-identified race/ethnicity, location of residence and ABO Group. Studies on changes in donors' antibody signal strength over time were conducted on the subset of donors with multiple test-reactive donations or those who seroconverted. Testing and data collection were considered by the ARC's Institutional Review Board (IRB) to be exempt as human subjects' research; each donor was provided with an information sheet prior to agreeing to donate.

Testing

All donations are tested routinely for transfusion-transmissible infections [3]. During the study period, each donation was also tested by the Ortho test under Emergency Use Authorization (EUA) by the Food and Drug Administration (100% sensitivity [95% confidence interval: 92.7-100.0%] in 49 SARS-CoV-2 PCR-confirmed patients 8 days or more following symptom onset, and 100% clinical specificity [95% confidence interval: 99.1-100.0%] in 400 presumed negative individuals) [4]. Each donation was tested singly; seroreactive donors were entered into this study. All available seroreactive samples were further tested, using the Roche Elecsys Anti-SARS-CoV-2 Test (identified as the Roche test) (Indianapolis, IN) [6], targeting a different SARS-CoV-2 protein (Subunit 1 of spike, S1, for Ortho vs nucleocapsid for Roche). Secondary testing provided more definitive information to donors and is reported here for 87.25% of seroreactive donors; Roche testing could not be performed when the approved sample storage time was exceeded per manufacturer's instructions. Reference to seroreactivity in the Roche test is identified as Roche-reactive. Test signal levels for Ortho and Roche are both reported as S/CO values.

Donor Survey

A survey of donors with seroreactive test results was conducted to understand their history of COVID-19 diagnosis, prior testing, disease symptoms and motivation for donation in the context of COVID-19 testing. Eligibility for the web-based questionnaire was based on blood donation during the study period. The survey was voluntary and approved by the ARC IRB without requirement for written or verbal consent. Survey exclusion criteria were applied to 64,633 seroreactive donors, removing 22% without an e-mail address, 20% requesting no e-mail contact, 3% less than 18 years old, and fewer than 2% Spanish-speaking, having incorrect e-mail addresses, or previously opting out of research. For each symptom, “yes” or “no” responses were recorded, but those identified as “Unsure” were omitted. The Supplement contains the survey instrument.

Analyses and Statistics

Analyses of donor characteristics were reported at the level of individual donors. Donor characteristics associated with seroreactivity, including age, sex, race/ethnicity, region of residence, and ABO blood group were assessed using bivariable and multivariable logistic regression. In analyses of temporal change in antibody signal strength in donors with repeated donations, data were assessed by Spearman rank correlation and Kruskal-Wallis test. Within that population, the 469,605 donors who gave more than once provided 1.13 million donations, or 2.41 per donor. For surveyed donors, comparison of mean counts of symptoms (maximum of 12) by comorbidity was performed using Wilcoxon rank sum test, with Bonferroni correction for multiple comparisons. We conducted all analyses using SAS software (version 9.4, SAS Institute Inc., Cary NC). P values of less than .05 were considered significant. Relevant evaluations were 2-sided.

Results

Donor Testing

Of 1,531,221 donors, 64,633 (4.22%) had one or more seroreactive results. Among the seroreactive donors, 56,397 (87.25%) were also tested by Roche; 51,335 (91.02%) were Roche-reactive. Supplemental Table 1 shows the distribution of Roche-reactive samples. Of note, Roche nonreactive signals span the entire nonreactive dynamic range and do not correlate with seroreactive (Ortho) signal levels (Supplemental Figure 1). Thus, we are not able to establish that seroreactive, Roche nonreactive results are falsely positive, and because the frequency of Roche reactivity was stable over time, we focused primarily on seroreactive results knowing that over 90% were concordantly reactive by both tests.

The overall frequency of seroreactive donations as a percentage of all weekly donations, by US Census region, increased from 1,169 among 98,729 in calendar week 25 (1.18%), corresponding to the week of June 15, to 6,095 seroreactive donations in 75,505 (8.07%), in week 48; a 6.8-fold increase (fig. 1). For the final day reported (November 30), there were 1,283 seroreactive donations of 13,268 tested (9.67%), an overall 8.2-fold increase. The greatest
increase occurred in the Midwest of 1.25% to 13.60% (10.9-fold). Both seroreactive and test nonreactive donors may have provided more than one donation during the study (1.19 donations/donor for reactives, and 1.44 donations/donor for nonreactives); thus, data by donation differ from the donor-based data in Table 1.

Demographics and ABO Distribution

Table 1 shows the demographic characteristics of the population of 1,531,221 donors, their distribution among seroreactive and results of bivariate and multivariable analyses. Of the 64,633 (4.22%) seroreactive donors, reactivity was 5.78% among the 19.46% first-time donors (donors for whom there was no record of prior donation), a multivariable odds ratio (OR) of 1.55 (95% Confidence Interval [CI], 1.46-1.65) relative to repeat donors.

Younger donors were more likely to have reactive results with the highest frequency among the 16-17 (OR 2.49 [95% CI, 2.15-2.89]) and 18-24-year-old (OR 3.02 [95% CI, 2.80-3.26]) groups, with all age groups significantly more likely to be seroreactive than the 55+ age-group. Relative to Caucasian donors, African American, Hispanic, and Native American donors had higher seroreactive rates, with respective odds ratios of 1.50 (95% CI, 1.24-1.80), 2.12 (95% CI, 1.89-2.36) and 1.65 (95% CI, 1.13-2.42). There are clear regional differences in the frequency of seroreactive donors, with significantly higher rates in the Midwest and South, relative to those in the West (Fig. 1: Supplemental Figure 2 shows a heat map of seroreactive donations for ARC collections within the US).

The frequency of ABO groups differed between nonreactive and seroreactive donors. Relative to Group O, Groups A, AB and B were seen more frequently among seroreactive donors, (P < .0001 in both bivariate and multivariable analyses; Table 1), to a somewhat

![Graph showing the distribution of Ortho S/CO levels](image)
lesser degree for Group B, but still highly significant. The odds ratios for seroreactivity among A, AB, and B, relative to Group O were 1.32 (95% CI, 1.25–1.38), 1.26 (95% CI, 1.12–1.41) and 1.16 (95% CI, 1.08–1.25). Group O frequency was 51.5% among nonreactives but 46.1% among seroreactive donors (P < .0001).

**Stability of Antibody Levels**

Within the population of 1,531,221 donors, 469,605 gave blood twice or more, for a total of 1,130,149 donations. Of these, 8,583 donors were seroreactive throughout the study with 19,449 donations. An additional 11,392 donors seroconverted with 27,533 donations (Fig. 2) for an estimated incidence of 11.6 (range 10.5–12.7) per hundred person-years (see Supplement for calculations); the greatest number of seroconversions occurred 31–90 days following the donors’ first tested donation, likely corresponding to the 56-day minimum interdonation interval for whole blood donations. A small number of additional donors (210 having 504 donations) became seronegative. Fig. 3A shows the mean and median log_{10} S/CO values for the antibody test signals by time between seroreactive donations (ie, for donors whose donation remained reactive during the entire study period); overall, the test signals increased signifi-
Fig. 4. Proportion of surveyed seroreactive donors reporting COVID-19 related symptoms 14 or more days prior to donation. Of 13,343 seroreactive donors completing the survey, 95% or greater responded to each symptom question; 45% of responding donors reported no symptoms. Specific data for each symptom for donors reporting symptoms are: fatigue, 5,215 of 12,902; headache, 4,297 of 12,838; myalgia, 4,074 of 12,845; loss of smell, 3,545 of 12,827; cough, 3,518 of 12,820; loss of taste, 3,338 of 12,814; runny nose, 2,739 of 12,705; fever, 2,493 of 13,335; sore throat, 2,145 of 12,696; shortness of breath, 1,821 of 12,709; nausea/vomiting, 1,752 of 12,685; and, congestion, 1,716 of 12,651.

Discussion

Despite the lack of evidence that SARS-CoV-2 is transmissible by blood transfusion [7,8], the pandemic has had a profound impact on the collection and distribution of blood for transfusion. Testing blood donors for SARS-CoV-2 antibodies was implemented in part to encourage donation and to support the need for convalescent plasma [2]. Consequently, millions of individual donors have been tested to date. The data from this study are illustrative of the distribution of viral infection and of the stability of the antibody response.

Blood donors are healthy and differ from the overall population in age, racial and ethnic distribution, and education level. Parallel to case counts of COVID-19, there was a continuous increase in the prevalence of seroreactive donations (Fig. 1). There was a greater than 8-fold increase in this rate overall, and almost 11-fold among donors in the Midwest in a period of just over 24 weeks, prior to the general availability of vaccines. As of the end of November 2020, 9.67% of donations showed evidence of past infection with 13.60% in the Midwest. Within our study, we have observed over 11,000 incident-reactive donors with 2 or more donations (Fig. 2), allowing for the estimation of incidence of new Reactivity as 11.6 cases per hundred person-years, while the estimated incidence of cases of COVID-19 in the adult US population up to November 30th, 2020 was estimated at 6.22 reported cases per hundred person-years (Supplement)., or 1.86 blood donor infections per clinical case reported. The detection of asymptomatic infections among blood donors likely accounts for this difference.

Seroreactivity is more frequent among first-time donors than routine, or repeat donors (Table 1). It seems likely that this is related to the availability of the test, particularly among those who were concerned about potential exposure, or prior symptoms. It is well-known that the prevalence of markers for transfusion-transmissible agents is lower among repeat donors, but this is in part because individuals who test reactive are deferred from further donation [9]. This is not the case for COVID-related antibody
tests as donors with reactive antibody-test results may continue to donate.

As reported previously, the frequency of seroreactivity varies significantly by age, race/ethnicity and location [2]. Noticeably however, in a multivariable analysis the impact of age is greater than reported earlier, while the impact of race and ethnicity is less apparent, although African-American and Hispanic donors continue to be at significantly greater risk than are Caucasian donors.

Several studies have suggested that the frequency of SARS-CoV-2 infection varies with blood group and is lower among individuals with Group O red cells while individuals with Group A may be associated with a higher risk of infection and severe disease [10]. The odds ratios for seroreactivity among A, AB, and B, relative to Group O were respectively 1.32, 1.26 and 1.16 and all were significant (P<.0001; Table 1). In fact, the percentage of Group O donors among test-negative donors was 51.5%, while among seroreactive donors, was 46.1%, supporting a lower rate of infection in those donors with Group O red cells. Also, the lower odds ratio for Group B as compared to Groups A and AB further supports the concept that the effect is likely attributable to the natural antibody response to the A antigen, which can be expressed on the SARS-CoV-2 virion [11].

In this study, we found that the signal levels of the antibody tests used were remarkably stable over a period of 120 days or more. The overall trend was towards an increase in signals with only a few (590 of 8,583, 6.9%) showing a downward trend; other reports have shown stable antibody profiles for 4-6 months [12-16]. Examination of individual signal-level profiles among those with three or more seroreactive donations suggests that large increases in signal levels were rare or absent, and thus, that reinfec-
tion was infrequent among those individuals [15,16]. Greater duration of antibody detectability has been observed with tests that use a total immunoglobulin format (direct antigen sandwich) versus those with an IgG format (second antibody detection) as true of the tests used in our study [16]. This likely contributed to the sustained antibody response that we observed.

A notable proportion (45%) of surveyed, seroreactive donors were asymptomatic. This figure may not be representative of the general population, as most seroreactive respondents reported a history of diagnosis or contact with COVID-19 or indicated that they were aware that they would be tested. In this context, it is of interest to note that about 73% of individuals who reported that they were unaware of the testing, and were not motivated by test availability, reported no symptoms compared to 48% in the Netherlands [17]. Most of these asymptomatic donors were reactive by both the Ortho and Roche tests (79%). Thus, a measurable proportion of the population has unknowingly been infected with SARS-CoV-2 [18,19].

The dynamics of the blood donor population who test reactive for SARS-CoV-2 antibodies is changing with the availability of vacc-
cination and consequently patterns of test reactivity will reflect the mixture of donors with natural and vaccine-induced seroposi-
tivity. In fact, in the 6 months following vaccine availability, approxi-
mately 80% of seroreactive donors acknowledged vaccination with antibody reactivity consistent with vaccination (nucleocapsid non-reactive). This contrasts with 9% having isolated seroreactivity prior to vaccine availability.

The COVID-19 pandemic affected different populations and countries differently and varied over time with second waves fol-
loving the summer of 2020. The overall 4.22% seroprevalence doc-
umented for the US blood donor population in this study from June to November 2020 was consistent with other studies; it rose significantly from 1.18% to 9.67% by the last reporting period. For example, of 8 studies in blood donors published in Europe [20], seroprevalence ranged from 0.91% in North-Western Germany to 23.3% in a high-transmission area in the Lombardy region, Italy. In

the UK, seroprevalence rates of 4.9% occurred after the first epi-
demic wave (June 2020 - September 2020), and similar to our study, found the highest prevalence in younger versus older indi-
viduals [21]. Declines in seroprevalence were documented, partic-
ularly for those 75 years or older and those who did not report a history of symptomatic infection (presumably asymptomatic infec-
tion) as a function of antibody waning. In our study, that used a robust total immunoglobulin test directed towards S1, no antibody waning was observed over 4 months. Antibody testing of ~50,000 blood donations from six US metropolitan regions (3 of which were the ARC) from March-August, 2020 demonstrated seroprevalence peaking at 15.8% in New York City to rates of 2% to 4% in the other 5 locations, with the highest rates in non-Hispanic Black and Black donors, and 1.3-5.6 estimated cumulative infections based on anti-
body testing per COVID-19 case reported to the CDC [22].

Limitations

The study does have some limitations. The data presented are based upon a single test without a formal confirmatory test or any repeat testing. A second (Roche) test was reactive among 91% of seroreactive donors (Supplemental Table 1), and it is probable that the Ortho test may be slightly more sensitive than the Roche test, possibly due to the selection of a spike vs nucleocapsid target, respectively [11]. This was seen in the large number of elevated, but nonreactive Roche results in seroreactive donors (Supplemental Figure 1), and by the greater increase in signal strength observed over time for Ortho vs Roche reactive donors (Fig. 3 and Supplemental Figure 4A). In the absence of a gold-standard confirmatory test, we elected to present seroprevalence data based upon reac-
tivity by the Ortho test.

The blood donor population is not representative of the popu-
lation as a whole; at any time, approximately 3% donate [23]. We do, however, note that the observed trends relating to age, race/ethnicity and location of seroreactive donors follow national trends for COVID-19 [18,24]. Survey data show that most donors were aware that antibody testing was available and that some (par-
ticularly first-time donors) were motivated to give blood, thus im-
peacting the extent to which our data are broadly representative. However, the response to the survey instrument represents a rela-
tively small proportion of all donors: only 13,343 of 64,633 (20.6%).

Conclusions

A large population of healthy, voluntary blood donors pro-
vided evidence of widespread and increasing SARS-CoV-2 sero-
prevalence: 1.18% to 9.67%, an 8.2-fold increase from June through November 30, 2020. Infections in the US are underreported as evi-
denced by 45% of those previously infected being asymptomatic and incidence estimates 1.86 higher than reported for clinical cases. Epidemiologic findings were similar to those among clin-
ically reported cases with seroreactive donors retaining antibody reactivity for at least 120 days. This study of over 1.5 million blood donors (2.19 million donations), representing the US population, uniquely ties together a wide range of data describing the impact of the COVID-19 outbreak in the US prior to vaccine availability.

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Conflict of Interest

The authors report no relevant conflict of interest.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.tmr.2021.07.001.

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