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Anti-SARS-CoV-19 antibodies in children and adults with sickle cell disease: A single-site analysis in New York City

Antibody response to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and/or vaccination reduces infection risk.¹ In the United States, people with sickle cell disease (SCD) have experienced considerable complications from SARS-CoV-2 infection.^{2,3} Functional hyposplenia, asplenia, or splenectomy in SCD can increase pathogen susceptibility and reduce vaccine responsiveness.⁴ We evaluated serum antibody levels for anti-nucleocapsid antibodies from past infection and anti-spike antibodies from vaccination and/or past infection in people with SCD. We hypothesized that, compared to non-SCD controls, people with SCD would have reduced antibody levels following exposure to SARS-CoV-2 vaccination and/or infection.⁵⁻⁷

In this single-site cross-sectional study, a convenience sample of patients undergoing routine care in our SCD centre in New York City were enrolled if they had previously received SARS-CoV-2 vaccination and/or probable or confirmed prior infection(s). Electronic health record review confirmed eligibility, prior surgical splenectomy, SCD therapies, SARS-CoV-2 vaccination and infections and lack of immune suppressants or cancer diagnosis. Participants were queried for relevant extra-institutional events. Anonymized control samples were obtained from benign haematology clinics, with data limited to age, sex and lack of SCD. Study protocols were approved by our Institutional Review Board, including written consent by patients or their parent/guardian.

Anti-nucleocapsid antibody levels generated by SARS-CoV-2 infection and anti-spike protein antibodies from infection and/or vaccination were tested using serial dilutions of subjects' serum made with Diluent MultiAssay (Roche, Rotkreuz, Switzerland) and the Elecsys® Anti-SARS-CoV-2 and Anti-SARS-CoV-2 spike tests run on a cobas® e602 immunoassay analyser (Roche).⁸ The lowest dilution, which provided a semi-quantitative measurement in the linear range of the assay between 0 and 250 arbitrary units (AU), was used to calculate antibody levels. Cutoff for positivity defined by the manufacturer is a level of 1.0 AU or above. Descriptive and statistical analyses were performed using Prism v9 (GraphPad, San Diego, CA, USA). Means of logtransformed antibody levels were compared using t-tests and Pearson correlations were performed. p-Values<0.05 were considered significant.

Samples from 36 SCD participants (20 adults, 16 children) and 22 non-SCD controls (20 adults, two children) were obtained from September to December, 2021 (Table 1). The sample collection period occurred during the dominance of the Delta variant, prior to that of the Omicron variant in New York City and environs.⁹ No participants had been hospitalized or received recent immune suppression. Mean sample age of the SCD participants was 22 ± 10 years (range 2-42 years), and 22 (61.1%) were female (Table 1). Mean age of the non-SCD controls was 43 ± 19 (Table 1); 20 (90.9%) were female. Among SCD participants, 32 had HbSS or HbB⁰ thalassaemia, 13 (36.1%) had HbSC; 20 (55.6%) were taking hydroxyurea (hydroxycarbamide) and five (13.9%) were on chronic blood transfusion or apheresis therapy. Five (13.9%) had undergone a documented splenectomy. At sample collection, all but four SCD participants had received one or more documented SARS-CoV-2 vaccine doses,¹⁰ and two had received a booster dose.

All SCD participants and 95% of controls had detectable anti-spike protein antibodies; 21 (58.3%) and 11 (50.0%) had anti-nucleocapsid antibodies, respectively. Six (16.7%) SCD participants were aware of or had a documented COVID-19 infection. Among vaccinated participants (n = 32), the most recent vaccination occurred 1–269 days (mean 120.7±9.4) prior to sampling. Of the two participants vaccinated only once, 6–44 days had elapsed, and both had detectable antinucleocapsid antibodies, with anti-spike antibody levels >30 000 AU.

Both anti-spike and anti-nucleocapsid antibody levels were indistinguishable between participants and controls (Figure 1A,B). Among SCD participants, antibody levels did not differ by age (Figure 1C,D). Anti-spike antibody levels significantly increased by exposure number (i.e., vaccination and/or infection, $R^2 = 0.34$, p < 0.001), but anti-nucleocapsid antibody levels did not (Figure 1E,F). Increased time from most recent vaccination correlated with decreased anti-spike antibody levels ($R^2 = 0.17$, p = 0.02; Figure 1G). Finally, neither anti-spike nor anti-nucleocapsid antibody levels differed by splenectomy, hydroxyurea therapy, or chronic red blood cell transfusion/exchange transfusion therapy (Figure 1H,I,J).

Our observations suggest that splenic function may not be required for generating sufficient anti-SARS-CoV-2 antibodies upon infection and/or mRNA-based vaccination. Specifically: (1) no differences by SCD status were found for anti-spike or anti-nucleocapsid levels; (2) antibody levels did not vary by age, hydroxyurea use, blood-transfusion therapy

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TABLE 1 Demographic, clinical and SARS-CoV-2 characteristics of our sample of participants with sickle cell disease and non-sickle controls. No SCD patients had been hospitalized for SARS-CoV-2 infection

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Abbreviations: AU, antibody units; SCD, sickle cell disease.

^aPrior splenectomy or suspected SARS-CoV-2 infection could not be confirmed for three adult SCD participants.

^bPer the assay manufacturer, $AU \ge 1.0$ are positive.

or splenectomy; (3) half of the SCD participants and controls had antibody evidence of past infection(s), suggesting high prevalence of SARS-CoV-2 exposure; and (4) anti-spike antibody levels among SCD participants increased with number of known exposures (infection and/or vaccination).¹¹ Our data suggest that anti-spike antibody levels waned over time after vaccination. However, the study design precluded assessment of whether the rate of antibody decrease was similar to those reported for people without SCD.¹² Nonetheless, our results were consistent with those of recent small studies examining antibody levels in SCD populations.⁵⁻⁷

Anti-spike antibody responses to SARS-COV-2 MRNA vaccination are variably reduced or absent in other types of immune dysregulation (e.g., from post-transplant immuno-suppression or primary antibody deficiencies).^{1,5,8} Whether antibody levels correlate with outcomes of SARS-COV-2 infection in people with SCD is not yet clear, as published reports to date have examined pre-vaccination outcomes.¹³ Nonetheless, among this modest sample of mostly vaccinated participants with SCD, none were hospitalized for pandemic-associated complications in the 22-month period from the initial pandemic wave in New York City in March 2020 through December 2021.

Study limitations include the modest sample size, potential sample bias and incomplete information about the timing of infections. Most participants were vaccinated. Comparable data for controls were not available. Among participants with SCD, attenuated or absent splenic function was presumed and probably heterogeneous. All vaccinated SCD participants had received mRNA-based vaccines, precluding comparisons to other vaccine types. Antibodies were assayed prior to widespread dominance of the Omicron variant. Neutralization titres were not tested, although antibody levels measured by commercial assays like the one used correlate reasonably well with neutralization titres.^{14,15} Additional critical anti-viral immune functions were not assessed.

In conclusion, despite presumed SCD-associated impaired or absent splenic function, antibody responses to SARS-CoV-2 mRNA-based vaccine and/or infections were comparable to those from non-SCD controls. Prevalence of prior infection (anti-nucleocapsid antibodies) in 58% of our cases tested, universal positivity for anti-spike antibodies and lack of pandemic-associated hospitalizations among the 36 participants with SCD may predict protection against severe SARS-CoV-2 infection in New York City. As individuals living with SCD are expected to have poor spleen function, it is reassuring to see that they were still able to mount an adequate antibody response. Our findings strongly suggest that people with SCD should be encouraged to receive all recommended SARS-CoV-2 vaccinations, as they appear likely to develop exposuredependent antibody-mediated protection comparable to people without SCD.

ns

(A)

(B)



3



ns

FIGURE 1 Antibody levels were only affected by number of exposures to SARS-CoV-2 antigens and time from last vaccination. Anti-spike and anti-nucleocapsid antibody levels were quantified in 36 patients with sickle cell disease (SCD) and 22 control patients without SCD seen in the same haematology adult and paediatric clinics. (A) Log anti-spike and (B) log anti-nucleocapsid levels comparing subjects with SCD and control. Cutoff for test positivity is indicated by dotted line. (C) Log anti-spike and (D) log anti-nucleocapsid levels by age. One child under the age of 5 years was removed from the analysis shown in panel C, because of ineligibility for vaccination. (E) Log anti-spike and (F) log anti-nucleocapsid levels plotted against number of exposures, which was defined as the sum of number of COVID-19 illnesses and vaccine doses. (G) Log anti-spike levels plotted against time since last vaccination in days. Pearson correlation and p-values as indicated. Log anti-spike and nucleocapsid antibody levels for patients with SCD with and without history of (H) splenectomy, (I) hydroxyurea use, and (J) chronic red blood cell (RBC) transfusion treatment.



AUTHOR CONTRIBUTIONS

All authors made meaningful contributions to the research reported here. Nancy S. Green, Layla Van Doren and Eldad A. Hod designed the research study; Nancy S. Green, Layla Van Doren, Maureen Licursi, Daniel D. Billings, Luke A. Sandoval and Yona M.Z. Feit played key roles in participant recruitment, enrollment and data collection; Nancy S. Green, Layla Van Doren and Eldad A. Hod analysed the data; Nancy S. Green, Layla Van Doren and Eldad A. Hod wrote the paper.

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KEYWORDS

antibodies, coronavirus 2019 (COVID-19), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), sickle cell disease, splenic function

CONFLICT OF INTEREST

The authors declare no conflict of interest.

Nancy S. Green^{1,2} Layla Van Doren² Maureen Licursi¹ Daniel D. Billings² Luke A. Sandoval³ Yona M. Z. Feit³ Eldad A. Hod³

 ¹Department of Pediatrics, Division of Hematology, Oncology and Stem Cell Transplantation, Columbia University Irving Medical Center – New York Presbyterian Hospital, New York, New York, USA
 ²Department of Medicine, Division of Hematology and Oncology, Columbia University Irving Medical Center – New York Presbyterian Hospital, New York, New York, USA

³Department of Pathology and Cell Biology, Columbia University Irving Medical Center – New York Presbyterian Hospital, New York, New York, USA

Correspondence

Nancy Green, Columbia University Medical Center, 650 West 168 St., Box 168, New York, NY 10032, USA. Email: nsg11@cumnc.columbia.edu

ORCID

Nancy S. Green D https://orcid.org/0000-0002-9877-1561

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