Very low incidence of *Clostridioides difficile* infection in pediatric sickle cell disease patients

Despite advances in the survival of pediatric patients with sickle cell disease (SCD), affected individuals continue to experience progressive end-organ damage, recurrent painful vaso-occlusive episodes and a shortened lifespan, highlighting the need for additional therapies.[1,2] Recent animal and human studies suggest that antibiotic treatment may actually ameliorate the severity of SCD by altering the gastrointestinal microbiome.[3,4] Specifically, depletion of intestinal microbiota by administration of broad-spectrum antibiotics significantly reduces the number of circulating pro-inflammatory, aged neutrophils and improves inflammation-related organ damage or endotoxin-induced septic shock in mouse models of SCD.[3,5] Furthermore, routine administration of penicillin propylaxis to SCD patients up to 5 years of age has also been associated with reductions in the percentage and absolute number of aged neutrophils.[3] A recent murine study showed the dependence of stress-induced vaso-occlusive episodes and inflammation, on the gut microbiota, mediated by aged neutrophil expansion.[6] These observations suggest that in addition to preventing infection, there may be a role for extended antibiotic "prophylaxis" to reduce SCD severity by modulating the gut microbiome to decrease inflammation.

One concern with the approach of increasing the exposure of SCD patients to antibiotics is the potential for increasing rates of *Clostridioides difficile* infection (CDI), which is strongly associated with antibiotic use.[7] Surprisingly, a low incidence of CDI was recently reported in adults with SCD.[8] We sought to determine the incidence of CDI among pediatric SCD patients at a tertiary-care urban children's hospital.

We performed a retrospective review of all children ≥2 years of age admitted to The Children's Hospital at Montefiore (Bronx, NY, USA) from 2008 to 2017. Children <2 years of age were excluded because of the known higher rates of asymptomatic *C. difficile* colonization in this age group.[9] Approval for this study was granted by the Albert Einstein College of Medicine, Institutional Review Board. Documentation of positive glutamate dehydrogenase and toxin tests was required to classify the patient as having CDI. Patients with *C. difficile* who also had inflammatory bowel disease or who had received a stem-cell transplant were excluded from the final analyses of patients with and without SCD, since these underlying conditions are significant independent risk factors for CDI.[10,11] Patients with CDI and/or SCD were identified using all applicable International Classification of Diseases (ICD)-9 and ICD-10 diagnosis codes. A Z-test was used to compare the number of CDI cases per 1,000 admissions and per 10,000 patient-days between SCD and non-SCD patients.

Over the 10-year period, there were 71,920 qualifying hospital admissions for non-SCD patients, corresponding to 376,482 patient-days; 183 cases of CDI were identified, yielding an incidence of 4.86/10,000 patient-days or 2.59/1,000 admissions. The mean age of the non-SCD cohort was 10.6 ± 7 years; 50% were male. Over the same 10-year period, there were 5,666 admissions for children with SCD, corresponding to 25,915 patient-days. The mean age of the SCD cohort was 10.6 ± 6.7 years; 51.7% were male. We identified three children with SCD and positive *C. difficile* testing who met our inclusion/exclusion criteria. In the SCD cohort, charts were also reviewed by three investigators for clinical signs of diarrhea. Of the three patients, two did not have clinical signs of diarrhea, which would generally be required by clinicians to define CDI, but are included in these analyses for consistency with the non-SCD group as chart review for the larger non-SCD cohort was not feasible. The patient with diarrhea was a 12-year-old who developed diarrhea and abdominal pain after a recent hospital admission for pneumonia. These three cases yielded a CDI incidence of 1.16/10,000 patient-days or 0.54/1,000 admissions among SCD patients – significantly less than that identified among the non-SCD cohort (P=0.0113) and less than published rates of 2.7/1000 among hospitalized adults with SCD.[8] Additionally, in a subset analysis from 2015 to 2017, we found that there were no cases of CDI in 957 SCD patients, including the 218 patients who were receiving daily penicillin prophylaxis.

This study was undertaken to determine CDI rates in children with SCD. To our knowledge, there are no published studies of CDI in children with SCD. Over a 10-year period, the rate of CDI among inpatients with SCD was significantly lower than that in non-SCD children. These findings are consistent with the recent study of adult SCD patients and are surprising given the many potential risk factors for intestinal dysbiosis in SCD, including frequent antibiotic use, frequent hospitalization, iron overload, hypoxia, and altered gut permeability.[8,12,13] However, the low rates of CDI in both pediatric and adult SCD patients are consistent with the theory that the SCD microbiome might protect against *C. difficile* disease, possibly due to changes in the intestinal metabolome.[10,14,15] It is hypothesized that hypoxia-reperfusion injury in SCD alters intestinal microbiota and use of opioids impairs intestinal motility, both leading to bacterial overgrowth and subsequently increased concentrations of intestinal butyrate which inhibits the proliferation of *C. difficile*.[14] The bacterial overgrowth due to opioids may also play a role in reducing the risks for hospital–onset CDI by creating a tight microbial biofilm which depletes the spatial opportunity for the colonization and proliferation of *C. difficile*.[14] Another potential hypothesis is a hemoglobin S-induced right shift of the oxygen–hemoglobin dissociation curve leading to increased oxygen availability, which may inhibit the proliferation of *C. difficile*.[8]

One study limitation was our inability to review charts for all non-SCD patients identified as having CDI. These data did not, however, have an impact on the low rate of CDI found among SCD patients; as the rate of CDI in the SCD cohort includes those without documented diarrhea, and remains significantly lower than the rate of CDI in the non-SCD patients (P=0.0113). Another limitation is perhaps a lower clinical suspicion in SCD patients leading to decreased frequency of CDI testing in the SCD population; however, we suspect that it is unlikely that CDI will be missed given the severity of the presentation of CDI and likely non-resolution of symptoms of CDI without appropriate treatment.

Studies in adults have demonstrated a high rate of CDI among non-SCD stem-cell transplant recipients;[10] it is possible that any protective features of the SCD microbiome may be eliminated by chemotherapy and other pre-transplant conditioning, allowing for mucosal barrier disruption and the development of CDI. Further study of the intestinal microbiome profiles of SCD patients and transplant recipients is needed in order to better understand the CDI rates relative to those in non-SCD patients in that sub-population.

Antibiotic prophylaxis, if proven to be efficacious in

modulating SCD end-organ damage, could have tremendous relevance in low-resource settings worldwide, where the burden of SCD is greatest. However, adequately powered studies would need to be conducted to rigorously evaluate the safety of this approach and closely assess the risk of CDI.

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doi:10.3324/haematol.2019.244582

Received: December 11, 2019.

Accepted: September 10, 2020.

Pre-published: September 14, 2020.

Disclosures: no conflicts of interest to disclose.

Contributions: PL and AS are the main manuscript authors; DLG, DM, PL, AS and VLS designed the study, reviewed the data, and did the majority of manuscript writing; OA performed related research and provided critical manuscript review that led to a redesign; TW provided statistical support; CC performed research and data analysis; BCH and PSF contributed to the study design and manuscript review.

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