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Scientific Letter

Bacteraemia Among Patients with Sickle Cell Disease in Nigeria: Association with Spleen Size and Function

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To the editor.

In Sub-Saharan Africa, infections are a leading cause of morbidity among sickle cell disease (SCD) individuals. The causes of the increased risk of infection are poorly documented, but the loss of splenic function is important. Previous studies have documented increased susceptibility to bacterial infections among SCD patients, evidenced by increasing markers of splenic dysfunction;[1,2] however, there are no data on the association between bacterial infections and splenic function among the SCD population in Sub-Saharan Africa, partly because most of the techniques required to assess splenic function are not readily available.[3] We recently employed the presence of two red cell-containing inclusions - Howell-Jolly bodies (HJB) and argyrophilic (silver staining) inclusion (AI) red cells - to assess splenic dysfunction among our SCD patients.[4] In the present study, we aimed to determine the prevalence and pattern of organisms causing bacteraemia among our acutely-ill SCD patients and to describe any association between bacteraemia with splenic status on ultrasound and two markers of splenic dysfunction (i.e., HJB and AI red cells)

Methods. This cross-sectional study was conducted at the University of Maiduguri Teaching Hospital, North-Eastern Nigeria, from October 2020 to May 2021. All febrile and/or acutely ill SCD patients presenting to the adult or paediatric emergency unit during the study period were invited to participate. A case report form was used to obtain baseline clinical characteristics from the patients (or their carers). Under aseptic conditions, between 3 and 8 ml of venous blood for cultures were collected directly into appropriate BACTEC plus bottles and incubated manually at 37°C. Positive blood cultures were sub-cultured on standard media using routine microbiological techniques. Non-pathogenic organisms commonly associated with contaminated blood cultures, such as coagulase-negative *staphylococci*, *Acinetobacter* species, *Bacillus* species, *Corynebacterium* species, *Micrococcus* species, and non-meningitidis *Neisseria* species were considered as contaminants.[5] The splenic function was assessed by manual estimation of Howell-Jolly bodies (HJB) and argyrophilic inclusion (AI) containing red cells from blood smears as previously described.[4] The splenic status was assessed using ultrasound. Data were analysed using Statistical Package for the Social Sciences (SPSS) (version 25; SPSS, Chicago, IL, USA). The data were summarized using descriptive statistics. The prevalence of bacteraemia was defined as the proportion of positive cultures in all the blood cultures. Clinical and laboratory features of patients with bacteraemia were compared to those without bacteraemia, using non-parametric analysis.

Ethics statement. Written informed consent was obtained from the adults and paediatric participants' parents/guardians. The study protocol was approved by the University of Maiduguri Teaching Hospital (UMTH/REC/ 20/606) and Liverpool School of Tropical Medicine (LSTM) (REC reference number: 20-010) Ethics Review Boards.

Results. Over the 7-month study period, 162 febrile episodes involving 140 SCD patients (median age 13.0 years; IQR 5.0 - 21.0) occurred during the visits to adult and paediatric emergency units. The predominant haemoglobin phenotype was HbSS (98.1%); only two patients (1.9%) had HbSC phenotype. A total of 113 (69.8%) blood cultures were obtained. Bacteraemia occurred in six (5.2%, 95% CI, 1% to 10%) of the culture samples. Two cultures from children aged 2 and 3 years grew *Salmonella* species. The remaining four positive cultures were among the adult patients (one culture each grew *Serratia Marcescens, Citrobacter spp, Enterobacter spp,* and an unidentified Gram-negative bacillus). Four other positive cultures were

Table 1. Clinical characteristics of SCD patients with positive blood cultures.

Isolates	Sex	Age (years)	Temp	Ill looking	WBC	Prior antibiotics use	Immunization completed	% AI red cells (median)	% HJB red cells (median)	Spleen size on ultrasound
Salmonella typhi	Female	2	38.5	Yes	34.7	Yes	Yes	65.6%	1.3%	5.6 cm
Salmonella typhi	Female	3	37.8	Yes	30.0	Yes	Yes	45.%	0.6%	8.2 cm
Enterococci sp	Female	16	37.2	Yes	33.5	No	No	61.1%	26.0%	6.8 cm
Serratia marcescens	Female	22	37.2	Yes	18.1	No	Yes	73.0%	3.5%	Autosplenectomy*
Citrobacter	Male	25	37.8	Yes	26.7	No	Not sure	74.6%	NR	Autosplenectomy
GNB (unidentified)	Male	17	36.0	Yes	22.8	Yes	Yes	53.2%	2.9%	Autosplenectomy

AI argyrophilic inclusion; HJB Howell-Jolly bodies. M: male; F: female. Temp: temperature. GNB: Gram-negative bacillus WBC: white blood cell count; NR not reported; * spleen was not visualized on ultrasound.

considered contaminants and excluded during the analysis.

The clinical characteristics and splenic status on ultrasound of patients with bacteraemia are shown in **Table 1**. The spleen was present in three patients (2 children and one adult) and absent in the remaining three (i.e., autosplenectomy). Bacteraemia was not significantly different in SCD patients with spleen present or absent spleen on ultrasound (P = 0.87). A comparison of clinical and laboratory parameters between patients with and without bacteraemia is shown in **Table 2**. The median HJB and AI red cell counts were 1.8% (IQR 0.5% - 7.3%) and 48.2% (IQR 27.2% - 63.5%) respectively among the study participants. There was a trend towards higher counts of red cells with HJB (median 2.4% vs. 1.9%) and AI (62.4% vs. 43.4%) in patients with bacteraemia compared to those without bacteraemia respectively; however, the result was not significant for either the HJB (P = 0.744) or AI red cell counts (P = 0.075) (**Table 2**). Patients with bacteraemia had significantly higher white blood cell counts (mean 34.9 vs. 21.6; P = 0.018) and raised neutrophil counts (19.4 vs. 11.3; P = 0.006) than those without bacteraemia. All other clinical and laboratory parameters were not significantly different between the two groups.

Discussion. In the present study, we determined the prevalence and pattern of bacteraemia among our patients with SCD.

Our study observed prevalence rate of 5.2% is comparable to previous studies across Africa, which ranged between 4.0% and 9.7%.[6-8] Our prevalence rate is also similar to studies beyond Africa, including 6.1% in Jamaica,[9] 5.2% in the USA,[10] and 3.4% in the United Kingdom.[11] Though our finding is comparable to other studies, it is not clear if the use of over-the-counter antibiotics may have contributed to the low rates observed in studies from Africa, where there is unrestricted access to over-the-counter antibiotics;[6,12] more than a quarter of our patients admitted using antibiotics prior to presentation at the health facility. Most isolates cultured in this study were Gram-negative, similar to reports among SCD patients in Nigeria.[13,14] In contrast, some studies from Africa have reported Gram-positive organisms like *Staphylococcus aureus* as the predominant bacteria isolated,[12,15] and other studies from Africa[8] and Western countries have identified the Gram-positive organism, *Streptococcus pneumonia,* as the major bacterial pathogen implicated in infection among their SCD patients.[10,16] Patients with SCD are susceptible to infection with encapsulated organisms (*S. pneumonia*, *H. influenza*) and *Salmonella* species due to their underlying splenic dysfunction.[17] The prevalence and pattern of pathogens implicated in SCD infections may differ in the various countries. This can be influenced by the local epidemiology of infections, available vaccines and population-specific vaccine efficacy, environment, health care systems, and cultural behaviors.[18] Furthermore, the frequency with which specific pathogens cause infections have been shown to follow an age-specific pattern. *Salmonella* bacteremia is common among younger patients with SCD, with a peak incidence between 2 and 10 years, and can be associated with an increased risk of osteomyelitis.[19,10] In the current study, *Salmonella sp* was isolated in two of the children less than five years old, one of whom had a previous history and management for osteomyelitis of both femurs. The expanded bone marrow in patients with SCD, with its sluggish blood flow, is vulnerable to thrombosis, infarction, and fibrosis; this can result in ischemic foci, allowing for salmonellae localization. The proliferation of previously dormant foci of infection can accompany a sickle crisis and, with local bone changes, can result in the passage of the organisms into the bloodstream.[20]

Despite the high morbidity and mortality among SCD patients attributed to loss of splenic function, no study has evaluate the presence of bacterial infection and markers of splenic dysfunction in SCD patients in

Table 2. Association of clinical and laboratory parameters with bacteraemia.

	Bacteraemia absent [n = 107/113 (94.8)]	Bacteraemia present [n = 6/113 (5.2)]	*P*
Clinical parameters			
Age, years, mean (SD)	12.8 (8.9)	17.1 (11.5)	0.565
Male, n/n (%)	56/107 (52.3)	2/6 (33.3)	0.611
Temperature, mean (SD)	37.5 (0.9)	37.5 (1.0)	0.938
Jaundice, n/n (%)	36/106 (34.0)	3/6 (50.0)	0.418
Pallor, n/n (%)	50/106 (47.2)	4/6 (66.7)	0.426
Immunization completed	58/107 (54.2)	3/6 (50)	0.340
Spleen parameters			
Spleen status on ultrasound: n/n (%) Spleen present. Spleen absent**	 53/99 (53.5) 46/99 (46.5)	 3/6 (50) 3/6 (50)	0.87
% AI red cells, median (IQR)	43.4 (35.5)	62.4 (22)	0.075
% HJB red cells, median (IQR)	1.9 (6.7)	2.4 (19.7)	0.744
Laboratory parameters			
White blood cell count (10^6/l, mean (SD)	21.6 (17.3)	34.9 (20.0)	0.018*
Hb (g/dl) mean (SD)	6.3 (2.1)	7.3 (2.1	0.209
Platelets (10^9/l), mean (SD)	418 (210)	516 (241)	0.274
ANC (10^9/l) mean (SD)	11.3 (8.8)	19.4 (6.0)	0.006*
Reticulocyte count (%), mean (SD)	5.7 (6.3)	10.2(7.2)	0.148
Bilirubin (total)(umol/l), mean (SD)	28.7 (27.7)	22.0 (10.4)	0.881
ASAT (iu/l) mean (SD)	17.5 (16.3)	30.8 (29.6)	0.218
Haemoglobin F, %, mean (SD)	8.3 (5.9)	6.9 (1.9)	0.172

ASAT: aspartate amino transferase; ANC: absolute neutrophil count; Hb: haemoglobin; SD: standard deviation; IQR interquartile range *Significant P value by Mann Whitney U test. **Autosplenectomy.

SSA.[3] We have recently used two markers of splenic dysfunction that required simple techniques and thus can easily be performed in most resource-poor settings; the proportion of both markers, HJB- and AI-containing red cells, were higher in patients with autosplenectomy than those with visible spleens.[4] In the current study, we noted that SCD patients with bacteraemia were more likely to have higher AI and HJB red cell counts. However, the difference for both markers failed to reach statistical significance. The small number of patients with bacteraemia (n=6) may have affected the power to detect any significant relationship, limiting our ability to make concrete conclusions on the relationship between these markers and the risk of bacteraemia.

Furthermore, although a high count of markers of splenic dysfunction is expected to be associated with an increased risk of bacterial infections, the ability of the spleen to filter the blood of pathogens depends on several other mechanisms, including complement activation, of humoral and cellular immune responses.[17] Therefore, it is unclear whether the HJB or AI counts alone can accurately reflect the spleen-related risk of bacterial infection. A larger study will be useful in providing more insight into the relationship between bacteraemia and splenic parameters.

This study has some limitations. The small number of patients with bacteraemia (5.2%) may have affected the power to identify an association with splenic parameters; the low prevalence of bacteraemia observed could be due to prior use of antibiotics by the patients. The use of one culture bottle per set rather than two for adults and the use of a manual incubator instead of the standard BAC/Alert system for our bacterial detection may have affected the identification of fastidious organisms and demonstrate the difficulties in performing clinical research in laboratories with limited resources characterizing the conditions in most Sub-Saharan African countries.

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