Effectiveness of the monthly malaria chemoprophylaxis using sulphadoxine-pyrimethamine plus amodiaquine and its safety when used in combination with hydroxyurea in children living with sickle cell disease: A quasi-experimental study

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Abstract

Background

Malaria infection in patients with sickle cell disease (SCD) is associated with increased risk of SCD related complications and mortality. As such, the World Health Organization (WHO) recommends the provision of malaria chemoprophylaxis (MCP) in patients with SCD. However, in Sub Saharan Africa (SSA) which bears the highest burden of SCD, and where malaria is endemic, MCP is neither universal nor standardized in part due to inadequate evidence.

Methods and analysis

This will be a multi-country, multi-center, quasi-experimental study employing the interrupted time series design whereby the effectiveness of monthly intermittent presumptive treatment using sulphadoxine/pyrimethamine + amodiaquine (SPAQ) and its implementation will be assessed versus local standard practice. The study will be conducted at the Sickle Pan-African Research Consortium sites in Uganda, Tanzania, Nigeria, Mali, Ghana, Zimbabwe, and Zambia. An estimated 2,345 children confirmed with SCD aged between 6 months and 17 years will be enrolled in the study and followed up for 24 months. Case report forms will be used to collect demographics, clinical and laboratory information, whereas the interview guide will be used to explore the implementation of the interventions. The primary outcome is the incidence of the first malaria episode pre-and post-intervention. The secondary outcomes include the frequency of adverse events and changes in hematological parameters associated with using SPAQ in patients taking hydroxyurea (HU). Acceptability of SPAQ with HU will also be assessed using focus group discussions (FGDs) with communities (parents/caretakers and children > 15 years) and key informant interviews with healthcare workers. Data management will be done using REDCap; quantitative data will be analyzed using R and STATA, while NVivo will analyze qualitative data.

Ethics and dissemination: All parents/ guardians of enrolled participants will provide written, informed consent. Ethical approval has been obtained from all participating countries' Research and Ethics Committees. Results will be disseminated by peer-reviewed open-access publications together with open data.

Trial registration number (from registration at ClinicalTrials.gov):

Strengths and limitations of this study

- Malaria is associated with increased morbidity and mortality in patients with SCD, therefore evidence of the effective chemoprophylaxis is pertinent.
- This multicountry, multicenter quasi-experimental study will employ an interrupted time series design to provide evidence of the effectiveness of MCP in children with SCD.
- Evidence on the safety of SPAQ among patients using HU will also be determined in this study.
- Each subject acts as their own control, which minimizes the risk of confounding caused by interindividual variability.
- One of the limitations of this study is that the primary outcome is the incidence of malaria which is affected by the transmission rate, which can change as a function of time. To mitigate this, we will monitor malaria prevalence at the study sites to ensure that the effect observed is due to intervention and not a decrease in transmission rate.

Introduction

The World Health Organization (WHO) estimates that 300,000 children are born with sickle cell disease (SCD) each year, 75% of whom are in sub-Saharan Africa (SSA) [Roucher et al. 2012, Silva-Nunes et al. 2007]. SCD patients are susceptible to severe infections, including malaria, with the most vulnerable group being children under 5 years, adolescents, and pregnant women [Makani et al. 2010, McAuley et al. 2010, WHO, 2010].

Malaria is a significant cause of hospitalization in children with SCD in endemic areas [Eleonore et al. 2020, Aloni et al. 2013]. It is associated with higher mortality in hospitalized SCD patients compared to hospitalized non-SCD patients [Makani et al. 2010, McAuley et al. 2010, Ambe et al. 2001]. Malaria has been incriminated in endemic regions as a significant cause of mortality in

people with SCD. The distribution of SCD parallels that of malaria, with both disorders being more frequent in Africa's tropical belt [Wiebe et al. 2017, Piel et al. 2013]. This parallel epidemiology is not a coincidence: instead, there is evidence that heterozygotes for the sickle gene are relatively protected against death due to malaria, probably through accelerated clearance by macrophages of *P. falciparum*-infected erythrocytes [Luzzatto, 2012]; as a result, it prevents the establishment of disease following infection [Sabeti et al. 2006].

As SCT offers relative protection against malaria, one might expect the protection to be at least as effective in the homozygous state (HbSS). On the contrary, HbSS SCD patients are at increased risk of dying from malaria [Luzzatto, 2012, Makani et al. 2010, McAuley et al. 2010]. Previous studies have shown malaria is associated with increased morbidity patients with SCD and is often life-threatening since it does not only worsen the preexisting anemia but also the abnormal splenic function in SCD patients hinders clearance of parasitized red blood cells (RBCs) [Demar et al. 2014, Chotivanich et al. 2012, Luzzatto, 2012].

Given the severe malaria complications and its high contribution to the mortality rate in SCD patients, the WHO recommends that SCD patients in endemic areas receive antimalarial prophylaxis to tackle the burden of malaria in SCD patients. Chemoprevention and chemoprophylaxis are strategies that use antimalarial medicines to prevent malaria infection and disease. The use of malaria chemoprophylaxis (MCP) in this group of patients, though included in most standard-of-care guidelines, is not universal nor standardized due to a lack of adequate evidence in this population [Ndegeulaya et al. 2019, Frimpong et al. 2018]. Children and adults with SCD receive monthly SP for malaria chemoprophylaxis in Uganda and Mali. In Nigeria, monthly sulphadoxine-pyrimethamine (SP) or daily paludrine is given, whereas no MCP is used in Ghana, Tanzania, and Zimbabwe. Evidence from rigorously performed multi-center studies on optimal MCP regimens in persons with SCD in SSA is lacking. Moreover, SCD patients are using other medications such as folic acid and, more recently, hydroxyurea (HU), which may have several interactions with the current antimalarial drugs used for prophylaxis.

A combination of SP plus amodiaquine (SPAQ) is one of the antimalarial drugs recommended for chemoprevention to reduce the risk of new infections among patients with SCD [WHO, 2023]. SPAQ has been evaluated in 12 studies of seasonal malaria chemoprevention and has been widely used in Africa. SPAQ is efficacious, safe, well tolerated, available, and inexpensive and is

a potential candidate for perennial malaria chemoprevention [WHO, 2023]. Furthermore, assessing the effectiveness SPAQ use for MCP is important given the growing evidence of resistance to SP(Jiang et al , 2019, Malaria journal). Therefore, urgent evidence is required on the acceptability and effectiveness of the monthly SPAQ for MCP and its safety when used in combination with HU in children living with SCD in SSA. This study will provide evidence on whether the combination of SPAQ provides more effective, safe, and tolerable antimalarial chemoprevention among patients with SCD in African countries.

Methods and analysis

Objectives

Primary objective

To assess the effectiveness of monthly SPAQ for MCP and its safety when used in combination with HU in children living with SCD.

Secondary objectives

1. To assess malaria incidence among sickle cell patients pre- and post-intervention.
2. To determine malaria-related complications among sickle cell patients pre and post-intervention.
3. To describe the proportion of patients with adverse drug events (ADEs) pre- and post-intervention.
4. To determine the proportion of patients with changes in haematological parameters (lower hemoglobin, white blood cells, and platelet count).
5. To describe the proportion of patients requiring dose reduction or who were required to stop taking HU after the intervention.
6. To explore the acceptability of the intervention among children, parents, and health care providers.

Trial design

Study sites

Seven African countries will participate in this study: Ghana, Mali, Nigeria, Tanzania, Uganda, Zambia, and Zimbabwe. The study sites for each country will be as follows: Tanzania (Bugando Medical Center in Mwanza region), Mali (Centre de Recherche et de Lutte contre la Drépanocytose), Ghana (), Nigeria (), Uganda (Jinja Regional Referral Hospital), Zambia (), and Zimbabwe ().

These countries are members of the Sickle Pan-African Research Consortium (SPARCo) and have well-established clinics specializing in managing patients with SCD. SPARCo works closely with the Ministry of Health of individual countries to provide evidence that informs policy and recommendations, essential in preparing the standard of care guidelines for patients with SCD.

The prevalence of malaria in these participating countries varies, ranging from low (Ghana) to high malaria prevalent countries (Zambia). Therefore, the availability of evidence from these countries will give more information on the usefulness of MCP in SCD patients living in areas of varying malaria endemicity.

Summary of trial design

This will be a multi-country, multi-center, quasi-experimental study employing the interrupted time-series design whereby the effectiveness and safety of MCP and its implementation will be assessed. Participants will be enrolled at each site on a rolling basis until the desired sample size of 2,345 is met. Each participant will be followed for 24 months. Local standard guidelines for malaria chemoprevention will be used for the first 12 months a participant is enrolled in the study. Then, for the subsequent 12 months, each participant will be given SPAQ for MCP.

HU will be administered as per local site guidelines. This implies that some participants will be on HU at the beginning of the study, and others may not. Some will be prescribed HU during the study period. We expect 20-40% of study participants will be on HU during the study period.

Study duration

The planned study duration is 3 years, whereby training of research assistance and patient community engagement will precede study execution at least 1 month before patients' enrollment.

The patient enrollment phase is expected to last 6 months. Data analysis and report writing are expected to take about 5 months.

Primary and secondary endpoints

Primary endpoint

The incidence of malaria cases before and after the intervention is defined as the presence of one or more symptoms of malaria that coincide temporally with a positive Giemsa-stained blood smear.

Secondary endpoints

1. Incidence of malaria-related complications (hospital admissions, VOC, severe anemia, blood transfusion need) before and after intervention.
2. Incidence of malaria-related mortality before and after intervention.
3. The proportion of participants experiencing ADEs deemed possibly or related to SPAQ use.
4. The proportion of participants experiencing ADEs deemed possibly or related to SPAQ + HU use.
5. The proportion of patients with significant clinical and hematologic abnormalities (lower hemoglobin, white blood cells and platelet count) deemed possibly or related to SPAQ + HU use.
6. The proportion of patients requiring dose reduction or who were required to stop taking HU after using SPAQ.
7. User and provider acceptability of SPAQ with a focus on the tolerability, dosing schedule, dose of the drugs, and ease of drug administration.
8. User and provider perception on the safety of SPAQ use for MCP in SCD patients.

Trial participants

This will include SCD patients aged 6 months to 17 years enrolled in the SPARCO database, including all new participants during the study period. Based on previous research, newborns and young infants are protected during the first few months of life, likely due to the trans-placental acquisition of maternal antibodies and relatively high fetal hemoglobin content [White, 2005]. Protection from these factors wanes after 6 months of age [White, 2005]. Therefore, the selected age range is the highest risk group for malaria mortality and would benefit from MCP use.

Inclusion criteria

- Agreement to participate and sign a written consent and assent form (where applicable)
- Age: 6 months to 17 years
- Confirmed and documented SCD status
- Attendance at one of the 7 SPARCO sites
- Willing to comply with study procedures.

Exclusion criteria

- Pregnancy, as determined by a positive urine test.
- Inability to comply with study procedures.
- Known allergies or contraindications against SP or AQ.
- HIV-positive individual under cotrimoxazole chemoprevention.
- Patients with acute febrile illness or severely ill patients who cannot take oral medication. These can be enrolled after 42 days, when we estimate they would have recovered to their steady state.
- Patients who have a positive malaria test at baseline. Patients can be enrolled after the malaria treatment wash-out period of 28 days.

Procedures

Screening and enrolment will be done at every sickle cell clinic by the trained study research assistant and a qualified healthcare provider at the proposed study sites. The participants will be

enrolled consecutively on each study site until the sample size is attained. The study participants will be followed for two years (24 months), and all planned assessments will be conducted at the predetermined time according to the participants' timeline shown in Table 1.

During the first 12 months after the enrollment, the participants will be under the current standard of care according to the guidelines for SCD management used in each participating site. This will be an observation phase. After these 12 months, all participants will be given SPAQ for MCP.

To explore the acceptability of implementing scheduled MCP in persons living with SCD, a qualitative assessment will be conducted using key informant interviews (KIIs) and focus group discussions (FGDs). The FGDs and KIIs will be conducted after the collection of quantitative data. This arm of the study will explore how the introduction of monthly SPAQ into national SCD standard of care guidelines will be viewed and accepted by different stakeholder groups.

Table 1: Participants timeline

Activities		**Post enrollment (Months)**								
		M0	**M3**	**M6**	**M9**	**M12**	**M15**	**M18**	**M21**	**M24**
Screening and enrollment	x									
Study flow										
Observation period		x	x	x	x	x				
Intervention period						x	x	x	x	x
Assessments										
Medical history		x	x	x	x	x	x	x	x	x
Physical exam		x	x	x	x	x	x	x	x	x
Laboratory test (CBC, Reticulocytes)		x				x				x

Parasite density count (thick and thin blood smear)		x	x	x	x	x	x	x	x	x
Dry blood spots on filter paper		x	x	x	x	x	x	x	x	x
Hb electrophoresis (HbF)		x				x				x
Buffy coat		x				x				x
Adverse events monitoring		x	x	x	x	x	x	x	x	x
SPAQ medication adherence						x	x	x	x	x
Quantitative sub-study										x

Sample size

In this study, we assume the prevalence of malaria among sickle cell patients to be 3.2% (19) and an incidence of 12 cases per 100 person-years to be the cumulative number of cases in four consecutive clinic visits. If we assume overall confirmed malaria incidence during the intervention period to decrease by a third (30%), then with intra-cluster correlation coefficient, k=0.11, a power of 80%, an alpha (type I error) of 0.05 and seven clusters (study sites), a total sample size of 279 will be required for evaluation in each of the sites. In this study, we anticipate an attrition rate of 20% due to the long follow-up period (two years), so with seven (7) clusters, a sample size of 335 SCD patients per site, and a total of 2,345 for the seven sites will be required. Table 2 below describes the sample sizes by different incidence rates.

Table 2: Distribution of sample size by different incidence rates and intra-cluster correlations

	Sample sizes*

Incidence rate	0.05	0.06	0.07	0.08	0.09	0.1	0.11	0.12	0.13	0.14
k=0.11	669	558	478	418	372	335	304	279	258	239
k=0.12	717	598	513	449	399	359	326	299	276	257
k=0.13	779	649	556	487	433	390	354	325	300	278
k=0.14	857	715	613	536	477	429	390	358	330	307

Sample size estimates as explained elsewhere: (20)

The sample size for qualitative study is estimated for 36 participants. We will conduct 6-10 FGDs each with 6-8 participants which would include adolescents and caregivers of children until saturation is reached for each country. The key informant discussion (3 per country) will be held with healthcare workers of patients with SCD. The final number of FGDs and KIIs/sites will however be guided by the principle of saturation [Fusch et al. 2015]. Purposive sampling will be used to identify potential participants for the FGDs and KIIs. For the FGDs this will be through the list of participants who were part of the intervention arm of the study, while for the KIIS we will select health care workers at the different study sites.

Ethics and Dissemination

Informed consent and assent

During the pre-visit of the study sites, which will be conducted before the enrolment of participants, we will engage the patient community where the trial will recruit participants. Before enrollment, the principal investigator or a trained research assistant will obtain consent (and assent where appropriate). The consent document will be provided in a local language understood by the parent/caretaker and participant.

The information provided will include the study's summary, implications and constraints, known side effects, and any participation risks. It will be clearly stated that participation is voluntary and that the participant is free to withdraw from the study at any time for any reason without any

interference to the care and management offered at the sickle cell clinic. The parent/caretaker and participant will be allowed as much time as possible to consider the information and take the opportunity to question the investigator or other independent parties to decide whether they will (or allow his/her child to) participate in the study. Written informed consent and assent will then be obtained by means of the parent/caretaker and participant name, dated signature or thumbprint (if unable to write), and dated signature of the person who presented and obtained the informed consent and assent.

Screening, eligibility, and assessments

Children will be enrolled in the study on the same day after the parents give informed consent and they accent to participate in the study. Initially, they will be screened, and eligible ones will be enrolled in the study. Screening for eligibility will be conducted at every sickle cell clinic until the required sample size is attained. Participants who cannot return for follow-up as per the schedule will not be enrolled. A screening log will be kept.

Demographics and clinical history

Demographic data (e.g., sex, age, ethnicity, residing region, address, education level, health behavior including bed net use) and a full medical history will be recorded by the study staff. History of blood transfusion, other comorbidities, and the current SCD complications such as stroke symptoms, priapism, chest pain, yellow eyes (Jaundice), dactylitis, and VOC will be documented. The medical history will be collected on every visit.

Physical examination and vital signs

Physical examination, such as the presence of splenomegaly will be performed and vital sign will be taken by a qualified study team member. Weight, height, respiratory rate and temperature will be documented. Physical examinations and vital signs will be conducted and documented at baseline and on every visit.

Medication history

All prescribed or over the counter and herbal medications used within the last 7 days will be recorded. Routine medications used by patients with SCD, such as penicillin V and folic acid, will be documented. The use of hydroxyurea will also be recorded, including the date when it

was initiated and the duration of its use. Any antimalarial drug used for chemoprophylaxis at baseline will be documented. Any drug allergies will be recorded. Medication history will be collected at baseline and on every visit.

Clinical malaria

All participants will be screened for malaria using malaria rapid diagnostic tests (MRDT) and blood smear (BS) at baseline and on every visit. Those who will be found to be malaria positive at baseline will be given antimalarial treatment following the country-specific malaria standard treatment guidelines. They will not be enrolled in the study. Those who will be found to be malaria positive after enrollment will be given antimalarial treatment following the country-specific malaria treatment guidelines and will continue as study participants. They will continue with the study procedures once they are confirmed to have an adequate clinical and parasitological response (cured). The number of malaria episodes will be recorded for all the enrolled participants for 24 months. All episodes of malaria will be classified as either complicated or uncomplicated based on the following criteria:

- **Uncomplicated malaria (all the following)**
    - Positive thick blood smear
    - Fever (> 38.0ºC tympanic) or history of fever in the previous 24 hours
    - Absence of complicated malaria
- **Complicated malaria (any of the following)**
    - Danger signs in children (Lethargy, inability to sit up or stand, more than 3 convulsions over 24 hours, inability to breastfeed or drink, and vomiting).
    - Evidence of severe malaria (characterized by cerebral malaria, severe normocytic anemia (Hb < 5 gm/dL), generalized convulsions (> 3 convulsions over 24 hours period), metabolic acidosis with respiratory distress, hypoglycemia, acute pulmonary edema, and respiratory distress syndrome, fluid and electrolyte disturbances, acute renal failure, shock, septicemia, circulatory collapse, abnormal bleeding, and jaundice).

Blood sample collection, testing, and storage

At least 5mls of venous blood sample is required for the protocol-mandated tests, which include complete blood count (CBC) and HbF electrophoresis, and 0.5mls of the buffy coat will be stored for future studies stored and used for immunological (e.g., antibodies against viral infections) and other SCD genetic studies. This sample will be collected at baseline and on months 12 and 24 after enrollment. Dried blood spots (DBS) will also be collected on every visit by finger pricking after collecting the blood smear sample for malaria microscopic diagnosis. DBS samples will be used in the future to detect submicroscopic malaria infection and genotype malaria parasites resistant to SPAQ.

Study drug

Sulphadoxine/Pyrimethamine + Amodiaquine

SPAQ-CO® Disp 76.5mg+12.5mg/250mg and SPAQ-CO® Disp 25mg/500mg+153mg manufactured by Guilin Pharmaceutical Co., Ltd. (a subsidiary of Shanghai Fosun Pharmaceutical (Group) Co., Ltd.) will be used as an intervention drug.

Target dose/range

After 12 months of the observation period, participants will be given SPAQ according to weight and age, as shown in Table 3 below. The first dose of a 3-day regimen will be given under direct observation during the clinic visit. The second and third doses will be taken at home. Subsequent 3-day courses will be taken monthly at home. When the participant returns for a scheduled visit, they will bring empty pill packages for review and documentation by the study team member. At each visit, the participants will be supplied with enough SPAQ for monthly dosing at home until the next scheduled visit.

Table 3: SPAQ dosing based on age and weigth

		Once each month		**Once daily x3 days each month**		
Age	**Weight**	**Sulfadoxine**	**Pyrimethamine**	**Amodiaquine**	**Number**	**Type of**

(mo.)	(kg)	(mg)	(mg)	(mg)	of packs	tablet
6-11	N/A	250	12.5	76.5	1	infant dispersible
12-59	N/A	500	25	153	1	child dispersible
60-215	15.0-24.9	500	25	153	1	non-dispersible
	25.0-34.9	500	25	153	1	non-dispersible
	35.0-44.9	1000	50	306	2	non-dispersible
	45.0-54.9	1000	50	306	2	non-dispersible
	> 55.0	1500	75	459	3	non-dispersible

Compliance with study drugs

Text messages will be sent to remind the parents/caretakers to give their children medications as described at the clinic. Suppose the participant vomits within 30 mins after drug administration; he/she must retake the medication and inform the site contact person immediately to refill it and record keeping.

All drug doses will be recorded in the CRF. To maximize adherence to study medication, the study will be preceded by a period of patient community sensitization and engagement, including information sessions on the importance of taking all doses of medication as directed.

Storage of study drug

It is recommended that SPAQ should not be stored above 30°C, the tablets should remain in blisters in the provided box/carton until they are required, and the product should be protected from light. All efforts will be made to store the study drug following the manufacturers' recommendations in a secure area.

Study Drug Accountability

All movements of study medication will be recorded. The pharmaceutical personnel will be responsible for the provision of the study drug and will maintain complete records of the study drugs received in the clinic. The pharmacist will record the number of pills given to each study participant, date of receipt, batch/lot number, and expiration date on every visit. A monthly inventory of the study medication will be conducted, and a record log of investigational medicine will be kept at the study sites.

Concomitant medication

Throughout the study, participants may be prescribed concomitant medications or treatments deemed necessary (e.g., painkillers, folic acid, or penicillin prophylaxis) to provide adequate supportive care except for antibiotics with an antimalarial activity unless unavoidable (e.g., doxycycline, azithromycin). If these are required, the participants will be kept in the study, and this will be noted as a protocol deviation. Hydroxyurea dosing and monitoring will be done based on national specific guidelines with dose adjustments as needed.

Antimalarials for symptomatic, confirmed malaria infections will be prescribed according to the country-specific standard treatment guidelines. Any medication other than the study medication taken during the study period will be recorded in the CRF.

Data collection and management

Plans for assessment and collection of outcomes

Before the study onset, all the research assistants (clinical, nursing, laboratory, pharmacy, and data technicians) will undergo study-specific training on study procedures. On-site training will also take place to ensure the study flow is smooth at each site, followed by the subsequent interim monitoring visits.

Following the enrolment visit, participants will return for follow-up at 3-month intervals until the required person-months of observation is achieved. The study visits will coincide with their routine sickle cell clinic scheduled visits. The study will provide a reasonable amount for time compensation. At each visit, all clinical events in the intervening period will be ascertained, and those previously not reported or for which the patients did not attend the study clinic will be documented.

Participants with < 80% adherence will have additional counselling at any point. To determine the outcomes, any participant with a history of fever in the previous 48 h or temperature ≥ 37.5°C on any visit will have a blood smear for malaria microscopy performed by a certified malaria microscopist. All other SCD-related and non-SCD-related events, such as painful crises and hospitalizations, will also be documented and reported. In this way, the malaria prevalence at each visit and the incidence can be estimated in the pre-intervention and post-intervention phases.

Participants will be sensitized to continue receiving care at the study sites in case they become sick and will be referred for appropriate care if the level of care required is beyond the capacity of the clinic. All these unscheduled visits will be recorded and reported.

Record Keeping

Patient data will be recorded into confidential CRFs by the PI/CoPI or study research assistants. Laboratory results will be recorded in a laboratory record book by the laboratory technologists and then transferred to the CRF by study PI/CoPI, who will review the CRFs frequently for completeness and accuracy. All other patients' information and laboratory data (Hb measurements) will be entered into the CRFs, and hard copies of the original results will be attached to the form. Data will be entered directly from CRFs into the REDCap database. All computerized data will be double-entered to verify the accuracy of entry. The team leader will review the collected data daily for precision and consistency and respond to queries generated by the data manager. Data cleaning and validation will be done continuously following the SPARCO SOPs.

Data Quality Assurance and Monitoring

The data manager will perform a quarterly audit to ensure data quality. For this audit, a 1% random sample of study forms entered into the data management system from the previous 2

weeks will be selected and compared for accuracy with the original CRFs and source documents. In addition, the study data manager will perform monthly reviews of the 100% double data entry, data verification logs, and the data management system audit trail log to identify potential data quality issues.

Identification and Management of ADRs Potentially Related to Study Drugs

The lead researcher or research assistants will assess participants according to a standardized CRF at each scheduled and unscheduled visit to the clinic. Adverse event monitoring will occur during the entire follow-up period. The following grades will be used to grade the reported/observed adverse events and hence manage accordingly:

Grade 1(Mild): Transient (stopped after a short while) or mild discomfort; did not limit participation in routine activities; did not require medical intervention/therapy.

Grade 2 (Moderate): There were mild to moderate effects on daily activities, which might have required some assistance, but no medical intervention was sought.

Grade 3 (Severe): There was a marked effect on daily activities, assistance was required, and medical intervention or therapy was used or hospitalized.

Grade 4 (Potentially life-threatening): There was an extreme limitation on daily activities, assistance was a must, and significant medical care or therapy services were made, plus hospitalization.

Participants who develop grade 1 or 2 adverse events during the intervention phase may continue taking SPAQ. The study clinicians will manage the grade 1 or 2 events according to standard practice. Participants who develop grades 3 or 4 after initiating SPAQ will immediately stop taking the drug, followed by management of the condition and close follow-up. If SPAQ is permanently discontinued, study participants will remain in the study, following our intention-to-treat analysis approach. Data will be captured and recorded in the CRF on the incidence of all adverse events, regardless of severity. The following information will be recorded for all adverse events that are reported in this study: 1) Description of event 2) Date of event onset 3) Date event reported 4) Maximum severity of the event 5) Maximum suspected relationship of the event to study drug (SPAQ).

The adverse events which are more likely to be associated with the study drug will be recorded in the 'MHRA yellow card' obtained from the British National Formulary https://yellowcard.mhra.gov.uk/-BNF and submitted to the drug authority for further compilation and reporting. Adverse effects may include nausea, vomiting, diarrhea, pruritus, fever, increased sensitivity of the skin to sunlight, irritation or soreness of the tongue, skin rash, and any of the hematological complications such as severe anemia (Hb < 6 g/dL), leukopenia (WBC < 2,000 per uL), neutropenia (neutrophils < 500 per uL) and thrombocytopenia (PLT < 100,000per uL).

The outcome will be measured as the total number of events across all three safety outcome categories and as the number of events within each category. The outcomes will be analyzed as the incident rate ratios of events occurring while on the combination of the prophylactic antimalarial and hydroxyurea divided by the number of events while on either drug alone.

Collection of qualitative data

We will pilot-test the semi-structured interview guides to improve clarity. FGD and KIIs will occur in a private, quiet, safe, and convenient room at a study facility or other community location. We may consider having some of the KIIs via Zoom or telephone based on the location and availability of the healthcare worker. FGDs and KIIs will last approximately 40–60 min and be audio recorded and transcribed verbatim. A trained moderator with expertise in qualitative research will lead the FGD. A trained note-taker will record content and non-verbal communication. Discussions will be in English/ French/ lingua franca (e.g., Pidgin, Swahili) or one of the local languages as appropriate. Where needed, an interpreter may be used to provide additional support.

The questions on acceptability of MCP for persons with SCD will be based on a theoretical framework on acceptability of health interventions comprising affective attitude, burden, ethicality, intervention coherence, opportunity costs, perceived effectiveness, and self-efficacy.

Retention plan

To retain the study participants, we will communicate the goals and expected benefits of participation, including the opportunity to recommend the IPT strategy to SCD patients to the Ministry of Health to reduce malaria-related morbidity and mortality. We will ensure each parent/guardian/participant understands the expected duration and extent of their involvement in

the study from the beginning. Also, the importance of adherence to IPT will be communicated to the child and parent/guardian whenever they attend the clinic, and participants will be compensated for their time. We anticipate that incorporating the study procedures into the routine sickle cell clinics will not overload the healthcare system and will facilitate easy monitoring of our study participants. Hence, whenever the participants are scheduled to come to the sickle cell clinic, sufficient SPAQ doses will be provided during the intervention period to cover three months. Each participant will be expected to make 8 visits during the years of participation, with additional ad-hoc visits to the clinic or hospital in case of ill health. We will use the patients' contact information in the REDCap database to remind attendance to scheduled visits.

Statistical design and power

Continuous variables will be tested for normality and, where appropriate, transformation will be done, e.g using log. Continuous variables such as age will be stratified into different age groups 0-4, 5-9 and 10-17 years. The age stratification has been selected based on the high risk of malaria infection in children under 5 years.

The primary analysis will be for the time to the first malaria episode, determined by the Cox regression model, while the secondary analysis will involve multiple events within individuals through the entire follow-up period. The latter will be analyzed using the negative binomial model. The incidence of malaria infection will be compared pre- and post-intervention. Secondary outcomes will include malaria-related frequency of complications and adverse drug events, changes in hematological parameters from baseline and dose reduction or stopping of hydroxyurea medication. A modified intention-to-treat approach to all analyses will be used, including all study participants who completed the protocol and those lost to follow-up. Kaplan-Meier curves will be used to compare the time to first malaria case pre- and post-intervention. Regression models will be used to determine the associations between the outcome variables with independent variables such as participants' age and gender. A two-tailed p-value of < 0.05 will be considered significant. Data from the FGDs and key informant interviews will be through thematic analysis (21). Immediately after each FGD and KI discussion, the moderator and research assistant will highlight the main emerging themes of the discussion and any pertinent information. All audio recordings will be transcribed verbatim, and the transcripts and field notes will be imported into NVivo 12 for thematic analysis.

The thematic analysis will adopt a hybrid (inductive and deductive coding) approach (22). Two transcripts will be coded (deductively) using a pre-defined coding framework developed from existing literature and the interview guide oriented by the theoretical framework. We will also look out for themes emerging from the data (inductive) that are not covered in the framework. This first round of coding will be done separately by two qualitative researchers. The transcript codings will be compared and discrepancies resolved with a third reviewer. Following this first round of coding, we will finalise the coding framework which would then be applied to the rest of the FGDs and KI transcripts. Once coding is complete, we will use the framework method of Gale et al (23) to analyse data by different dimensions and commonalities of themes, patterns and linkages, and by participant characteristics, including comparing facilitators and barriers across the CFIR domains. Based on the outcome of the data analysis, a selection of implementation strategies will be identified.

Qualitative analysis will occur independently and then will be integrated via joint analysis (24) and mapped onto different themes or illustrative stakeholder quotes. The advantage of this approach is that a joint analysis will contextualize barriers, facilitators, and acceptability of monthly malaria MCP/HU combination in children living with SCD. Put together, the joint analysis will provide a synergistically rich and broad understanding that would not have been possible with quantitative or qualitative designs alone.

Interim analysis

An interim analysis will be performed at least six months after enrolment of the first participant and at least 80% of the required sample size is enrolled into the first round of monitoring under the standard of care. This analysis will estimate the incidence of clinical malaria episodes and asymptomatic parasitemia at each site with the main aim of re-evaluating statistical power. After the interim analysis, the study statisticians can recommend increasing or decreasing enrollment.

Ethics and Dissemination

Declaration of Helsinki

The investigators will ensure that this study is conducted in compliance with the current revision of the Declaration of Helsinki.

ICH guidelines for GCP

The investigators will ensure that this study is conducted according to the participating countries National Regulations and that it will follow the principles of the ICH Guidelines for good clinical practice (GCP).

Approvals

The site-specific Institutional and National Research Ethics Committee will review and approve the protocol, the informed consent document, patient recruitment brochures, and any subsequent modifications. Permission will be requested to conduct the study at each site.

Confidentiality

To maintain subject confidentiality, all laboratory specimens, evaluation forms, reports, and other records will be identified by a coded number. Only research personnel will have access to participant records. The site investigators and staff can share clinical information for patient care reasons, e.g., local consultations.

Risks

This study will use SPAQ which has been studied for malaria chemoprophylaxis and for treatment and its toxicities are well described. In general, they are all well tolerated. In the event of any serious or severe ADEs, participants will be treated at the site or referred to the local referral hospital where best available care will be provided. The risk of participation is minimal and limited to the pain and bruising that may happen during the sample collection. We shall use experienced phlebotomists to draw blood and aseptic measures to minimize the risk of infections.

Possible SPAQ adverse events

- Blood dyscrasias (agranulocytosis, aplastic anaemia, thrombocytopenia),
- Allergic reactions (erythema multiforme and other dermatological conditions),
- Gastrointestinal reactions (glossitis, stomatitis, nausea, emesis, abdominal pain, hepatitis, diarrhea),
- Central nervous system reactions (headache, peripheral neuritis, convulsions, ataxia, hallucinations),
- Respiratory reactions (pulmonary infiltrates), and

- Miscellaneous reactions (fever, chills, nephrosis)
- Cutaneous reactions (rashes, itching, Steven Johnsons syndrome)

Benefits

There is no direct benefit to the patient, but the knowledge gained from this study will be of greater benefit to all SCD patients as it will inform our understanding of the effectiveness of MCP in SCD patients. The proposed study is pertinent for proper interventions on MCP among patients with SCD who are most vulnerable to malaria compliations.

Oversight and monitoring

Data and safety monitoring plan

A data and safety monitoring board (DSMB) will be constituted at the beginning of the study. It will constitute 8 independent members, with a representative from each site, the Sickle Africa Data Coordinating Center (SADaCC), and the SPARCo Clinical Coordinating Center (CCC). The DSMB will meet every 6 months via one of the online platforms. The Board will review the data and guide study conduct and continuance. The DSMB will be responsible for ensuring that study participants are not exposed to undue risk and hence will recommend for study or trial suspension or termination early if serious adverse events (SAEs) appear to differ between the pre and post-intervention period.

Stopping rules will be created for adverse events and effectiveness. All SAEs across the seven sites will be compiled and reviewed by the DSMB. The DSMB will have access to the details of the SAEs and will discuss them during the 6-monthly meetings. Rates of SAEs will be compared between the pre-and post-intervention periods by an independent statistician. Suppose the SAE rate post-intervention is outside the 95% confidence limits of the pre-intervention rate, indicating a strong chance that the two phases have different SAE rates. In that case, the study statistician will bring the issue of early stopping for safety reasons to the DSMB. Based on SAEs, the DSMB will then decide whether to recommend stopping the trial early.

Similarly, stopping rules for efficacy will be based on whether the interim analysis provides strong evidence in support of the efficacy of MCP on the incidence of malaria episodes. The

DSMB will then decide whether to recommend that the study be stopped early based on evidence of efficacy.

Study limitation and mitigation

Bias

The design used in the study will involve the evaluation of the effectiveness of interventions by comparing outcomes in year 1 (no intervention) to that of year 2 (intervention). External factors such as climatic and environmental factors may affect malaria transmission dynamics, which can affect outcome measures' evaluation. We propose recording the data from health facilities collected through the Health Management Information System (HMIS) to account for malaria transmission trends during the study period.

Study size

The study size is estimated to detect the change in malaria incidence rate before and after the intervention. Malaria incidence was derived assuming constant transmission throughout the year, and that on average, 3.2 cases per 100 will be detected in any of the four clinic visits making a total of 12 cases per 100 person-years. The sample size was estimated to meet the statistical power of 80%, assuming a modest intra-cluster correlation of 0.11, type I error of 5%, and reduced malaria incidence by 30% following the intervention. The sample size is not powered to determine the impact of HU since this will not be initiated outside the normal guidelines on HU in SCD. The study will only observe those already on HU or those who will be prescribed in case they meet the treatment guidelines of respective sites.

Dissemination plans

The study results will be published and disseminated at national and international conferences and in open access peer-reviewed scientific journals. Any data published will protect the identity of the participants. This trial will be registered in a web-based protocol registration scheme. A final report will also be submitted to the National Institute of Health (NIH) and the Ministry of Health of each participating country.

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