Tropical Medicine and International Health doi:10.1111/tmi.12056

VOLUME 18 NO 3 PP 313–327 MARCH 2013

Systematic Review

Systematic review of current and emerging strategies for reducing morbidity from malaria in sickle cell disease

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Abstract Sickle cell disease (SCD) is a chronic debilitating disorder affecting erythrocytes, which is especially prevalent throughout Sub-Saharan Africa and among individuals of African descent. Because malaria is thought to be a significant cause of morbidity and mortality in patients with SCD, malaria chemoprophylaxis is often recommended for these patients. In SCD, malaria chemoprophylaxis reduces malaria parasite count, anaemia and the need for blood transfusion, and improves clinical outcomes. However, the effectiveness of malaria chemoprophylaxis in the setting of SCD is based on a few studies conducted prior to the emergence of widespread antimalarial drug resistance. Consequently, it is uncertain what the optimal strategy for managing patients with SCD in malarious areas should be. Despite the widespread use of hydroxyurea in non-malarious regions, little is known about its effect in malaria-endemic areas or on malaria-related outcomes. On the one hand, hydroxyurea upregulates intercellular cell adhesion molecule 1 (ICAM-1), the cell surface receptor for adhesion of *Plasmodium falciparum*-infected erythrocytes, and theoretically, it could enhance parasite replication. On the other hand, hydroxyurea increases levels of foetal haemoglobin, which is protective against malaria. We explore what is currently known about the interactions between SCD and malaria and review the published literature on the efficacy of malaria chemoprophylaxis in SCD. We also consider alternative strategies, including hydroxyurea, in the reduction of malaria-associated morbidity and mortality in patients with SCD.

keywords malaria, sickle cell disease, malaria chemoprophylaxis, *Plasmodium falciparum*, Sub-Saharan Africa, hydroxyurea

Introduction

Sickle cell disease (SCD), an autosomal recessive disease, is one of the most common genetic disorders in the world. In its heterozygous form (sickle cell trait), the haemoglobin S gene provides substantial protection against malaria. In its homozygous form, SCD leads to substantial morbidity and eventually death, typically within the first 2–3 decades of life. The gene responsible for haemoglobin S is common among peoples living in malaria-endemic regions of Africa (Serjeant 1989; Angastiniotis & Modell 1998; Modell & Darlison 2008; Piel *et al.* 2010) and appears to have emerged due to evolutionary pressure exerted principally by *Plasmodium falciparum*. Accordingly, the largest public health burden is in Sub-Saharan Africa where more than 200 000 children are born with the disorder each year (Angastiniotis & Modell 1998). Through a variety of pathophysiological mechanisms, malaria and SCD each potentiate the effects of the other, thus increasing morbidity and mortality and treatment cost.

Although recommended by the World Health Organization (WHO African Region 2010), there is limited evidence that malaria chemoprophylaxis is beneficial in persons with SCD. It is not known how commonly chemoprophylaxis is used or how antimalarial drug resistance affects its effectiveness. As a consequence, the optimal policy for long-term management of patients with SCD living in malarious areas remains to be identified.

In this article, we review the interactions between malaria and SCD and the evidence regarding strategies

for mitigating the synergistic interactions between both conditions. We assess the effectiveness of chemoprophylaxis against malaria among patients with SCD, summarise the current limitations of our understanding and describe several promising novel approaches for co-managing these conditions.

Methods

We conducted a systematic review using MEDLINE, PubMed, Google Scholar and Cochrane review databases for all randomised controlled trials on malaria chemoprophylaxis in SCD in malaria-endemic zones ever published. The bibliographies of publications initially found were searched for other trials. We included publications of all levels of quality because so few trials were identified and we wanted to provide as much information as possible. We also searched the same databases for trials involving the use of non-conventional methods in potentiating the effects of malaria in SCD or the effects of SCD in malaria-endemic regions. A summary of the search method with search terms and their results is given in Table 1 and Figure 1.

We categorised our findings based on levels of evidence. Where possible, we included the quality of evidence and strength of recommendation based the Infectious Diseases Society of America–US Public Health Service Grading System for ranking recommendations in clinical guidelines explained in Table 2.

Sickle cell disease

Globally, an estimated 275 000 babies are born annually with SCD. In Africa, the prevalence of the beta-globin gene mutation that determines the incidence of sickle cell disorders is about 15% (Angastiniotis & Modell 1998). However, the prevalence varies widely with the highest rates in equatorial Africa, approaching 20% of the population, while the prevalence is <1% in North Africa. Outside of Africa, SCD and sickle cell trait follow the worldwide diaspora of African populations (Serjeant 1989; Piel *et al.* 2010). For example, in the United States, approximately 90 000 individuals have SCD, mainly African Americans and approximately 2.5 million are carriers of the mutant gene (Centers for Disease Control & Prevention 2011).

Sickle cell disease was the first disease conclusively linked to abnormalities in protein structure and function (Pauling *et al.* 1949). Inherited in a strict Mendelian fashion, haemoglobin S, the abnormal haemoglobin responsible for SCD, arises from a point mutation in the β globin gene, in which thymine is replaced by adenine in the 17th nucleotide and glutamic acid is replaced by valine in the resulting β globin chain (Ingram 1956; Bunn 1997; Rees *et al.* 2010). Individuals with a single mutant β chain are classified as having sickle cell trait; homozygotes are classified as having SCD.

Under conditions of low oxygen tension, Haemoglobin S (HbS) undergoes a sol–gel transformation and polymerises and distorts the red blood cell (RBC), causing it to be spiculated at the edges, a phenomenon known as sickling. Deformability of the erythrocytes is reduced, so is their ability to pass through the microcirculation. Sickling of numerous erythrocytes leads to vascular blockage and tissue ischaemia (White & Heagan 1970; Alexy *et al.* 2010). Depending on the tissue involved, this may manifest as a vaso-occlusive crisis of the bones, lungs, central nervous system or other tissues. Secondary sequelae include heightened susceptibility to bacterial infections due to repeated infarction and involution of the spleen (Johnston *et al.* 1975).

Sickle cell disease shows marked phenotypic variability, which has been largely ascribed to two major genetic factors – foetal haemoglobin concentrations and co-inheritance of α-thalassaemia (Steinberg 2005; Sebastiani *et al.* 2010; Thein 2011). While SCD and α-thalassaemia lead to anaemia via different mechanisms, paradoxically, co-inheritance of α-thalassaemia with SCD, which occurs in about 30% of patients with sickle cell anaemia, attenuates the phenotypic presentation of SCD itself. This happens because the abnormal haemoglobin gene created by the thalassaemia reduces the concentrations of HbS in each erythrocyte and in so doing inhibits HbS polymerisation (Steinberg 2005). This observation was in fact one of the key clues to devising strategies for treating individuals with SCD. For example, enhanced or persistent

Table 1 Systematic review search methodology

Sources	PubMed/MEDLINE, Web of Science, Cochrane Library of clinical trials
Search terms	Combinations of 'sickle cell disease (SCD)' and 'malaria', 'malaria parasite', 'chemoprophylaxis', 'prevention', '*Plasmodium falciparum*', 'antimalarial'
Limits	Studies conducted in humans, published in English, with access to full texts or abstracts
Eligibility	Clinical trials with one or more interventions involving malaria chemotherapeutic drugs or placebo
Outcomes	Malaria or SCD-related events
Inclusion criteria	All Studies regardless of methodological quality

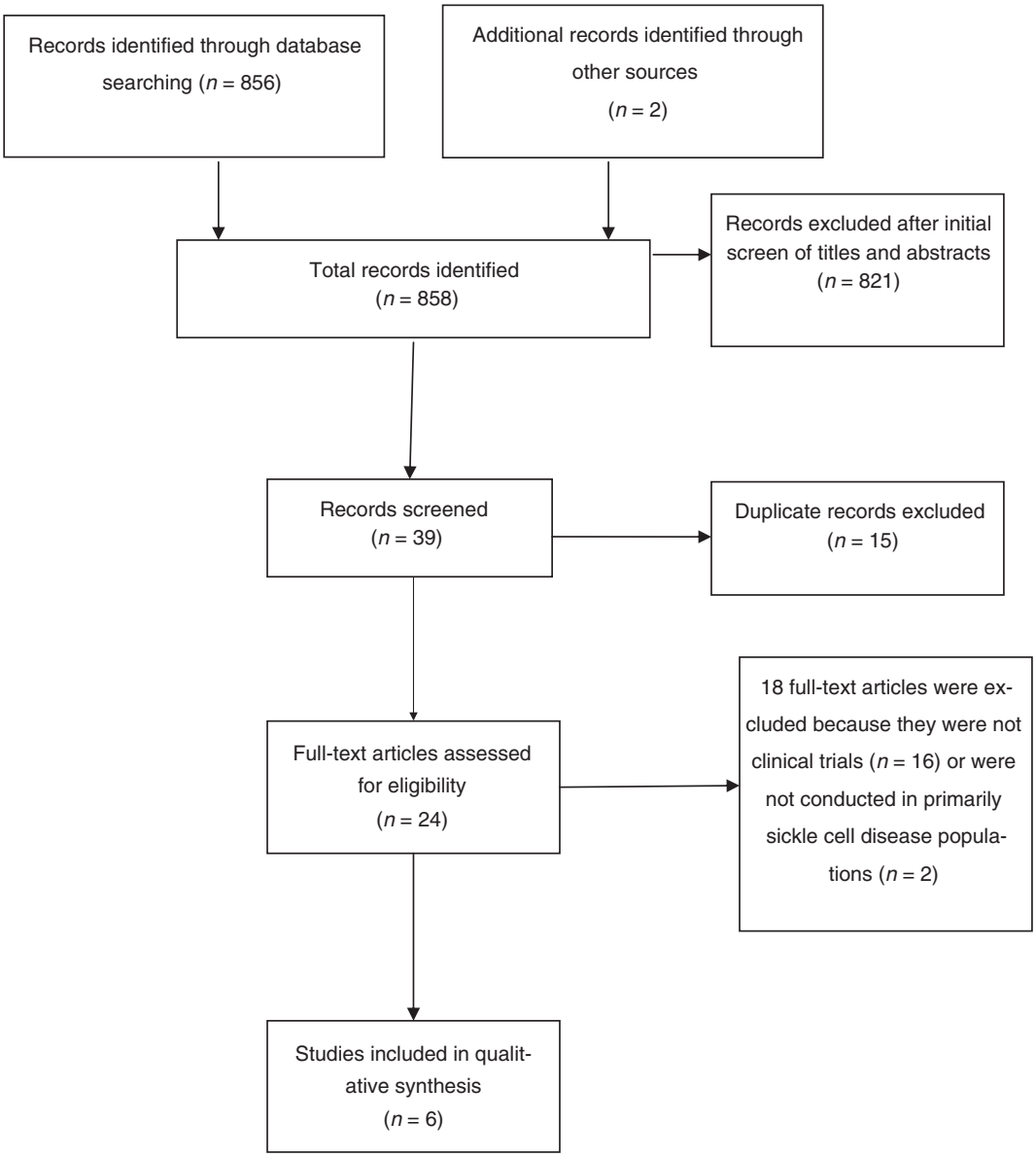

Figure 1 Systematic review flow diagram.

expression of foetal haemoglobin is now known to attenuate the severity of SCD – and explains why SCD does not manifest clinically in young infants (Stuart & Nagel 2004). Several therapeutic agents targeted at increasing the levels of foetal haemoglobin have been explored, the most widely used is the cytotoxic agent hydroxyurea (Trompeter & Roberts 2009). Hydroxyurea inhibits ribonucleotide reductase, the enzyme that catalyses the conversion of ribonucleotides to deoxyribonucleotides. This reduces the production of RBCs containing high HbS levels that arise from rapidly dividing progenitor cells, while favouring cells with high Haemoglobin F (HbF) levels (F cells) that arise from slow-dividing progenitor cells (Brun *et al.* 2003; Platt 2008). Clinically, hydroxyurea reduces haemolysis and the number of irreversibly sickled cells that are removed from circulation by the spleen. Additionally, hydroxyurea is metabolised to nitric oxide, which causes vessel dilation, reducing vascular blockage and improving tissue perfusion (Brun *et al.* 2003; Platt 2008). Hydroxyurea has been used successfully to reduce

Table 2 Infectious Diseases Society of America–US Public Health Service Grading System for ranking recommendations in clinical guidelines

Strength of recommendation
A. Good evidence to support a recommendation for use; should always be offered
B. Moderate evidence to support a recommendation for use; should generally be offered
C. Poor evidence to support a recommendation; optional
D. Moderate evidence to support a recommendation against use; should generally not be offered
E. Good evidence to support a recommendation against use; should never be offered
Quality of evidence
I. Evidence from ≥ 1 properly randomised, controlled trial
II. Evidence from ≥ 1 well-designed clinical trial, without randomisation; from cohort or case controlled analytic studies (preferably from 11 centres); from multiple time-series; or from dramatic results from uncontrolled experiments
III. Evidence from opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees

adverse events in SCD, and studies conducted in the United States suggest that it reduces morbidity and prolongs life (Dover & Charache 1989; Steinberg *et al.* 2003; Zimmerman *et al.* 2004).

The interaction between malaria and sickle cell disease

Malaria is the most common arthropod-borne infectious disease in the world. The WHO estimates there were 216 million malaria cases and 655 000 related deaths in 2010, 91% of which were in Africa. Most cases were due to *P. falciparum* and occurred in children under the age of five (World Health Organization 2011). Other mortality estimates for malaria in 2010 are as high as 1 238 000 with most deaths occurring in children under 5 in the WHO West African region (Murray *et al.* 2012). In contrast with *Plasmodium vivax* and *Plasmodium ovale*, *P. falciparum* is able to infect erythrocytes at multiple stages of development, a feature that may result in severe anaemia and other complications. Microocclusion of the vascular beds by infected erythrocytes causes ischaemia and direct tissue injury, manifesting as renal failure, cerebral malaria, pulmonary insufficiency and massive splenomegaly.

Malaria is a major environmental determinant of morbidity and mortality in SCD in most parts of Sub-Saharan Africa (Konotey-Ahulu 1971; Fleming 1989). It appears that malaria and the haemoglobin S gene are intimately connected being that they have similar geographic distributions (Serjeant 1989; Piel *et al.* 2010), and in the heterozygous state, the sickle cell gene confers substantial protection against clinical malaria (Ayi *et al.* 2004). Sickle cell trait is estimated to reduce malaria admission rates by 70% and is 90% protective against severe and complicated malaria (Williams *et al.* 2005). Similarly, sickle cell trait reduces severe malarial anaemia by 60% (Aidoo *et al.* 2002). Some of the suggested mechanisms by which the sickle cell gene protects against malaria include increased sickling and early senescence of phagocytosed erythrocytes, reduced parasite invasion and retarded development of *P. falciparum* in HbS erythrocytes at reduced oxygen tension, and the development of antibodies to the band 3 protein (Ayi *et al.* 2004; Kennedy 2010).

Somewhat paradoxically, the homozygous SCD state is associated with increased susceptibility to malaria (Uzoegwu & Onwurah 2003). Several laboratory and epidemiological studies have demonstrated that malaria plays a causal role in bacteraemia, particularly those due to Gram-negative enteric bacteria, including *Escherichia coli* and non-typhoidal salmonellae (Bronzan *et al.* 2007; Mackenzie *et al.* 2010; Were *et al.* 2011; Cunnington *et al.* 2012). This observation is hypothesised to result from sequestration of *P. falciparum* in the intestinal microvasculature, allowing for translocation of intestinal bacteria (Scott *et al.* 2011). Individuals with sickle cell trait have reduced incidence of bacteraemia in settings where malaria transmission is high, a benefit that is lost when leaving malaria zones. As with the risk of malaria, bacteraemia incidence was significantly higher among homozygotes (Scott *et al.* 2011).

The spleen plays an important role in the immunity that develops after repeated bouts of malaria episodes (Engwerda *et al.* 2005), and it is the central area of malaria parasite clearance. Parasite clearance is presumably prolonged in persons with impaired splenic function, and the hyposplenism that accompanies sickle cell anaemia may in part explain the reduced protection against malaria (Chotivanich *et al.* 2002; Engwerda *et al.* 2005; Buffet *et al.* 2011).

Several studies that compared individuals with surgical splenectomy to those with functioning spleens have demonstrated significantly more malaria parasitaemia in those with splenectomy (Boone & Watters 1995; Bach *et al.* 2005).

In the setting of SCD, malaria further reduces tissue perfusion by causing RBCs to adhere to the linings of

small blood vessels with resulting vaso-occlusion and by promoting RBC destruction and decrease in tissue oxygen levels. The reduced oxygen tension from hypoperfusion further promotes sickling with consequent worsening of vaso-occlusion and increased erythrocyte destruction. As both malaria and SCD exacerbate each other, a vicious cycle of pathogenesis is established, as summarised in the model proposed in Figure 2. The organ most directly affected by these malignant synergisms is the spleen, which is adversely affected by both conditions. As a consequence of reduced splenic function, patients with SCD have reduced immunity and are prone to various infections including encapsulated pathogens and malaria, thus creating a cycle of splenic damage, increased susceptibility to infections and malaria, and repeated bouts of sickle cell crisis.

Several observational studies provide support for this pathophysiological model. In a case–control study conducted in Kenya from 2001 to 2004, the risk of complicated and uncomplicated malaria was the same in patients with sickle cell anaemia compared with non-SCD patients, but those with SCD who developed clinical malaria had a higher risk of severe anaemia and of mortality from malaria (McAuley *et al.* 2010). In Tanzania, Makani *et al.* (2010) documented that patients with SCD were less likely to develop malaria than controls (non-SCD patients); however, patients with SCD who were hospitalised and had malaria parasitaemia had a much higher risk of death during that hospitalisation than hospitalised controls with malaria parasitaemia (OR = 4.9 95% CI 1.04–23.20; $P = 0.04$).

Strategies for mitigating the malignant synergism between sickle cell disease and malaria

Clinical evidence available in target group (persons with sickle cell disease living in malaria-endemic regions). Malaria chemoprophylaxis: In a bid to reduce the burden of malaria in patients with SCD, routine malaria chemoprophylaxis is recommended by physicians, ministries of health and by the World Health Organization for certain high-risk groups such as pregnancy and children under the age of 5 years (Konotey-Ahulu 1971; Onwubalili 1983; Kotila *et al.* 2007; WHO African Region 2010). However, the use of these drugs in accordance with local recommendations is unclear. Furthermore, several studies have suggested that persons with SCD may be protected from malaria in degrees similar to that established for those with HbAS bringing to question the relevance of antimalaria chemoprophylaxis (Komba *et al.* 2009; Makani *et al.* 2010). In addition, recent reports show widespread resistance to chloroquine and sulphadoxine–pyrimethamine (SP), two cost-effective drugs widely employed in malaria chemoprophylaxis for persons with SCD.

Based on the reasons above and the obvious gaps in knowledge concerning the role of chemoprophylaxis in SCD, we examined the role of malaria chemoprophylaxis in SCD, in the era of antimicrobial resistance and incongruous data on malaria in SCD.

We identified a total of six published randomised trials spanning a timeline of 45 years (1962–2008) all of which were conducted in eastern and western Sub-Saharan Africa (Table 3). The drugs involved were chloroquine, SP, proguanil, pyrimethamine and mefloquine.

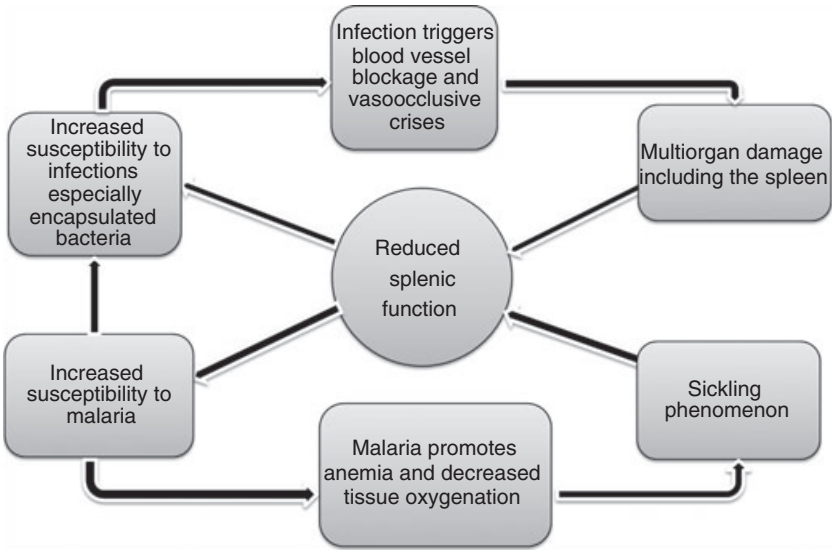

Figure 2 Suggested role of malaria in attenuating the morbidity from sickle cell disease.

Table 3 Randomized controlled trials of malaria prophylaxis published from 1962 to 2009

First author	Date and duration	Location	Sample size	Study design	Results	Limitations and generalisability
Lewthwaite C.J.	1962;Follow-up period aggregate of 1 year	Kampala, Uganda	24 children	Assigned to receive S/C CQ 5 mg/kg and ultra-long-acting BPG 1.2 MU I.M or 0.5 ml sterile water S/C	In the CQ+BPG group there was 1 death; no crises recorded 4 crises episodes in 4 patients in control group, 1 death. Blood transfusion and hospital admission were required in all 4 patients with crises. No significant difference in the mean Hb and WBC counts between groups MP positive 12× in CQ+BPG group, 13× in control group	Old study. Methods of randomisation unknown. Success at blinding uncertain as placebo was one (1) drug while treatment was with two (2). Small sample size, loss to follow-up of at least 11 (45.8%). No effect measures Only preliminary results available
Warley M.A.	August 1962–May 1965.	Kampala, Uganda	126 patients 75% were under 6 years largely of the same tribe	Prophylactic group given 1.2 MU long-acting BPG and 2 tablets of 200 mg CQ monthly with 7-day spacing Placebo received 0.5 ml sterile water S/C	Malaria: 7 episodes in the treatment group, 21 episodes in the control group (no *P*-value reported) Dactylitis: 1.8 attacks in treatment group, 5.2 attacks among control ($P < 0.1$). Mean Hb values: Significantly higher in the treatment group ($P < 0.02$)	Old study done with CQ. Combined two interventions therefore measured effects difficult to attribute to either therapy alone. Diagnostic criteria for malaria are not defined. Study is at best single blinded
Nwokolo C	1997 Six months	Multicentre, Nigeria	113 participants above the age of 5 years	Treatment groups were randomly assigned to weekly mefloquine or daily proguanil	Similar in malaria prevention efficacy, tolerability profile, and occurrence of adverse events. ($P > 0.05$) Greater reduction in ALT levels in the mefloquine group at the end of the study (*P*-value not reported)	Non-blinded

Table 3 (*continued*)

First author	Date and duration	Location	Sample size	Study design	Results	Limitations and generalisability
Eke F.U	Published 2003 Follow-up for 9 months	Port-Harcourt, Nigeria	101 patients aged between 1 and 16 years 97 of 101 completed the study	Randomly assigned into three groups Given either 0.5 mg/kg/week of pyrimethamine, 1.5 mg/kg/day of proguanil or placebo (vit. c) Patients were followed up at 2-week intervals for 3 months then monthly for the rest of the study	No significant difference in the prevalence of malaria parasite across the three groups Significant reduction in the mean parasite density in the proguanil compared with the pyrimethamine group ($P = 0.045$) Pyrimethamine group had significantly less parasite density than placebo. ($P < 0.05$). Sickle-related events: no significant difference. Splenomegaly: more frequent in those on proguanil or placebo. ($P < 0.05$) Blood transfusions: more in patients on placebo compared with the other 2 groups. ($P < 0.05$) Hospitalisations: More in the placebo group compared with the other 2 groups, although not statistically different across groups	Study underpowered Effect of malaria prophylaxis may be less pronounced in areas with less endemicity

Table 3 (*continued*)

First author	Date and duration	Location	Sample size	Study design	Results	Limitations and generalisability
Nakibuuka V.	October 2006–February 2007. 5 months	Kampala, Uganda	242 children between the ages of 6 months and 12 years	Children were randomised to receive either sulphadoxine–pyrimethamine (SP) (25/5 mg/kg) or CQ (5 mg/kg) monthly with Weekly follow-up for a month15 children were lost to follow-up (7-SP, 8-CQ)	14% of children in SP arm developed malaria compared with 26% in the CQ arm (OR 1.98, 95 CI 1.023–3.82). Higher proportion of malaria-related admissions in the CQ arm compared with the SP arm (5.7% *vs.* 2.5% OR 2.4 $P = 0.223$) No statistically significant difference in the all-cause admissions between both groups	Significantly more children on CQ were using bed nets as such observed results may be an underestimate
Diop S.	September 2007 to February 2008 Six months Follow-up	Dakar, Senegal	60 participants with a mean age of 24 years	Randomly assigned to receive IPT with SP (25 mg/kg/1.25 mg/kg) or placebo monthly	No statistically significant reduction in number of VOCs or hospitalisations 46% reduction in number of complaints ($P = 0.002$) 75% reduction in number of patients transfused ($P = 0.001$)	Short follow-up period Small sample size. Peak malaria transmission is from July to October but study included only two of these months

ALT, alanine transaminase; BPG, benzathine penicillin G; CQ, chloroquine; Hb, haemoglobin; MP, malaria parasite MU mega units; S/C, subcutaneous; S/P, sulphadoxine/pyrimethamine; VOC, vaso-occlusive crises.

Of particular relevance were the four studies that assessed the overall efficacy of malaria prophylaxis in the setting of SCD compared with no prophylaxis. Unfortunately, all of these suffered in varying degrees from insufficient sample size and/or duration of follow-up. The earliest study we located, conducted prior to the emergence of widespread antimalarial resistance, assessed the effect of chloroquine prophylaxis on Ugandan children with SCD (Lewthwaite 1962). While the sample size was small, leaving the study seriously underpowered (24 subjects), there was a trend towards lower rates of death and sickle cell crises among those who received prophylaxis *vs*. the control group (RR 0.24, 95% CI 0.03–1.7). In a similar study assessing the combined effect of benzathine penicillin and chloroquine, Warley *et al.* (1965) found significantly reduced rates of malaria parasitaemia and anaemia, as well as reduced incidence of dactylitis, one of the more common manifestations of sickle cell crises: 1.8 episodes per intervention child *vs*. 5.2 attacks in the control children. More recently, Diop *et al.* (2011) in Senegal have demonstrated a reduction in the need for blood transfusion in patients with SCD on SP prophylaxis compared with those who received placebo; no differences were seen in rates of vaso-occlusive crises, although their ability to detect these endpoints may have been limited by the sample size of sixty subjects followed for at most 4 months. These results are similar to the findings of Eke and Anochie who, in 2003, demonstrated that daily proguanil or weekly pyrimethamine, when compared with placebo, was effective in reducing malaria parasite density and the need for blood transfusion in patients with SCD. Collectively, these studies indicate that malaria chemoprophylaxis offers some protection against malaria in SCD and reduction in blood transfusion needs. However, the effects of prophylaxis on sickle cell endpoints are less clear. While several studies suggested a trend towards lower rates, none appeared to have had sufficient statistical power to reach any solid conclusions.

Two additional studies compared the effectiveness of different regimens of malaria chemoprophylaxis in patients with SCD (Table 3). The first demonstrated that SP was better than chloroquine at reducing malaria events in patients with SCD (Nakibuuka *et al.* 2009) This may have been due to the high rates of resistance to chloroquine compared with SP in the malaria parasites within the regions the study was conducted. Eke and Anochie noted that in persons with SCD, daily proguanil was more effective at reducing malaria parasite density than weekly pyrimethamine, although there were no significant differences in clinical outcomes between the two drugs. The study by Nwokolo *et al.* demonstrated similar clinical effectiveness and tolerance between mefloquine and proguanil, although significant biochemical profile differences were recorded between the two drugs.

In summary, based on guidelines shown in Table 2, malaria chemoprophylaxis appears to offer reduction in the following

- Anaemia: II/A.
- Clinical Malaria: II/B.
- Malaria Parasite Levels/Parasite Density: II/B.
- Sickle-Related Events: II/C.
- Malaria-Related Hospitalisations: II/C.

No clinical evidence available in target group but laboratory evidence suggests benefit. Hydroxyurea, the only drug that has been demonstrated to have long-term beneficial effects in SCD such as reducing hospital admissions, protecting against sickling-related events, increasing the quality of life and improving longevity, has no documented studies on the clinical effects of the drug on malaria or in malarious regions. However, experimental models suggest that hydroxyurea, like other ribonucleotide reductase inhibitors, possesses antimalarial properties that may be significant at the doses it is given in SCD (Holland *et al.* 1998; Pino *et al.* 2006). One of the major concerns about hydroxyurea use in malaria endemic-zones stems from its ability to upregulate intercellular adhesion molecule 1 (ICAM-1) expression in endothelial cells. As ICAM-1 is a cell surface receptor for the adhesion of erythrocytes infected with *P. falciparum*, it is theoretically possible that this could lead to enhanced replication in erythrocytes (Brun *et al.* 2003; Pino *et al.* 2006). It is somewhat reassuring that in contrast to this hypothetical concern, hydroxyurea-treated mice had significantly reduced levels of malaria parasitaemia and reduced mortality from malaria (Pino *et al.* 2006).

Haemoglobin F reduces malaria parasite proliferation in the human erythrocyte at degrees similar to that for HbS (Pasvol *et al.* 1976 Pasvol *et al.* 1977; Wilson *et al.* 1977; Shear *et al.* 1998). As persons with SCD on hydroxyurea have elevated HbF levels, the drug may offer these individuals substantial protection from malaria.

Several other studies have demonstrated that hydroxyurea therapy in SCD is associated with preservation of splenic function and retardation/improvement of splenic dysfunction (Claster & Vichinsky 1996; Santos *et al.* 2002; Hankins *et al.* 2005, 2008). This improvement in splenic function by hydroxyurea may be useful in improving immunity against malaria in persons with SCD residing in malaria-endemic regions. Details of these studies can be found in Table 4.

Table 4 Summary of studies in support of added benefit from HU in the setting of malaria

Mechanism	Author, date	Type of study	Summary of findings	Quality of evidence/ Strength of recommendation
Haemoglobin F (HbF) protects against Malaria	Pasvol *et al.* (1976)	Laboratory Experiment, Human Samples (in vivo)	*Plasmodium falciparum* distribution and growth was compared in erythrocytes containing either adult or foetal haemoglobin in vitro. There was a significant retardation of parasite growth in vitro in cells containing HbF	No clinical studies
	Pasvol *et al.* (1977)	Laboratory Experiment, Human Samples (in vivo)	Compared the rates of invasion and growth of *P. falciparum* in red blood cells in those containing HbA and those with HbF in an in vitro culture. There was significant retardation of parasite growth in HbF cells compared with HbA cells	
	Wilson *et al.* (1977)	Laboratory Experiment, Human Samples (in vivo)	Comparing *P. falciparum* parasitisation in cord blood (high in HbF) to that in adult blood (high in HbA) Rate of parasitisation was faster in cord blood than in adult blood Parasite growth was significantly retarded in cord blood compared with the adult blood	
	Shear *et al.* (1998)	Laboratory Experiment Transgenic mice (in vivo)	Rates of parasitisation and growth of 2 strains of rodent malaria were compared. Transgenic (γ) mice expressing 40% to 60% $\alpha_2{}^{M}\gamma_2$ haemoglobin (foetal haemoglobin) and normal mice infected with rodent malaria. When mice were infected with *Plasmodium chabaudi adami,* rates of parasitisation and parasite clearance were higher in the transgenic mice compared with their controls. When mice were infected with *Plasmodium yoelii* 17XNL (lethal form of malaria), parasitisation rates were similar; parasite clearance was quicker and complete in transgenic mice (HbF) compared with control mice. The faster rates of parasite clearance were also demonstrated in splenectomised transgenic mice compared with splenectomised control mice	

Table 4 (*continued*)

Mechanism	Author, date	Type of study	Summary of findings	Quality of evidence/ Strength of recommendation
HU possesses intrinsic antimalaria properties	Holland *et al.* (1998)	Laboratory studies	Hydroxyurea along with other RNR inhibitors was tested in an in vitro culture system for potential antimalarial activities. Hydroxyurea inhibited the growth of malaria parasite, although high concentrations were needed to achieve 50% inhibition of parasite (IC50)	No clinical studies
	Pino *et al.* (2006)	Laboratory studies	The effects of hydroxyurea on rodent malaria in mice (*Plasmodium berghei*) High dose HU (200 mg/kg/day) was administered to some mice. Others weren't treated with HU. Parasitaemia was significantly lower in treated mice compared with the untreated mice	
HU improves splenic function	Claster and Vichinsky (1996)	Case series	Report of 2 SS patients with reversal of splenic dysfunction after 30 months and 24 months. In both patients peak HbF levels ranged from 25% to 30%	II/B
	Santos *et al.* (2002)	Prospective Cohort	Twenty-one patients aged 3–22 years; 14 SS, 7 Sbeta (0) underwent splenic scintigraphy prior to HU initiation and after 6 and 12 months of treatment. At baseline, 9 SS and 1 Sbeta (0) were functionally asplenic, 5 SS and 6 Sbeta (0) patients had impaired splenic function. One year after treatment, splenic function improved in 10 patients, was unchanged in 8 patients and worsened in 3	
	Hankins *et al.* (2005)	Prospective study/ clinical trial extension	Twenty-one children originally involved in a pilot trial of hydroxyurea (HUSOFT) were offered continued therapy for a mean duration of about 5 years. After 4 years of hydroxyurea herapy, only 6 (43%) patients were functionally asplenic (absent radionucleotide uptake) upon study completion, in contrast to the expected 94% incidence of asplenia among untreated age-matched children with SCA based on red cell pit counts in the CSSCD ($P < 0.001$). Two infants with markedly diminished splenic function regained normal splenic uptake after 4 years of HU therapy.	

Table 4 (*continued*)

Mechanism	Author, date	Type of study	Summary of findings	Quality of evidence/ Strength of recommendation
	Hankins *et al.* (2008)	Retrospective cohort	Forty-three children who had radionuclide testing of the spleen before and during HU therapy were retrospectively assessed. Median follow-up period at maximum tolerated dose was 2.6 years. At baseline, 93% were asplenic or had markedly reduced splenic function. At follow-up, 14% (6) completely recovered splenic function, and 5% (2) had preserved splenic function. The Hb concentration in these eight children on hydroxyurea therapy was significantly higher than those without improved splenic function (9.1 *vs.* 8.6 g/dl, $P = 0.01$)	

Although we found no documented clinical studies on hydroxyurea in Sub-Saharan Africa, a prospective study of 47 Tunisian children with SCD followed up for average duration of 52 months showed significant reduction in hospitalisation, increase in haemoglobin levels and an overall improvement in the clinical picture of the patients (Mellouli & Bejaoui 2008). While this study suggests that hydroxyurea is beneficial in North African children, it was conducted in a country that is malaria-free, and therefore, the results cannot be extrapolated to children with SCD in Sub-Saharan Africa.

Hydroxyurea has been successfully used in very young children. A recent landmark clinical trial on the use of hydroxyurea in children as young as 9 months of age showed that hydroxyurea was both safe and efficacious in reducing clinical events such as pain and dactylitis, while increasing haemoglobin and foetal haemoglobin (Wang *et al.* 2011). Despite these, the fear of toxicity, inability to cope with adverse reactions, and the high cost of purchase and monitoring the use of this antineoplastic drug in most developing countries have hindered its use.

No clinical or laboratory evidence in target group but clinical evidence in other groups suggest benefit. Promising results from malaria vaccine trials have been recently published. The RTS,S/AS01E vaccine in children aged 6–10 weeks at first vaccination had a 1-year post-third-dose vaccine efficacy (VE) ranging from 61.6%, (95% CI 35.6–77.1; $P = 0.0003$) in children randomised to 0-, 1- and 2-month dosing schedule to 63.8% (40.4–78.0; $P < 0.0001$) in children randomised to 0-, 1- and 7-month dosing schedule in a phase 2 trial conducted in Mali and 45.8% (24.1, 64.3, $P = 0.0004$) over 15 months in a trial conducted on healthy Kenyan and Tanzanian children aged 5–17 months (Asante *et al.* 2011; Olotu *et al.* 2011). By contrast, the recent FMP2.1/AS02$_A$ trial did not show significant protection against clinical malaria, although it had a strain-specific VE of 64.3% (Thera *et al.* 2011). Despite these intriguing results, it will take several years for these vaccines to come in to commercial use, and their protective efficacy will need to be assessed in individuals with SCD.

The emphasis of malaria prevention programmes has shifted from only chemoprophylaxis to multiple strategies such as the use of long-lasting insecticide-treated nets (LLINs), intermittent preventive therapy in high-risk groups like pregnant women and young children and the use of environmental control, especially indoor residual spraying (World Health Organization 2011). Although these strategies have yielded some success generally, it is yet to be determined how effective they will be in primarily SCD populations with respect to SCD-related outcomes, cost-effectiveness and sustainability.

Discussion

We found that malaria chemoprophylaxis provides some benefits in patients with SCD, although the extent of the benefits differs across trials. In particular, the efficacy studies unanimously showed that malaria prophylaxis reduced the need for blood transfusion, an indication that it reduces the risk of severe malarial anaemia. The effect

of malaria chemoprophylaxis on sickle-related events was less evident, but much of this may reflect the limitations of the trials themselves (e.g. small sample sizes, short durations of follow-up, lack of blinding and inadequate allocation).

Nevertheless, decisions on malaria chemoprophylaxis in patients with SCD remain challenging for several reasons. Based on quality, only three of the trials, Warley *et al.*, Eke and Anochie, and Nakibuuka *et al.,* are of high-quality standards with regard to methodology and standards of reporting (Moher *et al.* 2001). The trials conducted were also in areas of high malaria endemicity and may be less applicable to other regions of Sub-Saharan Africa. More importantly, much of this literature is decades old, predating the emergence of high rates of drug resistance. Chloroquine resistance has been reported since the early 1960s (Young & Moore 1961) and is now widespread across all malaria-endemic areas (Guerin *et al.* 2002). Similarly, SP treatment failure rates range from about 19% to 53% in Africa (World Health Organization 2010). Although proguanil resistance has been reported, it is not as widely spread as chloroquine and SP resistance. However, it is more expensive and requires daily use and thus is more prone to problems with adherence. This creates a major barrier to devising a unified policy regarding prophylactic antimalarials in patients with SCD, as the alternative medications are either unsuitable for use in this role by reason of pharmacokinetics (artemesinin monotherapy) or toxicity and/or expense (mefloquine, amodiaquine, halofantrine and artemether–lumefantrine). Given the continually evolving nature of malaria drug resistance, developing consensus guidelines for chemoprophylaxis of SCD in the future may be difficult without new studies of safe and effective drugs with longer half-lives.

Consequently, there is, in our view, a pressing need to consider alternative approaches to reducing malaria-associated morbidity in patients with SCD. For example, hydroxyurea appears to provide clinical benefits both for SCD and malaria. Because there is little evidence of hydroxyurea therapy in malaria-prone SCD populations, its use would need to be defined by, and policy informed by, well-conducted studies designed to assess both sickle cell- and malaria-related outcomes, efficacy, long-term effectiveness and safety. Similarly, recent advances in malaria vaccines hold great promise for patients with SCD.

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