A randomized trial to compare the safety, tolerability and effectiveness of three antimalarial regimens for the prevention of malaria in Nigerian patients with sickle-cell disease

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Abstract

Background: Malaria prophylaxis is recommended for persons with sickle-cell disease (SCD) but the value of this has been questioned. The aim of this study was to find out if intermittent preventive treatment (IPT) with a fixed-dose combination of mefloquine-artesunate (MQAS) or sulfadoxine-pyrimethamine plus amodiaquine (SPAQ) was more effective than daily proguanil for malaria prevention in subjects with SCD.

Methods: Patients with SCD were randomized to receive daily proguanil, or IPT with either MQAS or SPAQ administered once every two months at routine clinic visits, and followed up for fourteen months.

Findings: 270 SCD patients were studied, 90 in each group. Adherence to the IPT regimens was excellent but 57% of patients took less than 75% of their daily doses of proguanil. IPT was well tolerated, the most common side effects were vomiting and abdominal pain. Protective efficacy against malaria, compared to daily proguanil, was 61%(95%CI 3%-84%) for MQAS and 36%(40%-70%) for SPAQ. There were fewer outpatient illness episodes in children who received IPT than in the proguanil group.

Conclusion: IPT with MQAS administered when sickle-cell disease patients come for routine clinic visits was well tolerated and more effective in preventing malaria than daily prophylaxis with proguanil.

Introduction

Although the incidence of *Plasmodium falciparum* infection is thought to be lower in children with sickle-cell disease (SCD) than in normal children [1], the consequence of malaria in SCD can be serious. Studies in Kenya [2] and Tanzania [3] in which confounding effects of prophylaxis for sickle-cell patients could be excluded, were consistent with a reduced prevalence of malaria parasitaemia and incidence of malaria infection in patients with HbSS compared to HbAA children. However, in the Kenyan study, mortality in children with SCD hospitalized with malaria was substantially higher than that among children without SCD [3]. Malaria can aggravate severe anaemia, and like other infections, can lead to crises in children with SCD and the complications of a crisis precipitated by *P. falciparum* infection can be fatal [4,5]. Malaria prevention in SCD has, therefore, been considered to be essential in regions where malaria is endemic, but the evidence base for current drug policies, which rely mainly on daily proguanil or weekly pyrimethamine or chloroquine, is extremely weak. A Cochrane review of malaria prophylaxis for patients with SCD concluded that prophylaxis is beneficial, with fewer episodes of malaria, higher mean haemoglobin concentration, fewer transfusions, reduced hospital admissions and sickle cell crises in patients on prophylaxis [6], but the review included only two small studies, one from Kampala in 1965, which involved administration of chloroquine plus benzathine penicillin for 12 months [7] and one from Nigeria in 2003, in which 97 patients were randomized to receive daily proguanil, weekly pyrimethamine or placebo for 9 months [8]. Studies not in the review include a placebo-controlled trial of 60 patients in Senegal that showed that monthly sulfadoxine-pyrimethamine (SP) during the transmission season was well tolerated and reduced morbidity [9]; a study in Uganda, with just one month of follow-up, suggesting weekly chloroquine was less effective than monthly sulfadoxine-pyrimethamine [10]; and a study in Nigeria, indicating that weekly mefloquine was well tolerated and more effective than daily proguanil [11]. Several small surveys of sickle cell patients in Nigeria who took daily proguanil or weekly pyrimethamine prophylaxis have found that malaria parasitaemia is common, attributed to poor compliance and resistance [12,13,14]. The prevalence of dhfr and dhps mutations has been little studied but appears to be high [15].

Adherence to a daily or weekly prophylactic regimen is likely to be poor and difficult to sustain over long periods. The national antimalarial policy in Nigeria for protection of patients with SCD against malaria at the time that this trial was conducted was daily proguanil and proguanil prophylaxis continues to be used widely in Nigeria although more recent guidelines (2011) state that, as proguanil may not be effective, SCD patients should rely on long-lasting insecticide-treated bednets, and prompt treatment if they have malaria.

Chemoprevention with drugs being given under supervision by health workers, is increasingly being used to prevent malaria in endemic countries. WHO now recommends Intermittent Preventive Treatment with sulphadoxine-pyrimethamine (IPTp-SP) for prevention of malaria in pregnant women, administered at antenatal clinic visits, Intermittent Preventive Treatment with SP in infants (IPTi), given when children come for routne vaccination and, in areas of seasonal transmission, Seasonal Malaria Chemoprevention with SP plus amodiaquine (SPAQ) for children under 5 years of age delivered monthly at home by community health worker. Sickle-cell patients who are stable are recommended to visit the clinic regularly, providing an opportunity to administer chemoprevention under supervision but for this approach to be effective a long-acting antimalarial drug combination is needed.

SPAQ is effective when administered monthly but protection wanes rapidly after about four weeks [16]. Mefloquine-artesunate (MQAS), given over three days, is highly effective in treating uncomplicated malaria. A fixed dose combination of mefloquine and artesunate developed by the Drugs for Neglected Diseases Initiative (DNDi) is better tolerated with a lower incidence of vomiting than the separate-tablet regimen [17]. When the duration of protection provided by different IPTi drug regimens given at 2, 3 and 9 months of age was estimated, mefloquine gave the longest period of protection, with estimated efficacy of 73% in the first month and 73% in the second month after treatment [18].

In Nigeria, sickle cell patients who are stable are recommended to attend the clinic once every two months. The aim of this trial was, therefore, to compare the efficacy, safety and tolerability of IPT with MQAS given over three days, or SPAQ (a single dose of SP plus AQ over three days), administered under supervision at routine bimonthly (once every two months) clinic visits, with the standard regimen of daily proguanil in patients with sickle cell disease.

Methods

Study design
Study population: This study was undertaken at the sickle-cell disease clinic of the Outpatient Unit of the University of Ilorin Teaching hospital, Kwara State, Nigeria. Patients aged 6 months or more and weighing 5kg or more with a documented genotype of SS or SC, were invited to participate. Those with an acute illness or any additional chronic disease, known allergy to any of the study drugs, or who had been treated during the two weeks prior to recruitment with SP, AQ or mefloquine, were excluded. Signed consent was sought from the participants or their parents after the aims and procedures of the study had been explained.

Randomization: Eligible patients were randomized, using a list of randomly permuted blocks of nine generated using Stata 11.1, to receive bimonthly IPT with MQAS, or with SPAQ, or the standard regimen of daily proguanil, and were asked to return to the clinic once every 2 months for the next year to receive their treatment and, in the case of the proguanil group, to be given the next supply of proguanil tablets. Treatment allocations were kept inside opaque sealed envelopes and, after enrolment the patient was assigned the next envelope in numerical sequence and their name and the date written on the envelope, before it was opened to determine treatment allocation. Participants were issued identification cards bearing the patient name, study number, and phone numbers of the investigators, and patients due to come to clinic were contacted by phone the day before to remind them to attend.

Procedures at each scheduled clinic visit: Participants were asked to record any illness in a diary and, at each clinic visit, patients were asked about symptoms experienced since their last visit. They were then given a clinical examination, a venous blood sample was taken for haematological and biochemical measurements, a finger-prick sample was taken for making thick and thin blood smears for microscopy, and a blood spot taken onto filter paper for PCR analysis of the presence of *P.falciparum* and analysis of molecular markers of drug resistance. Nested PCR was used to amplify fragments at the three gene loci, which encompass mutations associated with pyrimethamine resistance (dihydrofolate reductase (*dhfr*)-51, 59, 108, 164), sulfadoxine resistance (dihydropteroate synthase (*dhps)*-436, 437, 540, 581) and chloroquine resistance (multidrug resistance protein-1 (Pfmdr-1) mdr-81 [19,20].

Drug administration: For patients assigned to receive bimonthly IPT, the first dose of the three-day regimen was administered by study staff, and the remaining doses given to the participant or their carer to administer at home on each of the next two days. For those in the proguanil group, a dose of proguanil was administered, unused tablets were counted, and a supply of tablets for the next two months was given to the participant or their carer. All patients were also given folic acid and vitamin C one tablet daily, the standard of care for sickle cell disease in Nigeria. Study participants were asked to return three days after the clinic visit to be interviewed about adverse events over the previous three days and (for those in the IPT groups) to check adherence to the home doses by tablet counts. Those who were not able to be present for interview were contacted by phone and details cross-checked at their next visit. Participants were asked to come to the hospital if they were unwell at any time, where they were managed as outpatients or admitted to the paediatric ward. If a patient had suspected malaria, a rapid diagnostic test was performed to determine treatment.

The study was open label, patients and study physicians were unblinded, but laboratory staff were unaware of treatment allocations. Laboratory methods are described in the Supplement. The primary endpoint, for which the trial was powered, was the occurrence of any adverse event, secondary endpoints included the occurrence of vomiting, adherence to the regimen, malaria incidence, and the incidence of other illnesses treated as outpatients, and as inpatients. Statistical analysis was by intention-to-treat, including all patients who were randomized. Statistical methods are described in the Supplement.

Malaria case management

Any study subject who had malaria (an axillary temperature ≥37.5°C or a history of fever or vomiting within the last 24 hours together with a positive malaria Rapid Diagnostic Test), was treated with artemether/lumefantrine over three days. If this occurred at a scheduled clinic visit, they were not given the trial medications for that visit.

Ethics approvals

Ethical approval for the study was obtained from the ethics committee of the University of Ilorin Teaching Hospital and the London School of Hygiene & Tropical Medicine. An independent Data Safety Monitoring Board provided oversight for the trial.

Results
Characteristics of the study participants at enrolment

Patients were recruited from 26th September 2011 to 12th April 2012, and follow-up for the last patients ended in April 2013. 318 patients were screened and 270 who met inclusion criteria were randomized to receive MQAS once every two months, SPAQ once every two months, or daily proguanil in a 1:1:1 ratio. Baseline characteristics were similar in the three groups (Table 1 and Supplement, Table S1). Twenty-three of 270 samples (9%) were positive for *P.falciparum* by PCR at baseline, 9 of which (39%) carried the *Pfmdr1* mdr-81) mutation N86Y, 20 (87%) the *dhfr* triple mutations (S108N, N51I, C59R) and 18 (78%) the *dhps* double mutation (A437G, K540E). Participant flow is shown in Figure 1.

Adherence

Dosages of study drugs administered are shown in Table S2. Adherence to the once-every-two-months visits was good with most patients returning promptly at approximately 8-week intervals (Supplement, Table S3). Pill counts at the clinic suggested that all patients took both home doses of MQAS or SPAQ although this could not be verified. Adherence to daily proguanil doses, also based on tablet counts at the clinic, was poor with 57% of patients

using less than 75% of daily doses. Adherence was better in children under 10 yrs than in older children (Table S4).

Adverse events

Severe illnesses requiring hospital admission were common in all treatment groups (0.6 episodes per person during the period of the study, Table 2). The most common causes for admission were vaso-occlusive crisis and septicaemia (Table S6). Admissions were more frequent among female participants than males, in all three groups. Less severe illnesses treated as outpatients, were most frequent in the proguanil group, especially among females. The protective efficacy against outpatient illness episodes (the percentage reduction in the number of episodes), compared to the proguanil group, was 23% (95%CI 0%,43%) for MQAS and 25% (0%,44%) for SPAQ (Table 2). The excess number of outpatient illness episodes in the proguanil group was mainly among females (Table 3), (interaction between gender and treatment group P=0.023).

When participants were asked about side effects on day 3 after each bimonthly visit, vomiting, body pain and abdominal pain were the most frequently reported symptoms (Table 4) and were reported more commonly by patients who received MQAS and SPAQ than by those who received proguanil. Adverse events were mostly mild in intensity (Table S7). An adverse event during the 3 days following treatment was reported by 24% (95%CI 19%,21%) of patients who received MQAS, 14% (9.6%,18%) who received SPAQ and 5.4% (3.4%,17%) who received proguanil (risk ratio MQAS:proguanil 4.5, 95%CI 3.0-6.8 and for SPAQ:Proguanil 2.6, 95%CI 1.6-4.1). A similar proportion of male and female participants reported side effects on day 3. Among participants who received MQAS, the risk of vomiting was substantially higher in patients who received mefloquine doses in excess of 35mg/kg (Figure S1).

When patients were asked about side-effects and illness symptoms recorded in their diary in the two months prior to a routine clinic visit, 18% of participants in the proguanil group reported an adverse event, compared to 13% in each of the other treatment groups (Table 4). Females who received proguanil were more likely to report side effects than males (23% versus 14%, an adverse event risk ratio of 1.6, 95%CI 1.1-2.3), with body pain and abdominal pain being the symptoms more commonly reported.

Seven participants died during the study (a mortality rate of 25 per 1000 person years). Four of these had received MQAS (a girl aged 13 years, admitted with vaso-occlusive crisis precipitated by sepsis who had a blood culture positive for methicillin-sensitive *Staphylococcus aureus*; a girl aged 6 years who died at home with a suspected cerebro-

vascular accident; a boy aged 10 years admitted with severe anaemia and sepsis; and a boy aged one year with a febrile illness who died at home). Three deaths were in the proguanil group (a girl aged 16 years with upper gastro-intestinal bleeding associated with disseminated intravascular coagulation; a girl aged 9 years with acute chest syndrome with sepsis, and a girl aged 12 years who died at home from an unknown cause). There were no deaths in the SPAQ group.

Efficacy against malaria

Thirty-eight episodes of clinical malaria were recorded (15 inpatients and 23 outpatients). Incidence was lower in the MQAS group, with a relative protective efficacy of 61% (95% CI 3%, 84%) compared to the proguanil group. Relative efficacy of SPAQ compared to proguanil was 36% (-40%, 70%) (Table 5). The prevalence of *P.falciparum* infection in samples taken from all participants at each scheduled clinic visit was also lower in the MQAS group than the proguanil group, odds ratio 0.48 (95%CI 0.22,1.0) (Table S5). Mean haemoglobin concentration 12 months after enrolment in the trial was similar in all groups (Table S8).

Discussion

Intermittent treatment with MQAS administered when sickle-cell disease patients came for routine clinic visits was well tolerated, mild adverse events, the most common of which were were vomiting and abdominal pain, did not deter patients from returning for their clinic visits. MQAS given once every two months was more effective in preventing malaria than daily prophylaxis with proguanil. MQAS reduced the prevalence of parasitaemia at routine clinic assessments and reduced all-cause illness episodes treated as outpatients, but did not reduce the burden of severe disease requiring hospital admission. Adherence to daily proguanil prophylaxis was sub-optimal, and *P.falciparum* mutations associated with antifolate resistance were common. Therefore, it is likely that proguanil prophylaxis was ineffective although the study could not include a placebo group that would have allowed this to be assessed. We did not find that treatment with SPAQ once every two months was more effective than proguanil in preventing malaria episodes or reducing prevalence at routine visits, although there was a reduction in outpatient illness episodes in patients who took SPAQ. It is likely that the gap between treatments was too long (2 months) for this regimen to give a high degree of protection.

Mefloquine can cause neurological and psychiatric side effects, and should not be used in patients with psychiatric illness or epilepsy [21]. However, these side effects have not been common when MQ has been used for treatment in Asia, Latin America and in Africa [22, 23]. Vomiting is a common mefloquine side effect and dose-related, its incidence was reduced by using a split dose over three days. Tolerability of MQ in children for treatment and prophylaxis has been reviewed by Schlagenhauf et al. [24], and in pregnant women by Gonsalez et al. [25]. In a study of MQAS for the treatment of uncomplicated *Plasmodium falciparum* malaria undertaken in Nigeria, Agomo et al. [26] reported vomiting, dizziness, headache, abdominal discomfort, weakness and visceral pain as common adverse events, consistent with the findings of our study. Despite the occurrence of minor side effects the majority of the patients in the MQ arm when asked said they would prefer to continue with the bimonthly MQAS after the end of the study rather than revert to daily administration of proguanil.

Illnesses treatd as outpatients, and reports of illness symptoms recorded in patient diaries, were more common among female than among male participants who took daily proguanil. Some other studies have reported that girls with sickle cell disease can have more frequent pain episodes than boys [27]. Gender differences in adverse events have also been reported for antimalarials used for prophylaxis [28]. The excess illness in the proguanil group may reflect adverse reactions to proguanil, or a gender-specific increase in illness episodes associated with failure of prophylaxis.

The lack of an effect against hospital admissions in the MQAS group, despite a high efficacy against malaria, is consistent with the low prevalence of malaria in the study population, there was limited scope to demonstrate an impact on all-cause admissions, it is possible a greater benefit would be seen in areas with more intense malaria transmission. In the 2010 malaria indicator survey of Nigeria, the national prevalence of malaria in children under 5 yrs in urban areas was 23%, compared to 48% in rural areas [29].

Malaria prevention in sickle cell patients using IPT with long-acting antimalarials has a number of advantages. It is recommended that stable patients should attend a clinic every two months and the IPT the strategy can take advantage of these routine visits. The first dose of the 3-day regimen can be administered by clinic staff or directly observed and adherence in taking the further two doses is likely to be higher than that of a regimen that requires daily treatment. The cost of the MQAS regimen compares favourably with that of the proguanil regimen (Supplement). Our trial was done in an area with year-round transmission. In areas of highly seasonal transmission, chemoprevention could be limited to the transmission period, as is now recommended for children under 5 yrs in the Sahel

subregion[16]. In areas where Seasonal Malaria Chemoprevention (SMC) is provided, children under 5 yrs with SCD could receive SMC as an alternative to chemoprevention at SCD clinics.

Our study is larger than previous trials of malaria prophylaxis in sickle cell patients, with follow-up over a longer period, but had a number of weaknesses. We could not monitor adherence directly, our estimates of the number of daily doses used based on pill counts at the clinic probably overestimate actual adherence, and adherence to daily doses, and return rates to clinic, may be poorer outside the context of the trial. It was not feasible to blind our study due to difference in the number of tablets to be taken and difficulty in adequate taste masking, this could have biased our assessment of adverse events. Reported vomiting can include spitting out of a tablet or a gag reflex on administration and so figures on the incidence of vomiting may be an overestimate. The number of malaria events was low (the study was powered primarily to assess tolerability) and the estimates of efficacy correspondingly imprecise. We did not investigate pharmacokinetics of mefloquine used for bimonthly prophylaxis and the incidence of malaria was too low to be able to estimate duration of protection directly, such studies would be useful.

A high burden of severe disease was observed with a rate of 0.6 episodes requiring admission per person per year, and a mortality rate of 25 per 1000 per year. Additional strategies are needed to reduce the burden of severe disease in sickle cell patients in Africa. In the US, an improvement in survival of sickle cell patients has been attributed to introduction of pneumococcal and Hib vaccination. Nigeria introduced pentavalent vaccine including Hib in 2012 and plans to introduce pneumococcal vaccine, the impact of these vaccines on survival of sickle cell patients needs to be evaluated. A recent survey of sickle cell disease management practices in Nigeria [30] found that few provided penicillin prophylaxis or pneumococcal vaccination for sickle cell patients. All 18 clinics surveyed provided malaria prophylaxis but our study indicates that regimens that are more effective than daily proguanil need to be used.

[3006 words]

Footnotes:

Preliminary results were presented at the MIM meeting, Durban, 2013.

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FIGURE

Figure 1: Trial profile. Each visit represents a bimonthly visit to the sickle cell clinic.

TABLES

Table 1: Baseline characteristics of study participants

Variable	MQAS	SPAQ	Proguanil
Nunmber randomized	90	90	90
Female:Male	45:45	41:49	43:47
Age <5yrs	30	29	24
5-9yrs	28	28	31
10-14yrs	18	21	21
15-25yrs	14	12	14
Hb Genotype SC	1 (1.1%)	5 (5.6%)	6 (6.7%)
SS	89 (99%)	85 (94%)	84 (93%)
Haemoglobin concentration g/dL	7.4(1.3)	7.5(1.6)	7.5(1.7)
PCV, percentage mean (sd)	24.6 (3.7)	25.1 (3.9)	24.6 (4.2)
Weight, kg, median (sd)	22.9 (12.1)	21.9 (10.6)	22.9 (11.5)
Wt for age z-score <-2	35 (39%)	34 (38%)	29 (32%)
Slept under treated net previous night	49(54%)	45(50%)	51(57%)
Prevalence of parasitaemia by microscopy	0% (0/74)	0% (0/77)	1.4% (1/73)
by PCR	12.2% (11/90)	6.7% (6/90)	6.7% (6/90)

Table 2: Rates of inpatient admission and outpatient attendance for illness:

Variables	Inpatients and deaths			Outpatients		
	No. of events	Rate/yr	Adjusted rate ratio (95%CI)	No. of events	Rate/yr	Adjusted rate ratio (95%CI)
Proguanil	59	0.61	1	140	1.4	1
MQAS	60	0.66	1.1 (0.73,1.7)	107	1.2	0.77 (0.57,1.0)
SPAQ	56	0.59	1.0 (0.62,1.7)	104	1.1	0.75 (0.56,1,0)
Age <5	62	0.7	1	135	1.5	1
5-9	61	0.67	0.96 (0.62,1.5)	113	1.2	0.77 (0.59,1.0)
10-14	38	0.61	0.92 (0.52,1.6)	58	0.92	0.59 (0.41,0.84)
15+	14	0.34	0.51 (0.26,1.0)	45	1.1	0.65 (0.40,1.1)
Gender Male	75	0.51	1	148	1	1
Female	100	0.73	1.5 (1.0,2.2)	203	1.5	1.5 (1.2,1.9)

*rate ratios were estimated using Cox regression with a random effect to allow for repeat events in the same patient. adjusted for treatment group, age and gender.

Table 3: Outpatient attendance for illness in male and female patients according to prophylaxis group:

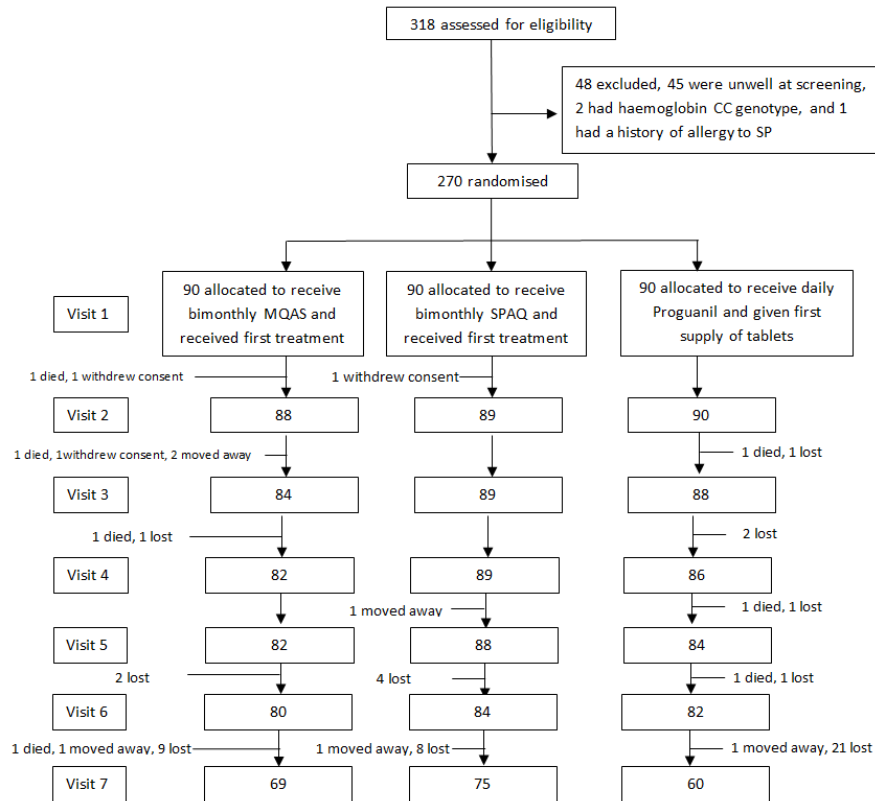|	Males			Females		
	Events (Person years)	Rate /year	Rate ratio (95%CI)	Events (Person years)	Rate /year	Rate ratio (95%CI)
Proguanil	47 (51.29)	0.9	1	93 (45.35)	2.1	1
SPAQ	54 (51.47)	1.0	1.1 (0.73,1.8)	50 (43.63)	1.1	0.56 (0.37,0.85)
MQAS	47 (43.59)	1.1	1.2 (0.74,1.9)	60 (47.51)	1.3	0.62 (0.42,0.92)

Table 4: Incidence of adverse events, for the 8 most commonly reported symptoms.

Symptom	Symptoms reported on Day 3 after each bimonthly visit:				Symptoms in the previous 2 months:		
	MQAS	SPAQ	Proguanil		MQAS	SPAQ	Proguanil
	N=583	N=608	N=595		N=554	N=591	N=579
Vomiting	8.9%	2.0%	0.0%	Body pain	3.2%	4.0%	7.6%
Body pain	2.7%	2.3%	2.4%	Fever	3.6%	3.6%	4.7%
Abdominal pain	5.8%	1.0%	0.3%	Cough	1.9%	2.9%	1.8%
Weakness	2.1%	4.4%	0.5%	Abdominal pain	2.2%	1.6%	2.6%
Nausea	4.3%	1.3%	0.3%	Headache	0.9%	2.4%	1.3%
Headache	2.7%	2.1%	0.3%	Other symptoms	0.8%	1.3%	2.5%
Fever	3.1%	0.7%	1.2%	Vomiting	1.1%	0.5%	0.9%
Dizziness	2.1%	2.0%	0.0%	Jaundice	0.5%	0.2%	1.0%
Any symptom	24.0%	13.8%	5.4%	Any symptom	12.5%	13.4%	18.1%
Risk ratio (95%CI)	4.5 (3.0,6.8)	2.6 (1.6,4.1)	1		0.68 (0.51,0.91)	0.74 (0.55,0.98)	1

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Table 5: Incidence of malaria episodes, and prevalence of *P.falciparum* infection:

Episodes of clinical malaria:				
Group	No. of individuals	Cases (person years at risk)	Rate/person/yr	Efficacy (95%CI)
Proguanil	90	19 (96.64)	0.20	-
SPAQ	90	12 (95.10)	0.13	36% (-39%,70%)
MQAS	90	7 (91.10)	0.08	61% (2%,84%)
Prevalence of *P.falciparum* infection at routine visits, by microscopy:				
	No. of visits	No. positive	% positive (95%CI)	Odds ratio (95% CI)
Proguanil	353	21	5.9%(3.3%,8.6%)	1
SPAQ	365	24	6.6%(4.3%,8.9%)	1.1 (0.61,2.0)
MQAS	340	10	2.9%(1.2%,4.6%)	0.48 (0.22,1.0)

318 assessed for eligibility

48 excluded, 45 were unwell at screening, 2 had haemoglobin CC genotype, and 1 had a history of allergy to SP

270 randomised

	90 allocated to receive bimonthly MQAS and received first treatment	90 allocated to receive bimonthly SPAQ and received first treatment	90 allocated to receive daily Proguanil and given first supply of tablets
Visit 1			

1 died, 1 withdrew consent — 1 withdrew consent—

| Visit 2 | 88 | 89 | 90 |

1 died, 1withdrew consent, 2 moved away — — 1 died, 1 lost

| Visit 3 | 84 | 89 | 88 |

1 died, 1 lost — 2 lost

| Visit 4 | 82 | 89 | 86 |

1 moved away — 1 died, 1 lost

| Visit 5 | 82 | 88 | 84 |

2 lost — 4 lost — 1 died, 1 lost

| Visit 6 | 80 | 84 | 82 |

1 died, 1 moved away, 9 lost— 1 moved away, 8 lost— 1 moved away, 21 lost

| Visit 7 | 69 | 75 | 60 |