

**SCHOOL OF PUBLIC HEALTH
COLLEGE OF HEALTH SCIENCES
UNIVERSITY OF GHANA**

**SYMPTOMATIC PLASMODIUM FALCIPARUM MALARIA FOLLOWING
ADMINISTRATION OF ARTEMETHER-LUMEFANTRINE IN HEALTHY
CHILDREN LIVING IN WESTERN KENYA: A TIME ANALYSIS**

BY

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**THIS DISSERTATION IS SUBMITTED TO THE UNIVERSITY OF GHANA,
LEGON, IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE
AWARD OF MASTER OF SCIENCE IN CLINICAL TRIALS DEGREE**

JULY 2010

DECLARATION

I, Ben Andagalu, declare that except for the other peoples investigations which have been duly acknowledged, this work is the result of my own original research, and that this dissertation, either in whole or in part has not been presented elsewhere for another degree.

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DEDICATION

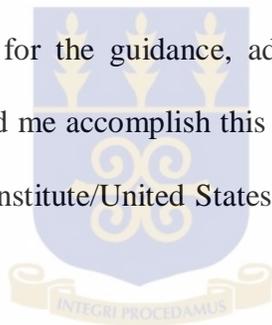
**To my wife Stella, and children Amy and Sid,
For their love, dedication, understanding and moral support**



ACKNOWLEDGEMENT

Glory and praises to God for strengthening me.

I thank my dear wife Stella, children Amy and Sid, family, friends and course-mates for their loving support, prayers and encouragement throughout my course. I owe my enormous debt of gratitude to the Malaria Clinical Trials Alliance (MCTA) who sponsored my Masters course at the University of Ghana; all the staff and lecturers who have taught and supported me through this Masters program. Special thanks to Dr Bernhards Ogutu, the principal investigator of the study whose data I used for this project, for his continued advice and encouragement. I am greatly indebted to my supervisor Prof. Fred Binka for the guidance, advice, countless discussions and vast knowledge, which has enabled me accomplish this work. I also acknowledge the support of Kenya Medical Research Institute/United States Army Medical Research Unit-Kenya (KEMRI/USAMRU-K).



ABSTRACT

Sulphadoxine-Pyremethamine (SP) has been an ideal choice of drug for Intermittent Preventive Treatment (IPT) of malaria; however, due to widespread resistance of the malaria parasite to this drug, there has arisen need to find alternative anti-malarial drugs for IPT to replace it. It has been suggested that short-acting drugs are unsuitable for IPT.

A longitudinal cohort study of the epidemiology of pediatric malaria was conducted in Kombewa Division, Western Kenya between 2003 and 2004, in which 270 healthy children were randomized to receive either Artemether-Lumefantrine (AL), a short acting anti-malarial, or placebo at the beginning of the study, and then followed up for one year. Data generated from this study was used to assess the effect of AL on the time to first clinical malaria episode and the risk of multiple clinical malaria episodes and thus evaluate the evidence against the use of short-acting drugs for IPT, and also assess the risk for rebound of clinical malaria episodes.

There was no significant difference in the time to first clinical malaria episode in the treatment groups (5.29 weeks (95% CI 2.86 to 11.14 weeks) for the placebo arm versus 5.43 weeks (95% CI 4.86 to 6.43 weeks), and no significant protective effect of AL against the first episode observed beyond 3 weeks post randomization. No rebound of clinical malaria episodes was observed in the treatment groups after 52 weeks of follow up – the rate ratio for multiple malaria episodes (AL/placebo) was 0.90 (95% CI 0.76 to 1.06, p-value=0.205) AL administered in healthy children therefore has little influence on the time to first clinical malaria episode and the risk of multiple clinical malaria episodes. Its short duration of protection makes it unsuitable for IPT.

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LIST OF ABBREVIATIONS

ACT – Artemisinin-based Combination Therapy

AL – Artemether-Lumefantrine

EIR – Entomologic Inoculation Rate

IPT – Intermittent Preventive Treatment

ITN – Insecticide-Treated Nets

SP – Sulphadoxine-Pyremethamine

WHO – World Health Organization

CHAPTER ONE: INTRODUCTION

1.1 Background

Intermittent Preventive Treatment (IPT) of malaria is the administration of a curative dose of an effective anti-malarial drug at pre-determined intervals to a target population, with the primary aim of reducing the burden of malaria. Pregnant women and children living in malaria-endemic areas are the target of this intervention, since they are considered to be at risk of adverse outcomes of *Plasmodium falciparum* infection (Grobusch et al., 2007).

IPT with Sulphadoxine-Pyremethamine (SP) was initially tried in pregnant women as an alternative to weekly Chloroquine chemoprophylaxis, with the purpose of reducing the risk of low birth weight and maternal anemia by clearing peripheral and placental parasitemia while intermittently protecting pregnant women against malaria between antenatal visits. SP was seen to be safe, efficacious and easy to administer – it was given as a single dose, thus allowing directly supervised treatment. The success of this strategy led to it being adopted by the WHO and its trial extended to infants and children as IPT in infants (IPTi), children (IPTc), and school children (IPTsc). The general aim of IPT in infants and children is to reduce the incidence of malaria and anemia in this population.

While only IPT in pregnant women (IPTp) has been formally adopted as policy by the WHO (World Health Organization, 2009), the principle of IPT is generally accepted as one of the malaria control strategies, and is regarded to be better than continuous chemoprophylaxis for a number of reasons. The requirement of fewer drug doses, fewer healthcare contacts and directly supervised treatment makes it easy to implement, and

ensures correct dosing, higher compliance and better tolerability than that of continuous long-term prophylaxis. Additionally, since the drugs are given at intervals only to high-risk groups, the selection pressure that promotes drug resistance may be minimized. Also, IPT may not greatly interfere with the development of anti-malarial immunity in children and infants, since blood stage malaria infection is not prevented between each treatment.

With the elimination and eradication of malaria once again becoming stated objectives of the global fight against malaria, alongside the reduction of disease burden in high-risk groups, IPT occupies a unique position since it can play an important role in achieving all these objectives. Elimination and eradication of malaria requires the reduction of carriage of malaria parasites in all individuals – the use of an effective anti-malarial drug for IPT would ensure this. Reduction of disease burden requires focus on treating clinical disease, or preventing disease in high-risk populations – IPT also has the ability to achieve this.

1.2 Rationale

SP was an ideal choice of drug for IPT – it was highly efficacious at the time, was administered as a single dose, thus allowing for directly observed treatment, and was generally well tolerated. However, widespread resistance of the malaria parasite to this drug has put its utility in question. Indeed, in one IPT efficacy trial, the failure to demonstrate the protective efficacy of SP was attributed to resistance (Cairns et al., 2010). New regimens for IPT are therefore needed to replace SP. Any new regimen that will replace SP, in addition to being efficacious in parasite clearance, will need to demonstrate protective efficacy over and above that already offered by preventive interventions of proven efficacy that are already in use, such as Insecticide Treated Bed-

Nets (Binka et al., 1996, Nevill et al., 1996, World Health Organization, 2009). The choice of any new regimens should also be informed by sound scientific evidence.

Artemisinin-based Combination Therapy (ACT) has replaced SP as the recommended first line treatment for uncomplicated malaria (World Health Organization, 2010). Artemether-Lumefantrine (AL) is one of the ACTs that has been approved and is widely used for the treatment of uncomplicated *P. falciparum* malaria, both in adults and children (World Health Organization, 2010, World Health Organization, 2009). Its efficacy, safety and tolerability in children has been demonstrated in several studies (Abdulla et al., 2008, Adjei et al., 2009, Juma et al., 2008, von Seidlein et al., 1998). The safety of AL in pregnant women, however, has not been fully established. It is administered as a 6-dose oral treatment course over approximately 3 days. Despite the requirement of multiple doses, AL when administered unsupervised (that is, given to the patient to take at home) has been shown to be as effective as when it is administered under the direct supervision of healthcare personnel (Piola et al., 2005). As such, AL would have been a good replacement for SP for IPT in children.

However, AL is classified as a short-acting drug – the half-life of Artemether is 3.9 hours, while that of Lumefantrine is 32.7 hours (Mwesigwa et al., 2009, White et al., 1999). Consequently, considering the requirement that drugs for IPT should have a long half-life, AL would theoretically not be suitable for IPT. However, little is actually known about the effect of AL on the risk of symptomatic malaria.

This study analyzed the effect of the administration of Artemether-Lumefantrine in healthy children on the time to first symptomatic *P. falciparum* malaria following the and

thus evaluated the strength of the evidence against the use short acting anti-malarial drugs for IPT. Additionally, the risk of multiple symptomatic *P. falciparum* malaria following the administration of Artemether-Lumefantrine was also analyzed, and thus the risk of a rebound of malaria episodes was assessed. This information may help inform future policy regarding IPT.

1.3 Objectives of the analysis

1.3.1 Primary objective

To compare the time to the first symptomatic *P. falciparum* malaria episode in children who received Artemether-Lumefantrine at randomization to those who received placebo.

1.3.2 Secondary objective

To compare the risk of multiple symptomatic *P. falciparum* malaria episodes in children who received Artemether-Lumefantrine at randomization to those who received placebo over a period of 12 months from the date of randomization.

CHAPTER TWO: LITERATURE REVIEW

IPT has both therapeutic and protective effects (Gosling et al., 2009a). Since a therapeutic dose of an efficacious anti-malarial drug is used for IPT, any circulating malaria parasites would be eliminated. This is important, especially considering that the prevalence of asymptomatic parasitemia in some endemic areas has been reported to be as high as 39%, and a significant proportion of these carriers in some of the areas studied were children and pregnant women (Ogutu et al., 2010). While such asymptomatic carriers may not present with overt signs of clinical malaria, they are at risk for malaria related morbidity such as anemia, which in turn, is related to poor pregnancy outcomes and poor child health and development. In addition, these asymptomatic carriers form a reservoir for the parasite and may serve as a source of new infections (Ogutu et al., 2010). Thus, the therapeutic effect serves an important public health role.

The mechanism behind the protective effects of IPT was the subject of debate in the recent past (Gosling et al., 2009a). One school of thought attributed the effects of IPT to immune mechanisms (Schellenberg et al., 2005). In this scenario, it was thought as the concentration of the anti-malarial drug in the blood waned over time, the sub-therapeutic levels thus achieved allowed the development of low-level asymptomatic parasitemia. This in turn was thought to stimulate the development of immunity in the IPT recipients and prevent new infections. In this case, drugs with efficacious parasite clearance and suppressive properties would not have been considered as appropriate for IPT. Another school of thought, however, attributed the protective effect of IPT to the chemoprophylactic properties of the drugs used for IPT (White, 2005). In this situation,

the recipient of IPT would not be susceptible to new malaria infections for as long as adequate blood levels of the drugs were present. Thus, the delay of the median time to first clinical episode of malaria from 38.5 days to 68 days in a study conducted in Mali was attributed to the long lasting effects of SP (Coulibaly et al., 2002). Even though the half-life of SP is approximately 7 days, chemo-suppressive levels are maintained in the blood for as long as 60 days (Barnes et al., 2006, White, 2005). In a recent study comparing mefloquine (a long-acting drug) and chlorproguanil-dapsone (a short-acting drug) for IPTI, both of which are efficacious at parasite clearance, only the long-acting drug showed protective efficacy against clinical episodes of malaria (Gosling et al., 2009b). This gives further credence to the fact that IPT works by and large through the chemoprophylactic mechanism. As such, it is expected that short-acting drugs should offer protective effect that would last no longer than their half-lives and that would make them unsuitable for IPT. This information is crucial for determining future policy regarding future alternative drug regimens for IPT.

The assessment of the efficacy of any drug regimen for IPT is expected to follow the routine methodology used for malaria preventive strategies. Endpoints that are usually assessed in efficacy trials of malaria preventive strategies include time to first malaria episode, risk of multiple episodes, risk of malaria-related morbidity and mortality (Moorthy et al., 2009). Several IPT efficacy trials have reported time to first clinical malaria episode as their primary objective (Macete et al., 2006, Massaga et al., 2003, Schellenberg et al., 2001). While this endpoint is useful for establishing the proof of concept, public health policy makers are usually more interested in the impact of the

intervention on the burden of disease, measured in this case as the impact on the risk for multiple episodes of malaria in an individual. In one IPT efficacy trial (Chandramohan et al., 2005), the risk for multiple episodes of malaria was reported as the primary endpoint. The evaluation of these endpoints is not as straightforward as it may seem. One of the difficulties is in the definition of clinical malaria. While most infectious diseases are diagnosed by establishing the presence of the infective agent along with a clinical syndrome, there has been considerable debate about the application of such criteria to define malaria in persons living in endemic areas where asymptomatic parasitemia is a common occurrence. It has been suggested that in the presence of a high prevalence of asymptomatic parasitemia and considering the significant overlap of malaria symptoms with symptoms of other disease, a positive malaria test could be an incidental finding in a sick resident of a malaria-endemic area. In this case, the likelihood of the symptoms being attributed to malaria would depend on the level of parasitemia – the higher the level of parasitemia, the more likely it is that malaria is the cause of symptoms. Consequently, cut-off points for levels of parasitemia above which a symptomatic person is considered to have malaria during the conduct of efficacy trials have been estimated using statistical methods with reference to the prevalence of asymptomatic parasitemia (Smith et al., 1994) and some of the IPT efficacy studies used these cut-offs (Kobbe et al., 2007, May et al., 2008). These cut-offs, however, do not exist in everyday practice – any practicing healthcare provider who encounters a sick patient with any parasitemia level regardless of whether the prevalence of asymptomatic parasitemia in the area is high or not, will make a diagnosis of malaria alongside any other differential diagnoses,

and treat as such. Therefore the use of any asexual parasitemia level of greater than 0 in endpoint definitions should result in higher sensitivity of the study, more conservative results and increase the validity of the study. The determination of the time to first episode of malaria has its own problems that are linked to the prevalence of asymptomatic parasitemia. While persons with malaria parasitemia may be asymptomatic at the time of conducting the malaria test, that fact does not preclude the possibility that the parasitemia was an incipient malaria infection, waiting to present clinically. As such, in some clinical trials evaluating preventive interventions against malaria, radical cure of malaria was performed prior to administering the intervention under investigation – this involved the administration of an effective anti-malarial drug regimen that would clear both blood and liver stages, and the participant tested for malaria at the end of the radical cure to confirm the absence of parasites (Baird et al., 2002). This procedure helps settle the problem concerning incipient malaria infection. However, in trials of IPT, this procedure has not been applied. While in everyday practice one would not run a malaria test in a healthy individual prior to administering IPT, it is still prudent, at least in clinical trials that have time to first malaria episode as an outcome, to deal with asymptomatic parasitemia, especially if one of the treatment groups will receive placebo. One way out is to exclude participants, especially those receiving placebo, with positive smears from the analysis of first events. The other problem area in endpoint determination is in the estimation of the risk for multiple malaria episodes. Usually, in studies that evaluate multiple failure events of the same type, assuming that the events occur independently, the time at risk for the next event usually begins soon after the subject has experienced

the preceding one, that is, the risk for event number two begins as soon as event number one has occurred. In the case of malaria, this may not be entirely true, since a person who has been infected with malaria is not considered to be at risk for a new episode for a particular period, and multiple malaria episodes occurring in one individual are not entirely independent, since an individual who experiences one episode of malaria is more likely to experience the next one (Moorthy et al., 2009). The duration of the period of “no-risk” is determined at least in part by the by the pharmacokinetic properties of the anti-malarial treatment used in the patient, and probably by the biological interactions between the human immune system and the parasite (Moorthy et al., 2009). It is expected that short-acting drugs would not influence the duration of this period significantly. In some of the IPT trials that evaluated multiple malarial episodes, the period of “no risk” was considered to be 21 days (Kobbe et al., 2007, May et al., 2008), while others used 28 days.(Dicko et al., 2008) (Odhiambo et al., 2010), and yet another conducted analysis using 0 day period of “no risk”, then repeated it using a 28 day period of “no risk” (Cairns et al., 2008). The latter study reported more conservative findings when the 0 day period of “no risk” was used. The justification for or against the use of such figures was not clearly reported by the investigators of the mentioned studies. The choice of 28 days has been used in several non-IPT studies, probably informed by the fact that most re-infections occur 28 days after a clinical episode – as such any new episode within this period is usually considered as recrudescence. This standpoint is supported by data from a study that was able to differentiate new from old infections using molecular methods (Cattamanichi et al., 2003). In addition, in order to differentiate between clinical visits for

the same malaria episode from those for a new episode at the analysis stage, the time gap is usually deemed necessary. The lack of independence between malaria episodes is usually handled at the analysis stage by using the random effects Poisson regression model (D'Agostino, 2004, Rabe-Hesketh and Everitt, 2004).

CHAPTER THREE: METHODS

3.1 Original study information

The source of data for this analysis was data generated from the longitudinal cohort study of the epidemiology of pediatric malaria that was conducted in Kombewa Division, Western Kenya between 2003 and 2004. The objectives of this study were to estimate the prevalence of malaria, describe the temporal patterns of symptomatic malaria and assess whether the administration of a curative dose of an anti-malarial treatment at the beginning of the study leads to a large change in the pattern of development of episodes of malaria during the follow-up period.

3.1.1 Study location

Kombewa is a 361 square kilometer rural area located near Lake Victoria in the western part of Kenya. The climate in Kombewa is characterized by two rainy seasons – the long rains (April through June) and the short rains (August through October). The vegetation is largely savanna in nature. The population of Kombewa Division is primarily Luo. The majority of the population in Kombewa speaks both Dholuo (the local dialect) and Kiswahili. Families are generally large with many small children in each household. Villages in this area are typically a loose conglomeration of family compounds near a family garden plot and grazing land. Houses are typically made of mud with thatched or corrugated roofs. In Kombewa Town, homes are often attached to family shops/businesses. Most homes do not have glass windows or screens. Water source is mainly from community wells and local streams though some households have their own wells and the lake for those living along the shores. Most water sources are not

chlorinated or covered. The majority of residents engage in either small scale farming or fishing.

The population of Kombewa is estimated to be 69382, with an under-five population of 8584. Primary outpatient health care is accessed at community health centers that are

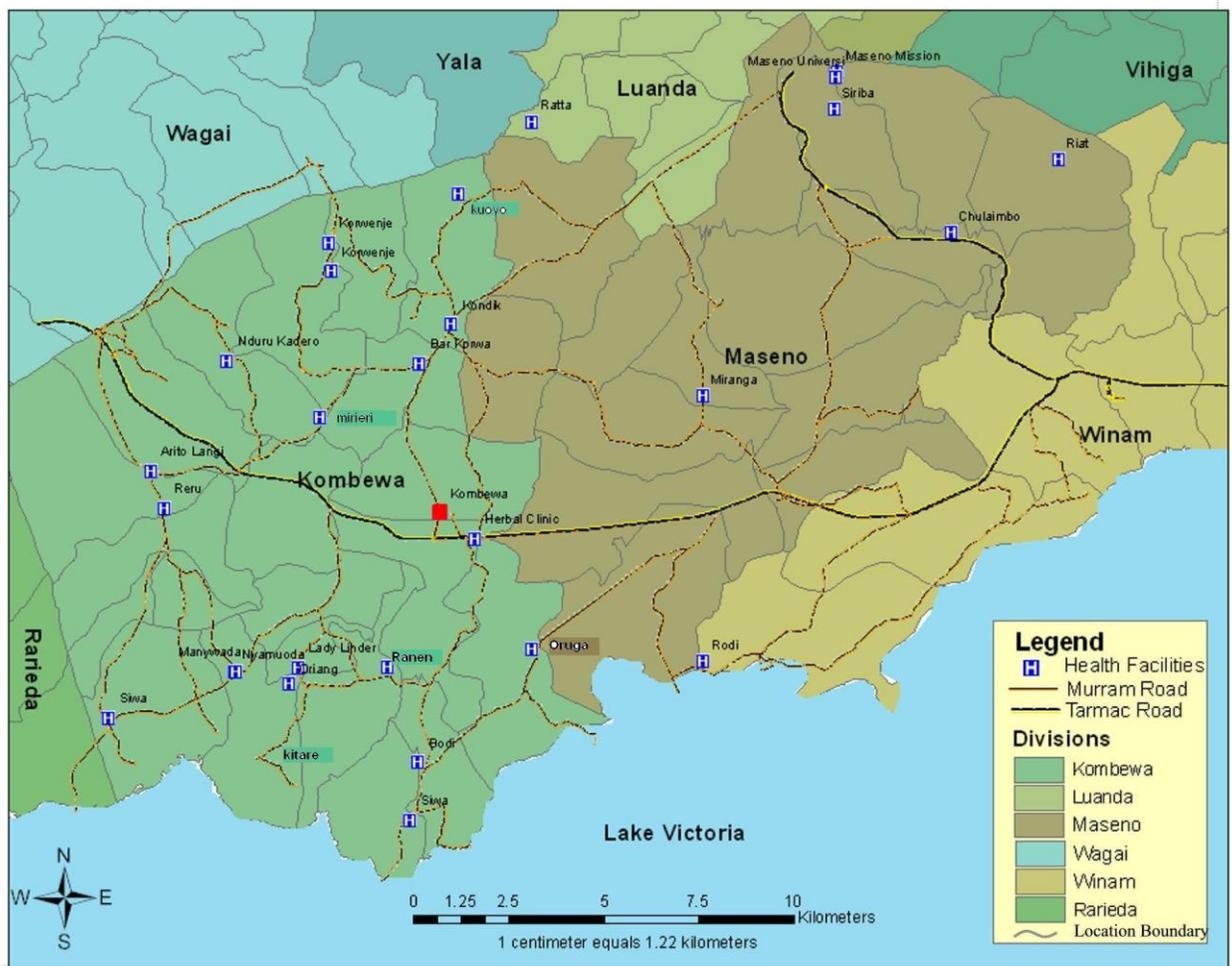


Figure 1: Map showing Kombewa Division and its health facilities, within Kisumu West District.

Note: Red box indicates location of the Kombewa clinical research center and the Kisumu West District Hospital.

Source: Kombewa Demographic Surveillance System, Walter Reed Project

operated by the Ministry of Health and faith based organizations and manned mainly by nurses. The area is also served by a district hospital having in-patient facilities and staffed with different cadres of government-employed healthcare workers. The poor transportation system within the area, however, makes this facility inaccessible to a significant proportion of the area residents. The provincial referral hospital is located in Kisumu City, approximately 30 km away from the district hospital. The Walter Reed Kombewa Clinical Research Center is located opposite the district hospital.

Kombewa is characterized by year round transmission of malaria, with the most intense transmission occurring during the long rains (April through June) and the short rains (August through October). *Anopheles gambiae* is the predominant mosquito species in this area, with an Entomologic Inoculation Rate (EIR) of 0.65 – 0.79 per person per night (Beier et al., 1994). Unpublished data estimate that the monthly clinical malaria attack rates in children aged 1 to 3 years in this area ranges from 20% - 55%. During the period when the original study was conducted, malaria preventive interventions such ITN and IPT had not been fully rolled out to most of the area. Additionally, at the time, the Ministry of Health was yet to implement the changeover of the first line treatment for uncomplicated malaria from SP to ACT.

For the purpose of organizing recruitment and follow-up of the volunteers, Kombewa was arbitrarily divided into 22 subdivisions. Within each subdivision in which a participant resided there was a satellite field site that was staffed 24 hrs/day, 7 days/week and with radio communication with the main research center. Each household that

participated in the study was thus located within a 1-mile radius of a satellite field site that was accessible during the rainy and dry seasons.

3.1.2 Study design and population

A total of 270 initially healthy, asymptomatic children aged between 12 months and 47 months living within the study area were enrolled in the longitudinal cohort study of the epidemiology of pediatric malaria after obtaining informed consent. Children who had significant pre-existing medical conditions, including homozygous sickle-cell disease were excluded. A blood sample for the preparation of a baseline malaria blood film and a full blood count was collected just before randomization – the results of this slide were not used to determine eligibility, and were not made available to the investigators. The children were then randomized to receive either a course of AL or placebo using a list prepared in advance using a blocked randomization scheme. The appropriate dosage of AL was crushed and mixed with chocolate-milk. The placebo was made of corn-starch and resembled the crushed AL tablets – this was also mixed with chocolate-milk. All study staff with the exception of the study pharmacists and the study coordinator were thus blinded to the allocations. The treatment was prepared by the study pharmacist at the clinic and its administration directly supervised by study team members – the first, third and fifth doses were administered at the clinic, while the second, fourth and sixth doses were administered by field workers at home. These six doses were administered at hours 0, 8, 24, 36, 48 and 60 respectively, with an allowance that ensured a minimum of 8 hours between doses. Each of the children was then followed up for 1 year. The follow-up consisted of active surveillance and passive surveillance. Active surveillance consisted

of weekly visits by field workers and monthly visits at the study clinic, during which samples for malaria blood films were collected. Blood smears were prepared, read and verified according to standard protocols. Hemoglobin assays were run only during the monthly visits. Results of the weekly malaria tests from the active surveillance were not made available to clinicians, unless the participant was unwell during a visit. Passive surveillance consisted of unscheduled visits, when participants had specific complaints. During such occasions, the participant was taken to the nearest satellite field site, and arrangements made to transport the participant to the study clinic, where all medical assessments were done. Malaria films were done if needed during unscheduled visits and the results made available to the attending clinician. Participants thus diagnosed with malaria were treated with AL, regardless of their randomization group.

3.1.3 Publications

The findings of the original study are yet to be published

3.2 Statistical methods

3.2.1 Definition of study endpoints

Definition of the primary endpoint

The primary endpoint was the time to the first or only episode of symptomatic *P. falciparum* malaria. Time of origin was the date of randomization, while the time of event was the date of the malaria film confirming the presence of asexual forms *P. falciparum* in a symptomatic child. Censoring occurred if no event had been recorded by 1 year following randomization or by the moment a participant had been lost to follow up.

Definition of the secondary endpoint

The secondary endpoint was the risk of multiple symptomatic *P. falciparum* malaria episodes occurring over a period of 12 months. The time of origin was the date of the first episode of clinical malaria after randomization. The time of end of observation was 12 months from the time of randomization. Censoring occurred if no event had occurred by 12 months following randomization or by the moment a participant had been lost to follow up. Multiple events from each participant were considered. The time at risk was defined as the period between the first clinical malaria episode after randomization and the end of observation or censoring. Participants were considered not to be at risk for symptomatic *P. falciparum* malaria for a period of 28 days following a symptomatic episode. Participants who did not experience any clinical malaria episode were excluded from the analysis.

For both endpoints, symptomatic *P. falciparum* malaria was defined as the presence of any clinical sign of malaria (World Health Organization, 2005) together with any level of asexual *P. falciparum* malaria parasitemia >0 as detected by microscopy of a malaria blood film.

3.2.2 Sample size and justification

Assuming that 85% of children living in a malaria endemic area will experience at least 1 symptomatic malaria episode per year, a sample of 270 children was considered to have 80% power to enable the detection of a 30% change in the risk of the first event at a two-sided significance of $\alpha=0.05$.

3.2.3 Data extraction

The study data was available as a Microsoft Access® database (Microsoft Corporation, Seattle, USA). Data was extracted by running queries and datasets fit for analysis in Stata/SE 10.0 for Windows (StataCorp LP, Texas, USA) were created.

The following baseline variables were extracted from the study database:

- Age
- Weight
- Gender
- Baseline parasitemia
- Use of bed-nets

Age, the use of bed-nets and baseline malaria parasitemia are factors that have been shown to greatly influence the risk of development of malaria. Younger children and those who have asymptomatic malaria parasitemia are considered at higher risk of developing symptomatic malaria as compared to older children and those without any parasitemia respectively. Bed-nets, on the other hand, have been shown to be protective against symptomatic malaria episodes. As such, these variables were considered as potential predictors of outcome in this analysis. They were extracted and categorized as follows:

- Age – group 1: 12 – 23 months; group 2: 24 –47 months
- Bed-net use – yes versus no
- Baseline asexual parasitemia – 0 versus >0

The following variables that enabled the determination of the analysis endpoints were also extracted:

- The date of randomization
- Dates of malaria blood films, both scheduled and unscheduled
- Results of malaria blood films
- Clinical findings during scheduled and unscheduled visits

3.2.4 Analysis of baseline characteristics

Categorical baseline variables (gender and use of bed-nets) were summarized by presenting their proportions by allocation group. Continuous baseline variables (age, weight and baseline parasitemia) were summarized by calculating the mean with standard deviation, or median with the range, as appropriate. No formal statistical tests were done to compare any differences in baseline characteristics between the treatment groups.

3.2.5 Primary endpoint analysis

Descriptive methods

A survival analysis of the first or only symptomatic *P. falciparum* malaria episode was carried out. In order to more accurately ascertain the time to first clinical malarial episode, placebo recipients who had a positive smear at randomization was excluded from analysis. This enabled fair comparison against the AL group, considering that AL already had proven parasite clearance capacity. The number of first or only events, together with the median and range of weeks to the first event were calculated according

to the allocation groups. The survival function for the allocation groups was estimated using the Kaplan-Meier method.

Regression model

The Cox regression model was used to estimate the hazard ratios between the allocation groups. The influence of the predictor variables on the time to first or only event was assessed using the same model, and adjusted hazard ratios calculated. The protective efficacy of AL was then estimated by subtracting the hazard ratio from 1.

3.2.6 Secondary endpoint analysis

Descriptive methods

The total numbers of symptomatic *P. falciparum* malaria episodes experienced in the allocation groups during the observation period were calculated at monthly intervals and for the whole observation period. The total person-time at risk was also calculated for the monthly intervals and the entire observation period, and thus event rates calculated.

Regression model

The relative risks for multiple symptomatic *P. falciparum* malaria in the allocation groups were estimated using the Poisson regression model that accounted for possible clustering of events among individual participants. The influence of the predictor variables on the relative risk were evaluated using the same model.

3.2.7 Significance levels

Statistical tests were two-sided and run at a significance level of $\alpha=0.05$. Where appropriate, 95% confidence intervals were estimated.

3.2.8 Missing data management

Participants who experienced failure on the randomization day were excluded from the analysis. For participants who dropped out or were lost to follow-up, any information contributed up to the moment of drop out or loss to follow-up was included in the analysis.

3.3 Ethical considerations

The original study was approved by the Kenya Ethics Review Committee and the Walter Reed Army Institute of Research Institutional Review Board, and conducted in accordance to the principles of Good Clinical Practice. The principal investigator authorized the use of the data from this study. The proposal for the analysis was reviewed and approved by the Ghana Health Service Ethics Committee.

CHAPTER FOUR: RESULTS

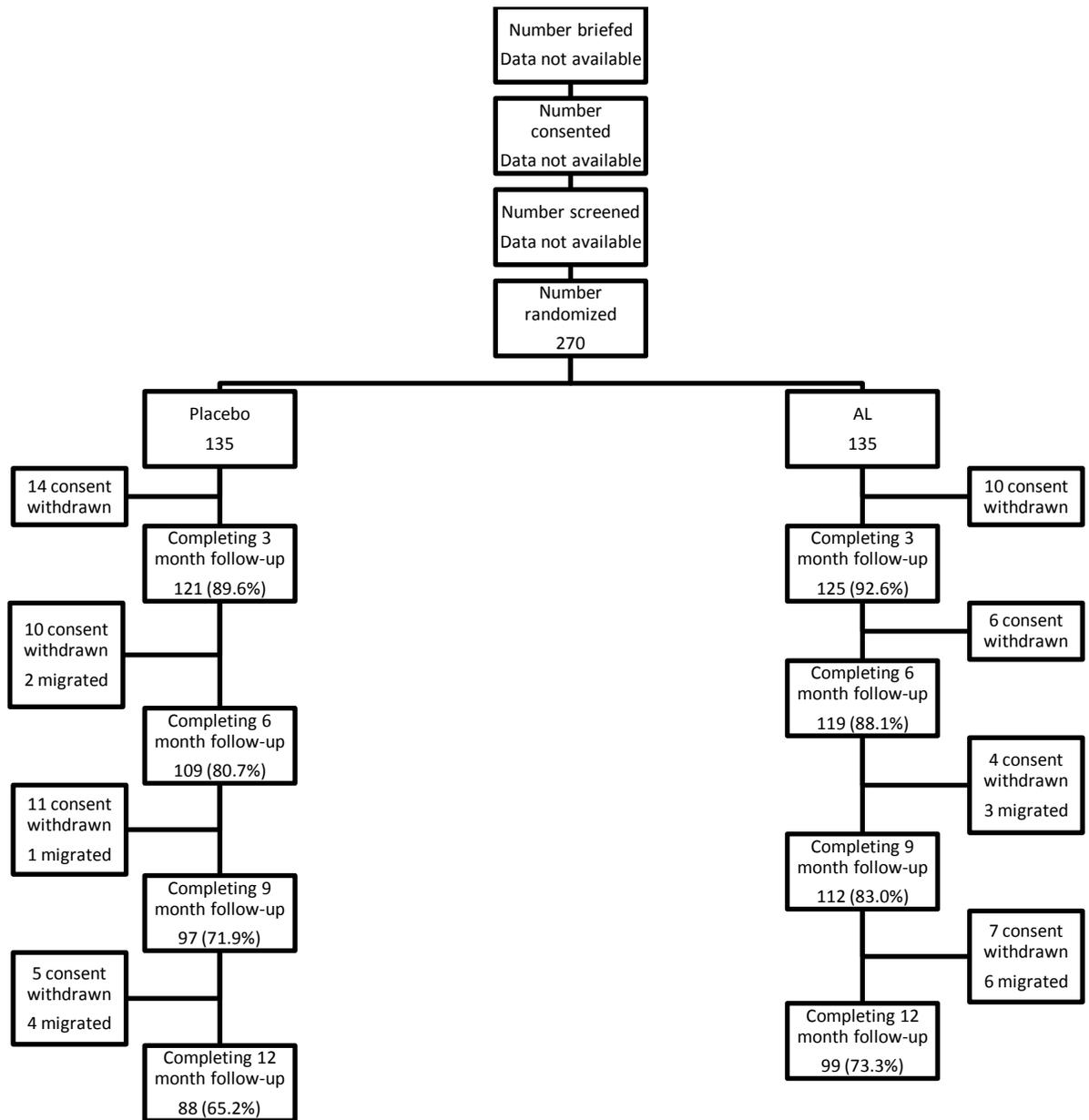


Figure 2: The trial profile

4.1 Study flow

A total of 270 children were enrolled into the study, 135 randomized to receive placebo and 135 randomized to receive AL at enrollment. Data on the total number of potential participants briefed, the number who consented and screened was not available. Overall, 187 out of 270 participants (69%), with 65.2% in the placebo arm and 73.3% in the AL arm, completed 1 year follow-up (Figure 2). 67 out of 83 participants (81%) who did not complete follow-up withdrew their consent, with 46 participants citing their discomfort with the weekly blood draws as the reason for consent withdrawal. Migration out of the study area accounted for the remainder of those who did not complete follow-up (Figure 3). No deaths were recorded among the study cohort for the duration of the study. 2 Serious Adverse Events (SAEs) were however recorded – 1 child in the AL arm was hospitalized with a diagnosis of severe malaria, while 1 child in the placebo arm was hospitalized with severe pneumonia. Both children recovered. All children completed the six doses of the study treatment.

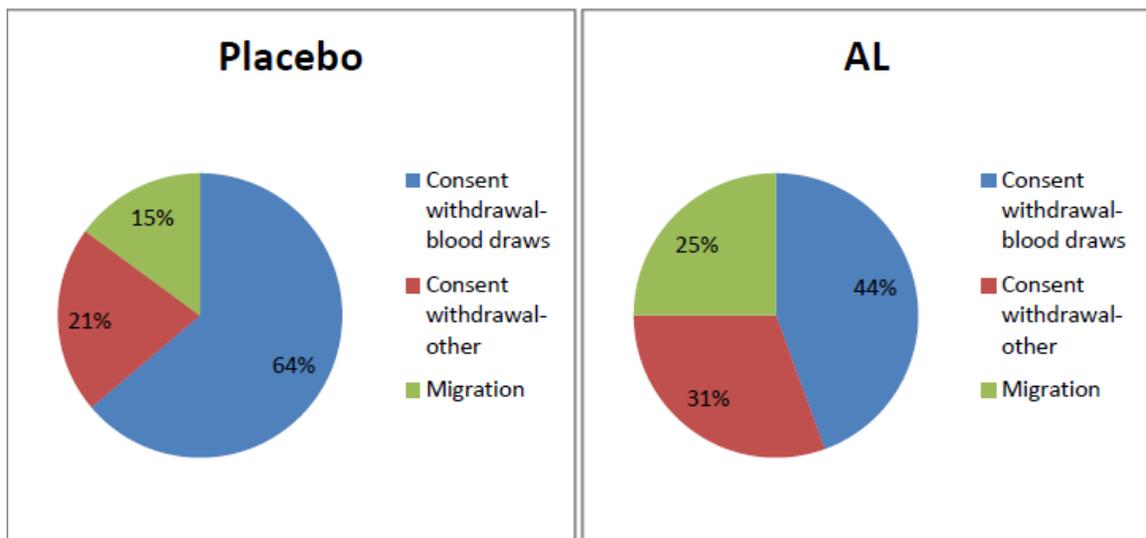


Figure 3: Reasons for not completing follow-up

4.2 Baseline characteristics

The baseline characteristics of both treatment arms were generally well balanced (Table 1). ITN use was low in the study cohort (33% overall) with a slight predominance in the placebo arm (36.3% vs. 29.6%). There was a general trend of higher ITN use in the 12-23 month age group than the 24-47 months age group (overall 44% vs. 27%). The prevalence of parasitemia at randomization was expectedly high, considering that enrollment took place over a 2 week period between the end of May and early June, coinciding with the peak of the malaria season. A total of 172 children out of 270 (63.7%) had a positive smear at randomization, with a prevalence of 63.0% and 64.4% in the placebo and AL arms respectively. The prevalence was lower in bed-net users (53.1% and 57.5% in the placebo and AL arms respectively), and higher in the older age group (70.8% and 73.6% in the two arms).

Table 1: Baseline characteristics

	Placebo N=135	AL N=135
Age – mean (SD)	29.1 (10.1)	28.7 (10.1)
Age 12-23 months n/N (%)	46/135 (34.1)	48/135 (35.6)
Age 24-47 months n/N (%)	89/135 (65.9)	87/135 (64.4)
Sex – female – n/N (%)	66/135 (48.9)	63/135 (46.7)
Sex – male – n/N (%)	69/135 (51.1)	72/135 (53.3)
Weight in kg – mean (SD)	11.9 (2.3)	11.8 (2.3)
Use of bed-nets – n/N (%)		
Overall	49/135 (36.3)	40/135 (29.6)
Female	25/66 (37.9)	20/63 (31.8)
Male	24/69 (34.8)	20/72(27.8)
Age 12-23 mo	24 /46 (52.2)	17/48 (35.4)
Age 24-47 mo	25/89 (28.1)	23/87 (26.4)
Parasitemia at randomization – geometric mean (range)		
Overall	2806.13 (0 - 642000)	4358.16 (0 - 220000)
Male	3602.24 (0 – 642000)	4295.51 (0 – 102000)
Female	2173.00 (0 – 84000)	4432.94 (0 – 220000)
Bed-net users	2276.37 (0 – 642000)	5091.67 (0 – 194000)
Bed-net non-users	3077.16 (0 – 88000)	4121.21 (0 – 220000)
Age 12-23 mo	3231.21 (0 – 642000)	4144.90 (0 – 102000)
Age 24-47 mo	2671.26 (0 – 88000)	4437.45 (0 – 220000)
Positive malaria smears at randomization – n/N (%)		
Overall	85/135 (63.0)	87/135 (64.4)
Male	43/69 (62.3)	47/72 (65.3)
Female	42/66 (63.6)	40/63(63.5)
Bed-net users	26/49 (53.1)	23/40 (57.5)
Bed-net non-users	59/86 (68.6)	64/95 (67.4)
Age 12-23 mo	22/46 (47.8)	23/48 (47.9)
Age 24-47 mo	63/89 (70.8)	64/87 (73.6)

4.3 First clinical malaria episodes

In view of the high prevalence of malaria parasitemia at randomization, and given the already proven efficacy of AL in parasite clearance, placebo recipients who had a positive malaria smear at randomization were excluded from the analysis of first clinical malaria episodes, in order to have a fair comparison between the two arms. Table 2 shows the characteristics of the cohort that excludes placebo recipients who had a positive smear at randomization.

Table 2: Baseline characteristics for the cohort that excludes placebo recipients who had positive malaria smear at randomization

	Placebo (N=50)	AL (N=135)
Age – mean (SD)	25.5 (9.5)	28.7 (10.1)
Age 12 – 23 months (%)	24/50 (48.0)	48/135 (35.6)
Age 24 – 47 months (%)	26/50 (52.0)	87/135 (64.4)
Sex – female – n (%)	24/50 (48.0)	63/135 (46.7)
Sex – male – n (%)	26/50 (52.0)	72/135 (53.3)
Weight in kg – mean (SD)	11.5 (2.1)	11.8 (2.3)
Use of bed-nets – n/N (%)		
Overall	23/50 (46.0)	40/135 (29.6)
Female	8/24 (33.3)	20/63 (31.8)
Male	15/26 (57.7)	20/72(27.8)
Age 12 – 23 months	14/24 (58.3)	17/48 (35.4)
Age 24 – 47 months	9/26 (34.6)	23/87 (26.4)

Of note is the imbalance in the total numbers in the treatment arms (50 vs. 135 in the placebo and AL arms respectively), and the proportion using ITN (46.0% vs. 29.6% for placebo and AL arms respectively). The mean age of the participants in the placebo arm is lower (25.5 months vs. 28.7 in the AL arm), with an almost equal distribution of the 2 age groups, unlike in the AL arm. The other characteristics are generally well balanced.

The proportions of participants experiencing the first clinical malaria episode in the 2 groups were not significantly different (79.6% vs. 89.6% in the placebo and AL arms respectively, p-value=0.077) (Table 3). The majority of first episodes in the placebo arm occurred in the first month (51.3%), while the majority of first events in the AL arm occurred in the second month after randomization (50.8%).

Table 3: Proportion of first clinical malaria episodes occurring at monthly intervals

	Placebo N=49 *	AL N=134 *
Overall proportion experiencing 1 st event **	39/49 (79.6)	120/134 (89.6)
Proportion of 1 st events occurring during Month 1	20/39 (51.3)	30/120 (25.0)
Proportion of 1 st events occurring during Month 2	7/39 (17.9)	61/120 (50.8)
Proportion of 1 st events occurring during Month 3	5/39 (12.8)	12/120 (10.0)
Proportion of 1 st events occurring after Month 3	7/39 (17.9)	17/120 (14.2)

* 1 participant in each arm failed on randomization day

** Chi-square p-value=0.077

The first event rates were thus higher in the first month in the placebo arm (12.92 vs. 5.78 events per 100 person-weeks) and higher in the second month in the AL arm (25.03 vs. 8.89 events per 100 person-weeks) (Table 4).

Table 4: First clinical malaria episode rates at monthly intervals

Month	Placebo			AL		
	P-time	Events	Rate	P-time	Events	Rate
(0 - 1]	154.86	20	12.92	519.29	30	5.78
(1 - 2]	78.71	7	8.89	243.71	61	25.03
(2 - 3]	57.14	5	8.75	114.14	12	10.51
> 3	220.71	7	3.17	442.86	17	3.84
Overall	511.42	39	7.63	1320	120	9.09

- P-time – person time in weeks
- Rate – (Events/P-time)x100

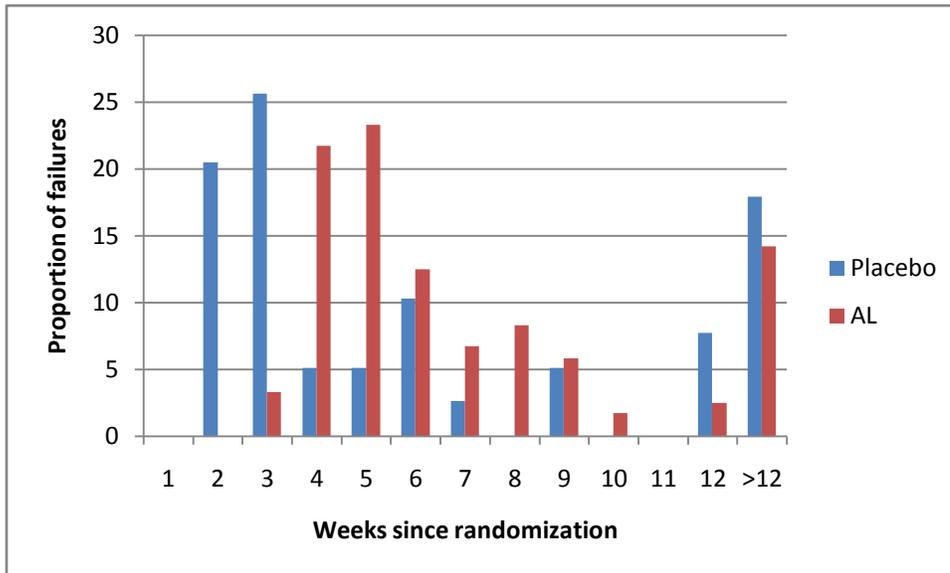


Figure 4: Proportion of first clinical malaria episodes occurring at weekly intervals

Further scrutiny reveals that a small proportion of participants in the AL arm started experiencing clinical episodes of malaria in the third week following randomization, with the bulk of participants getting clinical episodes in the fourth, fifth and sixth weeks, unlike participants in the placebo group, most of whom experienced their episodes in the second and third weeks after randomization (Figure 4). This is also evident in the Kaplan-Meier curve in figure 5.

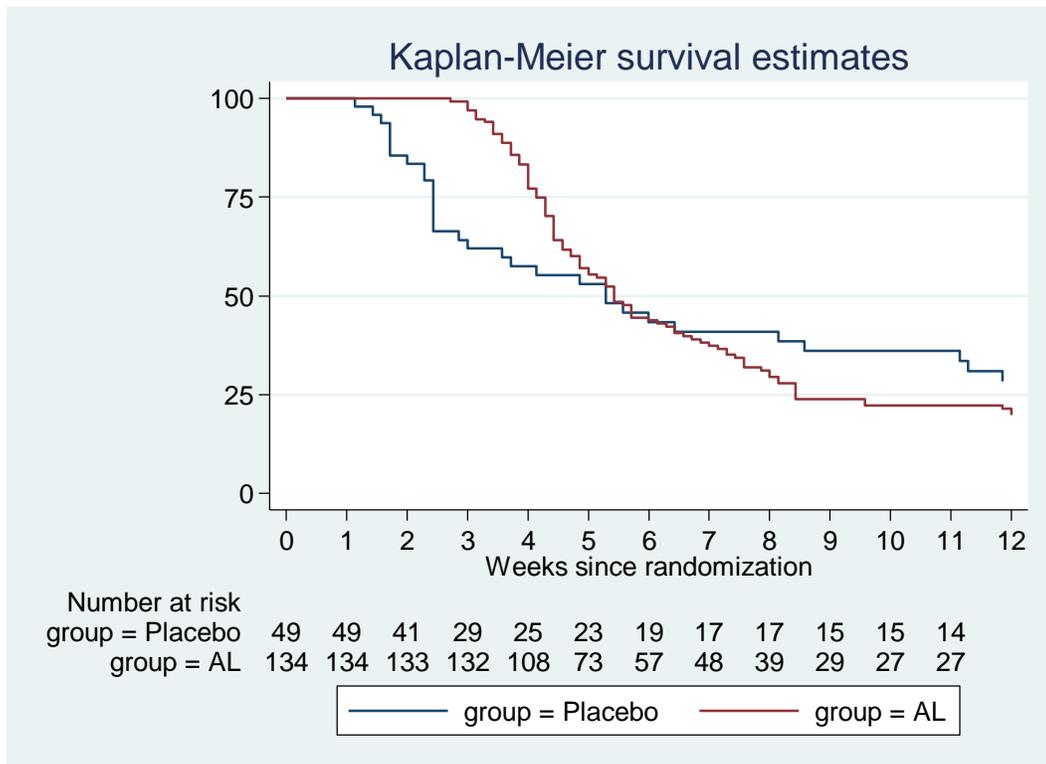


Figure 5: Kaplan-Meier curve showing the proportion of participants remaining event-free

The median time to clinical episode calculated using the Kaplan-Meier estimates was 5.29 weeks (95% confidence interval 2.86 to 11.14 weeks) for the placebo arm, while that of the AL arm was 5.43 weeks (95% confidence interval of 4.86 to 6.43 weeks). Adjusted hazard functions were calculated at weekly intervals, and these were used to estimate the protective efficacy of AL against the first clinical episode of malaria at weekly intervals (Figure 6). There were no events in the first week in both treatment arms; therefore the hazard ratio for the first week was not calculated.

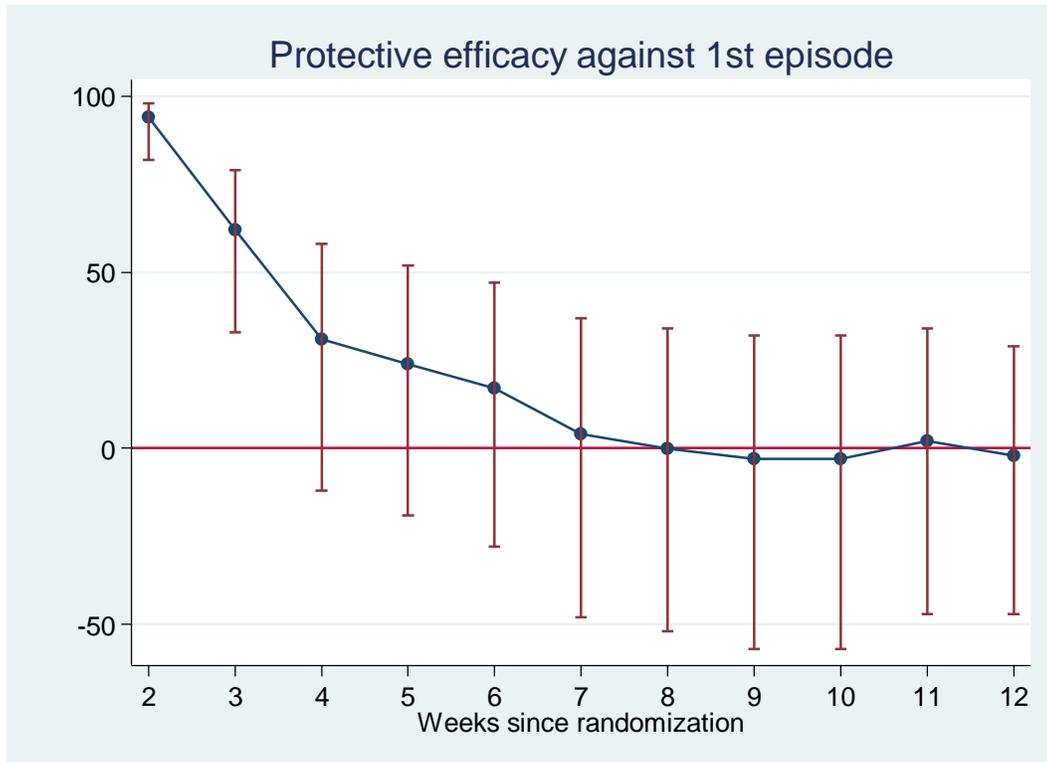


Figure 6: Protective efficacy of AL at weekly intervals

Error bars indicate 95% Confidence Intervals

The protective efficacy of AL was greatest during the second and third weeks - 94% (95% confidence interval 82 to 98%, p-value <0.001) and 62% (95% confidence interval 33 to 79%, p-value =0.001). From the fourth week onwards, there was little or no evidence of protection against clinical malaria being offered by AL.

4.4 Multiple clinical malaria episodes

Table 5: Number of multiple events

	Placebo	AL
Number randomized	135	135
Number at risk of multiple events	117	119
Proportion experiencing multiple events*	98 (83.8%)	102 (85.7%)
Total Number of Multiple events	328	344
Mean events per subject (range)	4 (0 – 8)	4 (0 – 6)

*Chi-square p-value=0.676

Observation for multiple clinical malaria episodes for each participant began after the participant experienced his first clinical malaria episode after randomization. Thus, 117 and 119 participants in the placebo and AL arms respectively were at risk of developing multiple events at the beginning of the observation period (Table 5). Those participants who did not experience any clinical episode during the study duration were therefore excluded from the analysis for multiple malaria episodes. A total of 328 and 344 multiple malaria episodes were recorded in the placebo and AL arms respectively over the observation period. The mean number of episodes per participant was 4 in both arms, with the number of episodes per participant ranging from 0 – 8 in the placebo arm and 0 – 6 in the AL arm (Table 5).

The proportion of participants experiencing at least one event in the two groups was not significantly different (83.8% vs. 85.7%, p-value =0.676), as were the proportions experiencing specific number of events during the observation period.

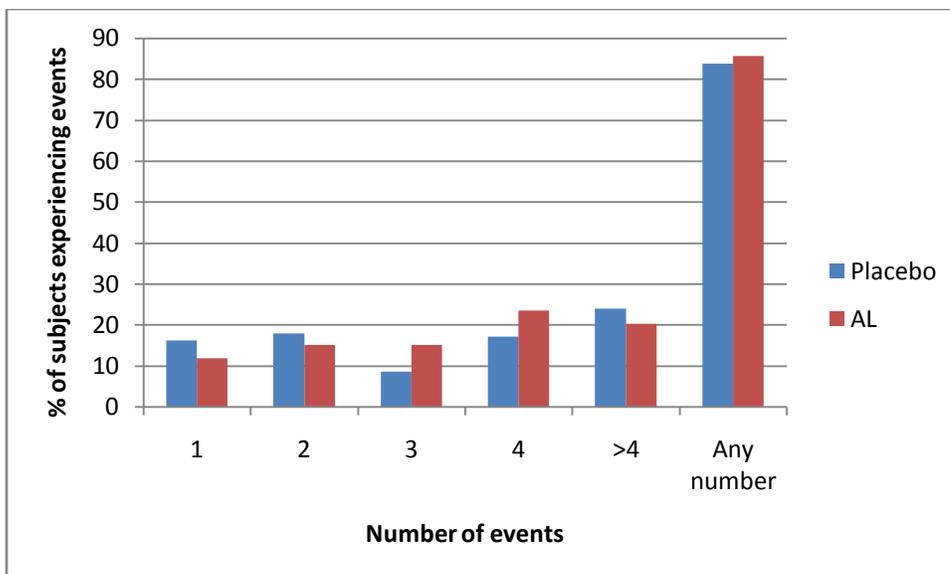


Figure 7: Proportion experiencing multiple clinical malaria episodes

Table 6: Multiple clinical malaria event rates at 4 week intervals

4 week interval	Placebo			AL		
	Person-weeks at risk	Number of events	Rate	Person-weeks at risk	Number of events	Rate
(0 - 4]	0	0	0	0	0	0
(4 - 8]	118.43	25	21.11	30.57	2	6.54
(8 - 12]	176.00	37	21.02	211.71	31	14.64
(12 - 16]	198.43	21	10.58	238.14	26	10.92
(16 - 20]	229.14	27	11.78	282.71	29	10.26
(20 - 24]	229.43	27	11.77	260.29	39	14.98
(24 - 28]	209.57	32	15.27	250.86	28	11.16
(28 - 32]	216.86	20	9.22	264.71	24	9.07
(32 - 36]	204.00	30	14.71	263.43	33	12.53
(36 - 40]	172.71	25	14.47	209.00	35	16.75
(40 - 44]	158.71	22	13.86	210.29	23	10.94
(44 - 48]	136.71	29	21.21	185.57	31	16.71
(48 - 52]	70.71	26	36.77	92.43	32	34.62
> 52	4.00	7	175.00	10.71	11	102.67
Overall	2125.86	328	15.43	2510.43	344	13.70

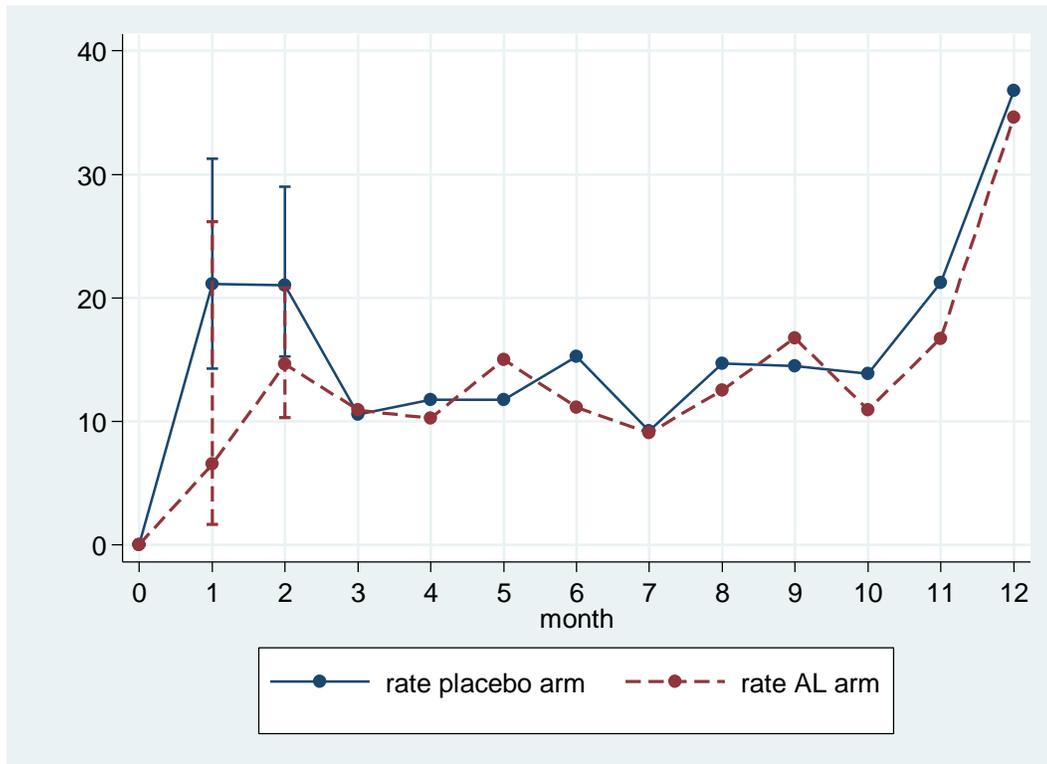


Figure 8: Multiple clinical malaria event rates at monthly intervals.

Error bars indicate 95% Confidence Intervals

The monthly event rates were lower in the AL arm up to the second month, though the difference was not statistically significant (Table 6, Figure 8). During the rest of the observation period, the rates were generally similar, with an overall rate of 15.43 episodes/100 person-weeks in the placebo arm and 13.70 episodes/100 person-weeks in the AL arm (Table 6). The ratios of the event rates in the AL arm to the placebo arm also showed no evidence of increased risk in either treatment arm at different time points,

with an overall adjusted rate ratio (AL/placebo) of 0.90 (95% confidence interval of 0.76 to 1.06, p-value=0.205).

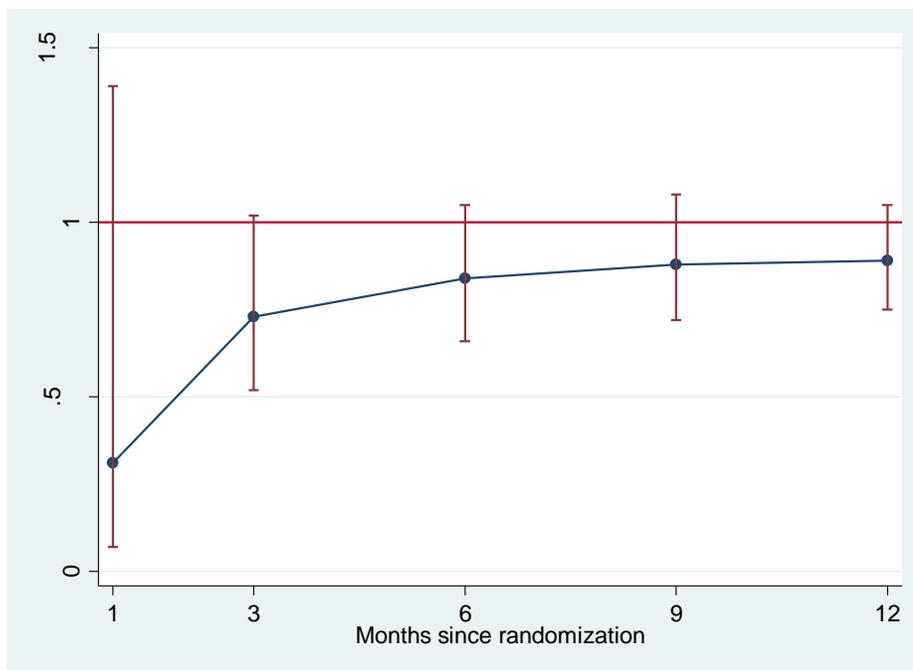


Figure 9: Rate ratios (AL/Placebo) of multiple clinical malaria episodes
Error bars indicate 95% Confidence intervals

Table 7: Rate ratios of multiple clinical malaria episodes at specific intervals

Months since randomization	Rate ratio	P-value
1	0.31 (0.07 to 1.39)	0.126
3	0.73 (0.52 to 1.02)	0.065
6	0.84 (0.66 to 1.05)	0.132
9	0.88 (0.72 to 1.08)	0.232
Overall (Unadjusted)	0.89 (0.75 to 1.05)	0.169
Overall (Adjusted)	0.90 (0.76 to 1.06)	0.205

CHAPTER FIVE: DISCUSSION

5.1 Main findings

This analysis describes the effect that the administration of Artemether-Lumefantrine (AL) in healthy children has on the risk of clinical episodes of malaria. The results of this analysis demonstrate that AL has little effect on the time to first clinical malaria episode – the median time to first clinical episode was 5.29 weeks (95% confidence interval 2.86 to 11.14 weeks) for the placebo arm, while that of the AL arm was 5.43 weeks (95% confidence interval of 4.86 to 6.43 weeks). This is explained by the fact that the protective effect of AL against the first clinical malaria episode is short lived – there was no evidence of protection beyond the third week after randomization. This is in turn explained by the relatively short half-life of AL – the Lumefantrine component has a half-life of 32.7 hours. Therefore, in order to achieve sustained protection, AL would have to be administered probably every two weeks or perhaps more frequently, which is clearly not a feasible venture. As such, despite the proven efficacy and tolerability of AL, its short acting properties make it unsuitable for IPT of malaria. This observation also suggests that IPT works by and large via the chemoprophylactic mechanism, since the short duration of protection is linked with the pharmacokinetic properties of the drug in question.

Additionally, the results of this analysis show no evidence of change of risk for multiple episodes of clinical malaria following the administration of AL in asymptomatic children – the overall rate ratio (AL/placebo) for multiple episodes, adjusted for age, parasitemia at randomization and ITN use was 0.90 (95% confidence interval of 0.76 to 1.06, p-

value=0.205). Hence, there was no rebound in the risk of multiple malaria episodes following the administration of AL.

5.2 Overall evidence

These findings are consistent with those of other studies that investigated the protective effect of short-acting drugs when administered as part of IPT of malaria and those that investigated the rebound of the risk of malaria after IPT. The results of an IPTi study conducted in Western Kenya had indicated that two long-acting combinations (Sulphadoxine-Pyremethamine/Artesunate and Amodiaquine/Artesunate), but not a short-acting combination (Chlorproguanil-Dapsone) provided significant protection against the first or only episode of clinical malaria and anemia in children living in an area with similar malaria transmission characteristics as Kombewa (Odhiambo et al., 2010). The study by Odhiambo et al demonstrated protective efficacy that lasted up to 8 weeks for the long acting drugs and no evidence of protective efficacy for the short acting drug. Another study that compared Mefloquine and Chlorproguanil-Dapsone for IPTi, but conducted in slightly different malaria transmission settings in Northern Tanzania showed that IPTi with the long acting drug mefloquine reduced the incidence of clinical episodes of malaria substantially in infants, whereas the short acting drug chlorproguanil-dapsone did not (Gosling et al., 2009b). Odhiambo et al also investigated the rebound effect of IPTi, and they did not observe any significant change in risk of clinical malaria episodes, defined by using any parasitemia level > 0 , following the administration of IPTi, which is consistent with the findings of several other studies that found no rebound effect following IPT (Odhiambo et al., 2010). A cluster randomized trial of IPTi

conducted in Northern Ghana however did observe a 20% rebound in high density malaria (defined as malaria with parasitemia >5000) effect following IPTi (Chandramohan et al., 2005).

5.3 Strengths and Limitations

The weekly malaria smears done as part of the active surveillance for the original study, together with the passive surveillance that was facilitated by the use of the field stations by the participants provided valuable data that enabled the exact determination of the time at which clinical malaria episodes occurred. This is in contrast with the Demographic Surveillance System that chiefly relies on the recall of the respondents.

Some limitations of the present analysis should however be noted. The sample size that was subsequently analyzed was considerably lower than what was initially intended due to two circumstances. First, the analysis of the time to first clinical malaria episode necessitated the exclusion of placebo recipients who had a positive malaria slide at randomization from the analysis, so as to allow for a fair comparison of the treatment groups. Participants who had asymptomatic parasitemia could have been cases having an incipient infection waiting to present clinically. AL was expected to clear any circulating parasites, and as such incipient malaria episodes were averted in participants in the AL group. The placebo recipients were not expected to receive a similar benefit, and hence they were excluded from the analysis. However, enrollment into the study took place at the peak of the malaria season and therefore the prevalence of asymptomatic parasitemia was quite high among the study cohort – more than 60% of the participants had positive smears at randomization. Consequently, the number of participants included in the

analysis for first clinical malaria episodes in the placebo arm was low (49 in total vs. 135 randomized), and some of this cohort's baseline characteristics differed from that of the AL group. The investigators of the original study failed to take this issue into consideration. Secondly, there was a high drop-out rate, with 31% of the subjects failing to complete the 12 month follow-up. A 10% loss to follow-up is usually considered acceptable in epidemiological studies, and previous studies conducted at the research center generally had a loss to follow-up of less than 10%. The majority of participants failing to complete follow-up were those who withdrew their consent, with the reason for withdrawal being primarily their discomfort with the weekly blood draws that were performed on the children during the study. Consequently it may be argued that the study cohort may not have been representative of the general pediatric population living in an area of perennial malaria transmission with seasonal peaks. Additionally, with smaller numbers being analyzed, there is a high likelihood of observing the findings purely by chance. However, the results observed from this analysis are consistent with those obtained from similar studies, thus providing some reassurance that the validity of the findings was not severely affected by the relatively small numbers analyzed.

Another limitation was that the original study was not specifically set up to investigate the suitability of AL for IPT. Hence the participants only received the equivalent of one dose of IPT, since AL was given to healthy children only at randomization – subsequent administration of AL was for actual treatment of clinical episodes. In other IPT studies, the participants would receive three doses of the anti-malarial under investigation.

Despite this limitation, the analysis still does provide information that could be useful when selecting drugs for use in IPT.

5.4 Future research

IPT will remain in the arsenal of weapons to be used in the fight against malaria for the foreseeable future. Now that the unsuitability of short-acting drugs for IPT is not in doubt, the problem of finding a suitable replacement for SP as the drug of choice for IPT still remains. Mefloquine, which is a long-acting anti-malarial drug that can be administered as a single dose, has been touted as a possible replacement, however its tolerability issues still raise doubt on its suitability. Amodiaquine also has a long half-life and its use has been suggested for IPT; however its drawback is the need to administer it as a three-day course. Piperaquine is another potential candidate – it is long-acting, and can be administered as a single dose, but it has not been rigorously and widely assessed as a potential replacement for SP. This drug thus needs to be further assessed, and probably combined with another long acting anti-malarial.

5.5 Conclusion

In conclusion, Artemether-Lumefantrine administered in healthy children has little influence on the time to first clinical malaria episode and the risk of multiple clinical malaria episodes.

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