THE EFFICACY AND SAFETY OF ARTEMISININ-BASED COMBINATION THERAPY FOR THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN NON-PREGNANT ADULTS AND CHILDREN: A SYSTEMATIC REVIEW

By

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The work described in this dissertation 'the effectiveness and safety of artemisinin-based combination therapy for the treatment of uncomplicated *Plasmodium falciparum* malaria in non-pregnant adults and children: a systematic review for the Master of Science was carried out in the School of Biochemistry, Genetics and Microbiology, University of KwaZulu-Natal, Pietermaritzburg, from July 2008 to June 2010, under the supervision of Professor Dean J.P. Goldring.

This study represents original work by the author and has not otherwise been submitted in any form for any degree or diploma to any tertiary institution. Where use has been made of the work of others it is duly acknowledged in the text.

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DETAILS OF CONTRIBUTION TO PUBLICATIONS that form part and/or include research presented in this thesis (include publications in preparation, submitted, *in press* and published and give details of the contributions of each author to the experimental work and writing of each publication)

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LIST OF ABREVIATIONS

A	artemisinin
ACT	artemisinin-based combination therapy
AE	arteether
AL	artemether-lumefantrine
ALAT	alanine aminotransferase
AM	artemether
AQ	amodiaquine
AS	artesunate
ATV	atovaquone
AZ	azithromycin
β-CDX	beta-cyclodextrin
BHC	benzenehexachloride
CENTRAL	Cochrane Central Register for Controlled Trials
CCT	quasi-randomized or controlled clinical trial
CD	chloroproguanil-dapsone
CI	confidence interval
CQ	chloroquine
CT	cotrimoxazole
D	deoxycycline
DDT	dichlorodiphenyltrichloroethane
DHA	dihydroartemisinin
DHPS	dihydropteroate synthetase
DHFR	dihydrofolate reductase
DNA	deoxyribonucleic acid
DRC	Democratic Republic of Congo
EM	environmental management
ETF	early treatment failure
FCT	fever clearance time
GDP	gross domestic product
GMEP	global malaria eradication programme
GST	glutathione-S-transferase
IRS	indoor-residual spraying
IUGR	intra-uterine growth retardation

ITN	insecticide treated net
KDR	knockdown resistance
LBW	low birth weight
LCF	late clinical failure
LLIN	longer-lasting insecticidal nets
LPF	late parasitological failure
LTF	late treatment failure
MB	Methylene-blue
MDR	multi-drug resistance
mRCT	*meta*Register for Controlled Trials
MQ	mefloquine
n	number of patients reporting an outcome
N	total number of patients examined
NR	not reported
PCR	polymerase chain reaction
PCT	parasite clearance time
PFCRT	*Plasmodium falciparum* chloroquine resistance transporter
PFMDR	*Plasmodium falciparum* multidrug resistance
PPQ	piperaquine
PQ	primaquine
PRG	proguanil
Q	quinine
QARCG	Qinghaosu Antimalarial co-ordinating research group
RBC	red blood cells
RevMan	Review Manager
RDT	rapid diagnostic tests
RR	relative risk
SD	standard deviation
SMP	sulfamethoxy-pyrimethamine
SP	sulfadoxine-pyrimethamine,
TF	treatment failure
tRNA	transfer-ribonucleic acid
WHO	World Health Organization
WMD	weighted mean difference

ABSTRACT

Effective case management of malaria is hampered by the spread of parasite resistance to non-artemisinin antimalarials. To counteract the impact of drug resistance, the World Health Organization (WHO) has endorsed artemisinin-based combination therapy (ACT) as the first-line treatment for uncomplicated *Plasmodium falciparum* malaria. Currently recommended ACTs are artemether-lumefantrine, artesunate plus amodiaquine, artesunate plus mefloquine, artesunate plus sulfadoxine-pyrimethamine and dihydroartemisinin-piperaquine.

This study sought to review evidence of the efficacy and safety of different non-artemisinin antimalarials in combination with artesunate, artemether or dihydroartemisinin for the treatment of uncomplicated *P. falciparum* malaria in non-pregnant adults and children. The search for randomized controlled trials (RCTs) was conducted in the Cochrane Central Register for Controlled Trials (CENTRAL), MEDLINE, EMBASE and in ClinicalTrials.gov in January 2009. The eligibility and the methodological quality of trials were assessed and data were extracted, using standard forms. Data were captured and analyzed in Review Manager Software, versions 4.2 and 5.0. The outcomes assessed were: treatment failure, fever and parasite clearance time, calculating the relative risk (RR) and a weighted mean difference (WMD) with a 95% confidence interval and p-values, indicating statistical significance at 0.05.

Thirty-seven trials with 6862 participants were included. Artesunate combined with amodiaquine had a statistically significant lower risk of treatment failure compared to the combination of artesunate with sulfadoxine-pyrimethamine (RR=0.57, 95% CI [0.33, 0.97],

p=0.04, seven trials, N=1341). In addition, treatment with artesunate plus mefloquine was significantly associated with a lower risk of treatment failure compared to artesunate plus azithromycin (RR=0.04, 95% CI [0.00, 0.64], p=0.02, one trial, N=54). There was no significant difference when either mefloquine or atovaquone-proguanil were combination partners with artesunate (RR=2.6, 95% CI [0.93; 7.24], p=0.07, one trial, N=1066). When artesunate was combined with chloroquine, primaquine or azithromycin and compared with artesunate monotherapy, there was no statistically significant difference in the risk of unadjusted treatment failure. Each of these comparisons had one trial each. Artesunate plus chloroquine was quicker at clearing fever compared to artesunate plus sulfadoxine-pyrimethamine (WMD= -7.20, 95% CI [-12.53, -1.87], one trial, N=132).

Few trials adequately reported adverse events. There was no significant difference observed in the risk of adverse events between artesunate plus amodiaquine compared with artesunate monotherapy, however, adverse events were significantly less in artesunate plus amodiaquine compared to artesunate plus methylene-blue. Artesunate plus amodiaquine on the other hand had significantly more adverse events reported compared to artesunate plus sulfadoxine-pyrimethamine.

The findings of this study support the implementation of artemisinin-based combination therapy for the treatment of uncomplicated malaria. Most crucially, this review found a greater advantage of combining amodiaquine with artesunate compared to sulfadoxine-pyrimethamine. The efficacy of artesunate plus mefloquine was superior to that of artesunate plus azithromycin. Furthermore, the combination of artemisinins with chloroquine, primaquine and azithromycin has shown very low efficacy and these combination therapies should not be

recommended. The reporting of efficacy was not standardized as many trials did not differentiate between re-infections and recrudescences. Adverse events were also not adequately reported.

CHAPTER 1: INTRODUCTION

1.1   The burden of malaria

Malaria remains one of the most devastating parasitic infections and a major cause of morbidity and mortality in tropical and sub-tropical regions of the world. It is closely intertwined with underdevelopment and flourishes in situations of social and environmental crisis and weak health systems (WHO, 2009). Malaria disease is due to blood infection by protozoan parasites of the genus *Plasmodium*, which are transmitted from one human to another by female *Anopheles* mosquitoes. Rarely transmission can be through accidents such as blood transfusion or through the placenta from an infected mother to her unborn child. There are four well known species of *Plasmodium* that cause malaria in humans; *Plasmodium falciparum, P. vivax, P. ovale* and *P. malariae*. The other *Plasmodium* parasite previously thought not to cause malaria in humans, *P. knowlesi* has also been identified as one of the causes of naturally acquired malaria in humans. Almost all severe disease and deaths are due to *P. falciparum* infections (Gillies, 1993; Gupta *et al*., 1994; Breman, 2001).

It is estimated that 2.2 billion people are exposed to the threat of malaria globally and there are about 515 (range 300-660) million clinical attacks per year attributed to *P. falciparum*. Most clinical events of malaria are concentrated in Africa, about 70.9%, 23.1% in South-East Asia, 2.9% in the Western Pacific region, 2.3% in the Eastern Mediterranean, 0.7% in America and 0.1% in Europe (Snow *et al*., 2005). In high transmission areas, pregnant women and children under the age of five years bear the heaviest burden of malaria compared to non pregnant adults (Murphy and Breman 2001; Breman 2001; Steketee *et al*., 2001).

The world malaria report (WHO, 2009) estimated that malaria accounted for an estimated 863 000 deaths in 2008, with 89% of those occurring in Africa, while South East Asia and the Eastern Mediterranean account for 4.64 and 6% of the malaria deaths, respectively. The Western Pacific region has 0.35% of the total malaria deaths whereas the Americas has 0.12%. Europe has the lowest burden of malaria, with no deaths recorded (WHO, 2009). In highly endemic areas, malaria is considered to be the main cause of death in children and in pregnant women (Murphy and Breman 2001; Steketee *et al.*, 2001).

Malaria poses an economic burden and is related to poverty. Geographically, malaria and poverty are both concentrated within the same boundaries. Malaria-endemic countries are not only poorer than non-malarious countries but they also have lower rates of economic growth. In 1995, the gross domestic product (GDP) *per capita* in malarious countries was estimated to be US$1.526 compared to US$8.268 in countries without intensive malaria transmission (Gallup and Sachs, 2001). Malaria impacts on trade, tourism, direct foreign investments and is associated with high fertility rates, which in turn reduces the likelihood of schooling in poor households (Sachs and Milaney, 2002). It is estimated that children already attending school miss 4.3 to 11% of school days due to malaria (Leighton and Foster, 1993) and up to 50% of medically related school absences are attributed to malaria (Brooker *et al.*, 2000). Absenteeism increases failure rates, repetition of school years and drop out rates (Sachs and Malaney, 2002). An African family may spend up to 25% of their income on malaria prevention and treatment and African countries may lose up to US$12 billion in lost GDP *per capita*, which may considerably retard economic development (Gallup and Sachs, 2001).

1.2   The life cycle of *Plasmodium* parasites and *Anopheles* mosquitoes

1.2.1   The *Plasmodium* parasite life cycle in the human host

Although the human malaria parasite species are closely related, there are major differences among them with regards to the severity of their infection and their geographical distribution. Infection caused by *P. vivax* is the most widely distributed and the most common in temperate regions of the world. *P. falciparum* is the most clinically dangerous and the most widespread in sub-Saharan Africa and throughout the world's tropics. In South Africa, the most prevalent plasmodium species is *P. falciparum* (Snow *et al*., 2003). *P. ovale* malaria is more common in West Africa and *P. malariae* has a similar geographic range as *P. falciparum*, although it is much less prevalent and occurs in more restricted zones. Infection with *P. vivax* may relapse months after the initial infection and if inadequately treated, *P. malariae* may persist for several years (Garnham, 1988; Oaks, 1991). *P. malariae* and *P. knowlesi* are difficult to distinguish microscopically, leading to misidentification of the two species (Cox-Singh *et al*., 2008).

Malaria parasites have a complex life-cycle involving vertebrate hosts and mosquito vectors as shown in figure 1.1. The life cycle starts when an infected *Anopheles* mosquito takes a blood meal, sporozoites are inoculated into the human blood, 15-20 sporozoites are injected at a bite (Bruce-Chwatt, 1985; Oaks, 1991). Once injected, the sporozoites infect the liver, entering the hepatocytes where they begin to divide into hepatic schizonts. This stage is called tissue schizogony. Each infected hepatic schizont ruptures and releases merozoites, which then invade erythrocytes or red blood cells (RBC). Approximately 30 000 merozoites are released after 5-6 days by each infected hepatocyte, a single schizont rupture releases 32 merozoites (Bruce-Chwatt, 1985; Oaks, 1991).

In the erythrocytes, the merozoites develop into early trophozoites, which are ring shaped, vacuolated and uni-nuclealated, these trophozoites consume haemoglobin in RBCs (Mauritz *et al*., 2009). The infected erythrocytes are eventually lysed by merozoites, which subsequently invade other erythrocytes, starting a new cycle of schizogony. The parasite burden expands logarithmically by approximately 10-fold per cycle. All the clinical symptoms of malaria (such as fever, malaise, anaemia) are associated with this multiplication of blood stage parasites, especially the bursting of infected RBCs (Bruce-Chwatt, 1985; Oaks, 1991).
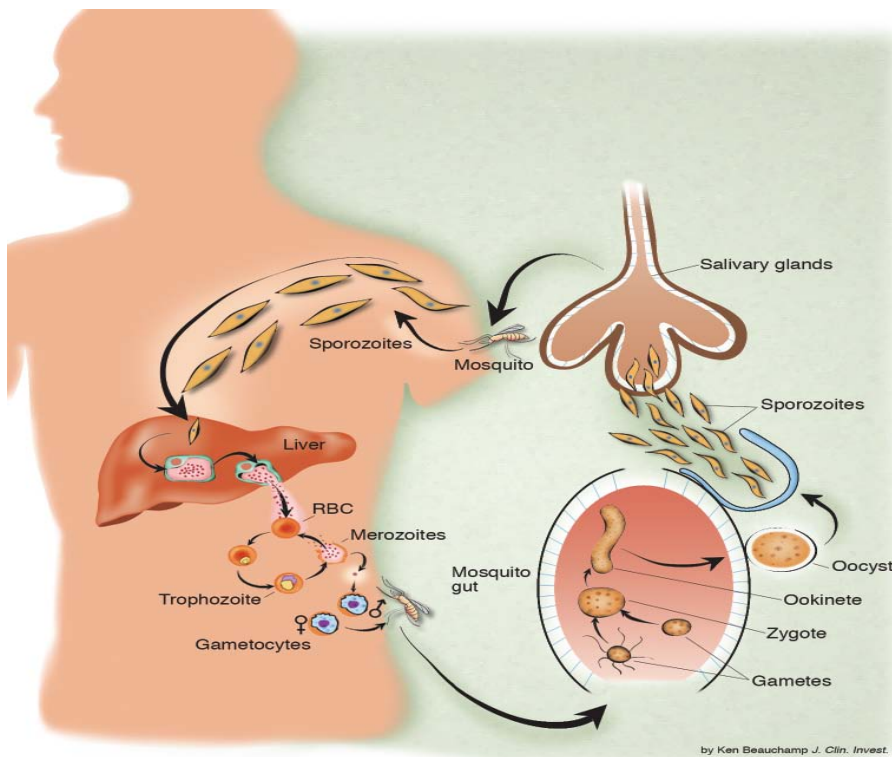
Figure 1.1: The life cycle of *Plasmodium* parasites in the human host and the mosquito vector. Adapted from White (2004).

After several cycles, some merozoites develop into gametocytes, the sexual forms of malaria parasites, which do not cause symptoms but are infective to mosquitoes. Gametocyte development continues in the human host over a period of approximately 10 days,

encompassing five morphologically defined gametocyte stages, ending with the formation of mature male and female gametocytes. In the first three stages, the sexual parasites are sequestered and they are potentially susceptible to drugs used to treat asexual stage parasites. In stage four, they re-enter the circulation and by stage five, they are resistant to most drugs (Bruce-Chwatt, 1985; Oaks, 1991).

1.2.2   The *Plasmodium* parasite life cycle in the mosquito vector

There are more than 400 species of *Anopheles* worldwide, however, only about 60 are vectors of malaria parasites and only 30 are vectors of major importance (Bruce-Chwatt, 1985). In a particular region, only one or a few *Anopheles* mosquito species serve as the predominant malaria vectors. The most efficient vectors for *P. falciparum* in the Afrotropical region (Sub-Saharan Africa, Madagascar, Seyhelles and Mauritius) are in the *A. gambiae* complex (which includes *A. gambiae, A. arabiensis, A. melas, A. merus, A. bwambae* and *A. quadriannulatus)* and *A. funetus* (Breman, 2001). *A. gambiae* has the highest rate of sporozoite development and exists in frost free regions or where the temperature remains above 5°C (Leeson, 1931; Gillies and De Meillion, 1968).

Among the *A. gambiae* complex, *A. gambiae sensu stricto* is the most important vector and it is probably the world's most efficient malaria vector. It bites humans both indoors (endophagic) and outdoors (exophagic), it rests mainly indoors (endophilic) but it may also rest outdoors (exophilic) (Service, 1996). The adult female *Anopheles* mosquitoes require protein from blood meals for their eggs to mature. They generally feed only between sunset and dawn and they prefer to feed near the ground level often selecting to feed on the lower leg rather than the arms and the upper body. About 81% of bites by *A. arabiensis* occur on the

ankles or feet. *A. arabiensis* has similar breeding and biting habits to *A. gambiae s.s.*, except that the mosquito tends to occur in drier areas and it is more likely to bite cattle and rest outdoors (Service, 1996).

The mosquito undergoes four stages of growth, namely egg, larva, pupa, and adult. Adult females mate once and store the sperm. The female may deposit a total of 200 to 1,000 eggs in three or more batches (Morrow, 2007). The actual egg production is dependent on blood consumption. After hatching, anopheline larvae lie along the water-air interface, where they feed on organisms along the surface film. Adult mosquitoes develop from the pupa stage within 2 to 4 days. An adult mosquito will emerge from the egg stage in 7 to 20 days, depending on the species of mosquito and environmental conditions (Oaks, 1991).

Female anopheline mosquitoes can survive at least a month under favorable conditions of high humidity and moderate temperatures. That is sufficient time for them to take a blood meal, for the parasite to develop, and the mosquito to take another blood meal and thus transmit the parasite to a second human host (Oaks, 1991). When mature gametocytes are taken up by a mosquito as part of a blood meal, sexual development continues in the mosquito gut; the male gametocyte undergoes three rounds of mitosis leading to the formation of eight highly motile haploid gametes. The process is called exflagellation. Female gametocytes enlarge to form female gametes. Fertilization of a single male and female gamete forms a diploid zygote, which then differentiates into a motile ookinete (Morrow, 2007). After traversing the peritrophic matrix, which is a chitin-containing extracellular layer surrounding the blood bolus, the ookinetes cross the midgut epithelium and lodge beneath the basal lamina facing the mosquito body cavity (hemocoel). During the next 10-15 days (depending on the parasite

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species and temperature), they differentiate into mature oocysts. Each oocyst produces thousands of sporozoites that are released into the hemocoel and invade the salivary glands. When the mosquito takes a blood meal, sporozoites leave with the saliva which includes an anti-coagulant, infecting the new vertebrate host where the asexual part of the life cycle takes place (Bruce-Chwatt, 1985; Oaks, 1991).

1.3   The transmission of malaria

Malaria is governed by a large number of environmental factors, which affect its distribution, seasonality and transmission intensity (Snow *et al*., 1999). The environmental drivers are determined by the sensitivity of anopheline vectors and its particular plasmodium parasite to climate and thus latitude and elevation (Macdonald, 1956; Molyneux, 1988; Oaks, 1991).

Ambient temperature plays a major role in the cycle of both the malaria parasite and its vector. The development of the parasite within the mosquito (sporogonic cycle) is dependant on temperature. On average, the sporogonic cycle takes about 9-10 days at temperatures of 28°C and stops at temperatures below 16°C. The daily survival of the vector is dependant on temperature as well. Generally, at temperatures between 16 and 36°C, the daily survival is about 90%. This survival drops rapidly at temperatures above 36°C. The highest propotion of vectors surviving the incubation period is observed at temperatures between 28 and 32°C (Mcdonald, 1956; Oaks, 1991)

Rainfall provides breeding sites for mosquitoes to lay their eggs. It is also related to humidity, thus affecting mosquito survival. Though flooding often causes destruction of breeding sites and a temporary reduction of vectors, it never eliminates the vectors. *A. gambiae* mosquitoes

breed readily in large and small collections of sun-exposed still water, which exist throughout tropical Africa (Mcdonald, 1956; Oaks, 1991). In regions where temperature is high but rainfall is limiting, mosquito populations increase rapidly at the onset of rain because of short development cycles. In areas where the temperature is low, mosquito populations increase slowly at the onset of rain, and parasite development takes a long time (Craig *et al*., 1999).

Regions can therefore be classified according to the suitability of conditions for transmission and disease patterns. There are regions with perennial transmission, where climatic conditions are always suitable for transmission and the disease is endemic. Some regions have seasonal malaria transmission, where conditions become most suitable during a certain period in a given year. There are epidemic regions, where conditions are unstable and suitable for transmission on an irregular basis and there are malaria free regions, where conditions are always unsuitable for malaria transmission (Craig *et al*., 1999).

The level of malaria transmission in a given area is measured using a number of malariometric indexes. These include the entomologic inoculation rate, vector capacity, reproductive rate, parasite prevalence and the disease incidence. The entomologic inoculation rate is expressed as the number of infective mosquito bites per person per unit time. The reproductive rate is the mean number of secondary cases (infections) that result from a single infective case introduced in a susceptible population (Martens, 1999). Vector capacity is defined as the average number of inoculations with a specified parasite, originating from one case of malaria in unit time, which the population would distribute to man if all the vectors biting the case became infected (Garrett-Jones, 1964). Parasite prevalence is defined as the proportion of a sampled population that is confirmed positive for malaria parasites, canonically by identifying

immature "ring stage" trophozoites in stained blood film slides (Gillies, 1993). Lastly, disease incidence is measured as the number of new infections in a defined period.

1.4 The symptoms of infection and immunity to malaria

1.4.1 The symptoms of malaria infection

The early signs and symptoms of uncomplicated malaria tend to be non-specific and they often resemble those of common viral infections. Common manifestations include fever, chills, malaise, abdominal discomfort and mild anaemia. Fever patterns are usually described with spikes every two days in *P. falciparum*. Other common symptoms include rigors, headache, cough, diarrhoea, dizziness, nausea, vomiting, sweating and weakness (Snow *et al*., 1999).

In a small proportion of *P. falciparum* infections, unrestricted parasite multiplication leads to heavy parasite burdens, leading to severe malaria. The major complications of severe malaria include cerebral malaria, coma or repeated convulsions, respiratory distress, pulmonary oedema, acute renal failure, severe anaemia, acidosis, hypoglycemia and hyperlactataemia. Any of these complications can develop rapidly and progress to death within hours or days (WHO, 2000; Murphy and Breman, 2001; Dzeing-Ella *et al*., 2005). However, the relationship between transmission intensity and disease outcome is not simple and straight forward and depends, among other things, on the immune status of the human host.

1.4.2 Immunity to malaria

People living in endemic areas appear to acquire some degree of immunity to malaria as a result of repeated exposure to *P. falciparum* parasites from infancy (Bunn *et al*., 2004). This immunity is acquired slowly, however, a state of sterile immunity against all infections is

never attained (White, 2004). Immunity is acquired at a variable rate and it is species-, stage-, strain- and variant-specific (Wipasa *et al*., 2002). Immunity to malaria is often rapidly lost when an individual moves away from endemic areas (Keenihan *et al*., 2003). In low transmission areas, adults do not get repeated exposure to malaria parasites from infancy, thus the risk of the severity of infection is equivalent among all age groups (Snow *et al*., 1999). In non-immune individuals, the majority of malaria infections are symptomatic and they often occur a day or two before parasites are detectable in the blood (White, 2004). Individuals in endemic areas often tolerate parasites without developing sysmptoms (Artavanis-Tsakonas *et al*., 2003). Epidemiological studies (Snow *et al*., 1997) have shown that under conditions of intense, perennial, stable transmission, the burden of morbidity and mortality is concentrated among the youngest age groups which are children under the age of five years.

1.4.2.1 Immunity in infants and young children

Young infants in malaria endemic areas are often protected from *P. falciparum* infection early in life, mainly as a result of trans-placentally acquired maternal antibodies (Akanmori *et al*., 1995). When infected, their infections tend to be of very low parasite density, frequently at the lower limit of detection by microscopy (Wagner *et al*., 1998). They also tend to be asymptomatic and infection is spontaneously cleared within four weeks (Franks *et al*., 2000). Achidi and co-workers investigated the duration of antibodies in infants in an endemic area in Nigeria and found that the concentration of maternal antibodies fell to minimal levels by the age of four months and infant antibody titres began to rise from about six months of age, following malaria exposure (Achidi *et al*., 1995). The passive transfer of antibodies from mother to child continues post-natally via breastfeeding as antimalarial antibodies are detectable in human breastmilk. Antibodies from breastmilk are believed to act only locally

within the gut as the bulk of breastmilk immunoglobulin is degraded in the intestines and very small amounts are absorbed in an active form into the systematic circulation (Leke *et al*., 1992).

1.4.2.2 Immunity in pregnant women

In pregnancy, malaria infection is recognized as the major public health problem throughout the world, causing serious complications especially in women who have a low level of acquired immunity before pregnancy (Nahlen, 2000). Its frequency and severity are greater during pregnancy than before pregnancy and among pregnant women than in non-pregnant ones (Diagne *et al*., 2000). The impact of malaria on pregnant women varies according to the intensity of transmission; this is also true for the level of immunity acquired by the mother (Cot and Deloron, 2003).

The most severe malaria infections in pregnant women are caused by *Plasmodium falciparum*. The other three human malaria parasites contribute to fewer infections and more moderate disease and relatively few deaths (Mendis *et al*., 2001; Steketee *et al*., 2001). However, a few reports of adverse consequences due to *P. vivax* in pregnancy exist (Nosten *et al*., 1999). Malaria infection in pregnancy increases the chance of maternal anaemia, abortion, stillbirths, premature birth, intrauterine growth retardation (IUGR) and low birth weight (LBW), which is defined as the weight <2500g, which is the greatest risk factor for neonatal mortality (Nahlen, 2000). These occur with great frequency in endemic areas and they threaten lives of both mother and child.

1.5   Malaria control

In general, malaria is a preventable and curable disease. The control of malaria infection has traditionally relied on two arms; controlling the anopheline mosquito vector and effective case management. A long hoped for third arm; an effective malaria vaccine has not yet materialized. For vector control the WHO's global malaria programme recommends indoor residual spraying (IRS) with an insecticide, where applicable dichlorodiphenyltrichloroethane (DDT), and the distribution of insecticide-treated bed nets (ITNs) to populations at risk as means to reduce and eliminate malaria transmission. For parasite control, prompt diagnosis of malaria cases and treatment with effective antimalarials is recommended (WHO, 2006a).

1.5.1   Malaria vector control

Vector control employs environmental management targeted at the larval stages of malaria vectors, prevention of human-vector contact via the use of insecticide-treated bed nets and indoor residual spraying with insecticides to reduce vector density. Chemicals used in vector control strategies broadly fall into five categories. The first are petroleum oils, which are sprayed into water thus forming a film which prevents the larvae and the pupae from breathing through the water surface. The natural constituents of flowers like pyrethrum, pyrethrins and synthetic derivatives like pyrethroids form another group. Lambda-cyhalothrin is also included in this group. Organochlorines which include benzinehexachloride (BHC), DDT and dieldrin, organophosphates such as malathion and temephos and carbamates, which include bendiocarb and propoxur constitute the three remaining groups (Phillips, 2001)

1.5.1.1 Environmental management for malaria vector control

Since the role of *Anopheles* mosquitoes was recognized in malaria transmission, malaria control experts also recognized the value of reducing mosquito larval habitats to eliminate malaria transmission (Bruce-Chwatt, 1985). The concept of modifying vector habitat to discourage larval development is generally referred to as environmental management (Walker, 2002).

Environmental management is used to reduce the number of breeding sites and therefore the overall population of vector species. It includes larviciding, drainage, flushing, filling and rendering river and lake margins unsuitable for Anophelene breeding (Shiff, 2002). However, in rural settings it is impractical to suggest source reduction as the only effective control effort for anophelines, particularly because breeding sites may be numerous and vectors are opportunists. Furthermore, their populations expand during rainy spells and they breed in a variety of situations, thus under such conditions attempting to limit the extent of suitable breeding habitats may not be successful. Nevertheless, when properly executed and maintained, its sustainability is relatively easy, particularly in urban areas (Killeen *et al*., 2004). Environmental management methods should be seriously considered in agricultural systems and in man-made environments it should be the first-line of defense in reducing the risk of malaria transmission (Najera and Zaim, 2002).

1.5.1.2 The use of insecticide-treated bed nets for malaria vector control

An insecticide-treated bed net is a mosquito bed net that repels, disables and/or kills mosquitoes that come into contact with the insecticide on the bed net material. There are two categories of insecticide-treated bed nets; the conventionally treated and the long-lasting

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insecticidal treated bed nets. Conventionally treated bed nets are the ones treated by dipping in an insecticide. The longer-lasting insecticide treated bed nets are factory-treated mosquito bed nets in which the insecticide is incorporated within or bound around the fibres of the netting material (WHO, 2007).

A treated mosquito bed net provides personal protection against malaria to the individual using it by acting as both physical and chemical barrier and therefore increases the efficacy of the mosquito bed net. The physical barrier prevents access by mosquitoes and reduces human-vector contact while the chemical barrier is provided by the insecticides and can kill adult mosquitoes directly or indirectly, further reducing contact with the human host (WHO, 2007). Although insecticide-treated bed nets are widely viewed as devices for personal protection, they may have community-wide effects as well. When full coverage is achieved, insecticide-treated bed nets are associated with reduced all-cause child mortality in sub-Saharan Africa (Binka *et al*., 1998; Howard *et al*., 2000; Lengeler, 2004; Gamble *et al*., 2006). This is because insecticide-treated bed nets can kill adult mosquitoes directly or force them to take longer routes in search of vertebrate blood. Overall, insecticide-treated bed nets have been shown to reduce the incidence of clinical attacks of malaria by an average of 50% in different epidemiological settings in Asia and Africa (Choi *et al*., 1995).

1.5.1.3 Indoor residual spraying for malaria vector control

Indoor residual spraying is the application of long-acting chemical insecticides on the walls and roofs of all houses and domestic animal shelters in a given area, in order to kill the adult vector mosquitoes that land and rest on these surfaces, it reduces the life span of mosquitoes

so that they are unable to transmit malaria parasites from one person to another (WHO, 2006a).

Indoor residual spraying became the mainstay of malaria control in the world following the availability of DDT and other insecticides in the 1940s. Its effectiveness against indoor resting mosquitoes led to the adoption of the Global Malaria Eradication Programme in 1955. The efforts of the Global Malaria Eradication Programme contributed significantly in reducing the burden of malaria; in the United States, Europe and in the former Soviet Union, malaria was completely eliminated. South East Asia, India and South America experienced a significant reduction in disease incidence. In Africa, malaria eradication was piloted from the 1950s to the 1970s in Benin, Burkina Faso, Burundi, Cameroon, Kenya, Liberia, Madagascar, Nigeria, Rwanda, Senegal, Uganda and the United Republic of Tanzania. These projects demonstrated that malaria was highly responsive to indoor-residual spraying, the vector population was decreased but transmission could not be interrupted, this led to a conclusion that malaria eradication was not possible and was substituted with malaria control (Najera, 1999). Only South Africa, Botswana, Namibia, Zimbabwe and Swaziland successfully sustained large scale malaria control operations based on indoor-residual spraying with insecticides (Mabaso *et al*., 2004).

Recently, there has been a renewed interest in scaling up indoor-residual spraying along with other vector control strategies (WHO, 2006a). It is noted, however, that even with the most effective vector control measures, the numbers of clinical cases remains substantial and effective case management is required for limiting morbidity, mortality and the economic impact of malaria (Winstanley *et al*., 2004).

1.5.2   Malaria parasite control

1.5.2.1 Effective case management for parasite control

Effective case management is a fundamental and an indispensable element for malaria control. It aims to limit the duration of the disease, prevent progression of mild to severe or complicated malaria, prevent death from severe malaria, prevent transmission of parasites and minimize the risk of selection and spread of drug resistance. Determinants of effective case management involve the early and accurate diagnosis of malaria as well as availability, affordability and accessibility of safe and effective antimalarials. Early and accurate diagnosis of malaria depends on high sensitivity and specificity of diagnostic tools and if implemented effectively, it will help to reduce unnecessary use of antimalarials (WHO, 2009).

Methods used for parasitological diagnosis are light microscopy and rapid diagnostic tests (Tagbor *et al*., 2008). In addition, there are techniques used to detect the parasite's deoxyribonucleic acid (DNA) based on polymerase chain reaction (PCR), which are highly sensitive and very useful for detecting mixed infections, in particular, at low parasite densities (Tagbor *et al*., 2008). PCR techniques are also useful for studies on drug resistance and other specialized epidemiological investigations but are not generally available in malaria endemic areas. According to the WHO guidelines, the results for parasitological diagnosis should be available within a short time (less than two hours) after presentation, if this is not possible, the patient must be treated on the basis of clinical diagnosis (WHO, 2006b).

There are different classes of antimalarials that act on the different stages of the parasite. The anti-plasmodial action of sulfonamides and sulfones was reported as early as 1937 (Wernsdorfer and Trigg, 1988) and they act on all multiplying stages in the parasite's life-

cycle. Their activity is limited by their slow and short lasting action and the need for high doses that may be toxic. Longer acting compounds like sulfadoxine and sulfadiazine have since been developed and are used in combination with dihydrofolate reductase inhibitors. Dihydrofolate reductase inhibitors like pyrimethamine and trimethoprim only affect the schizont stage of the parasite and they disrupt the folate pathway (Wernsdorfer and Trigg, 1988). Blood schizontocides such as chloroquine (CQ), Quinine (Q), mefloquine (MQ) and amodiaquine (AQ) only have effects on the erythrocytic stages of malaria parasites (Wernsdorfer and Trigg, 1988).

There are also antibiotics that have been used for the treatment of malaria. The first reports of antimalarial effect of antibiotics appeared in 1949 (Wernsdorfer and Trigg, 1988), when chlorotetracycline was found to be effective against avian and human malaria parasites. The antibiotics that were of potential value to malaria chemotherapy included chlorotetracycline, oxytetracycline, chloroamphenicol, clindamycin, erythromycin and doxycycline. Their schizontocidal and liver-stage activity has been shown, but their effects were slow to be manifested in comparison to other drugs such as chloroquine (Wernsdorfer and Trigg, 1988).

1.6   Challenges to malaria vector and parasite control

Despite the efficacy and effectiveness of interventions, malaria control is made difficult by several administrative and technical problems. Deficiencies in social structure, economic conditions, communication, administrative systems and health organization have posed difficulties in malaria control (Najera, 1999). Although the effectiveness of some control strategies has been proven, inadequate implementation, differing political and power views often come on the way of successful malaria control and elimination (Omari, 1988).

The main technical problems include, among other factors, insecticide and antimalarial resistance. The development and spread of insecticide resistance decreases the efficacy of interventions such as IRS and antimalarial resistance in parasites hinders effective management of malaria cases and contributes to the transmission of the disease.

1.6.1 The challenges of insecticide resistance in vector control

Insecticide resistance is defined as the ability of an organism to tolerate doses of a toxicant that would prove lethal to a majority of organisms in a normal population of the same species (Hemingway and Ranson, 2000). Resistance is genetically inherited and when an insecticide is applied, resistant organisms have a higher probability of surviving over susceptible ones, which increases the resistance gene and thus the resistant population over time (Coleman and Hemingway, 2007).

Two major mechanisms of resistance in mosquitoes have been shown, which are increased metabolism of insecticide which reduces the effective insecticide dose available at the target site and reduced target site sensitivity which leads to ineffective binding of a given dose of insecticide (Hemingway *et al*., 2004). Metabolic resistance mechanisms can be attributed to the enzyme glutathione-S- transferase (GST) and target site resistance can be attributed to nerve insensitivity governed by the knockdown resistance gene (*kdr* gene) (Chandre *et al*., 1999). Insecticides such as DDT and permethrin may also influence behavioral changes in insects by reducing the rate of mosquito entry into houses, by causing them to exit early from the house or by inducing a shift in biting times. The insecticide's penetration may be reduced by the alteration or thickening of mosquito's cuticle (Hemingway *et al*., 2004).

During the Global Malaria Eradication Programme in the 1950s and the 1960s, dieldrin resistance was recorded among most *A. gambiae s.l.* populations in Africa. In contrast, only a few cases of DDT resistance were reported at that time. The first report involved *A. gambiae s.s.* in Burkina Faso in 1967 and was attributed to the use of DDT in cotton pest control. After that, it was also recorded among *A. arabiensis* from Senegal (Brown and Pal, 1973; Mouchet, 1988; Lines, 1988). Resistance to DDT became wide-spread in the early 1970s and recently, it has been observed in Ghana, South Africa, Kenya and Cote-d'Ivore (Ranson *et al*., 2000; Hargreaves *et al*., 2003; Coetzee *et al*., 2006; Tia *et al*., 2006).

The first case of pyrethroid resistance in *A. gambiae s.l.* was reported in Cote-d'Ivore and was attributed to the domestic use of aerosols. Pyrethroid resistance was reported in Kenya and was attributed to the use of permethrin-impregnated bed nets (Chandre *et al*., 1999), although impregnated bed nets were also used in the Gambia, pyrethroid susceptibility in *A. gambiae s.s.* did not change (Hemingway *et al*., 1995). More recently, *A. gambiae* and *A. arabiensis* were found to be resistant to pyrethroids (deltamethrin and lambda-cyhalothrin) and carbamates (propoxur) but not to organochlorines (DDT) in Mozambique (Casamiro *et al*., 2006). In Burkina Faso and Benin, *A. gambiae* was resistant to both pyrethroids and DDT. Resistance was severe in Cote d'Ivore as *A. gambiae* mortality was as low as 7.1% with DDT, 7.3% with permethrin and 28.4% with deltamethrin (Chandre *et al*., 1999). Resistance to DDT has also been reported in several South American countries, and in Mexico, India and in Sri Lanka (Davidson, 1963; Georghiou and Lagunes-Tejeda, 1991; Hemingway *et al*., 1997; Penilla *et al*., 1998; Karunaratne, 1999).

1.6.2 Parasite resistance to antimalarials

Antimalarial drug resistance is defined as the ability of the parasite strain to survive and/or multiply despite the proper administration and absorption of an antimalarial drug in the dose normally recommended. The development of drug resistance can be considered in two parts; the initial spontaneous genetic event which produces the resistant mutant and the subsequent selection process where the resistant mutants have a survival advantage in the presence of the drug and are the ones transmitted (WHO, 2006b). Antimalarial drug resistance has emerged as one of the greatest challenges facing malaria control today and has been implicated in the spread of malaria to new areas and re-emergence in areas where the disease had been eradicated. Drug resistance has also played a significant role in the occurrence and severity of epidemics in some parts of the world (Bloland, 2001). Figure 1.2 shows molecular structures of different antimalarials, for which resistance is discussed.

1.6.2.1 Chloroquine Resistance

Chloroquine (CQ) is a 4-aminoquinoline that is thought to interfere with the parasite's haem detoxification pathway (White, 1999). CQ acts as a schizontocide by preventing the development of blood-stage malaria parasites. It is believed that CQ acts by binding to haem molecules in the parasite food-vacuole, which is a by-product of the parasite's digestion of haemoglobin (Mita *et al*., 2009). Heam can be degraded by glutathione when it exits the food vacuole, however, the ability of CQ to inhibit this glutathione mediated degradation enables the cytotoxic activity of this antimalarial (Ginsburg *et al*., 1998). Resistance to CQ in *P. falciparum* may be multigenic and is initially conferred by mutations in the gene that codes for the *P. falciparum* chloroquine resistance transporter (*pfcrt)* gene, this leads to the reduction in the parasite's accumulation of the drug (White, 1999).
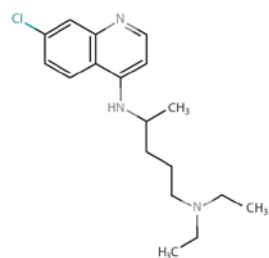
(a). Chloroquine
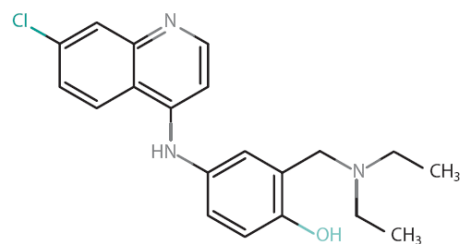
(b) Amodiaquine
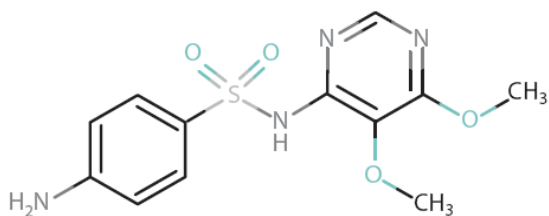
(c) Sulfadoxine
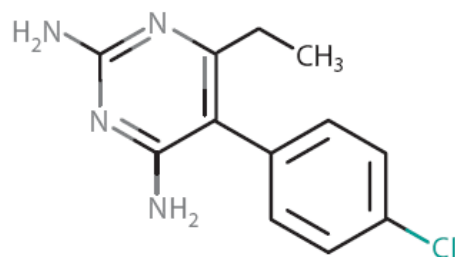
(d) Pyrimethamine
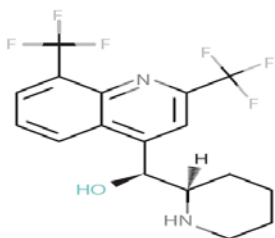
(e) Mefloquine
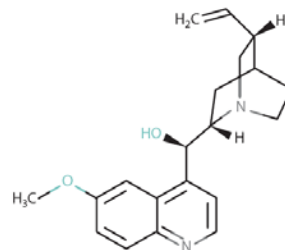
(f) Quinine
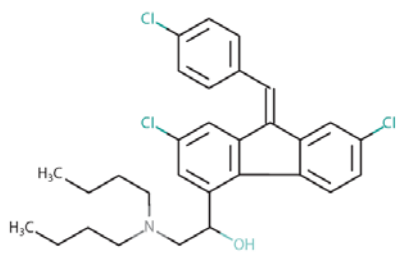
(g) Lumefantrine
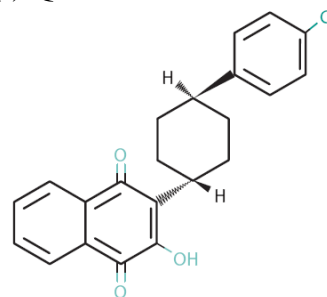
(h) Atovaquone
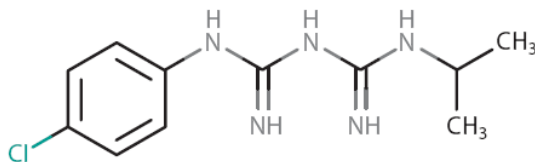
(i) Proguanil

Figure 1.2: The molecular stuctures of antimalarials which are possible combination partners to artemisinins. Adapted from WHO 2006b. Guidelines for the treatment of malaria. Geneva, Switzerland.

Point mutations in the *P. falciparum* multidrug resistance 1 (*pfmdr 1*) gene are also associated with CQ resistance, though to a lesser extent (Ochong *et al*., 2003). The first reports of CQ resistance occurred on the Thai-Cambodian border and in Columbia in the late 1950s, around 12 years after the drug's introduction. All endemic areas of South America were affected by 1980 and almost all Asia and Oceania by 1989.

In Africa, CQ resistance emerged in the east in 1978, spread to the central and southern parts of the continent before arriving in West Africa in 1983. By 1989, CQ resistance was widespread in sub-Saharan Africa (Wongsrichanalai *et al*., 2002). CQ resistance is less severe in west and central Africa than in east Africa, even in the west, its intensity varies from an advanced stage with severe effects on morbidity and mortality in focal areas of Senegal (Haruki *et al*., 1998; Trape, 2001) to a moderate level in Ghana (Landgraf *et al*., 1994) and Cameroon (Ringwald *et al*., 2000), and a low level in Mali (Djimde *et al*., 2001). The level of clinical failure after chloroquine treatment was estimated to be 44% in Madagascar (Menard *et al*., 2008).

1.6.2.2 Amodiaquine resistance

Amodiaquine (AQ) is a Mannich base 4-aminoquinoline with a mode of action similar to that of chloroquine. Although these two drugs are chemically related, AQ remains effective in areas of substantial CQ resistance (Van Dillen *et al*., 1999; Brasseur *et al*., 1999; Staedke *et al*., 2001). Cross resistance between CQ and AQ has been shown in some clinical and *in vitro* reports (Childs *et al*., 1989; Basco and Le Bras, 1993; Bloland and Reubush, 1996; White, 1996; Schellenberg *et al*., 2002) but the molecular mechanism of that cross resistance is not fully understood. Mutations in two genes that code for CQ resistance, the *pfcrt* and the *pfmdr1*

genes have been identified in AQ resistant isolates in southern Sudan (Ochong *et al*., 2003). Treatment failure rates of 18% have been observed in the Democratic Republic of Congo (Bonnet *et al*., 2009).

1.6.2.3 Sulfadoxine-Pyrimethamine Resistance

Sulfadoxine and pyrimethamine (SP) act synergistically, the former inhibits dihydropteroate synthetase (DHPS) and the latter inhibits dihydrofolate reductase (DHFR). These two enzymes are involved in folate synthesis. Point mutations in the *dhps* and *dhfr* genes are implicated to confer resistance by decreasing the drug-binding affinity of the enzyme (Wongrischanalai *et al*., 2002). Although the precise relation between mutations in the *dhps* and *dfhr* genes in clinical SP resistance is unclear, Sibley *et al*. (2001) showed that the presence of a sensitive *dhfr* allele is highly predictive of SP treatment success irrespective of the *dhps* allele.

Resistance to SP was first noted on the Thai-Cambodian border in the mid 1960s (Bjorkman and Phillips-Howard, 1990) and it became an operational problem in the same area within a few years of its introduction to the malaria control programme in 1975. High level resistance is found in South East Asia, southern China and the Amazon Basin. Lower degrees and frequencies of resistance are observed on the Pacific coast of South America, southern Asia east of Iran and western Oceania (Wongrischanalai *et al*., 2002).

In Africa, SP sensitivity started declining in the 1980s and started gaining ground more in the east than in the west. Low sensitivity to SP was observed in Kenya and in Tanzania from the late 1980s to the early 1990s, pyrimethamine resistant isolates were present in the 1980s even before SP was widely used (Ronn *et al*., 1996; Mberu *et al*., 2000). *In-vitro* SP resistance has long been documented in sub-Saharan Africa; in varying prevalence from 13-30%, in

Tanzania (Alin, 1997), Equatorial Guinea (Benito *et al*., 1995), Gabon (Philips *et al*., 1998) and Ghana (Landgraf *et al*., 1994). Clinical failure rates of more than 25% have already been reported in Liberia (Checchi *et al*., 2002), Guinea Bissau (Kofoed *et al*., 2002) and Malawi (Plowe *et al*., 2004). KwaZulu-Natal, South Africa has also observed treatment failure rates of almost 90% following treatment with SP (Bredenkamp *et al*., 2001). The risk of treatment failure of 51-79% has also been observed in Uganda (Nankabirwa *et al*., 2010).

1.6.2.4 Quinine resistance

Quinine (Q) is an alkaloid derived from the bark of the *Cinchona* tree, it acts principally on the mature trophozoite stage parasites and does not prevent sequestration or further development of circulating ring-stage parasites (Krugliak and Ginsburg, 1991; Bray *et al*., 1991). Quinine also does not kill pre-erythrocytic stages of malaria parasites and has no gametocidal effect. The rates of treatment failure greater that 10% at 28 days following quinine treatment have been shown in Sudan (Kofoed *et al*., 2007) and in Thailand (Pukrittayakamee *et al*., 2000). The mechanisms of quinine antimalarial activity are thought to involve the inhibition of parasite heam detoxification in the food vacuole (Krugliak and Ginsburg, 1991; Bray *et al*., 1991; WHO, 2006b).

Observations of clinical resistance to quinine began to accumulate during the mid 1960s, especially from the Thai-Cambodian border. Now it occurs sporadically in South East Asia and Western Oceania (Wernsdorfer and Payne, 2001). Resistance is less frequent in South America (Zalis *et al*., 1998) and Africa (Jelinek *et al*., 2001). Wide-spread use of quinine in Thailand in the early 1980s as an interim therapy in the face of declining SP efficacy resulted in significant reduction in parasite sensitivity (Wongsrichanalai *et al*., 2002). There is some

suggestion that *pfmdr1* mutations associated with chloroquine resistance may also account for reduced susceptibility to quinine (Wongsrichanalai *et al*., 2002). In a Brazilian study of *pfmdr1* mutations, chloroquine resistant strains were found to have low susceptibility to quinine (Zalis *et al*., 1998). In the Gambia, *pfmdr1* Tyr86 was weakly associated with decreased sensitivity to the drug (Duraisingh *et al*., 2000).

1.6.2.5 Mefloquine resistance

Mefloquine (MQ) is a 4-methanoquinoline and is related to quinine. Resistance to MQ was first observed near the Thai-Cambodian and the Thai-Myanmar borders in the late 1980s and its monotherapy is no longer effective there (Wongsrichanalai *et al*., 2002). There are also case reports of mefloquine resistance from the Amazon basin but the scope and degree of resistance in South America are still far below those of South East Asia. Though some *in-vitro* studies suggested the presence of *P. falciparum* strains with low MQ sensitivity in Africa, clinical resistance is rare (Wongsrichanalai *et al*., 2002).

Copy number and polymorphisms of the *P. falciparum* multidrug resistance 1 gene (*pfmdr1* gene) have been investigated as molecular markers of MQ resistance; the evidence on increased *pfmdr1* copy number as a molecular marker for MQ resistance remains conflicting. Some studies in Thailand suggested that a higher copy number confers MQ resistance (Price *et al*., 1999; 2004; Wilson *et al*., 1993) but other studies did not confirm that finding in Thailand (Chaiyaroj *et al*., 1999), Brazil (Zalis *et al*., 1998) and Africa (Basco *et al*., 1995). Some studies have shown increased sensitivity to mefloquine with the *pfmdr1* Tyr86 mutation in the Gambia (Durraisingh *et al*., 2000) and in Thailand (Price *et al*., 1999), suggesting a possible

inverse relation between sensitivity to MQ and to CQ. Sowunmi and colleagues (2009a) observed 11% resistance at day 42, after treatment with mefloquine monotherapy.

1.7 Alternative treatment for malaria

Due to an increasing level of resistance of malaria parasites to non-artemisinin antimalarials, new therapeutic approaches have been developed, these include the use of artemisinin-based combination therapy (ACT). In this approach, an artemisinin drug is combined with a non-artemisinin antimalarial to improve efficacy. The concept of combination therapy is based on synergistic or additive potential of two or more drugs with different mechanisms to improve therapeutic efficacy and to decrease drug resistance. This approach is well known to the treatment of bacterial and viral infections as well (Nosten and White, 2007).

1.7.1 Artemisinin and its derivatives

Artemisinin, also known as qinghaosu, is a sesquiterprene lactone extracted from the leaves of *Artemisia annua* (sweet wormwood). It was isolated by Chinese scientists in 1972. The plant has been used in China for the treatment of fever for over a thousand years (QARCG, 1979; WHO, 2006b; McIntosh and Olliaro, 1999). Artemisinins are potent and rapidly acting blood schizontocides, active against all *Plasmodium* species. They have an unusually broad activity against asexual parasites, killing erythrocytic stages from young rings to schizonts (Nosten and White, 2007). Artemisinins have a unique mode of action; they are well tolerated and have a gametocidal effect (Davis *et al*., 2005). Peak plasma concentrations occur around 3 hours following oral administration (WHO, 2006b).

When artemisinins are used alone over short periods (less than five days), clearance of parasites from the blood is only temporary (Meshnick, 1996; McIntosh and Olliaro, 1999) and they have a high rate of re-infections and recrudescences, although recrudescences occur less frequently (Yeka *et al*., 2005), hence they should be combined with other antimalarials to achieve maximum efficacy. Only four compounds of artemisinin have reached pharmaceutical development for use in humans; artesunate (AS), artemether (AM), dihydroartemisinin (DHA) and arteether (AE) (Davis *et al*., 2005), their molecular structures are shown in figure 1.3.
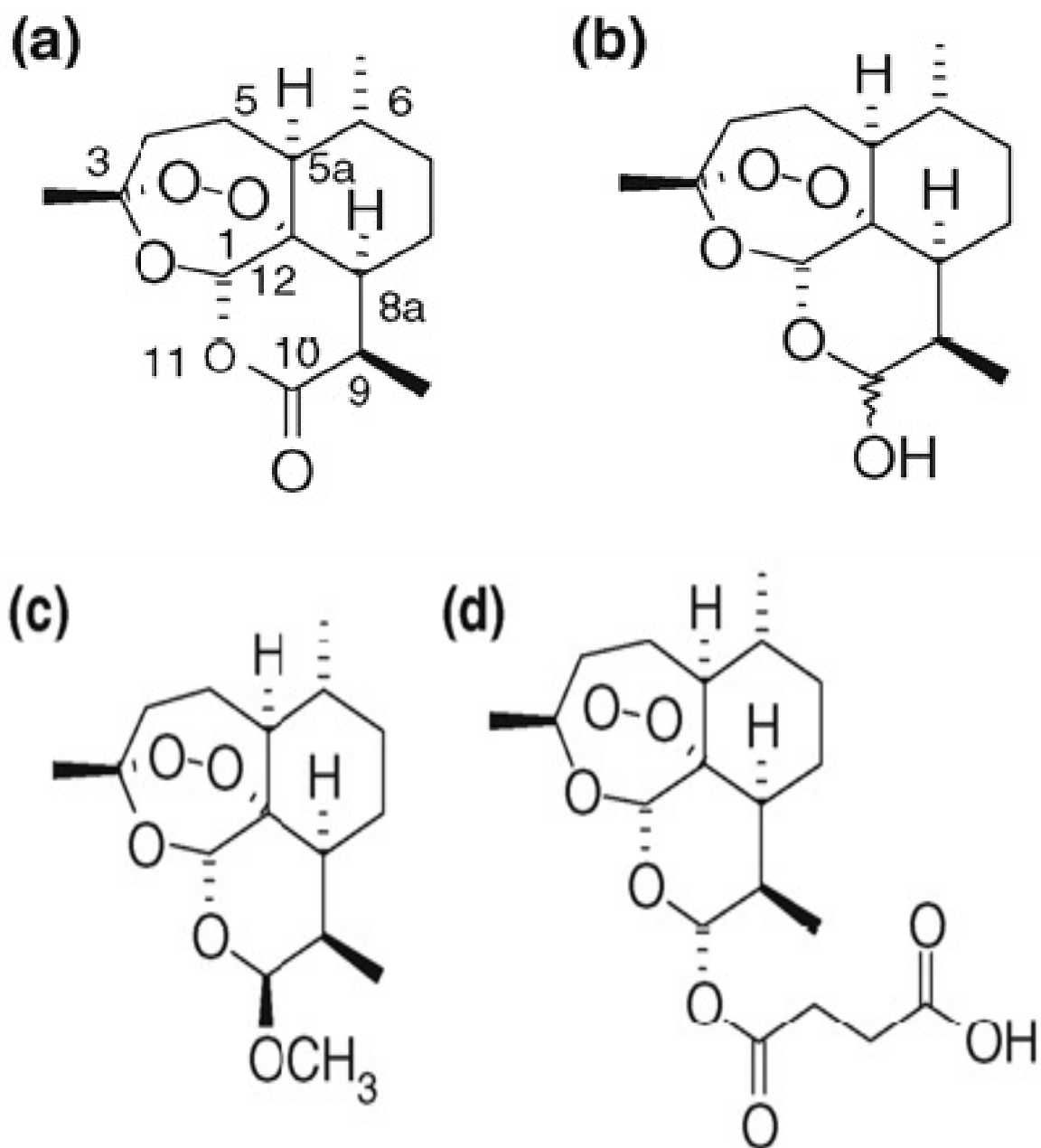
Figure 1.3: The molecular structures of artemisinin and artemisinin derivatives. Artemisinin (a), dihydroartemisinin (b), artemether (c) and artesunate (d). Adapted from Krishna *et al.* (2008).

1.7.1.1 Artesunate

Artesunate is one of the most commonly used artemisinin derivatives and can be given orally, rectally, intramuscularly or intravenously (WHO, 2006b). Artesunate is rapidly absorbed, with peak plasma levels occurring 1.5 hours after oral administration (Batty *et al*., 1998). It is almost entirely converted to dihydroartemisinin, the active metabolite (Navaratnam *et al*., 2000). Elimination of artesunate is very rapid and the duration of its antimalarial activity is determined by the eliminination of dihydroartemisinin (half-life approximately 45 min) (Hien *et al*., 2004).

1.7.1.2 Artemether

Artemether (AM) is the methyl ether of dihydroartemisinin. It is more lipid soluble than artemisinin or artesunate. It can be given as an oil-based intramuscular injection or orally. It is also co-formulated with lumefantrine (previously referred to as benflumetol) for combination therapy. Peak plasma concentrations occur around 2–3 hours after oral administration. Artemether is metabolized to dihydroartemisinin and its elimination half life is approximately one hour (Ezzet *et al*., 1998).

1.7.1.3 Dihydroartemisin

Dihydroartemisinin is the main active metabolite of the artemisinin derivatives, but can also be given orally and rectally as a drug in its own right. It is relatively insoluble in water and requires formulation with suitable excipients to ensure adequate absorption. It achieves cure rates similar to those of oral artesunate. Dihydroartemisinin is rapidly absorbed following oral administration, reaching peak levels after around 2.5 hours. Its elimination half-life is approximately 45 minutes via intestinal and hepatic glucuronidation (Newton *et al*., 2002).

1.7.1.3 Arteether

Arteether, more recently known as artemotil, is the ethyl ether of artemisinin. It is closely related to the more widely used artemether. It is oil-based, water-insoluble and is given by intramuscular injection only. Absorption of artemotil is slower, with some patients having undetectable plasma artemotil until more than 24 hours after administration (WHO, 2006b)

1.7.2 Artemisinin-based combination therapy for the treatment of malaria

Although there are some minor differences in oral absorption and bioavailability between the different artemisinin derivatives used for the oral treatment of uncomplicated malaria, there is no evidence of significant clinical differences in current formulations (WHO 2006b). The properties of a partner antimalarial are crucial and they determine the efficacy and choice of a combination therapy. The ACTs combine a derivative of the natural product of artemisinin with longer lasting partner drug that continues to reduce the parasite biomass after the short-lived artemisinin has dropped below therapeutic levels (Greenwood *et al*., 2008). Currently recommended ACTs are artemether-lumefantrine, dihydroartemisinin-piperaquine, artesunate plus amodiaquine, artesunate plus mefloquine and artesunate plus sulfadoxine-pyrimethamine. Artemether-lumefantrine was the first internationally recognized fixed-dose ACT in use but other fixed-dose combinations have later been developed (Nosten and White, 2007). Dihydroartemisinin-piperaquine has also been investigated and it is part of the WHO recommendations published (Sinclair *et al*., 2009; WHO, 2010).

1.7.2.1 Artemether-lumefantrine

Artemether-lumefantrine (AL) is currently available as co-formulated tablets known as Coartem^{TM} or Riatem^{TM}, it was previously recommended as a four-dose regimen (AL-4) but

now it is currently recommended at six-doses (AL-6), two doses a day for three days. Its advantage is that lumefantrine, previously referred to as benflumetol, is not available as a monotherapy and has never been used on its own. This has limited the potential of lumefantrine-resistant parasites (WHO, 2006b). A systematic review (Omari *et al*., 2004) reported a higher risk of treatment failure at days 28 and 63 with AL-4 compared to the combination of artesunate plus mefloquine (AS+MQ). There was no statistically significant difference in the risk of treatment failure at day 28 when AL-6 was compared to AS+MQ. On day 42, there was a significantly higher risk of treatment failure with AL-6 compared to AS+MQ. Compared to artesunate plus amodiaquine (AS+AQ), there was a significantly lower risk of treatment failure at day 28 in AL-6. The risk of parasitological failure was not significantly different when AL-6 was compared to dihydroartemisinin+napthoquine+ trimethroprim (Omari *et al*., 2005; Burkiwa *et al*., 2006).

The disadvantage of using AL is that it requires a complicated twice daily dose and has to be administered with milk or fat-containing foods. This could potentially be a problem in poor households, considering that the most malarious areas are also poverty stricken (Muheki *et al*., 2004; Kobbe *et al*., 2008).

1.7.2.2 Artesunate plus amodiaquine, mefloquine or sulfadoxine-pyrimethamine

Artesunate plus amodiaquine (AS+AQ) is currently available in blister packs as separate scored tablets containing 50mg of artesunate and 153 mg base of amodiaquine. Its co-formulation has recently been developed and the treatment is recommended for three days (Nosten and White, 2007). The combination is sufficiently efficacious only where 28-day cure rates with AQ monotherapy exceed 80% (WHO, 2006b). With the potential for cross

resistance with CQ, and the availability of both AQ and CQ as monotherapies, the efficacy of this ACT may be compromised. The co-formulated dose of artesunate plus mefloquine (AS+MQ) has been developed and the treatment is recommended for three days (Ashley *et al*., 2006). Artesunate plus sulfadoxine-pyrimethamine (AS+SP) is also currently available as separate scored tablets. High level of resistance to SP, the continued use of SP for intermittent preventive treatment of malaria in pregnancy, the wide spread availability of SP, sulfalene-pyrimethamine and cotrimoxazole (trimethoprim-sulfamethoxazole) are likely to compromise the efficacy of combination therapy using SP (WHO 2006b).

1.7.2.3 Dihydroartemisinin-piperaquine

The other promising form of ACT is Dihydroartemisinin-piperaquine (DHAP). It is well tolerated and has no significant cardio-vascular or metabolic side effects (Davis *et al*., 2005). This ACT which has been co-formulated as Artekin^{TM} has been shown to be safe and effective, and represents another option for malaria treatment (Sinclair *et al*., 2009). Piperaquine is a bisaminoquinoline synthesized in China and France in the 1960s (Chen *et al*., 1982), frequently used in China in the 1970s and 1980s and was re-discovered in the 1990s as a candidate for ACT (Davis *et al*., 2005). Although the long half-life of piperaquine (3-4 weeks) may allow resistant parasites to be selected, this has not been of concern in areas of Thailand where transmission is low (Davis *et al*., 2005). The cost of using Artekin^{TM} is lower than that of using Coartem^{TM} so it could be an attractive option (Denis *et al*., 2002).

1.7.3   Other potential artemisinin based combinations

The partner drug to artemisinins should be well tolerated and nontoxic, it should be present in the blood at therapeutic concentrations for at least several times the duration of the parasite

life cycle, which is 48 hours in the case of *P. falciparum* (Davis *et al*., 2005). Primaquine which also has a gametocidal effect against *P. falciparum* reaches peak plasma concentration around 1-2 hours after administration and has an elimination half-life of 3-6 hours (Mihaly *et al*., 1984) and is effective even against stage five gametocytes (Pukrittayakamee *et al*., 2004). Similar to lumefantrine, atovaquone needs to be administered with fat-containing foods due to poor absorption (McGrey *et al*., 2003). It is available in co-formulation with proguanil. Proguanil on the other hand is poorly metabolized by approximately 3% of the African population (Helsby *et al*., 1990; Kaneko *et al*., 1999). The elimination half-life of proguanil and its active metabolite, cycloguanil is approximately 20 hours (Wattanagoon *et al*., 1987; Hussein *et al*., 1996). Chlorproguanil has similar properties to proguanil and is co-formulated with dapsone as Lapdap^{TM}. Peak plasma concentrations of dapsone occur 2-8 hours after an oral dose, its elimination half-life is 10-50 hours (WHO, 2006b). Chlorproguanil-dapsone has been withdrawn due to safety concerns (Burkiwa *et al*., 2004).

Tetracyclines are a group of antibiotics originally derived from certain *Streptomyces* species, but mostly used in synthetic form. Tetracycline itself is water soluble and an inhibitor of aminoacyl-tRNA binding during protein synthesis. The target for tetracyclines is the apicoplast, a chloroplast-like organelle with uncertain functions. Tetracyclines cause modest antimalarial effects initially but are much more potent against the progeny of treated parasites. Blocking production of apicoplast proteins causes the delayed death effect. Although too slow acting to be used as monotherapy, antibiotics targeting apicoplast functions could be ideal partners for combination therapy with artemisinins (Dahl and Rosenthal, 2008).

Peak plasma concentrations occur 1-3 hours after ingestion and its elimination half-life is around eight hours. Administration with antacids, iron preparations and dairy products should be avoided. Doxycycline, which is also a tetracycline, has a longer half-life (10-24 hours) (Newton *et al.*, 2005) and is better absorbed. It should not be given to pregnant or lactating women or children aged up to eight years. Doxycycline has a lower binding affinity with calcium so it can be taken with food or milk. Clindamycin is very soluble in water and it inhibits the early stages of protein synthesis. Peak plasma concentrations are reached in one hour in children and three hours in adults. Its elimination half-life is 2-3 hours (WHO, 2006b).

Other combinations with artemisinins include combination with methylene blue (MB), Cotrimoxazole (CT), Sulfamethoxy-pyrimethamine (SMP), atovaquone-proguanil (ATV+PRG), β-cyclodextrin (β-CDX) and others. Methylene is a specific inhibitor of *P. falciparum* glutathione reductase; it inhibits the heme polymerization within the parasite's food vacuole and prevents methaemoglobinaemia in clinical malaria (Zoungrana *et al.*, 2008). Cotrimoxazole, a combination of sulfamethoxazole and trimethoprim, has an antimicrobial spectrum that includes bacterial infections and protozoan infections such as malaria. Compared with sulfadoxine-pyrimethamine, CT supports less gametocyte generation. Cotrimoxazole and AS are often used together and are both easily available (Fehintola *et al.*, 2008). A long-acting sulfonamide, SMP, has a long but stable elimination half-life of approximately 80 hours and its low plasma-binding capacity (65%) enables use of a low dosage with a long-lasting effect on parasites (Rulisa *et al.*, 2007).

β-cyclodextrin has been shown to improve the absorption of artesunate (Wong *et al.*, 2003). Atovaquone-proguanil is very well tolerated and effective against multidrug-resistant *P. falciparum* isolates (Van Vugt *et al.*, 2002). When atovaquone was used alone, high level

resistance was selected rapidly. The addition of proguanil reduced this considerably, but because proguanil is a relatively weak antimalarial drug, the degree of protection against resistance may not be sufficient to prevent the development of resistance to the combination if it is used widely (Van Vugt *et al*., 2002).

1.7.4 Resistance to ACTs

Although artemisinins are effective and rapidly acting, their widespread use has raised questions with regards to emerging drug resistance (Duffy and Sibley, 2005). *In-vitro* drug susceptibility data as well as reports of failurerates associated with ACTs suggest the possibility of clinical artemisinin resistance. In the Thai-Cambodian border, an artesunate-mefloquine 28-day cure rate of 78.6%-85.7% has been observed (Vijaykadga *et al*., 2006; Mey Bouth *et al*., 2006). These studies suggest declining susceptibility to this ACT. However, it is not yet clear whether these failures are a result of true parasite resistance or not (Alker *et al*., 2007). Dondorp *et al*. (2009) observed 30% PCR confirmed recrudescence in Pailin, Cambodia after 21-35 days of treatment with artesunate monotherapy, which could not be explained by pharmacokinetic or other host factors. There was also no consistant pattern of genetic mutations in this study, despite clear evidence of *in-vivo* artesunate resistance. Although there has been some decline in the efficacy of ACTs, the relative contributions of resistance to artemisinins, the partner drug and other factors have not been clarified (Dondorp *et al*., 2009).

1.8   Rationale for the study

The World Health Organization has endorsed ACT as the 1st-line treatment for uncomplicated *Plasmodium falciparum* malaria (Davis *et al*., 2005) and many countries are changing their treatment policies in that regard. By June 2006, 39 African countries had changed their policies to recommend ACTs as the first line treatment for uncomplicated malaria, to reduce the effects of failing monotherapies and to limit the development of drug resistance (Zurovac *et al*., 2007). South African treatment guidelines have also adopted ACTs for the treatment of uncomplicated malaria and artemether-lumefantrine is the treatment of choice (Olumese, 2006). Several clinical trials have proven the superior efficacy of ACTs compared to non-artemisinin based regimens (McIntosh and Olliaro, 1999). Reviewing different ACTs, there is no consensus on which antimalarial is better suited for combination with the various artemisinin derivatives. In a Cochrane review, DHAP was equivalent to AL, ASMQ, ASAQ and ASSP in reducing the risk of treatment failure. In the same review, AL was also equivalent to ASMQ and ASAQ, however, it was superior to ASSP (Sinclair *et al*., 2009). The above review evaluated ACTs compared to each other; however, the ACTs have not been evaluated when the same artemisinin derivative is combined with different non-artemisinin drugs, thus only evaluating the non-artemisinin partner of the combination therapy.

The aim of this study was to review the evidence of efficacy and safety of different non-artemisinin antimalarials when they are combined with the same artemisinin derivative, in reducing the risk of treatment failure in non-pregnant adults and children with uncomplicated *P. falciparum* malaria.

Included trials were randomized controlled trails in non-pregnant adults and children with uncomplicated *P. falciparum* malaria, evaluating the efficacy and safety of non-artemisinin antimalarials like amodiaquine, mefloquine, chloroquine and others, combined with artemisinin, artesunate, artemether or dihydroartemisinin in reducing the risk of treatment failure. The intervention was compared with a different non-artemisinin antimalarial in combination with the same artemisinin derivative given in the intervention arm, or monotherapy of the same artemisinin derivative without the non-artemisinin antimalarial.

Objectives

- To systematically review the evidence of the efficacy and safety of different non-artemisinin antimalarials when combined with the same artemisinin derivative for the treatment of uncomplicated *P. falciparum* malaria in non-pregnant adults and children.

CHAPTER 2: METHODS

2.1 Criteria for including studies in the review

2.1.1 Types of studies

This review considered only randomized controlled trials (RCTs), quasi-randomized controlled trials (CCT) were excluded.

2.1.2 Participants

The participants were non-pregnant adults and children with uncomplicated *Plasmodium falciparum* malaria, confirmed by a blood slide using thick smears. The focus of this study was uncomplicated malaria, particularly because the vast majority of malaria infections cause uncomplicated disease with approximately 1-2% of these episodes becoming severe. Pregnant women were excluded as artemisinins are not recommended for women in their first trimester of pregnancy due to limited safety data (WHO 2006b).

2.1.3 Interventions

The interventions were non-artemisinin antimalarials like amodiaquine, mefloquine, sulfadoxine-pyrimethamine and others, combined with artemisinin, artesunate, artemether or dihydroartemisinin.

2.1.4 Controls

The interventions were compared with a different non-artemisinin antimalarial in combination with the same artemisinin derivative given in the intervention arm, or monotherapy of the same artemisinin derivative without the non-artemisinin antimalarial. Since the aim of this

review was to compare different non-artemisinin antimalarials combined with the same artemisinin derivative, RCTs that compare two different artemisinin derivatives combined with the same or two different non-artemisinin antimalarials were excluded, for example, artemether-lumefantrine was not compared with artesunate-amodiaquine, since the artemisinin derivatives are different in a single trial, irrespective of the difference in the combination non-artemisinin antimalarial.

2.1.5   Outcomes

The definitions of treatment failure used in this review were first drawn from the WHO 2006 treatment guidelines (WHO, 2006), then combined to be consistent with the WHO 2010 treatment guidelines (WHO, 2010) and a Cochrane review by Sinclair *et al*., (2009). The primary outcome was treatment failure adjusted by polymerase chain reaction (PCR), refered to as adjusted treatment failure, which includes early treatment failure or late parasitological or clinical failure on or before day 28. In late treatment failure, new infections and recrudescences were differentiated by PCR where data was provided. When PCR was not used and it was not possible to clarify whether failure was a result of new infections or recrudescence, treatment failure was presented as unadjusted treatment failure.

- Early treatment failure (ETF) defined as danger signs or complicated malaria or failure to adequately respond to therapy on days 0–3

- Late clinical failure (LCF) defined as danger signs or complicated malaria or fever and parasitaemia on days 4–28 without previously meeting criteria for ETF,

- Late parasitological failure (LPF) defined as asymptomatic parasitaemia day 28 without previously meeting criteria for ETF or LCF

- Late treatment failure defined as failure to respond to treatment between day 4 and day 28 not categorized as clinical or parasitological.

Secondary outcomes were:

- Fever clearance time defined as the time between starting treatment and the temperature returning back to normal and remaining normal for more that 48 hours.

- Fever on day two

- Fever on day three

- Parasite clearance time (PCT) defined as the time between starting treatment and the first negative blood test, if negativity persists for more than 48 hours.

- PCT 50 defined as the time it takes for the parasites to be reduced to 50% of the first value

- PCT 90 defined as the time it takes for the parasites to be reduced to 10% of the first value

- Anaemia on day 28 defined as hemoglobin level < 11 g/dL (Kayentao *et al*., 2009)

- Gametocyte carriage on days seven, 14 and 28

Adverse events leading to discontinuation of treatment, hospitalization and death were noted as well as side effects not leading to discontinuation of treatment as defined by trial authors.

2.2   Search strategy for the identification of studies for inclusion in the review

The search for studies attempted to identify all relevant RCTs regardless of language or publication status. The first search was conducted in June 2007 and the last one in January 2009. Only RCTs that were published and indexed before the end of January 2009 were

included. The search for published RCTs was conducted on the Cochrane Central Register of Controlled Trials (CENTRAL) (*The Cochrane Library* 2008, Issue 4), MEDLINE (1966-January 2009) and EMBASE (1980-January 2009) using 'malaria', 'artemisinin', 'artesunate', 'artemether' and 'dihydroartemisinin' as search terms. The detailed search strategies are included in Appendix A. Ongoing RCTs were searched for from the trials registry; ClinicalTrials.gov (http://clinicaltrials.gov).

Abstracts of conference proceedings were also searched from the following meetings:

- Fifth European Congress on Tropical medicine and International Health, 24-28th May 2007, Amsterdam, The Netherlands.

- Sixth European Congress on Tropical medicine and International Health, 6-10th Spetember 2009, Verona, Italy.

- Fifth AMANET Biennial Conference, 26-28th February 2007, Zanzibar, Tanzania.

- Gordon Research Conference on Malaria, 9-14th September 2007, Oxford, United Kingdom.

- Gordon Research Conference on Malaria, 6-11th September, 2009 Magdalen College Oxford, United Kingdom

- Fourth Cost B22 Anual Congress, 10-13th June 2007, Dundee.

- Malaria 2007, 21st-27th April 2007, The Gambia.

- 4th MIM Pan African Malaria Conference, 13-18th November 2005, Youde, Cameroon.

- 5th MIM Pan African Malaria Conference, 2nd-6th November 2009, Nairobi, Kenya.

The reference lists of all RCTs identified by the above methods were checked.

2.3 Methods for selecting studies for inclusion in the review

The results of the search were scanned applying the eligibility criteria and the full articles of all potentially relevant studies were retrieved. Studies were scrutinized for possible multiple publications and assessed for inclusion in the review using standard eligibility forms based on the inclusion criteria. The eligibility form is included in Appendix B.1. Studies that did not meet the eligibility criteria were excluded. Authors of unpublished conference abstracts and those obtained from the trials registry were contacted for full data. If data was not successfully obtained or the trial was still ongoing, those RCTs were included as RCTs with no data extracted.

2.4 Assessment of the methodological quality of included studies

Each RCT's methodological quality was assessed using standard methodological quality forms. The methods used to generate the sequence of allocating patients to different treatments and to conceal allocation sequence were classified as adequate, if the sequence could not be predicted, unclear, if the methods were not described or inadequate if the next allocation could be predicted using adopted guidelines (Juni, 2001). Blinding of participants, care givers or outcome assessors to the interventions in each trial was noted. Furthermore, the percentage of randomized participants excluded in the analysis was also noted and reported as loss to follow-up. The methodological quality form is also included in Appendix B.2.

2.5 Extraction of data from studies included in the review

Data from included studies were extracted using standard data extraction forms (Appendix B.3). The following information was gathered from each included study:

- Administrative details - Identification; author(s); publication status; year of publication; year in which study was conducted; details of other relevant papers cited.

- Details of study - study design; inclusion and exclusion criteria; number of participants analyzed; type, duration, frequency and completeness of follow-up; country and location of the study; setting in which the study was performed (e.g. urban or rural), local malaria transmission and local drug resistance. Local malaria transmission and resistance to antimalarials were extracted as reported in the literature at the time the study was conducted or in a related study.

- Characteristics of participants - age and sex.

- Details of intervention - drugs administered, route of administration, duration of use, size and frequency of dose.

- Details of outcomes - number of people experiencing a specific outcome.

- Details of study ethics- independent ethics review board and type of informed consent.

Authors of trials with insufficient or missing data were contacted for more information. For dichotomous outcome measures, the number of participants experiencing the event (n) and the number analyzed in each group (N) were recorded. For continuous outcome measures, means and standard deviations (SD) were extracted for each group together with the numbers analyzed in each group.

2.6   Analysis of data extracted from studies included in the review

Data were synthesized and analyzed using Review Manager 4.2 and 5.0. Dichotomous data were presented and combined using relative risks (RR). Continuous data were summarized by

means and standard deviations and combined using weighted mean differences (WMD). Both effect measures were accompanied by 95% confidence intervals (CI) and p-values. In cases where data were not normally distributed, thus means not reported, data were descriptively analyzed. Heterogeneity amongst trials was assessed by visually inspecting forest plots, applying the chi-squared test with a p-value of 0.1 indicating statistical significance and using the I^2 test (Higgins and Green, 2006) where a value of 75-100% was used to denote considerable heterogeneity. In cases where moderate heterogeneity was detected but it was still appropriate to combine the results, the random-effects model was used and potential sources of heterogeneity were explored and discussed (DerSimonian and Laird, 1986). In cases where the meta-analysis was not possible to conduct due to considerable heterogeneity, the results were only described. Differences in malaria transmission, local drug resistance and age groups of participants enrolled were investigated as potential sources of heterogeneity, and where considerable differences were found, subgroup analyses were performed. Due to inadequate reporting of the methods used in clinical trials, the sensitivity analysis for this review was not performed. The sensitivity analysis would have been conducted by subgroup analysis of RCTs according to the risk of bias and by imputing data on loss to follow up, either as treatment failures or treatment successes.

CHAPTER 3: RESULTS

3.1 Description of the studies included in the review

The search for literature provided 2354 abstracts and titles across the databases, 2245 were clearly described in the abstract and title, and did not meet the criteria for inclusion in the review and were, therefore, excluded. These studies were either clearly not Randomized Controlled Trials (RCTs), participants had severe malaria, had other forms of malaria not caused by *Plasmodium falciparum* or the interventions compared did not include the same artemisinin derivative in both arms, with only a differing combination antimalarial. The abstracts were re-screened two months from the initial screening to ensure that no abstracts or titles within the inclusion criteria were discarded. There were 109 potentially relevant studies that were identified. These abstracts were screened for duplicate studies identified in more than one database, thus excluding 43 studies. It was not clear if 66 remaining studies qualified for inclusion or not, thus full-text articles were sought and they were further evaluated for eligibility using a standard eligibility form. Eighteen of these were unpublished studies obtained from conference proceedings and clinical trials registries. Data and the description of methods for unpublished studies were sought from the investigators. There were 47 RCTs that met the inclusion criteria, one of them was in Spanish and interpretation for it was sought. Nineteen were excluded and the reasons for excluding them are shown in Table 3.1. Data for 10 unpublished RCTs could not be obtained, the available data has been captured in Table 3.2e, leaving 37 included and extracted RCTs. Figure 3.1 shows the flow diagram with exclusion and inclusion of studies.

Table 3.1: Characteristics of the studies that did not meet the eligibility criteria.

Reference	Reasons for exclusion
Tall *et al*., 2005	Quasi-randomization
Nahum *et al*., 2007	The trial compared ACT with a non-ACT antimalarial
Osorio *et al*., 2007	The trial compared ACT with a non-ACT antimalarial
Van den Broek *et al*., 2005	Quasi-randomization
Price *et al*., 1999	The study was a review
Krudsood *et al*., 2002	Quasi-randomization
Sowunmi *et al*., 2001	Children had severe malaria
Von Seidlein *et al*., 2003	The control group received placebo only
Targett *et al*., 2001	The trial compared different AS doses with the same partner drug
Looareesuwan *et al*., 1992b	Study not a randomized controlled trial
Sabchareon *et al*., 1998	The trial compared AS with AS suppository with the same partner drug
Newton *et al*., 2001	Patients required intra-venous treatment (sign of severe malaria)
Ibrahium *et al*., 2007	Not described as randomized
Odhiambo, 2009	The trial was for prevention of malaria instead of treatment.
Mworozi *et al*., 2009	The participants received different artemisinin derivatives with different combination antimalarials (Artemisinin vs Artemether)
Tona *et al*., 2009	Intervention drug not identified
Kabongo *et al*., 2009	Not a trial
Maiga *et al*., 2009	The trial was for prevention of malaria instead of treatment.
Aina *et al*., 2009	Not described as randomized
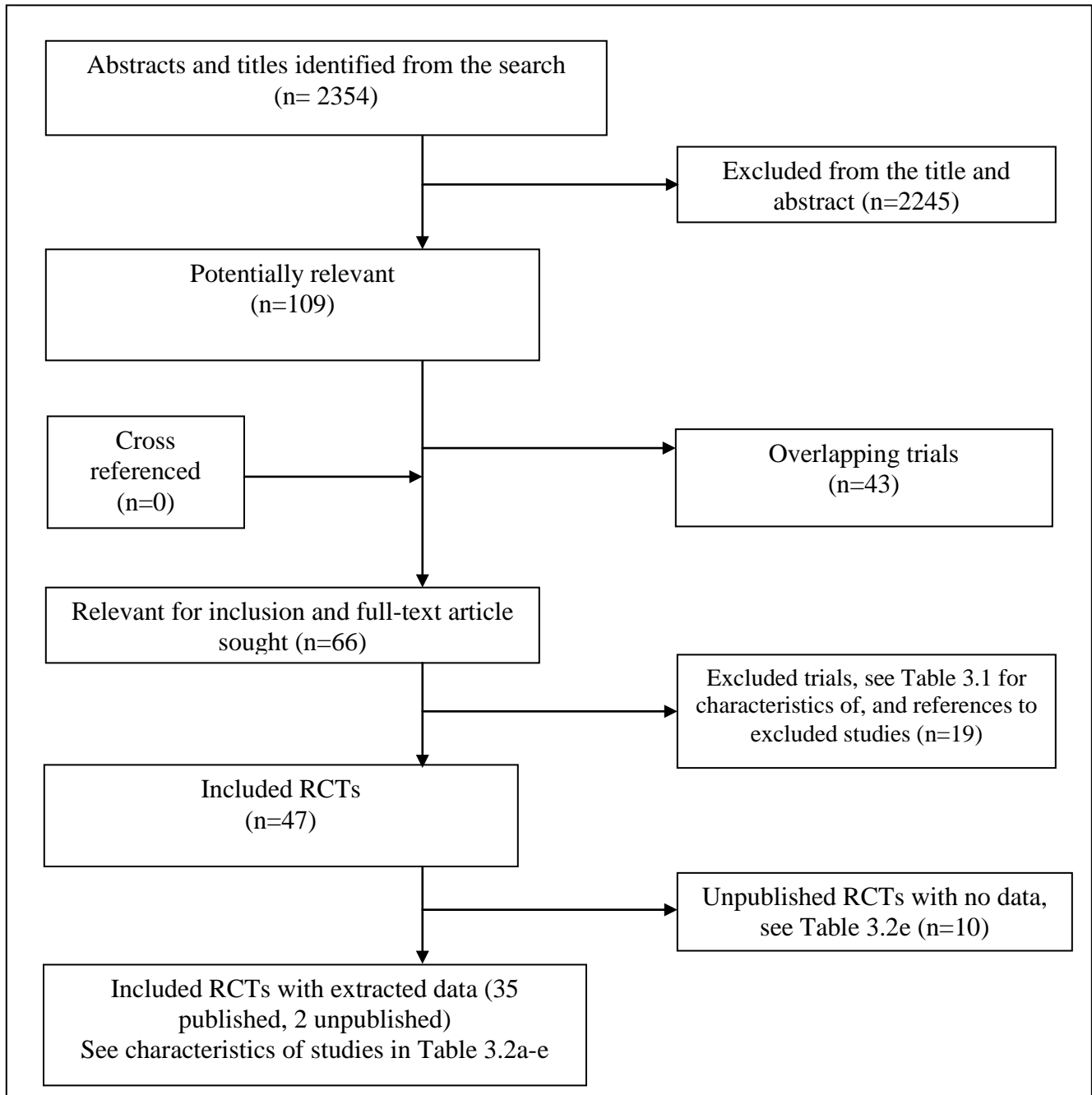
Figure 3.1: Flow diagram showing the studies that were included or excluded in this review.

The thirty-seven included RCTs had a total of 6862 analyzed participants, the number of participants ranged from 34 to 1066 in these trials. Tables 3.2 (a) to (e) show the details of included trials.

3.1.1   Location, transmission intensity and drug resistance

Twenty-one of the RCTs were conducted in Africa, one in each of Angola, Guinea, Guinea-Bissau, Ghana, Mozambique, Rwanda, Senegal and Sudan (Tables 3.2a-e). There were two RCTs conducted in Burkina-Faso, in the Democratic Republic of Congo, Mali, Sudan and in Tanzania. Three RCTs were conducted in Nigeria. There were 14 RCTs conducted in Asia, nine in Thailand, three Vietnam, one in China and one in Malaysia. Only two trials were conducted in South America, in Colombia and in Ecuador. These trials were conducted in areas of varying malaria transmission. Twenty-two of them were conducted in endemic regions; three in areas with seasonal malaria transmission and eleven were conducted in areas of low to moderate transmission (Tables 3.2a-e). Chloroquine and sulfadoxine-pyrimethamine resistance was reported in all the locations. Furthermore, multi-drug resistance was reported in Thailand, Vietnam and in Sudan. Resistance to amodiaquine was rare, reported only in the DRC (Van den Broek *et al*., 2006) while halofantine resistance was reported in Burkina Faso (Barennes *et al*., 2004; Zoungrana *et al*., 2007) and proguanil resistance reported in Malaysia (Wong *et al*., 2003).

Table 3.2 (a): Characteristics of the studies that were included- artesunate plus amodiaquine combination therapy.

REFERENCE	CONTROL	DURATION OF ARTEMISININ DOSES	LOCATION	TRANSMISSION	RESISTANCE	AGE	FOLLOW-UP (days)
Abacassamo *et al.*, 2004	AS+SP	3 days	Mozambique	Seasonal	CQ	6-59 months	14
Bonnet *et al.*, 2007	AS+SP	3 days[$]	Guinea	Seasonal	CQ	6-59 months	28
Carmona-Fonseca *et al.*, 2008	AS+SP	3 days	Colombia	Low	CQ, SP, AQ	1 year and older	28
Guthmann *et al.*, 2005	AS+SP	3 days	Angola	Hyper-endemic	CQ, SP	6-59 months	28
Hamour *et al.*, 2005	AS+SP	3 days	Sudan	Seasonally endemic	MDR	6-59 months	28
Kayentao *et al.*, 2009	AS+SP	3 days	Mali	Endemic	CQ, SP	5-59 months	28
Swarthout *et al.*, 2006	AS+SP	3 days	DRC	Endemic	CQ, SP	6-59 months	28
Van den Broek *et al.*, 2006	AS+SP	3 days	DRC	Highly endemic	CQ, SP	6-59 months	28
Djimde *et al.*, 2008	AS+SP AS	3 days 5 days	Mali	Hyper-endemic	CQ, SP	0.5-38 years	28
Faye *et al.*, 2007	AS+MQ	3 days	Senegal	Moderate	CQ	Not reported	28
Owusu-Agyei *et al.* 2008	AS+CD	3 days	Ghana	Endemic	CQ, SP	0.5-10 years	28
Zoungrana *et al.*, 2008	AS+MB	3 days	Burkina-Faso	Endemic	CQ, SP, Halofantrine	0.5-10 years	28
Fehintola *et al.*, 2008	AS+CT	3 days	Nigeria	Endemic	CQ, SP	0.5-10 years	28
Barennes *et al.*, 2004	AS	3 days	Burkina Faso	Endemic	CQ, SP, Halofantrine	1-15 years	28
Sowunmi *et al.*, 2007	AS	7 days (both arms)	Nigeria	Endemic	CQ, SP	0.5-11 years	21
Sowunmi *et al.*, 2009	AS	3 and 7 days (monotherapy)	Nigeria	Endemic	CQ, SP	0.5-11 years	42

AS = artesunate, SP = sulfadoxine-pyrimethamine, MQ = mefloquine, CD = chlorproguanil-dapsone, MB = methylene-blue, CT= cotrimoxazole, CQ = chloroquine, MDR= multidrug resistance, DRC= Democratic Republic of Congo, [$]route of dosage oral and intramuscularly, * baseline mean (age range not reported)

Table 3.2 (b): Characteristics of studies that were included- artesunate plus mefloquine combination therapy.

REFERENCE	CONTROL	DURATION OF ARTEMISININ DOSES	LOCATION	TRANSMISSION	RESISTANCE	AGE	FOLLOW-UP (days)
Gomez *et al*., 2003	AS	In combination therapy- 3 days[$$]. In monotherapy- 6 days[$$].	Ecuador	Endemic	CQ & MQ	1-12 years	28
Karbwang *et al*., 1996	AS	In combination therapy- single dose In monotherapy- 5days	Thailand	Low	MDR	15-58 years	28 & 42
Looareesuwan *et al*., 1992	AS	5days	Thailand	Low	MDR	16-60 years	28
Price *et al*., 1998	AS	5days	Thailand	Low	MDR	Not reported	42
Krudsood *et al*., 2000	AS+AZ AS	3 days.	Thailand	Low	MDR	>14 years	28
Li *et al*., 1984	AS+MQ+SP AS	In combination therapy-single dose In monotherapy- 3days	China	Low	CQ & SP	8-60 years	28
Van Vugt *et al*., 2002	AS+ATV+PRG	3 days	Thailand	Low	MDR	2-70 years	42

AS = artesunate, AZ = azithromycin, MQ = mefloquine, SP = sulfadoxine-pyrimethamine, ATV = atovaquone, PRG = proguanil, CQ = chloroquine, MDR = multidrug resistance, [$$] route of administration rectal

Table 3.2 (c): Characteristics of studies that were included- artemisinin derivatives in monotherapy.

REFERENCE	TREATMENTS	DURATION OF ARTEMISININ DOSES	LOCATION	TRANSMISSION	RESISTANCE	AGE (years)	FOLLOW-UP (days)
Hassan Alin *et al.*, 1996	A+MQ vs A	In combination therapy-3 days In monotherapy- 5days	Tanzania	Holo-endemic	CQ & SP	15-45	28
Karbwang *et al.*, 1995	AM+MQ vs AM	In combination therapy- 1day In monotherapy- 5	Thailand	Low	MDR	13-47	28 & 42 *
Looareesuwan *et al.*, 1997	AM+MQ vs AM	In combination therapy- 1day In monotherapy- 5 or 7days	Thailand	Low	MDR	16-60	28
Krudsood *et al.*, 2000	AS+AZ vs AS	3days	Thailand	Low	MDR	>14	28
Li *et al.*, 1984	AS+MQ+SP vs AS	In combination therapy- single dose In monotherapy- 3days	China	Low	CQ & SP	8-60	28
Kofoed *et al.*, 2003	AS+CQ vs AS	3days	Guinea-Bissau	Seasonal	CQ	0.5-15.5	35
Pukrittayamee *et al.*, 2004	AS+PQ vs AS	7days	Thailand	Low	MDR	14-62	28
Wong *et al.*, 2003	A+β-CDX vs A	5days oral	Malaysia	Endemic	CQ, SP & PRG	15-61	35
Diem Thuy *et al.*, 2007	DHA+MQ vs DHA	5 days in monotherapy 2 days in combination therapy	Vietnam	Endemic	MDR	≥15	28 & 42*

A = artemisinin, AM = artemether, AS = artesunate, MQ = mefloquine, AZ = azithromycin, CQ = chloroquine, PQ = primaquine, β-CDX = beta-cyclodextrin, DHA = dihydroartemisinin, MDR= multidrug resistance, PRG = proguanil. *extended follow-up in combination therapy only.

Table 3.2 (d): Characteristics of the studies that were included - other combination therapies.

REFERENCE	TREATMENTS	DURATION OF ARTEMISININ DOSES	LOCATION	TRANSMISSION	RESISTANCE	AGE	FOLLOW-UP (days)
Huong *et al.*, 2003	AS+SP vs AS+CQ	3days	Vietnam	Highly endemic	CQ & SP	4-65 years	28
Rulisa *et al* 2007	AS+SP vs AS+SMP	3 days	Rwanda	Endemic	CQ & SP	3 months - 12 years	28
Shekalaghe *et al.*, 2007	AS+SP vs AS+SP+PQ	3 days	Tanzania	Hyper-endemic	CQ & SP	3-15 years	42
Bich *et al.*, 1996	A+Q vs A+D	single dose	Vietnam	Hyper- and Holo-endemic	MDR	8-65 years	28
Na-Bangchang *et al.*, 1996	AM+D vs AM+AZ	In AM+D- 5days. In AM+AZ- single dose	Thailand	Low	MDR	15-59 years	28

AS = artesunate, A = artemisinin, AM = artemether, CQ = chloroquine, SP = sulfadoxine-pyrimethamine, Q = quinine, D = doxycycline, AZ = azithromycin, PQ = primaquine, SMP = sulfamethoxypyrimethamine, MDR = multidrug resistance

Table 3.2e. Characteristics of unpublished included studies.

REFERENCE	TREATMENTS	DURATION	LOCATION	TRANSMISSION	RESISTANCE	AGE	FOLLOW-UP
Newman, unpublished	AS+AQ vs AS+SP	3 days	Mali	Hyperendemic	CQ, SP	6-59 months	28 days
Lameyre, unpublished	AS+AQ vs AS+SP	3 days	Mali	Endemic	CQ, SP	≥6 months	28 days
Graham, unpublished	AS+AQ vs AS+SP vs AS+CQ	3 days	Parkistan	Endemic	CQ, SP	5-70 years	28 days
D'Alesandro, unpublished	AS+AQ vs AS+CD	3 days	Multinational	Differs	Differs	6-59 months	63 days
Bronzan, unpublished (a)	AS+AQ vs AS+CD	3 days	Malawi	Endemic	CQ, SP	6-59 months	28 days
Bonzan, unpublished (b)	AS+AQ vs AS+CD	3 days	Malawi	Endemic	CQ, SP	≥5 years	28 days
Mueller, unpublished	AS+AQ vs AS+MB	3 days	Burkina Faso	Endemic	CQ, SP	6-59 months	28 days
Borghini-Fuhrer, unpublished	AS+MQ vs AS+PR	3 days	Multinational	Differs	Differs	3-60 years	28 days
Fofana, unpublished	AS+AQ vs AS+SP	3 days	Mali	Endemic	CQ, SP	Unclear	28 days
Rueangweerayut, unpublished	AS+MQ vs AS+PR	3 days	South East Asia and Africa	Differs	CQ, SP	Unclear	28 days
Dembele, unpublished (a)	AS+AQ vs AS+SP	3 days	Mali	Endemic	CQ, SP	Unclear	28 days
Dembele, unpublished (b)	AS+AQ vs AS+SP	3 days	Mali	Endemic	CQ, SP	Unclear	28 days

AS = artesunate, AQ= amodiaquine, SP=sulfadoxine-pyrimethamine, CQ = chloroquine, CD, chlorproguanil-dapsone, MB=methylene-blue, MQ = mefloquine, PQ = primaquine, PR=pyronadine

3.1.2 Participants included

Participants in these trials were male and female participants, in 11 trials only children aged between 6 and 59 months were included, one trial included children from the age of three months (Rulisa *et al*., 2007). Twenty-two RCTs included adults and in one, up to 70 years old (Tables 3.2a-d). All the participants included in these RCTs were febrile or had a history of fever in the previous 24 hours. They were also included if they had parasitaemia of 1000 or 2000 up to 100 000 or 200 000 parasites/µL. Pregnancy, mixed plasmodium infection, severe malaria and other concomitant febrile or severe illnesses were exclusion criteria common in all trials.

3.1.3 Interventions investigated

Four trials had three intervention arms relevant for this review (Djimde *et al*., 2008; Krudsood *et al*., 2000; Li *et al*., 1984; Graham, unpublished), these were split into three comparisons and the rest of the trials had only two intervention arms relevant in this review. In 14 trials, there were intervention arms where participants were given non-artemisinin treatments, or artemisinins not relevant to the objectives of this review and data on these participants were not extracted (Fehintola *et al*., 2008; Guthmann *et al*., 2005; Van den broek *et al*., 2006; Abacassamo *et al*., 2004; Looareesuwan *et al*., 1992; Sowunmi *et al*., 2007; Barennes *et al*., 2004; Faye *et al*., 2007; Van Vugt *et al*., 2002; Kofoed *et al*., 2003; Bich *et al*., 1996; Li *et al*., 1984; Kayentao *et al*., 2009; Carmona-Fonseca *et al*., 2008). In three trials which employed more than two interventions, two intervention arms were similar with regards to the drugs used, with minor differences in dosages; these were combined into one intervention due to their similarity for this review (Gomez *et al*., 2003; Looareesuwan *et al*., 1997; Kofoed *et al*., 2003).

3.1.3.1 Artesunate combined with amodiaquine

The efficacy of artesunate combined with amodiaquine (AS+AQ) was evaluated in 17 published and 10 unpublished trials (Table 3.2a and e), in 14 of those AS+AQ was compared with artesunate plus sulfadoxine-pyrimethamine (AS+SP). One trial compared the efficacy of AS+AQ with that of artesunate plus mefloquine (AS+MQ) and three trials compared AS+AQ with artesunate (AS) monotherapy. Artesunate plus methylene-blue (AS+MB), artesunate plus chlorproguanil-dapsone (AS+CD) and artesunate plus cotrimoxazole (AS+CT) were comparisons for AS+AQ in one trial each. There was one trial evaluating the efficacy of adding mefloquine to dihydroartemisinin (DHA) compared to DHA monotherapy (Diem Thuy *et al*., 2007), this and other comparisons are shown in Tables 3.2b-d. Doses for AQ, SP and MQ were given over 3 days but there were variations in the duration of AS doses. Artesunate was given over three days in all the trials that compared AS+AQ with AS+SP or AS+MQ. Contrary to that, a longer duration of seven days was used in one trial for both combination- and monotherapy (Sowunmi *et al*., 2007). On the other hand, artesunate was given for three days in both combination and monotherapy in one trial (Barennes *et al*., 2004). Diem Thuy and colleagues (2007) gave two doses of DHA over two days in combination therapy. Treatment doses were not described in one trial (Swarthout *et al*., 2006). All doses were supervised but in one trial (Abacassamo *et al*., 2004), the second and third doses were given at home. Data from the included unpublished studies was not complete for this descriptive analysis, however, the available data have been presented in Table 3.2e.

3.1.3.2 Artesunate combined with mefloquine

Artesunate was combined with mefloquine (AS+MQ) in nine trials (Table 3.2b). In four of those it was compared with AS monotherapy, with artesunate plus azithromycin (AS+AZ) in

one trial, with artesunate plus atovaquone-proguanil (AS+ATV+PRG) in one and with artesunate plus mefloqine+sulfadoxine-pyrimethamine (AS+MQ+SP) in one. Artesunate plus pyronadine (AS+PR) was a comparison in two unpublished RCTs. Doses for artesunate also varied in this comparison. In monotherapy, AS was given for 5-6 days, with the exception of two trials in which AS was given for three days (Li *et al*., 1984; Krudsood *et al*., 2000). In combination therapy, treatment was given for a shorter duration. AS was given as a single dose in two trials (Li *et al*., 1984; Karbwang *et al*., 1996), while in other trials AS doses were given over three days (Gomez *et al*., 2003; Krudsood *et al*., 2000; van Vugt *et al*., 2002; Borghini-Fuhrer, unpublished; Rueangweerayut, unpublished). Treatment was given for a longer duration in two trials (Looareesuwan *et al*., 1992; Price *et al*., 1998), where AS was given over five days in combination therapy.

3.1.3.3 Artemisinin or artemether combined with mefloquine

The other combinations of mefloquine were with artemether (Karbwang *et al*., 1995; Looareesuwan *et al*., 1997) and with artemisinin (Hassan Alin *et al*., 1996). These combination therapies were compared with AM and A monotherapies, respectively. AM was given as a single dose in combination therapy and over five to seven days in monotherapy (Table 3.2c).

3.1.3.4 Artesunate or artemisinin monotherapy

Artesunate monotherapy was used as a control for artesunate plus mefloquine plus sulfadoxine-primethamine (AS+MQ+SP) (Li *et al*., 1984), for artesunate plus chloroquine (AS+CQ) (Kofoed *et al*., 2003), for artesunate plus primaquine (AS+PQ) (Pukrittayakamee *et al*., 2004) and for artesunate plus azithromycin (AS+AZ) (Krudsood *et al*., 2000). In addition,

artemisinin monotherapy was evaluated against the combination of artemisinin plus beta-cyclodextrin (A+β-CDX) in one trial (Wong *et al*., 2003) and one trial evaluated dihydroartemisinin (DHA) monotherapy compared to DHA given with mefloquine (DHA+MQ) (Dien Thuy *et al*., 2007) (Table 3.2c).

The duration of treatment with artemisinin derivatives was three days for both combination and monotherapy in two trials (Kofoed *et al*., 2003; Krudsood *et al*., 2000), five days in one trial (Wong *et al*., 2003) and seven days in another (Pukrittayakamee *et al*., 2004). In another trial, monotherapy was given over three days and combination therapy was given as a single dose (Li *et al*., 1984).

3.1.3.5 Other combinations

Artesunate was combined with chloroquine (AS+CQ) and compared to artesunate plus sulfadoxine-pyrimethamine (AS+SP) in one trial (Huong *et al*., 2003). Other interventiosn compared with AS+SP were artesunate plus sulfadoxine-pyrimethamine plus primaquine (AS+SP+PQ) (Shekalaghe *et al*., 2007) and artesunate plus sulfamethoxypyrimethamine (AS+SMP). One RCT compared artemisinin plus quinine (A+Q) with artemisinin plus doxycycline (A+D) (Bich *et al*., 1996) and artemether plus doxycycline (AM+D) was compared with artemether plus azithromycin (AM+AZ) in one trial (Table 3.2d) (Na-Bangchang *et al*., 1996). In one trial (Huong *et al*., 2003), artesunate doses were given over three days in both treatment arms while in another, artemisinin treatment was a single dose in both treatment arms (Bich *et al*., 1997). A single dose of artemether was given in the AM+AZ treatment arm while the other participants in the treatment group received AM over five days (Na-Bangchang *et al*., 1996).

3.1.4 Length of follow-up

The length of follow-up was 28 days for these RCTs, but in one RCT (Abacassamo *et al.*, 2004) a follow-up of 21 days was reported while the reported results were up to the 14th day of follow-up. In another RCT, the length of follow-up was 28 days but treatment failure rates were up to 21 days (Sowunmi *et al.*, 2007). The two trials were not excluded since the length of foloow-up was not listed as the exclusion criteria and results for some outcomes, like early treatment failure and fever clearance times could be obtained irrespective of the length of follow-up. In one RCT (Faye *et al.*, 2007), there were no participants lost to follow-up at dat 28, however, 30% of participants were followed up for 42 days. Five of the trials that gave MQ followed up for 42 days (Diem Thuy *et al.*, 2007; Karbwang *et al.*, 1995; 1996; Price *et al.*, 1998; Van Vugt *et al.*, 2002). The duration of follow-up was 35 days in two trials (Kofoed *et al.*, 2003; Wong *et al.*, 2003).

3.1.5　Outcomes

3.1.5.1 Analyzed outcomes

Ten of the RCTs that reported treatment failure excluded new infections detected with the polymerase chain reaction (PCR) (Bonnet *et al.*, 2007; Djimde *et al.*, 2008; Fehintola *et al.*, 2008; Guthman *et al.*, 2005; Hamour *et al.*, 2005; Kayentao *et al.*, 2009; Owusu-Agyeyi *et al.*, 2008; Swarhout *et al.*, 2006; Van den Broek *et al.*, 2006; Zoungrana *et al.*, 2008) while in two RCTs patients were admitted to health facilities for the duration of follow-up (Looareesuwan *et al.*, 1992; 1997). This method was also used to exclude the possibility of re-infection in patients who were treated, thus these were also categorized as true treatment failure. When new infections were excluded, treatment failure was reported as adjusted treatment failure. If re-infections were not excluded, unadjusted treatment failure was reported. In other RCTs, re-

infections were not excluded in treatment failure. Events of early treatment failure were reported in four RCTs (Bonnet *et al*., 2007; Guthman *et al*., 2005; Owusu-Agyeyi *et al*., 2008; Kayentao *et al*., 2009).

Eleven trials reported both fever and parasite clearance times in hours (Barennes *et al*., 2007; Bich *et al*., 1996; Diem Thuy *et al*., 2007; Fehintola *et al*., 2008; Huong *et al*., 2000; Sowunmi *et al*., 2007; Li *et al*., 1984; Looareesuwan *et al*., 1992; 1997; Krudsood *et al*., 2000; Wong *et al*., 2003), while Pukrittayakamee and colleagues (2004) reported only parasite clearance time in hours. Two trials also reported time taken to clear 50% and 90% of parasites (PCT 50 and PCT 90, respectively) (Bich *et al*., 1996; Diem Thuy *et al*., 2007). There were also three trials that reported parasite clearance at day 3 (Bonnet *et al*., 2007; Hamour *et al*., 2005; Kayentao *et al*., 2009). Only two trials reported anaemia on day 28 (Guthman *et al*., 2005; Kayentao *et al*., 2009). Gametocyte carriage was reported at day 7 in two trials (Shekalaghe *et al*., 2007; Owusu-Agyeyi *et al*., 2008), at day 14 in three (Guthman *et al*., 2005; Hamour *et al*., 2005; Owusu-Agyeyi *et al*., 2008) and at day 28 in three trials as well (Bonnet *et al*., 2007; Guthman *et al*., 2005; Hamour *et al*., 2005).

3.1.5.2 Other reported outcomes

In addition to the outcomes reported above, there were other outcomes that were analyzed in trials but not reported in this review. These include clinical failure at day 21 (Faye *et al*., 2007) parasite reduction ratios on day two and gametocyte carriage after treatment (Sowunmi *et al*., 2007). Parasitaemia on day one and decrease in heamatocrit values (%) were reported in one trial (Barennes *et al*., 2004) whereas one trial (Van Vugt *et al*., 2002) reported the decrease in heamatocrit values on day seven (%) and gametocyte carriage in person-gametocyte

weeks/1000 person-weeks. One trial reported a 60-day cure rate (Gomez *et al*., 2003) and another one reported parasitaemia on day two and day 35 (Kofoed *et al*., 2003). Lastly, clearance of 95% of the baseline parasites burden (PCT 95) was reported in one trial (Li *et al*., 1984).

With an indication that other outcomes were assessed but not adequately reported, additional data were sought from the authors. The requested data were:

- Fever clearance time, presence of fever on day three, anaemia on day 28 and gametocyte carriage at day 14 (Abacassamo *et al*., 2004).

- Gametocyte carriage on days 14 and 28 (Guthmann *et al*., 2005).

- Parasite clearance time of 90% of the baseline burden (Looareesuwan *et al*., 1992).

- Fever and parasite clearance time (Na-Bangchang *et al*., 1996).

- Cure rates for the AS and AS+PQ treatment groups and the number of patients with gametocyte appearances (Pukrittayakamee *et al*., 2004).

- Proportions of patients who cleared fever on day two or who had fever on day three, gametocyte carriage on day 14 and anaemia on day 28 in (Van den Broek *et al*., 2006), and

- Anaemia at day 28 (Swarthout *et al*., 2006).

Data were successfully obtained from Guthmann *et al*., (2005).

3.1.5.3 Adverse events

Most of the trials did not adequately and systematically reported adverse events and side effects, however, there were ten trials adequately reporting adverse events and comparing the

different intervention arms (Bich *et al*., 1996; Djimde *et al*., 2008; Karbwang *et al*., 1995; 1996; Li *et al*., 1984; Looareesuwan *et al*., 1992; Na-Bangchang *et al*., 1996; Price *et al*., 1998; Van Vugt *et al*., 2002; Zoungrana *et al*., 2008).

3.2   Methodological quality of studies that were included

3.2.1   Generation of the sequence used to allocate participants into different groups

All 37 trials were reported as randomized. Only eight RCTs reported methods to generate the allocation sequence that were likely to be free of bias. These included a random permuted block method (Abacassamo *et al*., 2004; Swarthout *et al*., 2006; Diem Thuy *et al*., 2008) and computer generated codes (Bich *et al*., 1996; Barennes *et al*., 2004; Shekalaghe *et al*., 2007; Owusu-Agyeyi *et al*., 2008; Zoungrana *et al*., 2008). In one RCT, randomization started only after the 30th patient was enrolled (Guthmann *et al*., 2005) which is likely to have introduced bias. The rest of the RCTs were not clear on the methods used to generate the allocation sequence. Table 3.3 shows the methodological quality of included studies.

3.2.2   Concealment of the allocation sequence before allocating participants into groups

The methods used to conceal the allocation sequence were likely to be free of bias in eight RCTs; these were sealed and numbered envelopes containing the treatment code (Table 3.3). It was, however, not stated whether the envelopes were opaque or not (Swarthout *et al*., 2006; Van Vugt *et al*., 2002; Bich *et al*., 1996; Abacassamo *et al*., 2004; Diem Thuy *et al*., 2007; Harmour *et al*., 2005; Kofoed *et al*., 2003; Zoungrana *et al*., 2008). In three RCTs, it was stated that the envelopes were opened or drawn only after informed consent was given or after inclusion (Van Vugt *et al*., 2002; Swarthout *et al*., 2006; Bich *et al*., 1996). In two RCTs, the

allocation sequence was reported as not concealed (Guthmann *et al*., 2005; Bonnet *et al*.,

2006). The rest of the trials were unclear on allocation concealment.

Table 3.3: Methodological quality of the studies that were included.*

Reference	Generation of allocation sequence	Allocation concealment	Blinding	Loss to follow-up treatment (%)	Loss to follow-up control (%)
Abacassamo *et al*., 2004	Random permutted block method	Sealed envelopes	Open	13	11
Barenness *et al*., 2004	Computer generated	Unclear	Participants, care givers and outcome assessors	0	0
Bich *et al*., 1996	Computer generated	Envelopes drawn after inclusion	Open	2.2	7.5
Bonnet *et al*., 2007	Unclear	Not concealed	Unclear	2.7	3.6
Carmona-Fonsecca *et al*., 2008	Unclear	Unclear	Unclear	0	0
Diem Thuy *et al*., 2007	Codes in blocks of 4	Sealed and numbered envelopes	Open	0	0
Djimde *et al*., 2008	Unclear	Unclear	Unclear	1.6	3.6 & 1.6
Faye *et al*., 2007	Unclear	Unclear	Open	0	0
Fehintola *et al*., 2008	Unclear	Unclear	Unclear	0	0
Gomez *et al*., 2003	Unclear	Unclear	Unclear	0	0
Guthmann *et al*., 2005	Started after 30th patient	Not concealed	Unclear	13	7
Hamour *et al*., 2005	Unclear	Sealed envelopes	Unclear	0	2.5
Hassan Alin *et al*., 1996	Unclear	Unclear	Outcome assessor	15	5.5
Huong *et al*., 2003	Unclear	Unclear	Unclear	0	0
Owusu-Agyei *et al*., 2008	Computer generated	Unclear	Unclear	2.9	2.9
Karbwang *et al*., 1995	Unclear	Unclear	Unclear	10.7	5.7
Karbwang *et al*., 1996	Unclear	Unclear	Unclear	17.6	7
Kayento *et al*., 2009	Unclear	Unclear	Participants and outcome assessors	1.5	1.5
Kofoed *et al*., 2003	Unclear	Sealed envelopes	Unclear	21.3	11.3

Table 3.3 (continued): Methodological quality of the studies that were included*

Reference	Generation of allocation sequence	Allocation concealment	Blinding	Loss to follow-up treatment (%)	Loss to follow-up control (%)
Krudsood *et al.*, 2000	Unclear	Unclear	Unclear	18.9	10.3
Li *et al.*, 1984	Unclear	Unclear	Unclear	0	0
Looareesuwan *et al.*, 1992	Unclear	Unclear	Unclear	7.1	4.8
Looareesuwan *et al.*, 1997	Unclear	Unclear	Open	17	11.2
Na-Bangchang *et al.*, 1996	Unclear	Unclear	Unclear	0	10
Price *et al.*, 1998	Unclear	Unclear	Open	2	0
Pukrittayakamee *et al.*, 2004	Unclear	Unclear	Unclear	7.4	8.7
Rulise *et al.*, 2007	Unclear	Unclear	Open	5.8% replaced	3.7% replaced
Shekalaghe *et al.*, 2007	Computer generated	Unclear	All staff	1.9	1.9
Sowunmi *et al.*, 2007	Unclear	Unclear	Unclear	0	7.5
Sowunmi *et al.*, 2009	Unclear	Unclear	Unclear	15	3.3
Swarthout *et al.*, 2006	Computer generated	Envelopes drawn after inclusion	Outcome assessor	7.7	10
Van den Broek *et al.*, 2006	Unclear	Unclear	Unclear	4	4.6
Van Vugt *et al.*, 2002	Unclear	Envelopes drawn after inclusion	Open	0	0
Wong *et al.*, 2003	Unclear	Unclear	Outcome assessor	0	0
Zoungrana *et al.*, 2008	Computer generated	Sealed envelopes	Outcome assessors	0	1.6

*Two unpublished and included RCTs had no data on methodological quality

3.2.3 Blinding of research personnel and participants to allocated interventions

Participants, care-givers and outcome assessors were blinded to the study treatments in one RCT (Barennes *et al*., 2004) and in one RCT, all research staff were blinded but there was no mention of blinding the patients though it is likely that they were blinded as well (Shekalaghe *et al*., 2007). Four RCTs blinded only the outcome assessors (Swarthout *et al*., 2006; Hassan Alin *et al*., 1996 and Wong *et al*., 2003; Zoungrana *et al*., 2008). Authors of seven RCTs (Abacassamo *et al*., 2004; Diem Thuy *et al*., 2007; Faye *et al*., 2007; Bich *et al*., 199; Looareesuwan *et al*., 1997; Price *et al*., 1998; Van Vugt *et al*., 2002; Rulise *et al*., 2007) reported not blinding participants and care-givers. The rest of the studies did not describe blinding (Table 3.3).

3.2.4 Inclusion of all randomized participants in the assessment of outcomes

In nine RCTs, all randomized participants were included in the analysis (Barennes *et al*., 2004; Diem Thuy *et al*., 2007; Faye *et al*., 2007; Fehintola *et al*., 2008; Gomez *et al*., 2003; Huong *et al*., 2003; Li *et al*., 1984; Van Vugt *et al*., 2002; Wong *et al*., 2003). The inclusion of randomized participants in the analysis was more than or equal to 90% in 19 trials and seven trials lost more than 10% of the participants during follow-up (Table 3.3).

3.3 The efficacy and safety of different treatments

3.3.1 Artesunate plus amodiaquine combination therapy

3.3.1.1 Treatment Failure

Adjusted treatment failure at day 28 was reported in seven trials comparing AS+AQ with AS+SP (Figure 3.2). Treatment with AS+AQ was associated with a 43% lower risk of adjusted treatment failure compared to AS+SP (RR=0.57, 95% CI [0.33, 0.97], p=0.04, 7 trials, N= 1341).

	AS+AQ		AS+SP			Risk Ratio	Risk Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Fixed, 95% CI	M-H, Fixed, 95% CI
Bonnet *et al.*, 2007	1	107	1	106	3.0%	0.99 [0.06, 15.63]	
Djimde *et al.*, 2008	1	115	0	115	1.5%	3.00 [0.12, 72.88]	
Guthmann *et al.*, 2005	1	84	1	84	2.9%	1.00 [0.06, 15.73]	
Hamour *et al.*, 2005	4	72	5	71	14.8%	0.79 [0.22, 2.82]	
Kayentao *et al.*, 2009	6	131	6	130	17.7%	0.99 [0.33, 3.00]	
Swarthout *et al.*, 2006	5	83	13	79	39.1%	0.37 [0.14, 0.98]	
Van den Broek *et al.*, 2006	1	84	7	80	21.1%	0.14 [0.02, 1.08]	
Total (95% CI)		676		665	100.0%	0.57 [0.33, 0.97]	
Total events	19		33				
Heterogeneity: Chi² = 5.19, df = 6 (P = 0.52); I² = 0%							
Test for overall effect: Z = 2.06 (P = 0.04)							

AS = artesunate, AQ = amodiaquine, SP = sulfadoxine-pyrimethamine, CI = confidence interval

Figure 3.2: The risk of treatment failure in patients when artesunate plus amodiaquine combination therapy was compared with artesunater plus sulfadoxine-pyrimethamine combination therapy.

The risk of early treatment failure was lower in AS+AQ (RR=0.26, 95% CI [0.04, 1.59], P=0.15; 3 trials, N= 658), however, the difference between treatments was not statistically significant. With regards to the risk of re-infection there was tremendous heterogeneity

between the RCTs, with two RCTs conducted in Mali significantly in favour of AS+SP (Djimde *et al.*, 2008; Kayentao *et al.*, 2009). In other RCTs in the DRC and in Guinea, there was no significant difference observed between the two treatment groups. Due to this level of heterogeneity ($I^2 = 83\%$, $p < 0.0001$), it was not correct to combine data in a meta-analysis. The potential sources of heterogeneity between these five trials were explored; even though these RCTs were conducted in areas with SP resistance, resistance in Mali is lower than in the DRC (Djimde *et al.*, 2008; Swarthout *et al.*, 2006).

Compared to AS+CD and AS+MB, AS+AQ had a significantly lower risk of adjusted treatment failure but there was no statistically significant difference when compared to AS+CT (Table 3.4). Furthermore, treatment with AS+AQ was significantly associated with a lower risk of re-infection compared to other combination therapies, with the exception of AS+MQ, which showed no significant difference (Table 3.5).

Table 3.4: Adjusted treatment failure at day 28 when artesunate plus amodiaquine was compared with other combination therapies or artesunate monotherapy.

Reference	Comparator	Number of patients (n/N)		Relative Risk (95% CI)	P-value
		AS+AQ	comparator		
Fehintola *et al.*, 2008	AS+CT	4/61	11/121	0.72 (0.24, 2.17)	0.31
Owusu-Agyeyi *et al.*, 2008	AS+CD	11/149	25/147	0.43 (0.22, 0.85)	0.01
Zoungrana *et al.*, 2008	AS+MB	11/61	22/60	0.49 (0.26, 0.92)	0.03
Djimde *et al.*, 2008	AS	1/114	4/115	0.25 (0.03, 2.22)	0.21

AS= artesunate, CT= cotrimoxazole, CD= chlorproguanil-dapsone, MB= methylene-blue n= number of patients reporting an outcome, N= total number of patients examined, CI= confidence interval

Table 3.5: The risk of re-infection when artesunate plus amodiaquine was compared with other combination therapies or artesunate monotherapy.

Reference	Comparator	Number of patients (n/N)		Relative Risk (95% CI)	P-value
		AS+AQ	comparator		
Fehintola *et al.*, 2008	AS+CT	2/61	22/121	0.18 (0.04, 0.74)	0.02
Owusu-Agyeyi *et al.*, 2008	AS+CD	11/148	23/144	0.47 (0.24, 0.92)	0.03
Zoungrana *et al.*, 2008	AS+MB	10/61	21/60	0.47 (0.24, 0.91)	0.03
Faye *et al.*, 2007	AS+MQ	9/360	3/145	1.21 (0.33, 4.40)	0.77
Djimde *et al.*, 2008	AS	18/114	44/115	0.41 (0.25, 0.67)	<0.001

AS= artesunate, CT= cotrimoxazole, CD= chlorproguanil-dapsone, MB= methylene-blue, MQ= Mefloquine, n= number of patients reporting an outcome, N= total number of patients examined, CI= confidence interval

In one trial comparing AS+AQ with AS monotherapy, there was a 95% lower risk of PCR unadjusted treatment failure at day 21 in AS+AQ (RR= 0.05, 95% CI [0.01, 0.34], p=0.002; N=231).

3.3.1.2 Fever clearance time

Two trials reported the number of patients who cleared fever on day two, there was no statistically significant difference between AS+AQ and AS+SP (RR=1.03, 95% CI [0.09, 1.08], p= 0.32; 1 trial, N=161), and between AS+AQ and AS monotherapy (RR=0.92, 95% CI [0.75, 1.13], p=0.44; 1 trial, N=60). Another trial reported the number of patients who were still febrile at day three when treated with AS+AQ compared to AS+SP, and there was no statistically significant difference between the two treatments (RR=0.67, 95% CI [0.15, 2.91], p= 0.59; N=191). When AS+AQ was compared to AS monotherapy, two trials reported fever clearance time in hours. Treatment with AS+AQ was significantly quicker in clearing fever compared to AS monotherapy, however, there was no statistically significant difference when AS+AQ was compared to AS+CT (Figure 3.3).

3.3.1.3 Parasite clearance time

Three studies reported the number of patients who cleared parasitaemia at day three,

comparing AS+AQ with AS+SP. In both treatments, most patients cleared parasitaemia

showing no statistically significant difference between treatments (RR=1.01, 95% CI [0.96,

1.07], p=0.58; N=649). Two trials that compared AS+AQ with AS monotherapy reported the

time taken to clear parasitaemia in hours. These showed no difference between treatments.

However, when AS+AQ was compared with AS+CT, AS+AQ was significantly slower in

clearing parasitaemia (Figure 3.4).
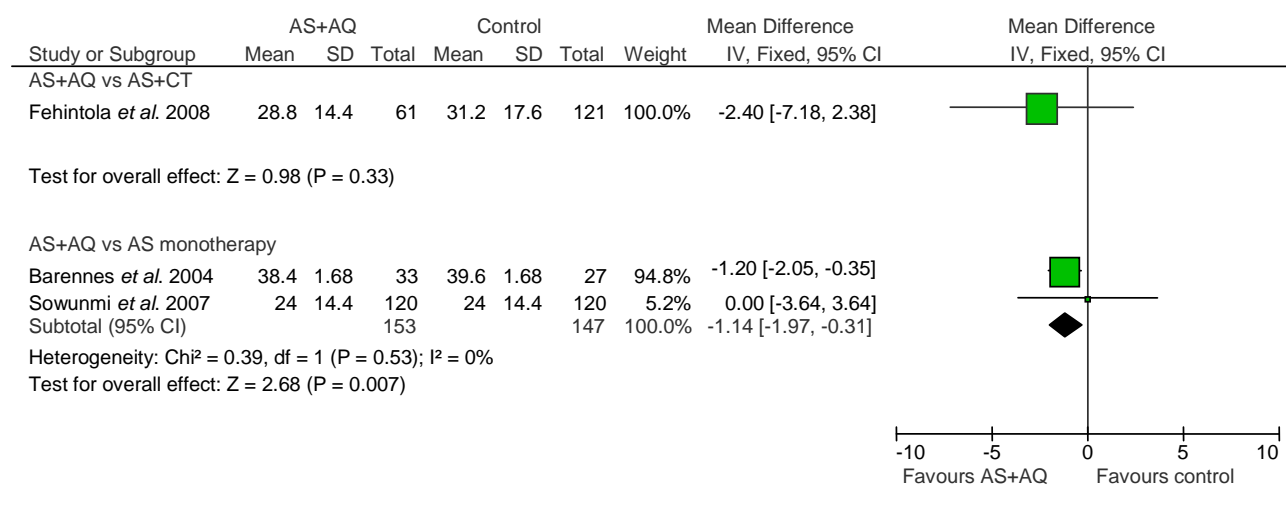
Study or Subgroup	AS+AQ Mean	SD	Total	Control Mean	SD	Total	Weight	Mean Difference IV, Fixed, 95% CI
AS+AQ vs AS+CT								
Fehintola *et al.* 2008	28.8	14.4	61	31.2	17.6	121	100.0%	-2.40 [-7.18, 2.38]
Test for overall effect: Z = 0.98 (P = 0.33)								
AS+AQ vs AS monotherapy								
Barennes *et al.* 2004	38.4	1.68	33	39.6	1.68	27	94.8%	-1.20 [-2.05, -0.35]
Sowunmi *et al.* 2007	24	14.4	120	24	14.4	120	5.2%	0.00 [-3.64, 3.64]
Subtotal (95% CI)			153			147	100.0%	-1.14 [-1.97, -0.31]
Heterogeneity: Chi² = 0.39, df = 1 (P = 0.53); I² = 0%								
Test for overall effect: Z = 2.68 (P = 0.007)								

AS= artesunate, AQ= amodiaquine, CT= cotrimoxazole, SD= standard deviation, CI= confidence interval

Figure 3.3: Fever clearance time (hours) in patients treated with artesunate plus amodiaquine, artesunate plus cotrimoxazole or with artesunate monotherapy.

Study or Subgroup	AS+AQ Mean	SD	Total	Control Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI	Mean Difference IV, Random, 95% CI
AS+AQ vs AS+CT									
Fehintola *et al.* 2008	44.6	15.84	61	31.44	11.52	120	100.0%	13.16 [8.68, 17.64]	

Test for overall effect: Z = 5.76 (P < 0.00001)

AS+AQ vs AS monotherapy									
Barennes *et al.* 2004	27.2	1.2	33	27.2	1.2	27	52.3%	0.00 [-0.61, 0.61]	
Sowunmi *et al.* 2007	33.6	14.4	120	33.6	12	120	47.7%	0.00 [-3.35, 3.35]	
Subtotal (95% CI)			**153**			**147**	**100.0%**	**0.00 [-0.60, 0.60]**	

Heterogeneity: Tau² = 0.00; Chi² = 0.00, df = 1 (P = 1.00); I² = 0%
Test for overall effect: Z = 0.00 (P = 1.00)

```
        -20   -10    0    10    20
     Favours AS+AQ      Favours control
```
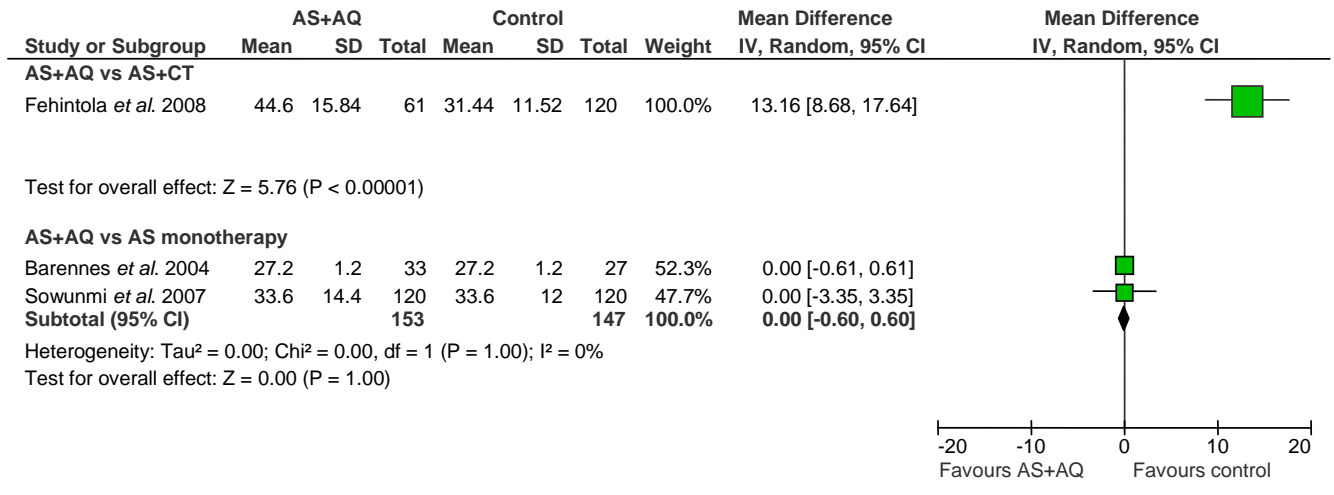
AS= artesunate, AQ= amodiaquine, CT= cotrimoxazole, SD=standard deviation, CI= confidence interval

Figure 3.4: Parasite clearance time (hours) in patients treated with artesunate plus amodiaquine, artesunate plus cotrimoxazole or with artesunate monotherapy.

3.3.1.4 Anaemia at day 28

There were two RCTs reporting the number of patients with anaemia at day 28, although the risk of having anaemia was slightly lower in patients treated with AS+AQ compared to AS+SP, there was no statistically significant difference between the two treatments (RR=0.95, 95% CI [0.79, 1.14], p=0.46; N=429).

3.3.1.5 Gametocyte carriage at days 7, 14 and 28

Gametocyte carriage at day seven, 14 and 28 was reported in one, two and three trials, respectively (Figure 3.5).
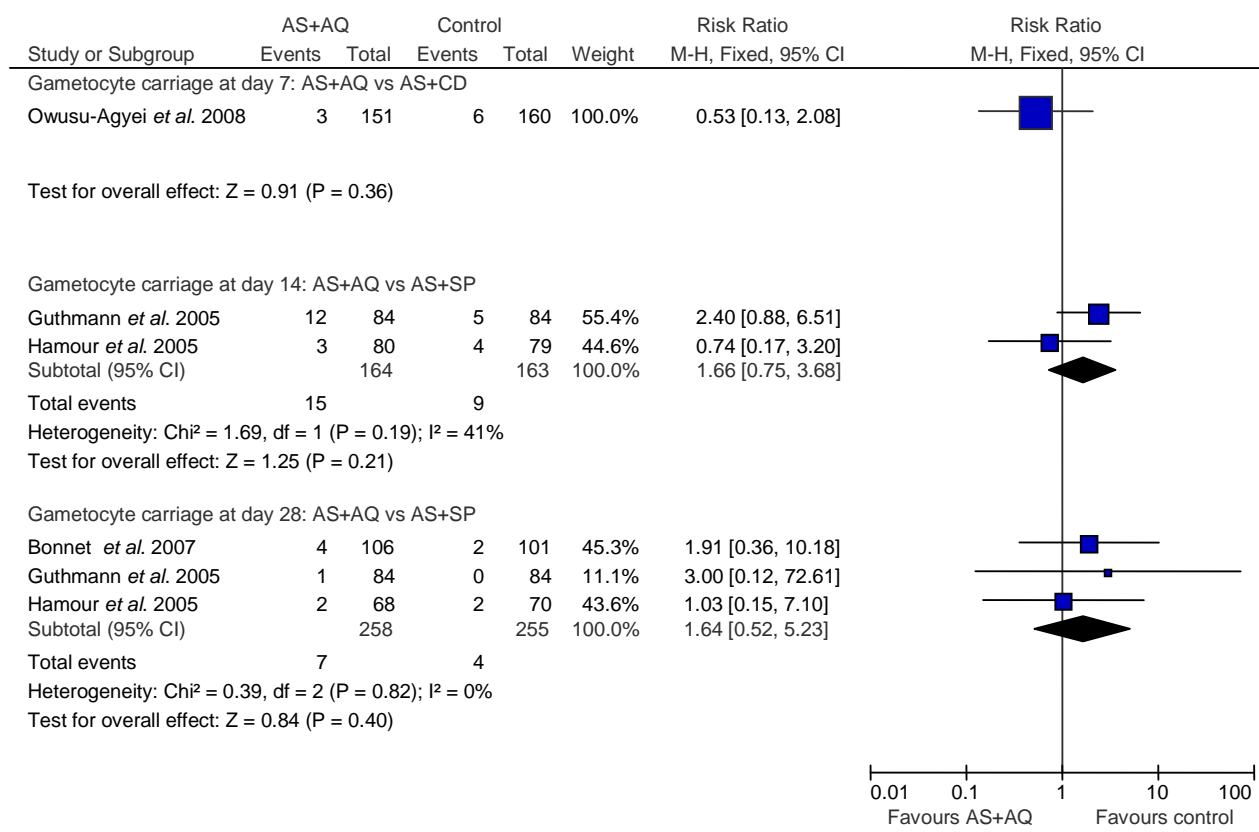
AS=artesunate, AQ= amodiaquine, SP=sulfadoxine-pyrimethamine, CD= chlorproguanil-dapsone, CI= confidence interval

Figure 3.5: Gametocyte carriage at days 7, 14 and 28 in patients treated with artesunate plus amodiaquine, artesunate plus sulfadoxine-pyrimethamine or artesunate plus chlorproguanil-dapsone.

Treatment with AS+AQ was associated with a lower risk of gametocyte carriage at days seven compared to AS+CD, whereas the risk of gametocyte carriage at days 14 and 28 was greater in AS+AQ compared to treatment with AS+SP. There was, however, no statistically significant difference between the treatments.

3.3.1.6 Adverse events and side effects

The most commonly cited side effect in all treatments was repeated dose vomiting or vomiting (Hamour *et al*., 2005; Sowunmi *et al*., 2007; Guthman *et al*., 2005; Ibrahium *et al*., 2007; Owusu-Ayei *et al*., 2008; Djimde *et al*., 2008; Van den Broek *et al*., 2006; Faye *et al*., 2007; Zoungrana *et al*., 2008). In one trial a patient treated with AS+AQ was hospitalized due to severe vomiting (Zoungrana *et al*., 2008). A statistically significant difference in the risk of inducing vomiting was observed when AS+AQ was compared to AS+MB (Zoungrana *et al*., 2008) with a 64% lower risk in AS+AQ (RR=0.36, 95% CI [0.23, 0.57] $\leq$p.001; 1 trial n=122). Other common side effects associated with the digestive system included nausea, diarrhoea, inability to suck or drink, loss of appetite, anorexia, ulcers and mild gastralgia, with no difference in their frequency between treatment groups in all trials.

Pruritus was the most common dermatological side effect in patients treated with AS+AQ, AS+SP, AS+MQ, AS+CD or AS monotherapy (Sowunmi *et al*., 2007; Owusu-Agyei *et al*., 2008; Zoungrana *et al*., 2008). In other trials urticaria, skin itching, macopapular rash, asthenia, dysuria, bronchitis, difficulty in breathing, body pains, joint pains and abdominal pain were also reported. Dizziness was reported in patients treated with AS+AQ, AS+SP or AS+MQ (Ibrahium *et al*., 2007; Faye *et al*., 2007). Other common side effects associated with the nervous system included headache and difficulties in sleeping (Owusi-Agyei *et al*., 2008; Zoungrana *et al*., 2008).

One patient treated with AS+AQ had febrile convulsions at day 16 and in the same trial, biochemical analysis revealed a transient increase in alanine aminotransferase (ALAT) at day 28 in one patient treated with AS+SP (Barennes *et al*., 2004). In another trial a patient treated with AS+SP had a slight increase in creatinine levels (Faye *et al*., 2007). One trial reported the risk of grade 1 ALAT toxicity and abnormal ALAT during follow-up, when comparing AS+AQ with AS+SP or AS monotherapy (Djimde *et al*., 2008). The risk of grade 1 ALAT toxicity was four times greater in AS+AQ compared to AS+SP (RR=4.03, 95% [1.17, 13.94], p=0.03, 1 trial, N=149) but there was no significant difference when AS+AQ was compared with AS monotherapy. Abnormal ALAT during follow-up was three times greater in ASAQ compared to AS+SP and AS monotherapy, and the difference between treatments was at the borderline of statistical significance. In both comparisons, RR=3, 95% CI (1.00, 9.02), p=0.05; 1 trial, N=226)

3.3.2 Artesunate plus mefloquine combination therapy

3.3.2.1 Treatment failure

Treatment failure at day 28 was reported in six trials comparing AS+MQ with AS monotherapy. Treatment with AS+MQ was significantly associated with a 66% lower risk of PCR unadjusted treatment failure compared to AS monotherapy (Figure 3.6). However, there was no significant difference in adjusted treatment failure between the two treatments (RR=0.09, 95% CI [0.01, 1.63], p=0.1, one trial, N=79).

Study or Subgroup	AS+MQ Events	Total	Control Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI
AS+MQ vs AS						
Gomez *et al.*, 2003	5	100	12	150	19.0%	0.63 [0.23, 1.72]
Karbwang *et al.*, 1996	6	28	2	31	3.8%	3.32 [0.73, 15.13]
Krudsood *et al.*, 2000	1	28	17	31	32.0%	0.07 [0.01, 0.46]
Li *et al.*, 1984	0	10	4	9	9.4%	0.10 [0.01, 1.65]
Looareesuwan *et al.*, 1992	0	39	5	40	10.8%	0.09 [0.01, 1.63]
Price *et al.*, 1998	2	34	13	36	25.1%	0.16 [0.04, 0.67]
Subtotal (95% CI)		**239**		**297**	**100.0%**	**0.33 [0.19, 0.56]**
Total events	14		53			

Heterogeneity: Chi² = 15.55, df = 5 (P = 0.008); I² = 68%
Test for overall effect: Z = 4.06 (P < 0.0001)

AS= artesunate, MQ = mefloquine, CI = confidence interval

Figure 3.6: The risk of unadjusted treatment failure in patients when artesunate plus mefloquine combination therapy was compared with artesunate monotherapy.

In one trial comparing AS+MQ with AS+AZ, the risk of PCR unadjusted treatment failure at day 28 was 96% lower in AS+MQ. Another trial comparing AS+MQ with AS+ATV+PRG reported treatment failure at day 42, the risk of PCR unadjusted treatment failure in AS+MQ was almost twice the risk in AS+ATV+PRG, and when re-infections were separated with recrudescences, no statistically significant difference was observed in the risk of adjusted treatment failure between AS+MQ and AS+ATV+PRG (Table 3.6).

Table 3.6: The risk of treatment failure when artesunate plus mefloquine combination therapy was compared with artesunate plus azithromycin or artesunate plus atovaquone plus proguanil.

Reference	Comparison	Outcome	Number of patients		RR (95% CI)	p-value
			AS+MQ (n/N)	Control (n/N)		
Krudsood *et al*., 2000	AS+AZ	Unadjusted treatment failure at day 28	0/27	12/27	0.04 (0.00, 0.64)	0.02
Van Vugt *et al*., 2002	AS+ATV+PRG	Unadjusted treatment failure at day 42	35/533	18/533	1.94 (1.12, 3.39)	0.02
Van Vugt *et al*., 2002	AS+ATV+PRG	Adjusted treatment failure at day 42	13/533	5/533	2.60 (0.93, 7.24)	0.07

AS= artesunate, ATV= atovaquone, PRG= proguanil, MQ= mefloquine
n= number of patients with an outcome, N= number of patients examined, RR= relative risk, CI= confidence interval

3.3.2.2 Fever clearance time

Fever clearance time was longer with AS+MQ by 3.76 hours compared to AS monotherapy, by 5.2 hours compared to AS+AZ and by 8.4 hours compared to artesunate plus mefloquine plus sulfadoxine-pyrimethamine (AS+MQ+SP), but the difference between treatments was not statistically significant (Figure 3.7). Most patients cleared fever on day two when comparing AS+MQ with AS+ATV+PRG and the difference between these two treatments was marginally significant (Table 3.7).

Comparisons or references	AS+MQ N	AS+MQ Mean (SD)	Control N	Control Mean (SD)	WMD (fixed) 95% CI	Weight %	WMD (fixed) 95% CI
AS+MQ vs AS							
Li *et al.*, 1984	10	22.00(12.90)	9	15.50(11.00)		39.99	6.50 [-4.25, 17.25]
Looareesuwan *et al.*, 1992	39	37.50(24.60)	40	35.10(23.40)		41.19	2.40 [-8.19, 12.99]
Krudsood *et al.*, 2000	28	38.80(32.10)	31	37.90(29.00)		18.82	0.90 [-14.77, 16.57]
Subtotal (95% CI)	**77**		**80**			**100.00**	**3.76 [-3.04, 10.56]**
Test for heterogeneity: Chi² = 0.44, df = 2 (P = 0.80), I² = 0%							
Test for overall effect: Z = 1.08 (P = 0.28)							
AS+MQ vs AS+AZ							
Krudsood *et al.*, 2000	**27**	**38.80(32.10)**	**28**	**33.60(27.20)**		**100.00**	**5.20 [-10.55, 20.95]**
Test for overall effect: Z = 0.65 (P = 0.52)							
AS+MQ vs AS+MQ+SP							
Li *et al.*, 1984	**10**	**22.00(12.90)**	**10**	**13.60(11.60)**		**100.00**	**8.40 [-2.35, 19.15]**
Test for overall effect: Z = 1.53 (P = 0.13)							

-100   -50   0   50   100

Favours AS+MQ   Favours Control

AS = artesunate, MQ = mefloquine, AZ = azithromycin, SP = sulfadoxine-pyrimethamine

n= number of patients with an outcome, N= number of patients examined, SD= standard deviation, CI= confidence interval, WMD= weighted mean difference

Figure 3.7: Fever clearance time when artesunate plus mefloquine was compared with artesunate monotherapy, artesunate plus azithromycin or artesunate plus mefloquine plus sulfadoxine-pyrimethamine.

Table 3.7: The number of patients who cleared fever and parasites at the given times when artesunate plus mefloquine combination therapy was compared with artesunate plus atovaquone plus proguanil.

Reference	Comparison	Outcome	Number of patients		RR (95% CI)	p-value
			AS+MQ (n/N)	Control (n/N)		
Van Vugt *et al.*, 2002	AS+ATV+PRG	Fever on day 2	6/533	15/533	0.40 (0.16, 1.02)	0.06
Van Vugt *et al.*, 2002	AS+ATV+PRG	Parasite clearance on day 3	531/533	511/533	1.04 (1.02, 1.06)	<0.001

AS= artesunate, ATV= atovaquone, PRG= proguanil, MQ= mefloquine
n= number of patients with an outcome, N= number of patients examined, RR= relative risk, CI= confidence interval

3.3.2.3 Parasite clearance time

The time taken to clear parasites was not significantly different when AS+MQ was compared with AS monotherapy (WMD=-0.09, 95% CI [-1.43, 1.26], p=0.9), or with AS+AZ (WMD=1.80, 95% CI [-6.67, 10.27], p=0.68) and with AS+MQ+SP (WMD=5.2, 95% CI [-10.76, 21.16], p=0.52 (Figure 3.8). However, more patients cleared parasitaemia on day three when treated with AS+MQ compared to AS+ATV+PRG (Table 3.7).

Comparisons or references	AS+MQ		Control		WMD (fixed) 95% CI	Weight %	WMD (fixed) 95% CI
	N	Mean (SD)	N	Mean (SD)			
AS+MQ vs AS							
Li *et al.*, 1984	10	60.10(22.40)	9	44.70(13.00)		0.68	15.40 [−0.88, 31.68]
Looareesuwan *et al.*, 1992	39	37.50(10.30)	40	35.90(10.10)		8.94	1.60 [−2.90, 6.10]
Krudsood *et al.*, 2000	28	45.30(14.70)	31	43.70(13.60)		3.44	1.60 [−5.65, 8.85]
Gomez *et al.*, 2003	100	8.75(4.15)	50	9.20(4.30)		86.93	−0.45 [−1.89, 0.99]
Subtotal (95% CI)	**177**		**130**			**100.00**	**−0.09 [−1.43, 1.26]**
Test for heterogeneity: Chi² = 4.47, df = 3 (P = 0.22), I² = 32.9%							
Test for overall effect: Z = 0.13 (P = 0.90)							
AS+MQ vs AS+AZ							
Krudsood *et al.*, 2000	**27**	**45.30(14.70)**	**28**	**43.50(17.30)**		**100.00**	**1.80 [−6.67, 10.27]**
Test for overall effect: Z = 0.42 (P = 0.68)							
AS+MQ vs AS+MQ+SP							
Li *et al.*, 1984	**10**	**60.10(22.40)**	**10**	**54.90(12.70)**		**100.00**	**5.20 [−10.76, 21.16]**
Test for overall effect: Z = 0.64 (P = 0.52)							

```
        -100   -50    0    50   100
       Favours AS+MQ        Favours Control
```

AS= artesunate, MQ= mefloquine, AZ= azithromycin, SP= sulfadoxine-pyrimethamine, N= number of patients examined, SD= standard deviation, WMD= weighted mean difference, CI= confidence interval

Figure 3.8: Parasite clearance time in patients treated with artesunate plus mefloquine or other treatments.

3.3.2.4 Adverse events and side effects

There was no statistically significant difference in the risk of developing dizziness, diarrhea, nausea and vomiting in patients treated with AS+MQ compared to AS monotherapy (Table 3.8) (Looareesuwan *et al*., 1992; Karbwang *et al*., 1996; Li *et al*., 1984; Price *et al*., 1998).

Table 3.8: Adverse events when artesunate plus mefloquine was compared with artesunate monotherapy.

Number of RCTs	Outcome	Number of patients		Relative Risk (95% CI)	P-value
		AS+MQ (n/N)	AS (n/N)		
Three	Dizziness	6/126	8/124	0.76 (0.29, 2.00)	0.58
Three	Nausea	11/86	7/84	1.52 (0.65, 3.56)	0.33
Four	Vomiting	18/136	13/134	1.37 (0.72, 2.59)	0.58
Two	Diarrhoea	1/92	4/92	0.33 (0.05, 2.07)	0.24

AS= artesunate, MQ= mefloquine, n= number of patients reporting an outcome, N= number of patients assessed, CI= confidence interval

In other trials, abdominal pain was reported in two patients treated with AS+MQ and two with AS monotherapy (Looareesuwan *et al*., 1992; Li *et al*., 1984). There was one report of heamoglobinuria in a patient treated with AS+MQ (Price *et al*., 1998). Elevated transaminase enzymes were reported in two patients treated with AS+MQ and six treated with AS monotherapy. There was no statistically significant difference in the risk of enzyme increase between the two treatments (RR=0.31, 95% CI [0.07, 1.44], p=0.14, 1 trial, N=66) (Karbwang *et al*., 1996). The risk of developing headache (RR=0.86, 95% CI [0.45, 1.63], p=0.64) and skin itching with rash (RR=0.33, 95% CI [0.04, 3.08], p=0.33) was lower in patients treated with AS+MQ compared to AS monotherapy, but the difference between these treatments was not statistically significant (Looareesuwan *et al*., 1992).

In one trial, there was a significantly increased risk of vomiting within one hour in patients treated with AS+ATV+PRG (RR=0.11, 95% CI [0.01, 0.87], p=0.04, N=1066) compared to AS+MQ. Contrary to that, the risk of nausea at days one and two was significantly greater in patients treated with AS+MQ (RR=1.92, 95% CI [1.26, 2.93], p=0.003, N=664). Although the difference between treatments was not statistically significant, the risk of vomiting after one hour (RR= 1.26, 95% CI [0.85, 1.86], p=0.24, N=817) and of chills and rigors on days one and two (RR=1.03, 95% CI [0.64, 1.65], p=0.92, N=589) was a higher in patients treated with AS+MQ compared to AS+ATV+PRG (Van Vugt *et al*., 2002).

3.3.3 Other artemisinin derivatives plus mefloquine combination therapy

3.3.3.1 Treatment failure

Treatment with AM+MQ was significantly associated with less unadjusted treatment failure when compared with AM monotherapy. However, when re-infections were excluded, the difference in the risk of treatment failure was not statistically significant. Treatments with A+MQ and A monotherapy were also not significantly different in reducing the risk of unadjusted treatment failure (Table 3.9).

Table 3.9: The risk of treatment failure when artemethe plus mefloquine combination therapy was compared with artemether monotherapy and when artemisinin plus mefloquine was compared with artemisinin monotherapy.

Reference	Intervention	Control	Outcome	Number of patients		RR (95% CI)	p-value
				Intervention (n/N)	Control (n/N)		
Karbwang *et al.*, 1995; Looareesuwan *et al.*, 1997	AM+MQ	AM	UTF	4/94	17/137	0.32 (0.11, 0.98)	0.05
Looareesuwan *et al.*, 1997	AM+MQ	AM	ATF	1/44	11/87	1.08 (0.02, 1.35)	0.1
Hassan Alin *et al.*, 1996	A+MQ	A	UTF	0/17	7/17	0.07 (0.00, 1.08)	0.06

AM= artemether, A= artesunate, MQ= mefloquine, UTF= unadjusted treatment failure, ATF= adjusted treatment failure, n= number of patients with an outcome, N= number of patients examined, RR= relative risk, CI= confidence interval

3.3.3.2 Fever clearance time

There was no statistically significant difference in reducing the time to fever clearance between treatments with AM+MQ and AM monotherapy (WMD=0.9, 95% CI [-5.84, 7.64)], p=0.79, 1 trial N=131) and between DHA+MQ and DHA monotherapy (WMD=2.9, 95% CI [-4.14, 9.94], p=0.42), 1 trial, N=89).

3.3.3.3 Parasite clearance time

Although patients treated with AM+MQ cleared parasites 3.7 hours earlier than patients treated with AM monotherapy, the difference between treatments was not statistically significant (WMD= -3.70, 95% CI [-7.57, 0.17], p=0.06, 1 trial, N=131). Adding mefloquine to DHA had no additional benefit in PCT as there was no significant difference when compared with DHA monotherapy (WMD=2.5, 95% CI [-5.12, 10.12], p=0.52, 1 trial, N=89). Clearance of 50% of the burden of parasitaemia (PCT50) was also not statistically different comparing DHA+MQ and DHA monotherapy, though slightly in favour of combination therapy (WMD=-0.4, 95% CI [-3.00, 2.20], p=0.76, 1 trial, N=89).

3.3.3.4 Adverse events and side effects

One patient treated with A+MQ reported restlessness, dysphoria, lack of concentration and insomnia. In the same trial, two patients treated with artemisinin monotherapy reported skin itching which appeared one week after treatment and disappeared after two weeks (Hassan Alin *et al*., 1996). In another trial, headache, dizziness, nausea, vomiting, abdominal pain and diarrhoea were reported but with no specifications about the treatment given and the number of patients reporting such events (Looareesuwan *et al*., 1997). When AM+MQ was compared with AM monotherapy in one trial, there was a higher risk of vomiting and dizziness in patients treated with AM+MQ, on the other hand, the risk of nausea was higher in patients treated with AM monotherapy. Nevertheless, there was no statistically significant difference between these treatments (Table 3.10) (Karbwang *et al*., 1995).

Table 3.10: The risk of side effects in patients treated with artemether plus mefloquine or with artemether monotherapy.

Reference	Outcome	Number of patients		Relative Risk (95% CI)	P-value
		AM+MQ (n/N)	AM (n/N)		
Karbwang *et al* 1995	Nausea	7/56	7/53	0.95 (0.36, 2.52)	0.91
Karbwang *et al* 1995	Vomiting	9/56	3/53	2.84 (0.81, 9.92)	0.1
Karbwang *et al* 1995	Dizziness	13/56	6/53	2.05 (0.84, 5.00)	0.11

AM= artemether, MQ= mefloquine, n= nimber of patients reporting an outcome, N= number of patients evaluated, CI= confidence interval.

3.3.4 Artesunate and artemisinin monotherapy

3.3.4.1 Treatment failure

There was no statistically significant difference in reducing the risk of unadjusted treatment failure when AS+MQ+SP, AS+CQ, AS+AZ and AS+PQ were compared with AS monotherapy (Table 3.11).

Table 3.11: The risk of unadjusted treatment failure when different combination therapies were compared with artesunate monotherapy.

Reference	Comparison	Number of patients		RR (95% CI)	p-value
		Treatment (n/N)	AS (n/N)		
Li *et al.*, 1984	AS+MQ+SP	0/10	3/8	0.12 (0.01, 1.98)	0.14
Kofoed *et al.*, 2003	AS+CQ	15/180	10/72	0.60 (0.28, 1.27)	0.18
Pukrittayakamme *et al.*, 2004	AS+PQ	4/25	2/21	1.68 (0.34, 8.28)	0.52
Kroodsood *et al.*, 2000	AS+AZ	12/28	17/30	0.76 (0.45, 1.28)	0.30

AS = artesunate, MQ = mefloquine, SP = sulfadoxine-pyrimethamine, CQ = chloroquine, PQ = primaquine, AZ = azithromycin, n = number of patients with an outcome, N = number of patients examined, RR= relative risk, CI = confidence interval

3.3.4.2 Fever clearance time

Treatment with A+ß-CDX was associated with a significantly decreased fever clearance time compared to artemisinin monotherapy. This maybe attributed to the ability of ß-CDX to increase the solubility of artemisinins in water (Illapakurthy *et al.*, 2010), but there was no statistically significant difference when AS+MQ+SP and AS+AZ were compared with AS monotherapy (Table 3.12a).

3.3.4.3 Parasite clearance time

Artemisinin monotherapy was quicker than A+ß-CDX in clearing parasites but AS+MQ+SP, AS+AZ and AS+PQ were not significantly different to AS monotherapy (Table 3.12b).

3.3.4.4 Adverse events and side effects

When AS+MQ+SP was compared with AS monotherapy, two patients treated with AS+MQ+SP experienced nausea and one experienced vomiting, with no events reported in patients treated with AS monotherapy (Li *et al*., 1984). In one trial, there were five hospital admissions and four of them were due to repeated vomiting. The authors did not report the reason for admission of the fifth patient. Four patients were treated with AS+CQ and one was treated with AS monotherapy (Kofoed *et al*., 2003).

Table 3.12a: Fever clearance time when patients were given different artemisinin-based treatments.

Reference	Treatment	Control	Treatment		Control		WMD (95% CI)	p-value
			N	Mean (SD) (hours)	N	Mean (SD) (hours)		
Li *et al.*, 1984	AS+MQ+SP	AS	10	13.60 (11.60)	8	15.50 (11.00)	-1.90 (-12.38, 8.58)	0.72
Krudsood *et al.*, 2000	AS+AZ	AS	27	33.60 (27.20)	30	37.90 (29.00)	-4.30 (-18.89, 10.29)	0.56
Wong *et al.*, 2003	A+β-CDX	A	50	17.60 (1.90)	50	21.70 (2.60)	-4.10 (-4.99, -3.21)	<0.001*

AS= artesunate, A= artemisinin, MQ= mefloquine, SP= sulfadoxine-pyrimethamine, AZ= azithromycin, β-CDX= beta-cyclodextrin, FCT= fever clearance time, N= number of patients examined, SD= standard deviation, WMD= weighted mean difference, CI= confidence interval, * statistically significant at p<0.05

Table 3.12b: Parasite clearance times when patients were given different artemisinin-based treatments.

Reference	Treatment	Control	Treatment		Control		WMD (95% CI)	p-value
			N	Mean (SD) (hours)	N	Mean (SD) (hours)		
Li *et al.*, 1984	AS+MQ+SP	AS	10	54.90 (12.70)	8	44.70 (13.00)	10.20 (-1.76, 22.16)	0.09
Krudsood *et al.*, 2000	AS+AZ	AS	27	43.50 (17.30)	30	43.70 (13.60)	-0.20 (-8.34, 7.94)	0.96
Wong *et al.*, 2003	A+β-CDX	A	50	48.70 (2.80)	50	46.60 (2.50)	2.10 (1.06, 3.14)	<0.001*
Pukrittayakamee *et al.*, 2004	AS+PQ	AS	27	63.00 (18.00)	27	69.00 (19.00)	-6.00 (-16.31, 4.31)	0.25

AS= artesunate, A= artemisinin, MQ= mefloquine, SP= sulfadoxine-pyrimethamine, AZ= azithromycin, β-CDX= beta-cyclodextrin, PQ= primaquine
PCT= parasite clearance time, N= number of patients examined, SD= standard deviation, WMD= weighted mean difference, CI= confidence interval, * statistically significant at p<0.05

3.3.5 Other combination therapies

3.3.5.1 Treatment failure

The combination of A+Q was significantly associated with a 59% lower risk of unadjusted treatment failure compared to A+D. On the other hand, the risk of unadjusted treatment failure was almost twice as much in AM+AZ when compared to the combination of AM+D. Unadjusted treatment failure was significantly higher in AS+CQ when compared with AS+SP. No statistically significant difference was observed when AS+SP+PQ and AS+SMP were each compared with AS+SP (Table 3.13). When re-infections were excluded, the difference in risk of adjusted treatment failure between AS+CQ and AS+SP was marginally significant, and not statistically significant when AS+SP+PQ and AS+SMP were compared with AS+SP (Table 3.13).

Table 3.13: The risk of treatment failure in patients treated with different artemisinin-based combination therapies.

Reference	Treatment	Control	Outcome	Number of patients		RR (95% CI)	p-value
				Treatment (n/N)	Control (n/N)		
Bich *et al.*, 1996	A+Q	A+D	UTF	9/32	29/42	0.41 (0.23, 0.73)	0.003
Na-Bangchang *et al.*, 1996	AM+AZ	AM+D	UTF	23/27	14/30	1.83 (1.21, 2.76)	0.004
Huong *et al.*, 2003	AS+CQ	AS+SP	UTF	33/61	21/61	1.57 (1.04, 2.38)	0.03
Shekalaghe *et al.*, 2007	AS+SP+PQ	AS+SP	UTF	17/53	15/53	1.13 (0.63, 2.03)	0.67
Rulisa *et al.*, 2007	AS+SMP	AS+SP	UTF	11/109	18/103	0.58 (0.29, 1.16)	0.12
Huong *et al.*, 2003	AS+CQ	AS+SP	ATF	32/61	21/61	1.52 (1.00, 2.32)	0.05
Shekalaghe *et al.*, 2007	AS+SP+PQ	AS+SP	ATF	5/48	2/49	2.55(0.52, 12.52)	0.25
Rulisa *et al.*, 2007	AS+SMP	AS+SP	ATF	4/102	10/95	0.37 (0.12, 1.15)	0.09

AS= artesunate, A= artemisinin, AM= artemether, CQ= chloroquine, Q= quinine, D= doxycycline, AZ= azithromycin, SP= sulfadoxine-pyrimethamine, SMP= sulfamethoxy-pyrimethamine, UTF=unadjusted treatment failure, ATF= adjusted treatment failure

3.3.5.2 Fever clearance time

The combination of AS+CQ cleared fever significantly quicker than AS+SP with more than seven hours (WMD= -7.20, 95% CI [-12.53, -1.87], p= 0.008) whereas there was no statistically significant difference in fever clearance time between A+Q and A+D (WMD= 3.00, 95% CI [-5.54, 11.54], p= 0.49) (Table 3.14).

3.3.5.3 Parasite clearance time

Treatment with AS+CQ was associated with a statistically significant shorter parasite clearance time of six hours compared to AS+SP (Table 3.14). Both A+Q and A+D were equally efficacious in clearing parasites (WMD= 2.00, 95% CI [-4.74, 8.74], p= 0.56) and in reducing the density of parasites to 50% of the initial parasitaemia (PCT 50) (WMD=0, 95% CI [-2.26, 2.26], p=1) as well as to 10% of the initial parasitaemia (PCT 90) (WMD= 1.00, 95% CI [-2.15, 4.15], p= 0.53) (Table 3.14).

3.3.5.4 Gametocyte carriage at day 7 and 14

The risk of carrying gametocytes was significantly reduced by 78% at day 7 and by 94% at day 14 when AS+SP+PQ was compared with AS+SP. The difference between these treatments was statistically significant with a p-value <0.001. This comparison was done in one trial with 104 participants at day 7 and 102 at day 14 (Figure 3.9).

Table 3.14: Fever and parasite clearance times in patients treated with different combination therapies.

Reference	Treatment	Control	Outcome	Treatment		Control		WMD (95% CI)	p-value
				N	Mean (SD) (hours)	N	Mean (SD) (hours)		
Bich *et al.*, 1996	A+Q	A+D	FCT	44	34.00 (19.00)	49	31.00 (23.00)	3.00 (-5.54, 11.54)	0.49
Huong *et al.*, 2000	AS+CQ	AS+SP	FCT	61	32.40 (13.20)	62	39.60 (16.80)	-7.20 (-12.53, -1.87)	0.008*
Bich *et al.*, 1996	A+Q	A+D	PCT	44	43.00 (14.00)	49	41.00 (19.00)	2.00 (-4.74, 8.74)	0.56
Huong *et al.*, 2000	AS+CQ	AS+SP	PCT	61	40.80 (15.60)	62	46.80 (15.60)	-6.00 (-11.51, -0.49)	0.03*
Bich *et al.*, 1996	A+Q	A+D	PCT 50	44	9.00 (6.00)	49	9.00 (5.00)	0.00 (-2.26, 2.26)	1
Bich *et al.*, 1996	A+Q	A+D	PCT 90	44	18.00 (9.00)	49	17.00 (6.00)	1.00 (-2.15, 4.15)	0.53

A= artemisinin, AS= artesunate, Q= quinine, CQ= chloroquine, D= doxycycline, SP= sulfadoxine-pyrimethamine, FCT= fever clearance time, PCT= parasite clearance time, PCT 50= time to clearing 50% of parasite, PCT 90= time to clearing 90% of parasites, N= number of participants assessed, WMD= weighted mean difference, * indicates statistical significance

Study or Subgroup	AS+SP+PQ Events	Total	AS+SP Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI	Risk Ratio M-H, Fixed, 95% CI
Gametocyte carriage at baseline							
Shekalaghe *et al.*, 2007	48	53	45	53	39.4%	1.07 [0.92, 1.23]	
Test for overall effect: Z = 0.88 (P = 0.38)							
Gametocyte carriageat day 7							
Shekalaghe *et al.*, 2007	8	51	38	53	32.6%	0.22 [0.11, 0.42]	
Test for overall effect: Z = 4.52 (P < 0.00001)							
Gametocyte carriage at day 14							
Shekalaghe *et al.*, 2007	2	51	32	51	28.0%	0.06 [0.02, 0.25]	
Test for overall effect: Z = 3.95 (P < 0.0001)							

0.01   0.1   1   10   100
Favours AS+SP+PQ   Favours AS+SP

AS= artesunate, SP= sulfadoxine-pyrimethamine, PQ= primaquine, CI= confidence interval

Figure 3.9: The risk of gametocyte carriage in patients when artesunate plus sulfadoxine-pyrimethamine plus primaquine was compared with artesunate plus sulfadoxine-pyrimethamine.

3.3.5.5 Adverse events and side effects

There was a 75% lower risk of mild nausea, abdominal discomfort and/ or loss of appetite in patients treated with AM+AZ compared to the AM+D, (RR=0.25, 95% CI [0.08, 0.80], p=0.02 (Na-Bangchang *et al*., 1996). Although the risk of developing dizziness, tinnitus, impaired hearing and dry mouth was higher in patients treated with A+Q was compared to A+D, the difference between these two treatments was not statistically significant (Table 3.15) (Bich *et al*., 1996).

Table 3.15: Adverse events and/ or side effects in patients treated with artemisinin plus quinine or artemisinin plus doxycycline.

Reference	Treatment	Control	Outcome	Number of patients		RR (95% CI)	p-value
				Treatment (n/N)	Control (n/N)		
Bich *et al*., 1996	A+Q	A+D	Dizziness	4/45	4/53	1.18 (0.31-4.44)	0.81
Bich *et al*., 1996	A+Q	A+D	Tinnitus	5/45	1/53	5.89 (0.71-48.57)	0.10
Bich *et al*., 1996	A+Q	A+D	Impaired hearing	4/45	0/53	10.57 (0.58-191.09)	0.11
Bich *et al*., 1996	A+Q	A+D	Dry mouth	1/45	1/53	1.18 (0.08-18.30)	0.91

A= artemisinin, Q= quinine, D= doxycycline, RR= relative risk, n= number of patients with an outcome,
N= number of patients evaluated

4.1 The challenge of parasite resistance to antimalarials

Effective case management of malaria is severely crippled by the development and spread of parasite resistance to antimalarials. To combat the impact of drug resistance, antimalarials with different modes of action are combined. This improves drug efficacy and may reverse the impact of drug resistance (Nosten *et al*., 2000). It also reduces the risk of selection for resistant mutants in the plasmodial parasites. The World Health Organization (WHO) now recommends artemisinin-based combination therapy (ACT) for the treatment of uncomplicated *P. falciparum* malaria. The WHO also recommends that the cure rates should be at least 90% and preferably $\geq$95% assessed at 28 days (WHO, 2010). Although artemisinins are effective and rapidly acting, their widespread use has raised questions with regards to emerging drug resistance (Duffy and Sibley, 2005). Declining clinical cure rates in patients treated with artemisinins have been observed (Vijaykadga *et al*., 2006; Mey Bouth *et al*., 2006). Of concern is the 30% PCR confirmed recrudescence observed in Cambodia, in patients treated with artemisinin-monotherapy 21-28 days after treatment.

4.2 Artemisinin-based combination therapy for the treatment of malaria

Currently recommended ACTs are artemether-lumefantrine, artesunate plus amodiaquine, artesunate plus mefloquine and artesunate plus sulfadoxine-pyrimethamine. Artemether-lumefantrine was the first internationally recognized fixed-dose ACT in use but other fixed-dose combinations have later been developed (Nosten and White, 2007). In a recent

Cochrane review, dihydroartemisinin-piperaquine has been investigated and it is now included in the updated WHO guidelines (Sinclair *et al*., 2009; WHO, 2010). Other ACTs that can possibly be used in the near future include artesunate-chlorproguanil-dapsone, artesunate-atovaquone-proguanil and artesunate-pyronaridine (Nosten and White, 2007).

4.3 The focus of the present study

Several clinical trials have proven the superior efficacy of ACTs compared to non-artemisinin based regimens (McIntosh and Olliaro, 1999). Reviewing different ACTs, there is no consensus on which antimalarial is better suited for combination with the various artemisinin derivatives. A Cochrane review (Sinclair *et al*., 2009) compared different ACTs; however, the ACTs have not been evaluated when the same artemisinin derivative is combined with different non-artemisinin drugs, thus only evaluating the non-artemisinin partner of the combination therapy.

This study, therefore, sought to review the evidence of efficacy and safety of different non-artemisinin antimalarials when they are combined with the same artemisinin derivative, in reducing the risk of treatment failure in non-pregnant adults and children with uncomplicated *P. falciparum* malaria.

4.4 The findings of this review and the existing knowledge on artemisinin-based combination therapy

The thirty-seven trials with a total of 6862 participants met the inclusion criteria; 21 of these RCTs were conducted in Africa, 14 in Asia, and two in South America (Tables

3.2a-d). Artesunate was the most commonly used artemisinin derivative in combination with antimalarials including amodiaquine, sulfadoxine-pyrimethamine, mefloquine, azithromycin and atovaquone-proguanil. Artemether was used in combination with mefloquine only. One trial analysed the efficacy of a dihydroartemisinin based combination therapy.

4.4.1 The efficacy of artesunate plus amodiaquine combination therapy

The results show that the efficacy of artesunate combined with amodiaquine was higher than the efficacy of artesunate combined with sulfadoxine-pyrimethamine for the treatment of uncomplicated *P. falciparum* malaria. Although there is a possibility of cross-resistance between amodiaquine and chloroquine, amoqiaquine has been found to be efficacious even in areas of high chloroquine resistance (Van Dillen *et al*., 1999; Brasseur *et al*., 1999; Staedke *et al*., 2001). The WHO recommends that artesunate plus amodiaquine and artesunate plus sulfadoxine-pyrimethamine can be used in areas where the efficacy of their non-artemisinin partner exceeds 80% (WHO, 2010). Prior to this review there has been insufficient evidence on the efficacy of artesunate plus amodiaquine compared to artesunte plus sulfadoxine-pyrimethamine. In addition, the findings of this review show artesunate plus amodiaquine to be more efficacious than artesunate monotherapy. There was only one trial comparing artesunate plus amodiaquine with artesunate plus mefloquine (Faye *et al*., 2007) and there were very few outcomes reported while showing no significant difference between the two treatments.

Previously, the efficacy of artesunete plus amodiaquine has been evaluated and compared with that of artemether-lumefantrine and there was a significantly lower risk of treatment failure at day 28 in artemether-lumefantrine given at six doses (Omari *et al*., 2005). However, a recently published Cochrane review showed no significant difference in the risk of treatment failure when artemether-lumefantrine was compared with artesunate plus amodiaquine (Sinclair *et al*., 2009). In the unavailability of artemether-lumefantrine and considering the requirements of having to consume it with fat-containing foods or milk (Muheki *et al*., 2004; Ashley *et al*., 2007), which may not be readily available in poor communities or house-holds and given the findings of the present study, combination therapy with artesunate and amodiaquine should be considered.

The combination of artesunate with amodiaquine is currently available in blister packs as separate scored tablets containing 50 mg of artesunate and 153 mg base of amodiaquine and its co-formulation has recently been developed. There are no plans for developing a fixed dose of artesunate with sulfadoxine-pyrimethamine (Nosten and White, 2007). This could be due to the increasing level of resistance to SP and the differing dosage times for AS and SP. Artesunate plus sulfadoxine-pyrimethamine is currently being used in parts of South America, the Middle East and South Asia, where sulfadoxine-pyrimethamine susceptibility remains high. Since sulfadoxine-pyrimethamine, sulfalene–pyrimethamine and trimethoprim–sulfamethoxazole (co-trimoxazole) are still widely used as monotherapies, resistance is likely to worsen and this is likely to compromise the efficacy of ACTs using sulfadoxine-pyrimethamine as a combination antimalarial (Nosten and White, 2007).

4.4.2 The efficacy of artesunate plus mefloquine combination therapy

This study showed that artesunate combined with mefloquine was more efficacious compared to the combination of artesunate plus azithromycin for the treatment of uncomplicated *P. falciparum* malaria. There is no basis for recommending atovaquone-proguanil over mefloquine as there were few trials identified comparing them and the efficacy of artesunate plus mefloquine and artesunate plus atovaquone-proguanil did not differ significantly. This study found that efficacy of artesunate monotherapy was found to be low and this is in agreement with previous findings (McIntosh and Olliaro, 1999), thus artesunate monotherapy cannot be recommended. Although mefloquine resistance is wide-spread and severe in Asia and low levels have been detected in South America (Wongsrichanalai *et al*., 2002), the results showed that the combination of artesunate plus mefloquine is still efficacious in these areas.

The WHO recommends artesunate plus mefloquine combination therapy in areas of high AQ and SP resistance and when AL is not available (WHO, 2006b). A systematic review reported a higher risk of treatment failure on days 28 and 63 with artemether-lumefantrine given at four doses compared to the combination of artesunate plus mefloquine (Omari *et al*., 2004). It also found that there was no significant difference in the risk of treatment failure at day 28 when artemether-lumefantrine at six doses was compared to artesunate plus mefloquine. On the other hand, there was a significantly higher risk of treatment failure on day 42 with six-dose artemether-lumefatrine compared to artesunate plus mefloquine. Artesunate plus mefloquine and six doses of artemether-lumefantrine seem to have maintained equivalence in a recent Cochrane Review (Sinclair

et al., 2009). A fixed dose of artesunate plus mefloquine has been developed and is dispensed as tablets containing 200 mg artesunate and 400 mg mefloquine (Nosten and White, 2007). The combination of atovaquone-proguanil with artemisinins is not recommended due to its high cost, though its safety and efficacy has been proven (WHO, 2006a).

4.4.3 The use of artemisinins in monotherapy

With regards to the artemisinin derivatives used in monotherapy, their efficacy has once again been confirmed to be very low. In three trials, artesunate monotherapy given over three days had failure rates of 13.8-56.7% (Li *et al.*, 1984; Krudsood *et al.*, 2000; Kofoed *et al.*, 2003). While artemisinin derivatives used as monotherapy in Thailand given over five to seven days had failure rates slightly lower than the 10% recommended by the WHO (Karbwang *et al.*, 1995; Looareesuwan *et al.*, 1997; Pukrittayakamee *et al.*, 2004). The low efficacy of artemisinin derivatives used in monotherapy was also observed in Vietnam and Tanzania with AS monotherapy given for five to seven days (Hassan Alin *et al.*, 1996; Giao *et al.*, 2001). Contrary to these observations, polymerase chain reaction (PCR) adjusted failure rates in Gabon were 10%, when artesunate monotherapy was given for five days (Schwarz *et al.*, 2005).

In the present study, the low efficacy of artesunate monotherapy was not significantly different to treatment with artesunate plus mefloquine plus sulfadoxine-pyrimethamine, artesunate plus chloroquine, artesunate plus primaquine and artesunate plus azithromycin (Li *et al.*, 1984; Krudsood *et al.*, 2000; Kofoed *et al.*, 2003; Pukrittayakamee *et al.*,

2004). This can be attributed to a small sample size in one trial (n=60) (Li *et al*., 1984), which does not give enough power to detect the difference between treatments. It is also worth noting that in the same trial, AS doses in combination therapy were given for one day. In the other trials this may be due to the efficacy of combination treatment being similar to that of monotherapy.

4.4.4 The efficacy of ACTs for fever clearance and on gametocytes

Fever clearance time in hours is the other commonly reported outcome employed for assessing the efficacy of treatment. In this analysis, a significant difference was observed in favour of artesunate combined with amodiaquine compared to AS monotherapy. However, the difference was just more than one hour in this comparison. The clinical significance of fever clearance time becomes doubtful when the difference between the treatments is just a few hours or less. A difference of more than four hours was observed when artemisinin combined with β-cyclodextrin was compared with artemisinin monotherapy, in favour of combination therapy (Wong *et al*., 2003). This maybe due to the ability of β-cyclodextrin to increase the solubility of artemisinins in water (Illapakurthy *et al*., 2010). Furthermore, artesunate combined with chloroquine was more than seven hours quicker in clearing fever than artesunate combined with sulfadoxine-pyrimethamine (Huong *et al*., 2000). The addition of mefloquine to dihydroartemisinin did not significantly improve the time taken to clear fever or parasitaemia. In another review, fever clearance time did not differ when six-dose artemether-lumefantrine was compared with artesunate-mefloquine in a systematic review (Omari *et al*., 2005).

This study also assessed the efficacy of treatment based on its ability to reduce gametocyte carriage. Artemisinins have displayed an ability to reduce gametocyte carriage by clearing the asexual parasites before they fully develop to gametocytes and by their action against matured gametocytes (Guthmann *et al*., 2005). Treatment that reduces gametocyte carriage eventually reduces malaria transmission, especially in low transmission areas. Gametocytes persist when the initial burden of parasites is not eliminated due to the failure of treatment. The results show that there was no significant difference observed in the ability of different therapies to reduce gametocyte carriage, although the number of patients who carried gametocytes after treatment was low. Treament with primaquine is known to be effective in reducing the risk of gametocytes, however, none of the trials that used a combination with primaquine reported gametocyte carriage. Omari *et al*. (2004) made similar observations when comparing six-dose artemether-lumefantrine and artesunate plus mefloquine. Therefore, ACTs are equally efficacious in reducing gametocyte carriage, and in another study they were even better when compared with quinine (Price *et al*., 1996).

4.5 Limitations and strengths of the study

While this review clearly identifies antimalarials that can be considered for combination with artemisinin derivatives, it is also important to highlight the limitations of the study. To assess the efficacy of treatment in high transmission areas, clinical trials should exclude new infections using polymerase chain reaction (PCR). Not all the trials reported PCR-corrected failure rates and in some of the trials where PCR was performed, not all the participants had results available. This could be problematic as it does not give a clear

picture of the efficacy of the treatment, in accordance with the WHO recommendations. The information on the efficacy of ACTs against new infections is crucial at it will inform the policy makers of the effectiveness of the non-artemisinin combination antimalarials. The elimination halflife of these antimalarials sustains the effectiveness of ACTs against new infections. It should be taken into account, however, that antimalarials given in routine settings should be effective even against new infections. Policy makers require information on the efficacy of antimalarials against both the initially diagnosed infection, and the new infections in high transmission areas. Whenever PCR corrected failure rates were reported in trials, they were used in this review. The WHO recomendations also emphasize that the antimalarials of choice should be safe, this information has not been well presented in these RCTs. The methods of detecting and reporting adverse have not been standardized, thus the safety information presented in this review is inadequate. Clinical trials are usually not powered enough to detect adverse events and the duration of follow-up is short. Nevertheless, this information should be properly recorded and presented in a standardized manner.

The duration of follow-up was also not the same between trials but 37 of them had a follow-up of 28 days. Most of the mefloquine comparisons had a follow-up of 42 days. There were also differences in doses given to participants. In some trials, the duration of treatment was shorter than recommended (WHO, 2010), this lead to the high rate of treatment failure. The duration of treatment was not stated as the exclusion criteria, thus trials that gave artemisinin treatment for shorted durations were not excluded, although the short-duration treatment is no recommended. In other trials, artemisinin derivatives

were given over five to seven days even in combination therapy and this is likely to have over-emphasized the efficacy of ACTs and such long-duration treatments cannot be implemented. There is lack of information on two important ACTs; artemether-lumefantrine and dihydroartemisinin-piperaquine on this review. The two artemisinin derivatives, artemether and dihydroartemisinin, have not been evaluated in combination with different non-artemisinin antimalarials, thus evaluating only the non-artemisinin partner. However, the two ACTs, artemether-lumefantrine and dihydroartemisinin-piperaquine have been evaluated in a Cochrane review (Sinclair *et al*., 2009). There was also inadequate reporting of the methodology of the trials included in this review, which did not allow for the sensitivity analysis in this review. Furthermore, the autors of most RCTs did not respond or were unreachable to provide unpublished data. There is limited knowledge with regards to the effects of ACTs on pregnant women and infants, as well as on *P. vivax* malaria.

Nevertheless, evidence presented in this study confirms the efficacy of ACTs for the treatment of uncomplicated *P. falciparum* malaria in non-pregnant adults and children. This is inline with WHO recommendations (WHO, 2010). The choice of ACT to be implemented in policy recommendations in different settings has been reviewed here. Artesunate plus amodiaquine is the combination of choice for Africa over artesunate plus sulfadoxine-pyrimethamine and other ACTs evaluated in this review. This ACT has been evaluated in ten trials and its efficacy was superior to that of artesunate plus sulfadoxine-pyrimethamine and artesunate monotherapy. Although artesunate plus amodiaquine was not compared with artemether-lumefantrine in this review, a Cochrane review found the

two ACTs to be equivalent in efficacy (Sinclair *et al*., 2009). Although artesunate plus mefloquine is also efficacious, it has not been well researched in Africa. Artesunate plus mefloquine is the combination of choice in Asia (Sinclair *et al*., 2009). The efficacy of this combination was high but there was only one trial comparing artesunate plus mefloquine with artesunate plus atovaquone-proguanil (Van Vugt *et al*., 2002), artesunate plus azithromycin (Krudsood *et al*., 2000) and artesunate plus mefloquine plus sulfadoxine-pyrimethamine (Li *et al*., 1984). This review included both the published and unpublished studies, however, some of the data obtained from both published and unpublished studies was inadequate and the authors of such studies were unreachable. There were no language limitations employed in searching for studies.

CONCLUSION

The findings of this study support the implementation of artemisinin-based combination therapy for the treatment of uncomplicated malaria. This work also argues strongly against the use of artemisinin-based monotherapy, which has been shown to be associated with high rates of treatment failure in this review. Most crucially, this review found a greater advantage of combining amodiaquine with artesunate compared to sulfadoxine-pyrimethamine. Treatment with artesunate plus amodiaquine should also be considered in situation where artemether-lumefantrine is not convenient. The efficacy of artesunate plus mefloquine was also superior to that of artesunate plus azithromycin. Furthermore, the combination of artemisinins with chloroquine, primaquine and azithromycin has shown very low efficacy and these combination therapies should not be recommended.

Artesunate plus atovaquone-proguanil needs to be further investigated globally. In addition, a more detailed description of the methods used in clinical trials is suggested. Investigators need to implement a more vigorous approach in generating and concealing the allocation sequence, in blinding the participants, care givers and outcome assessors. This lowers the chances of bias in clinical trials (Higgins and Altman, 2008). These trials were not designed to investigate adverse events as there was no systematic reporting of such events. This raises a need for clinical trials to consider the safety of different combination therapies and to uniformly collect data regarding adverse events.

In this review, the efficacy of ACTs has been confirmed in agreement with present recommendations, systematic reviews and previous trials (WHO, 2006b; Sinclair *et al*., 2009). Furthermore, the potential antimalarials for combination with different artemisinin derivatives have also been identified as well as antimalarials that are not suitable for combination with artemisinins. This was done by collating findings of different randomized controlled trials performed in different settings globally. The methods used in this study enable the determination of consistencies and variations in available evidence, thus providing more reliable findings from which conclusions can be drawn.

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APENDIX A: Search strategy

The following databases were searched using the search terms and strategy described in Table 01.

- Cochrane Central Register of Controlled Trials (CENTRAL), published in *The Cochrane Library,* 2008; Issue 4

- MEDLINE (1966 to January 2009).

- EMBASE (1974 to January 2009).

Search set	CENTRAL	MEDLINE**	EMBASE**
1	malaria	malaria	Malaria
2	Plasmodium	Plasmodium	Plasmodium
3	1 or 2	1 or 2	1 or 2
4	artemisinin	artemisinin	artemisinin
5	artesunate	artesunate	artesunate
6	artemether	artemether	artemether
7	arteether	arteether	arteether
8	dyhydroartemisinin	dyhydroartemisinin	dyhydroartemisinin
9	4 or 5 or 6 or 7 or 8	4 or 5 or 6 or 7 or 8	4 or 5 or 6 or 7 or 8
10	3 and 9	3 and 9	3 and 9
		Limit 10 to human	Limit 10 to human

**Search terms for malaria were used in combination with the search strategy for retrieving trials developed by The Cochrane Collaboration, described in: Higgins JPT, Green S (editors). *Cochrane Handbook for Systematic Reviews of Interventions* Version 5.0.1 [updated September 2008]. The Cochrane Collaboration, 2008. Available from www.cochrane-handbook.org.

APPENDIX B.1: STUDY ELIGIBILITY FORM

Date:	
Extractor (initials):	
Trial ID:	
Trial Name:	
Journal:	

(1) Design		
(a) Described as randomized? If 'No', exclude. If 'Yes', go to question (2)	Yes No Unclear (Circle)	
(2) Participants		
(a) Did the participants have microscopically confirmed P. falciparum malaria?	Yes No Unclear (Circle)	
(b) Did the participants have uncomplicated malaria?	Yes No Unclear (Circle)	
If (a) or (b) answer 'No', exclude. If 'Yes' go to question (3)		
(3) Interventions		
(a) Was one group given an ACT as treatment?	Yes No Unclear (Circle)	
(b) Did another group receive an alternative ACT or monotherapy with the same artemisinin derivative?	Yes No Unclear (Circle)	
If (a) or (b) answer 'No', exclude. If 'Yes' go to question (4)		
(4) Outcomes		
Did the trial report any of the following outcomes?		
(a) Treatment failures	Yes No Unclear (Circle)	
(b) Fever clearance time	Yes No Unclear (Circle)	
(c) Parasite clearance time	Yes No Unclear (Circle)	
(d) Measure of anaemia	Yes No Unclear (Circle)	
(e) Measure of gametocytaemia	Yes No Unclear (Circle)	
(f) Adverse events	Yes No Unclear (Circle)	
If **all** (a) to (f) answer 'No', exclude.		
Final Decision		
Include	Yes No (Circle)	
Exclude	Yes No (Circle)	
Unclear	Yes No (Circle)	
Excluded or unclear because:		
If 'Unclear', action taken:		

APPENDIX B.2: METHODOLOGICAL QUALITY FORM

Date:	
Extractor (initials):	
Trial ID:	
Trial Name:	
Journal:	

(1) Sequence generation	
Method:	
Reviewers judgement:	Adequate Inadequate Unclear (circle)

(2) Allocation concealment	
Method:	
Reviewers judgement:	Adequate Inadequate Unclear (circle)

(3) Blinding of participants, personnel and outcome assessors	
Description:	
Reviewers judgement:	Adequate Inadequate Unclear (circle)

(4) Loss to follow-up	
Description:	
Reviewers judgement:	All included More than 90% Less than 90% Unclear (circle)

APPENDIX B.3: DATA EXTRACTION FORM A- CHARACTERISTICS OF STUDIES

Date:	
Extractor (initials):	
Trial ID:	
Trial Name:	
Journal:	

Trial details	
Trial Dates (from-to: dd/mm/yyyy):	
Country:	
Setting (urban/rural/hosp/clinic):	
Malaria transmission pattern:	
Local antimalarial drug resistance:	
Frequency and duration of follow-up: Include details of activity at each follow up visit e.g. temp/blood slide/ symptom questionnaire/ LFTs	

Participants	
Inclusion Criteria:	Exclusion Criteria:

Characteristics:	Group 1:	Group 2:	Group 3:
Sex ratio (Male:Female):			
Age Range (years/months):			
Mean/Median age (years/months):			
Body Weight (kg) mean/median:			
Haemoglobin (g/dl) mean/median:			
Were all treatment groups comparable at baseline? If 'No/unclear' Describe:	Yes No Unclear (circle)		

Interventions			
	Treatment Group 1:	Treatment Group 2:	Treatment Group 3:
Antimalarial 1:			
Formulation:			
Timing and frequency of dose:			
Duration:			
Total dose (target):			
Antimalarial 2:			
Formulation:			
Timing and frequency of dose:			
Duration:			
Total dose (target):			
Treatment supervised?	Yes No Unclear (circle)		
Dosing details: Weight based or age based? Tablets or suspension? Tablets cut into halves or quarters?			

APPENDIX B.3: DATA EXTRACTION FORM B- OUTCOMES REPORTED

Date:	
Extractor (initials):	
Trial ID:	
Trial Name:	
Journal:	

Participants with/without outcomes:			
	Group 1:	Group 2:	Group 3:
Participants randomised:			
Participants with no treatment Outcome (total):			
• Excluded after randomisation			
• Lost to follow up			
• Other reasons			
Notes:			

Primary outcome			
Early Treatment Failure			
Late Treatment failure: day 28 (n/N)			
• PCR unadjusted failure			
• PCR confirmed recrudescence			
• Re-infection			
• PCR indeterminate			
Late Treatment failure: day 42 (n/N)			
• PCR unadjusted failure			
• PCR confirmed recrudescence			
• Re-infection			
• PCR indeterminate			
Notes:			

Secondary outcomes			
	Group 1:	Group 2:	Group 3:
Fever clearance			
Fever clearance time (hrs) (N: mean ±SD)			
Fever on day 2 (n/N)			
Fever on day 3 (n/N)			
Parasite clearance			
Parasite clearance time (hrs) (N: mean ±SD			
PCT 50 (N: mean ±SD			
PCT 90 (N: mean ±SD			
Parasite clearance at day 2 (n/N)			
Parasite clearance at day 3 (n/N)			
Parasite clearance at day 7 (n/N)			
Parasite clearance at day 14 (n/N)			
Parasite clearance at day 21 (n/N)			
Parasite clearance at day 28 (n/N)			
Gametocyte carriage (n/N)			
Gametocyte carriage at baseline			
Gametocyte carriage at day 7			
Gametocyte carriage at day 14			
Gametocyte carriage at day 28			
Anaemia			
Anaemia at day 28 (n/N)			

Adverse events		Group 1:	Group 2:	Group 3:
Serious adverse events				
Biochemical/haematological monitoring				

Other important information	
Relevant papers cited:	
Other reported outcomes not analyzed:	
Additional Information required from authors:	
Authors contacted?	Yes No (circle)
Address:	
E-mail:	
Telephone:	
Data obtained?	Yes No Awaiting response (circle)
Comments:	

APPENDIX C: INTERVENTIONS AND OUTCOMES

Appendix C.1: Adjusted treatment failure

Outcome: Adjusted treatment failure	Interventions and results												
Reference	AS+AQ	AS+SP	AS+MQ	AS+ATV+PRG	AS+CT	AS+CD	AS+MB	AM+MQ	AS+CQ	AS+SP+PQ	AS+SMP	AS	AM
Bonnet et al 2007	1/107	1/106											
Djimde 2008	2/229	0/229										8/231	
Guthman 2005	1/83	1/84											
Hamour 2005	4/72	5/71											
Kayentao 2009	6/131	6/130											
Swarthout 2006	5/83	13/79											
Van den Broek 2006	1/84	7/80											
Looaresuwan 1992			0/39									5/40	
Van Vugt 2002			13/533	5/533									
Looaresuwan 1997								1/44					11/87
Huong 2003		21/61							32/61				
Shekalaghe 2007		4/49								5/48			
Rulisa 2007		10/95									4/102		
Fehintola 2008	4/61				11/121								
Owusu-Agyeyi 2008	11/149					25/147							
Zoungrana 2008	11/61						22/60						

Appendix C.2: Un-adjusted treatment failure

Outcome: Un-adjusted treatment failure	Interventions and results								
Reference	AS+AQ	AS+SP	AS+MQ	AS+ATV+PRG	AS+CT	AS+CD	AM+MQ	AS+PQ	AS+CQ
Bonnet et al 2007	6/107	7/100							
Carona-Fonseca	0/52	2/98							
Djimde 2008	44/235	12/232							
Hamour 2005	29/80	27/79							
Kayentao 2009	58/131	12/130							
Swarthout 2006	14/83	28/81							
Van den Broek 2006	31/97	21/85							
Looaresuwan 1992			0/39						
Van Vugt 2002			35/533	18/533					
Looaresuwan 1997							1/44		
Huong 2003		21/61				33/61			
Shekalaghe 2007		15/53							
Rulisa 2007		18/103							
Fehintola 2008	6/61				33/121				
Owusu-Agyeyi 2008	22/150					45/157			
Zoungrana 2008	21/61						43/60		
Gomez 2003			5/100						
Karbwang 1995							3/50		
Karbwang 1996			6/28						
Krudsood 2000			1/55						
Li 1982			0/20						
Price 1998			2/34						
Koefoed 2003									38/229
Pukrittayakamee 2004								4/25	
Bich 1996									
Na-Bangchang 1996									
Faye 2007	3/145		9/360						
Sowunmi 2007	1/120								

Appendix C.2 (table continued): Un-adjusted treatment failure

Outcome: Un-adjusted treatment failure	Interventions and results									
Reference	AS+SP+PQ	AS+MQ+SP	AS+SMP	AS+AZ	AM+AZ	A+Q	A+D	AM+D	AS	AM
Bonnet et al 2007										
Carona-Fonseca										
Djimde 2008									99/234	
Hamour 2005										
Kayentao 2009										
Swarthout 2006										
Van den Broek 2006										
Looaresuwan 1992									5/40	
Van Vugt 2002										
Looaresuwan 1997										11/87
Huong 2003										
Shekalaghe 2007	17/53									
Rulisa 2007			11/109							
Fehintola 2008										
Owusu-Agyeyi 2008										
Zoungrana 2008										
Gomez 2003									12/150	
Karbwang 1995										6/50
Karbwang 1996									2/31	
Krudsood 2000				24/55					34/61	
Li 1982		0/20							7/17	
Price 1998										
Koefoed 2003									45/112	
Pukrittayakamee 2004									2/21	
Bich 1996						9/32	29/42			
Na-Bangchang 1996					23/27			14/30		
Faye 2007										
Sowunmi 2007									20/111	

Appendix C.3: Re-infection

Outcome: Re-infection	Interventions and results										
Reference	AS+AQ	AS+SP	AS+MQ	AS+ATV+PRG	AS+CT	AS+CD	AS+MB	AS+CQ	AS+SP+PQ	AS+SMP	AS
Bonnet et al 2007	5/107	7/106									
Djimde 2008	37/229	9/229									88/231
Hamour 2005	17/72	14/71									
Kayentao 2009	52/131	6/128									
Swarthout 2006	9/83	13/79									
Van den Broek 2006	17/84	9/80									
Van Vugt 2002			22/533	13/533							
Huong 2003		0/62						1/61			
Shekalaghe 2007		9/49							7/48		
Rulisa 2007		8/103								7/109	
Fehintola 2008	2/61				22/121						
Owusu-Agyeyi 2008	11/148					23/144					
Zoungrana 2008	10/61						21/60				
Faye 2007	9/360		3/145								

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Appendix C.4: Fever and parasite clearance times (hours)

Outcome: Fever and parasite clearance times		Interventions and results																	
		DHA+MQ			DHA			AS+AQ			AS+CT			AS					
Reference	Outcome	N	mean	SD	N	mean	SD	N	mean	SD	N	mean	SD						
Barennes 2004	Fever clearance time							33	38.4	1.68				27	39.6	1.68			
Barennes 2004	Parasite clearance time							33	27.2	1.2				27	27.2	1.2			
Fehintola 2008	Parasite clearance time							61	44.6	15.84	120	31.44	11.52						
Fehintola 2008	Fever clearance time							61	28.8	14.4	121	31.2	17.6						
Sowunmi 2007	Fever clearance time							120	24	14.4				120	24	14.4			
Sowunmi 2007	Parasite clearance time							120	33.6	14.4				120	33.6	12			
Diem Thuy 2007	Parasite clearance time	44	37.8	19.2	45	35.3	17.4												
Diem Thuy 2007	Parasite clearance time 50	44	7.9	5.3	45	8.3	7.1												
Diem Thuy 2007	Fever clearance time	44	26.2	19.8	45	23.3	13.4												

Appendix C.5: Other outcomes

Outcomes	Reference	Interventions and results						
Early treatment failure		AS+AQ	AS+SP	AS+CD	AS+SP+PQ	AS+MQ	AS+ATV+PRG	AS
	Bonnet et al 2007	0/107	1/106					
	Guthman 2005	0/97	1/87					
	Kayentao 2009	0/131	2/130					
	Owusu-Agyeyi 2008	1/151		3/160				
Anaemia at day 28	Guthman 2005	33/84	31/84					
	Kayentao 2009	71/131	78/130					
Gametocytes at day 7	Shekalaghe 2007		38/53		8/51			
	Owusu-Agyeyi 2008	3/151		6/160				
Gametocytes at day 14	Guthman 2005	12/84	5/84					
	Hamour 2005	3/80	4/79					
	Shekalaghe 2007		32/51		2/51			
Gametocytes at day 28	Bonnet et al 2007	4/106	2/101					
	Guthman 2005	1/84	0/84					
	Hamour 2005	2/68	2/70					
Parasite clearance at day 3	Bonnet et al 2007	105/110	103/117					
	Hamour 2005	79/80	80/81					
	Kayentao 2009	129/131	129/130					
Fever clearance at day 2	Barennes 2004	27/33						24/27
	Hamour 2005	79/80	78/81					
	Van Vugt 2002					527/533	518/533	
Fever at day 3	Van den Broek 2006	3/101	4/90					

PRISMA Checklist for reporting systematic reviews

TITLE				
Title	1	Identify the report as a systematic review, meta-analysis, or both.	
ABSTRACT				
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	
INTRODUCTION				
Rationale	3	Describe the rationale for the review in the context of what is already known.	
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	
METHODS				
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	

PRISMA Checklist for reporting systematic reviews

RESULTS		
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).
DISCUSSION		
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.
FUNDING		
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.

For more information, visit: **www.prisma-statement.org**.