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IMPROVED MALARIA CASE MANAGEMENT IN UNDER-FIVES IN THE ERA OF ARTEMISININ-BASED COMBINATION THERAPY IN TANZANIA

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ABSTRACT

Early parasite-based diagnosis of malaria prior to treatment is critical for rational drug use, optimal outcome of febrile illness, patient adherence, prevention of parasite drug resistance, and reduced costs of health services. However, most primary health care (PHC) facilities in sub-Saharan Africa have no laboratory support and malaria diagnosis is based on clinical symptoms and signs, which are nonspecific. This results in over-diagnosis of malaria, overuse of antimalarial drugs, and failure to identify and treat other causes of fever appropriately.

Artemether-lumefantrine (AL) is the most widely adopted artemisinin-based combination therapy (ACT) for treatment of uncomplicated malaria in Africa. However, there is still limited data on its efficacy, effectiveness and safety during extended follow-up and after repeated treatment. Importantly, the effectiveness when used in the home management of malaria (HMM) strategy needs to be assessed.

The main aim of this thesis is to improve the quality of malaria case management in government health facilities and specifically to assess the efficacy, effectiveness and safety of AL in under-five children with acute uncomplicated *Plasmodium falciparum* malaria in Tanzania.

In a diagnostic intervention study, antimalarial drug prescriptions were significantly reduced in microscopy-based diagnosis arm (61%) compared to clinical algorithm arm (95%) and no training arm (100%) (both $p < 0.001$). Of the patients with positive blood smears, 99% were prescribed antimalarial drugs and so were 11% of children with negative readings. Those with positive blood smear readings were less likely to be prescribed antibiotics than those with negative results (relative risk = 0.66, 95% confidence interval: 0.55, 0.72). However, the training did not improve accuracy of microscopy at PHC level when compared to the reference level.

In a randomized study on the efficacy of AL and sulphadoxine-pyrimethamine, including methodological objectives regarding the use of PCR-corrected cure rates in antimalarial drug trials, PCR analysis from blood samples collected on consecutive days both at study inclusion and parasite recurrence during follow-up improved identification of recrudescences by about 20% compared with the WHO recommended standard single-day blood sampling protocol.

In a randomized trial of supervised versus unsupervised AL intake, the PCR-corrected cure rates were similarly high in both groups, i.e. 98% versus 96% by day 56 after initial treatment, and 93% versus 98% by day 42 after retreatment, respectively, despite that the median blood lumefantrine concentrations on day 7 were significantly higher in the supervised compared with the unsupervised group (after initial treatment [304 versus 194 ng/mL; p<001] and after retreatment [253 versus 164 ng/mL; p=001]). Both regimens were safe and well tolerated after both initial and retreatment.

In a single arm effectiveness study of unsupervised AL intake in the context of HMM, the PCR-corrected cure rate by day 42 was 93 %.

In conclusion, microscopy-based diagnosis of malaria at PHC facilities reduced prescription of antimalarial drugs and appeared to improve appropriate management of non-malaria fevers. Interpretation of PCR-corrected cure rates in high endemic areas is influenced by methodological aspects such as the blood sampling protocol. AL was highly efficacious and safe for the treatment of uncomplicated malaria. The high effectiveness of AL provides evidence for scaling-up its use within the HMM strategy in Tanzania.

Key words: malaria microscopy, case management, efficacy, effectiveness, artemether-lumefantrine, retreatment, lumefantrine concentrations, randomized trial, Tanzania.

LIST OF PUBLICATIONS

This thesis is based on the following papers, which will be referred to by their Roman numerals:

I. **Ngasala B**, Mubi M, Warsame M, Petzold MG, Massele AY, Gustafsson LL, Tomson G, Premji Z, Bjorkman A.
Impact of training in clinical and microscopy diagnosis of childhood malaria on antimalarial drug prescription and health outcome at primary health care level in Tanzania: a randomized controlled trial. *Malar J* 2008, **7**:199.

II. Mårtensson A, **Ngasala B**, Ursing J, Veiga MI, Wiklund L, Membi C, Montgomery SM, Premji Z, Farnert A, Björkman A.
Influence of consecutive-day blood sampling on polymerase chain reaction-corrected parasitological cure rates in an antimalarial-drug trial conducted in Tanzania. *J Infect Dis* 2007, 195(4):597-601.

III. **Ngasala B**, Carlsson AM, Malmberg M, Petzold MG, Blessborn D, Bergqvist Y, Gil JP, Premji Z, Björkman A, Mårtensson A.
Efficacy and effectiveness of artemether-lumefantrine after initial and retreatment in underfive children with acute uncomplicated malaria in rural Tanzania: a randomized trial. *Manuscript*

IV. **Ngasala B**, Carlsson AM, Malmberg M, Bergqvist Y, Petzold MG, Premji Z, Mårtensson A.
Effectiveness of artemether-lumefantrine provided by community health workers in underfive children with uncomplicated malaria in rural Tanzania: an open label prospective study. *Manuscript*

CONTENTS

LIST OF ABBREVIATIONS

ACO	Assistant clinical officer
ACT	Artemisinin-based combination therapy
AIDS	Acquired Immune Deficiency Syndrome
AL	Artemether plus lumefantrine combination
AMFm	Affordable Medicines Facility – malaria
AMO	Assistant medical officer
AS+AQ	Artesunate plus amodiaquine combination
ATP	Adenosine triphosphate
AQ	Amodiaquine
CI	Confidence Interval
CO	Clinical officer
CQ	Chloroquine
DHA+PPQ	Dihydroartemisinin plus piperaquine combination
EIR	Entomological inoculation rate
g/dL	Grams per deciliter
GLURP	Glutamate rich protein
Glurp	Glutamate rich protein gene
G6PD	Glucose-6-phosphate dehydrogenase
HIV	Human immunodeficiency virus
HMM	Home management of malaria
Ht	Hematocrit
IMCI	Integrated Management of Childhood Illness
IPTp	Intermittent Preventive Treatment in pregnancy
IRS	Indoor Residual Spraying
ITNs	Insecticide-treated bed nets
LLINs	Long lasting insecticide nets
MCHA	Mother and child health aides (MCHA),
MMV	Medicines for Malaria Venture
MSP	Merozoite surface protein
Msp-1	Merozoite surface protein gene 1
Msp-2	Merozoite surface protein gene 2
Ng	Nanogram
PHC	Primary health care
PCR	Polymerase chain reaction
PfATP6	*Plasmodium falciparum* adenosine triphosphatase 6 protein
Pfcrt	*Plasmodium falciparum* chloroquine resistance transporter gene
Pfdhfr	*Plasmodium falciparum* dihydrofolate reductace gene
Pfdhps	*Plasmodium falciparum* dihydroptereroate synthetase gene
Pfmdr 1	*Plasmodium falciparum* multidrug resistance gene 1
Pfmrp1	*P. falciparum* multidrug resistance protein 1 gene
QA	Quality assurance
RBC	Red blood cell
RDT	Rapid diagnostic test
RFLP	Restriction fragment length polymorphism
RR	Relative Risk
SP	Sulphadoxine-pyrimethamine
SNP	Single nucleotide polymorphism
µL	Microliter
UNICEF	The United Nations Children's Fund
WHO	World Health Organisation

1 BACKGROUND

1.1 GENERAL INTRODUCTION TO MALARIA

Malaria is one of the most important infectious diseases in the world. Although malaria is preventable and curable, it still causes significant morbidity and mortality. Approximately half of the world's population is at risk of malaria, which is endemic in more than 100 countries[1]. The World Health Organisation (WHO) estimates that in 2008, there were 243 million cases of malaria and 863 000 deaths, mostly among underfive children in sub-Saharan Africa[1].

The global campaign to eradicate malaria in Africa between 1955 to1978 were unsuccessful, since then the focus has been to improve effective case management using inexpensive widely available antimalarial drugs mainly chloroquine (CQ) and sulphadoxine-pyrimethamine (SP). However, resistance of *Plasmodium falciparum* to CQ and SP resulted in global resurgence of malaria morbidity and mortality in the 1980s and 1990s [2-4].

Currently available tools to control malaria include early diagnosis and treatment with highly effective artemisinin-based combination therapies (ACTs) Insecticide-treated bed nets (ITNs), Indoor Residual Spraying (IRS) and Intermittent Preventive Treatment in pregnancy (IPTp). An effective malaria vaccine is not yet available; although there are encouraging results from clinical trials of vaccines [5, 6]. In countries, where these interventions have been fully scaled up, the number of reported malaria cases and deaths have been significantly reduced[1]. However, access to these interventions is still limited in many countries particularly in sub-Saharan Africa[1].

More recent efforts by the governments of malaria endemic countries, foundations, bilateral donors, multilateral organizations, private companies and nongovernmental organizations have played an important role to increase access to effective treatment and prevention[7, 8]. For example, the Roll Back Malaria Partnership, the Global Fund and the AMFm (Affordable Medicines Facility - malaria) are working with ACT manufacturers to ensure affordable antimalarial drugs are accessible to the patients in both public and private sectors[9].

1.1.1 Malaria parasites

Malaria is a parasitic disease caused by a unicellular protozoan of the genus *Plasmodium* and transmitted by the bite of an infected female mosquito of the genus *Anopheles*. The French physician, Charles Louis Alphonse Laveran, discovered the parasite under the microscope in 1880, in a patient with intermittent fever in Algeria[10]. There are five malaria species that infect humans: *P. falciparum, P. vivax, P. ovale, P. malariae* and the recently described *P. knowlesi* [11-13]. Many other species of *Plasmodium* are found in other animals (mammals, reptiles and birds). *P. falciparum,* found mainly in tropical regions accounts for the majority of the clinical cases and mortality.

1.1.2 Malaria vectors

There are over 500 hundreds species of mosquitoes of the genus *Anopheles*, but only about 80 can transmit malaria to humans, of which approximately 40 are significant vectors. *Anopheles gambiae, A. arabiensis* and *A. funestus* transmit most of human malaria and are all found in tropical Africa [14-16]. The female anopheline mosquito needs a blood meal and a water site for laying eggs. Some vectors have anthropophilic behaviours, which include preference for humans as a source of blood meal and endophily (the tendency to enter and rest inside houses) [14]. Most vectors are nocturnal feeders, but timing varies with species.

1.1.3 Life cycle of the parasites

(i) V**ector phase** (the definitive host)

Female anopheline mosquitoes ingest **gametocytes** with human blood meal, where after the ingested blood ends-up in the mosquito's mid-gut (stomach). The male and female gametocytes escape from the human red blood cells (RBCs) and differentiate to microgametes (male) and macrogametes (female). Fertilization takes place when a microgamete penetrates a macrogamete to form a **zygote** (the only diploid stage during the life cycle of the *Plasmodium* parasite). In the mosquito mid-gut, about18 hours after blood meal, the zygote transform to an **ookinete,** an elongated mobile cell. The ookinete penetrates the gut wall (epithelial wall) and attaches to the outer aspect of the mosquito gut, then the ookinete differentiate to a spherical, **oocyst**. In the oocyst, multiplication takes place, i.e. meiosis, and the parasite becomes haploid again. This stage of multiplication is called **sporogony**, when thousands of **sporozoites** are produced. When the oocyst is mature, it ruptures to release sporozoites which migrate

and accumulate in the salivary glands and complete maturation. The average duration of the sporogony is about 2 weeks with a range of 1-5 weeks, depending on species and temperature. Inoculation of the sporozoites into a new human host continues the malaria life cycle.

(ii) **Human phase** (intermediate host: hepatic and erythrocytic schizogony)
Sporozoites enter blood with mosquito saliva; usually an infected mosquito injects on average 10-100 sporozoites per blood meal. The sporozoites take few minutes to an hour to reach the liver and invade liver cells (hepatocytes). In the hepatocytes, the sporozoites transform to trophozoites which grow and divide by schizogony, to produce thousands of infective **merozoites,** about 10,000 for *P. vivax and* 30,000 for *P. falciparum*. The duration of **hepatic schizogony** is about 5 days for *P. falciparum*, but 16 days for *P. malariae.* Clinical symptoms of malaria are not apparent during liver-stage maturation. In *P. vivax* and *P. ovale*, sporozoites may become dormant in hepatocytes, as **hypnozoites**, which reactivate at variable intervals (most often 3-45 weeks; determined by parasite genetics) to cause clinical and parasitological relapses[17]. The hepatic schizonts rupture and merozoites are released into the blood stream and rapidly invade RBCs.

In the RBC, the merozoite transform to **a trophozoite,** it feed on the content of RBC and form food vacuoles where hemoglobin is digested. After a period of growth, the mature trophozoite starts dividing **(erythrocytic schizogony)** and produces a new generation of merozoites. A mature schizont of *P. falciparum* contains circa16 merozoites. The erythrocytic cycle lasts for about 48 hours for *P. falciparum, P. vivax* and *P.ovale,* but 72 hours for *P. malariae* and only 24 hours for *P. knowlesi* . When this stage is reached, the schizonts rupture releasing merozoites and other parasite byproducts (pyrogenic molecules) into the bloodstream. The pyrogenic molecules are responsible for sequence of events leading to fever and other clinical symptoms and signs of malaria. Immediately after the release, the merozoites invade uninfected RBCs and repeat the cycle of erythrocytic schizogony. Some merozoites differentiate into sexual stages, i.e. gametocytes (**gamogony**) - as early as 4 days (equivalent to 2 RBC cycles) with *P. vivax* and 10 days with *P. falciparum* infections.

Adherence of infected RBCs to endothelial cells in various organs is called s**equestration**, which is characteristic of *P. falciparum* infections. Sequestration

protects the parasite from clearance mechanisms operating in the spleen. Sequestration plays an important role in pathophysiology of severe malaria particularly cerebral malaria and malaria during pregnancy[18, 19].

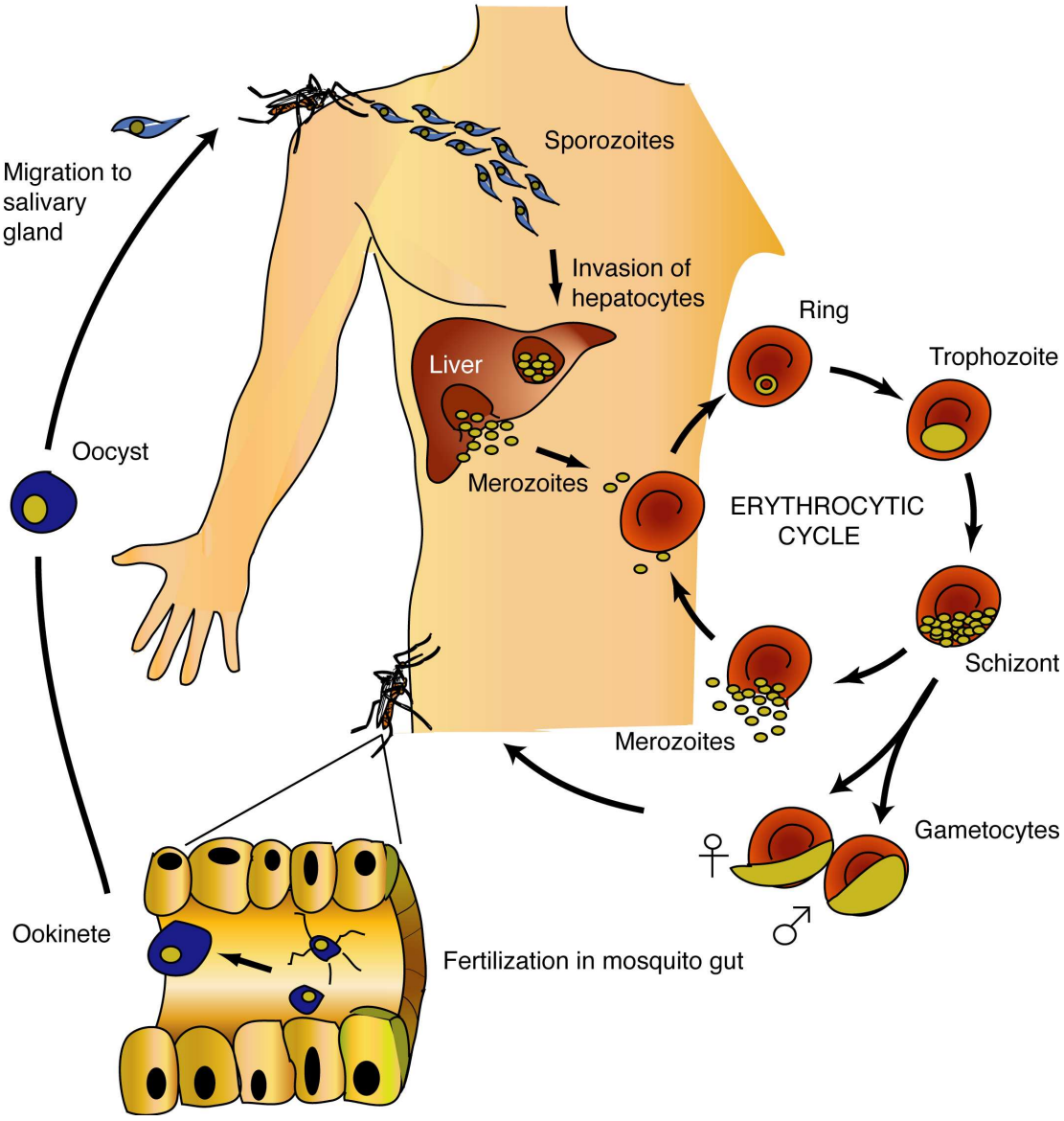

Figure 1. Illustration of the life cycle of the malaria parasite
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1.1.4 Malaria transmission and epidemiology

Vectors are key determinants of malaria transmission. The distribution and abundance of the mosquito vectors depend on environmental conditions such as temperature (optimal condition between 20ºC and 30ºC), altitude, humidity and seasonal pattern of rainfall, and suitable water for larval breeding [20-22].

1.1.4.1 Basic indices of malaria transmission

The intensity of malaria transmission is assessed by how often people are bitten by anopheline mosquitoes carrying sporozoites. The entomological inoculation rate (EIR) is the number of infective bites received per person per unit time, usually measured annually[23]. Methods of measurement include human bait catch, light and exit traps, and pyrethrum spray collections[24]. Measurement of sporozoites index from samples of mosquitoes can be determined either microscopically by dissecting the sampled mosquitoes for their salivary glands or using immunological assays which detect *Plasmodium*-specific circumsporozoite antigens[24].

The vectorial capacity is a transmission probability index, i.e. the expected number of humans infected per infected humans, per day, assuming perfect transmission efficiency[20] or number of new infections the population of a given vector would distribute per case per day at a given place and time, assuming conditions of non-immunity[25]. The vectorial capacity is an indicator of stability of transmission, and depends on relative density of female anophelines, probability of mosquito to take blood meal, proportion of mosquitoes surviving incubation period and probability of daily survival. The key factor in determining intensity of transmission is the probability of daily survival of mosquitoes [24].

1.1.4.2 Levels of endemicity

Geographical areas can be classified by intensity of transmission based upon percent of children, aged 2-9 years with splenomegaly or malaria parasitaemia:

Holo-endemic: (>75%) transmission occurs all year long;

Hyper-endemic: (50-75%) intense, but with periods of no transmission during dry season;

Meso-endemic: (11-50%) regular seasonal transmission;

Hypo-endemic: (<10%) very intermittent malaria transmission.

Malaria transmission can also be classified as stable, if the populations are continuously exposed to a fairly constant rate of malarial inoculations (EIR>10/year) or unstable (EIR<5/year) and where the level of acquired immunity determines patterns of disease[26]. Stable and high malaria transmission occurs in holoendemic and hyperendemic areas, such as in tropical Africa. The clinical profile in the area is characterized by:

- Most malaria infections are due to *P. falciparum;*
- Malaria associated anaemia and mortality affects mainly underfive children;
- Pregnant women, particularly primigravida, and their unborn infants are at high risk of morbidity;
- Older children (> 5 years) and adults are semi-immune and therefore the incidence rate of malaria is low; the clinical picture is characterized by fever and moderate parasitaemia and they are at low risk of developing severe malaria.

Unstable and low to moderate malaria transmission occurs in meso and hypoendemic areas, such as much of tropical Asia, Latin America and Central Asia.

- Both *P. falciparum* and *P. vivax* malaria are prevalent;
- Severe morbidity and mortality occur in all age groups;

1.1.5 Clinical presentation of *P. falciparum* malaria

1.1.5.1 Clinical presentation of uncomplicated P. falciparum

Malaria is an acute illness. The common first symptoms are nonspecific and include: fever, headache, joint aches, malaise, vomiting, diarrhea, body weakness, chest pain, poor appetite and body weakness. This is the typical picture of uncomplicated malaria; usually appears 10 to 15 days after a person is infected. The symptoms of malaria vary according to state of immunity, species of infecting parasites, and the epidemiology of malaria[27]. *P. falciparum* infections constitutes >90% of malaria cases in Tanzania[28].

1.1.5.2 Clinical presentation of severe P. falciparum

Clinical features of uncomplicated malaria may progress to severe malaria if a patient is treated with ineffective drugs or treatment is delayed. The WHO has developed specific diagnostic criteria for severe malaria [29]. A patient is classified to be suffering from severe malaria if one or more of the following features are present: Severe

anaemia (haemoglobin <5g/dL or Ht<15%), a common feature in infants and young children in malaria endemic areas; Cerebral malaria (unrousable coma), with a Blantyre coma scale <3 (in children) and Glasgow coma scale <10 (in adults), a common feature in adult and children in areas of low transmission; Prostration; Respiratory distress induced by metabolic acidosis. Other criteria include: Convulsions (>1 episode/ 24hours); Hypoglycaemia (<2.2 mmol/L); Extreme weakness; Acidosis (plasma bicarbonate <15mmol/L); Renal failure (serum creatinine above normal range for age); Shock; Macroscopic haemoglobinuria; Spontaneous bleeding; Pulmonary edema; Clinical jaundice; Hyperlactataemia (venous lactic acid > 5 mmol/L); and Hyperparasitaemia (>250000/µL or >5% of RBCs). For patients with severe malaria receiving treatment case fatality rate is about 15-20%, and if untreated severe malaria is fatal in a majority of cases[25]. A study in Kilifi, Kenya showed that African children with malaria who had impaired consciousness or respiratory distress were at high risk for death[30].

1.2 MALARIA DIAGNOSIS

Prompt and accurate diagnosis of malaria is an essential component of malaria case management as well as a key for reducing morbidity and mortality[25-27]. Accurate diagnosis is also important for differential diagnosis of other febrile illnesses including sepsis, meningitis and pneumonia in which delayed diagnosis and treatment may be a cause of mortality [31-36]

The diagnosis of malaria is based on recognition of symptoms and signs of malaria and confirmation by detection of parasites in the blood (parasitological diagnosis).

1.2.1 Clinical diagnosis

In sub-Saharan Africa, many primary health care (PHC) facilities lack diagnostic equipment, such as microscopes, and staff for high-quality laboratory diagnosis. Malaria diagnosis is therefore still based on clinical features such as fever or history of fever, and presence of anaemia [1, 25]. However, symptoms and signs of malaria are nonspecific. The symptoms of malaria overlaps with many other acute febrile illnesses and co-infections are common [33-35, 37-40] As a result malaria over-diagnosis is very common in sub-Saharan Africa[41, 42],

Efforts have been made to improve the management of illnesses in children through use of clinical algorithms. The WHO/UNICEF strategy for Integrated Management of Childhood Illness (IMCI) algorithm has shown poor specificity in diagnosing malaria which results in over-treatment with antimalarial drugs[43, 44]. Several research groups that developed and evaluated clinical algorithms for uncomplicated malaria found that clinical features could not accurately predict malaria diagnosis and algorithms were site and time specific depending on the intensity of transmission and prevalence of malaria [45, 46].

1.2.2 Parasitological diagnosis

The need for confirmation of parasites in the blood before therapy has become acute because of resistance of *P. falciparum* to the widely available monotherapies, CQ and SP, which are now replaced by the more costly ACTs[25-27]. Advantages of confirmed parasitological diagnosis of malaria include improved adherence to treatment and management for other febrile illnesses; preserved drug supply and efficacy by decreasing the exposure of parasite populations to antimalarial drugs hence reducing the selection of resistance; monitor trends in malaria prevalence; and reduced costs at household and national level, although this depends on cost of antimalarial drugs and epidemiology of malaria [25-27, 47].

The laboratory methods for malaria diagnosis, which have a potential for widespread routine use in Africa include light microscopy and parasite antigen detection (rapid diagnostic tests [RDTs]).

1.2.2.1 Light microscopy

Laboratory diagnosis by microscopic examination of stained blood smears remains the gold standard for case management, epidemiological studies, clinical trials of antimalarial drugs and vaccines, and quality assurance of other malaria diagnostics tests. Microscopy of Giemsa stained blood films has high sensitivity and specificity when used by well-trained staff, which can detect as low as 50 parasites/μL blood under field conditions [48, 49]. Other advantages of microscopy include low cost to maintain, determination of parasite densities and distinction between parasite stages, differentiation between malaria species and diagnosis of other diseases. However, its accuracy and usefulness depends on for example the quality of microscope, reagents, experience of the technician, effective quality control and quality assurance.

The quality of laboratory diagnosis in many malaria endemic countries is poor [50-52] and as a result clinicians often treat patients despite negative results [53-56]. Improved quality of microscopy depends on for example political commitment, adequate funding, in-service training and the implementation of quality assurance (QA) programmes [57, 58].

1.2.2.2 Rapid diagnostic tests

RDTs employ lateral flow immune-chromatographic technology to detect antigens derived from malaria parasites[59-61]. Malaria antigens currently used as diagnostic targets include histidine-rich protein II (HRP-II) specific for *P. falciparum*[62]. Another antigen is parasite lactate dehydrogenase (pLDH) for detection of *Plasmodium* spp. (pan-malaria), monoclonal antibodies against pLDH can differentiate between *P. falciparum* and *P. vivax*[62]. There is limited data on the use of RDTs to identify *P. knowlesi*; findings from a recent study show that the pLDH antibodies that detect *P. falciparum* and *P. vivax* can also be used to detect and distinguish *P. knowlesi* [63]. *Plasmodium* aldolase enzyme can also be used as a universal antigen target for all malaria parasites [62, 64]. Unlike HRP-II, pLDH is cleared from the blood about the same time as the parasites following successful treatment [65, 66]. However both pLDH and aldolase are expressed in gametocytes, limiting their use in monitoring response to therapy [67].

RDTs offer a useful alternative to microscopy particularly in PHC facilities, where reliable microscopic service is often not available[49, 64], and can be used safely in underfive children [68]. RDTs are simple to perform and do not require skilled personnel[69]. Limitations of RDTs include high cost, lack of density determination and persistent positivity (HRP-II in blood 1-3 weeks or more after effective treatment), heat and humidity instability, which lead to variation in individual test performance[64, 65, 70]. Also of concern with RDTs are the reported low sensitivity to detect non- *P. falciparum* species, failure to detect infections with low but clinically relevant parasite densities, and limited ability to monitor responses to therapy [62, 64, 65, 70]. Recently the WHO launched a program of product testing and quality assurance of RDTs that would assist national malaria control programmes in making procurement decisions [71-73]. Similar to microscopy, issues of ignoring test results and prescribing anti-malarial drugs to patients with negative results have been reported in Africa [74-

76]. However, results from recent studies with overall good adherence also to negative test results are encouraging [77, 78].

1.3 MALARIA CASE MANAGEMENT

The goal of malaria case management is to cure the infection and to reduce morbidity and mortality, whereas an important public health goal is to reduce the infectious reservoir from which other persons can become infected and thus reducing transmission[25-27]. Key components of malaria case management include prompt diagnosis, effective treatment, counseling, follow-up, and referral if necessary[27]. The WHO guidelines for the treatment of malaria provide evidence based guidelines for the formulation of policies and protocols for the treatment of malaria[25, 26]. These guidelines have recommendations addressing each component of malaria case management at different levels of health care delivery, intensity of malaria transmission (high or low risk areas), and for different populations at risk of malaria. The guidelines also provide a framework for national malaria control programmes in different countries to develop specific diagnosis and treatment protocols depending on drug resistance pattern and health services capacity.

1.3.1 Malaria diagnosis recommendations

Previously, the WHO recommended that antimalarial treatment should be based on parasite confirmed diagnosis except for underfive children in areas of high endemicity, epidemics and suspected severe malaria when parasitological diagnosis was not available[26]. The results of recent studies are in contrast to this WHO position on testing of underfive children, indicating that it is safe to withhold antimalarial treatment in febrile children with negative malaria test results based on accurate parasite-based diagnosis (microscopy or RDTs) [68, 78-80]. We have also shown that microscopy-based treatment is safe and effective in underfive children, even when non-expert microscopists were used for screening, provided that patients are followed-up and clinicians are trained to look for and treat other febrile illnesses [81]. There is also more evidence on decreased malaria prevalence and malaria-related mortality and morbidity in sub-Saharan African countries, which have scaled up malaria control interventions[1, 82-85], and decline of prevalence of malaria parasitaemia among children aged two to ten years in sub-Saharan Africa[86]. In Tanzania, only about 40% of febrile underfive children are parasitaemic in high transmission areas [87-89].

Now, the WHO recommendations include more recent evidence, suggesting antimalarial treatment should be provided based on parasitological confirmation in all patients [25, 27]. Treatment based on clinical diagnosis should now only be given when parasitological diagnosis is not available or is likely to delay treatment, particularly in high risk groups (underfive children, pregnant women and in severe malaria cases).

1.3.2 Malaria treatment recommendations

Due to development and spread of *P. falciparum* resistance to conventional monotherapies (CQ and SP)[90-92], the WHO guidelines recommend combination treatment with antimalarial drugs with different modes of action and different biochemical targets in the parasite, preferably ACTs [25, 26]. Artemisinin derivatives are extremely potent antimalarial drugs with a rapid onset of action. Artemisinins may also decrease malaria transmission considering their effect on gametocytes [93-95]. When combined with slowly eliminated partner drugs (e.g. lumefantrine) short courses of treatment are effective [96, 97]. The WHO currently recommends the following ACTs: artemether-lumefantrine (AL), artesunate-amodiaquine (AS+AQ), artesunate-mefloquine, artesunate-SP and dihydroartemisinin- piperaquine (DHA+PPQ) [1, 25, 27]. Many countries in Africa have recently adopted ACTs, which are being deployed for treatment of uncomplicated malaria [1, 98]. The choice of ACT in a country or region depends on a number of factors including level of underlying resistance to the long acting partner drug in the combination, availability, cost, presence of co-formulation, and safety profile [26, 99].

The management of severe malaria includes clinical assessment of the patient, specific antimalarial treatment, adjunctive therapy and supportive care [29, 100]. The specific antimalarial treatment for severe malaria include the cinchona alkaloids (quinine and quinidine) and artemisinin derivatives (artesunate, artemether and artemotil)[25, 27].Quinine is also a drug of choice for malaria in the first trimester of pregnancy, and malaria in children weighing <5 kg. Artesunate suppositories, which can be administered rectally, are recommended for pre-referral management of severe malaria in areas where parenteral antimalarial treatment would not be feasible [25, 27].

1.4 THE TANZANIAN CONTEXT

The United Republic of Tanzania is located in eastern part of Africa between longitude 29^{o} and 41^{o} East, and latitude 1^{o} and 12^{o} South [101]. The United Republic of Tanzania was formed by the union between two states, Tanganyika and Zanzibar in 26th April, 1964. Tanganyika gained independence in 9th December, 1961. Zanzibar became independent on 10th December, 1963, and later the Zanzibar Revolutionary Government was established after the revolution 12th January, 1964. There are two ministries of health, for Mainland Tanzania (previously Tanganyika) and Zanzibar. In 2008, the Tanzanian population was estimated to be over 42 million people, with 18% of the population being children below 5 years of age[1].

Almost all population in Tanzania is at risk of malaria, the main malaria parasite is *P. falciparum*, and transmission occurs in majority of the country all year round. According to the World Malaria Report 2009, between 2003 and 2008; about 11 million malaria cases and 17 000 deaths were reported annually[1]. In the report, trends in morbidity and mortality could be only analysed for Zanzibar, because of good quality of information. In Zanzibar, the number of cases and deaths decreased significantly from 2003 and 2008 following wide scale deployment of antimalarial interventions (ACTs, LLINs and IRS) [1].

1.4.1 Current antimalarial drug policy

In September 2006, Mainland Tanzania introduced AL as the official first line drug for uncomplicated malaria. Quinine is the second-line drug and is also recommended for treatment of severe malaria and malaria in pregnancy in the first trimester[102]. In Zanzibar since 2003, the combination, AS+AQ is used as first line drug for uncomplicated malaria. AL is an alternative drug for uncomplicated malaria for patients who cannot tolerate AS+AQ [103]. Quinine is the second-line and is also recommended for severe malaria and malaria in pregnancy in the first trimester[103]. SP is used for IPTp in both Mainland Tanzania and Zanzibar.

1.4.2 The formal health system in Tanzania

Health services in Tanzania are mainly provided by the government, over 70% of health facilities are in rural areas where a majority of the population lives. The private health providers include non-governmental organization (e.g. religious institutions) and private for profit health facilities[104]. Levels of health care delivery in Tanzania start

with Home/Village/Community PHC Post/Shopkeeper Health Service, followed by Dispensaries, Health Centers, District Hospitals, Regional Hospitals and the highest level is Referral/Consultant Hospitals[28]. The PHC facilities are accessible within 5-10 km from rural communities [105]. Malaria diagnosis is primarily based on clinical symptoms. Microscopy is available at hospitals, health centers, and some dispensaries. Currently the national malaria programmes are scaling up distribution of RDTs in PHC facilities.

1.4.3 Quality of malaria case management in Tanzania

In many PHC facilities in Tanzania the quality of malaria diagnosis and treatment suffers from inadequate clinical examination, lack of diagnostic equipment, such as microscopes, and non compliance with standard malaria treatment guidelines [52, 74, 88, 106-108]. This results in over-prescriptions of antimalarial drugs and prevents optimal management of other febrile illnesses. A qualitative study in northeast Tanzania that aimed to identify reasons of malaria over-diagnosis and over-prescriptions found that clinicians decisions to prescribe were influenced by three mindlines: malaria is easier to diagnose than alternative diseases; malaria is a more acceptable diagnosis; and missing malaria is indefensible[109] However, recent studies have shown that training of health workers and provision of microscopes or RDTs improved targeting antimalarial treatment to patients with confirmed parasitological diagnosis[77, 78, 81].

1.5 ANTIMALARIAL DRUG RESISTANCE

Spread of resistance to antimalarial drugs is a major obstacle to effective case management; CQ-resistance in Africa resulted in increase in morbidity and mortality due to malaria [2-4, 110, 111].

1.5.1 Definition of antimalarial drug resistance

In 1973, the WHO defined antimalarial drug resistance as "the ability of a parasite strain to survive and/or multiply despite the proper administration and absorption of an antimalarial drug in the doses equal to or higher than those normally recommended but within the tolerance of the subject"[112]. This definition was modified in 1986 to include the phrase "drug must gain access to the parasite or the infected red blood cell for the duration of the time necessary for its normal action" [112]. Recent development of techniques for *in vitro* drug-sensitivity testing, high-performance liquid

chromatography and molecular biology have improved our understanding of the mechanisms of drug resistance. In this definition it is important to consider pharmacokinetic properties of antimalarial drugs, which vary widely in different individuals e.g. lumefantrine has inter-individual variation in bioavailability up to 20-fold [113, 114]. Today, to confirm true resistance we need to consider the blood concentration of the drug and/or its metabolites, whether parasites are recrudescent, results of *in vitro* drug *s*ensitivity tests and genetic markers of resistance [115, 116].

1.5.2 Development of antimalarial drug tolerance and resistance

The development of resistance to an antimalarial drug is considered to starts with an intermediate stage of drug tolerance, i.e., the selection of parasites less sensitive to the drug, although still inside the therapeutic window[117]. This intermediate stage is important in evolution of resistance to antimalarial drugs deployed as combination therapy, although complete resistance for certain antimalarial drugs like atovaquone can result from a single mutation [118].

The genetic events (single nucleotide polymorphisms [SNPs] or increased gene copy numbers), which lead to drug resistance occur independently of drug effect [119, 120]. These genetic events relate to the drug's target or pumps that affect intraparasitic concentrations of the drug. Use of antimalarial drugs creates a selection process in which drug-tolerant/resistant parasites survive and spread [91, 119, 120]. Interaction of various factors in the host, parasites, drugs, vectors and environment, contribute to the development and spread of drug resistance [96, 119, 120]. For example, the host immune response influences the speed of spread of drug resistance, acquired immunity in high transmission areas reduces parasite burden and kills drug-resistant mutant malaria parasites, which have not been cleared by the antimalarial drug [121-123]. In low transmission areas, the degree of acquired immunity is low or non-existing, and the majority of the infections are symptomatic and associated with a higher parasite biomass, therefore low drug concentrations may select for resistance [96]. Also selection of tolerant parasites, which occurs among reinfections during the elimination phase of the slowly eliminated partner drug in ACT [124-126], may play an important role in the evolution of parasite resistance in high transmission areas [127, 128].

1.5.3 Monitoring of antimalarial drug efficacy and resistance

Effective surveillance for the recognition of tolerant/resistant parasites to antimalarial drugs is of particular importance in the era of ACTs. Emergency of resistance may go unnoticed if there is no effective surveillance system. Recently, there have been reports of reduced susceptibility to artemisinin derivates and ACTs with prolonged parasite clearance time in Southeast Asia [129, 130]. The goal of surveillance is to ensure efficacious drugs are available for management of clinical cases, allow for early detection of changing patterns of resistance and control the spread of resistance strains [26, 131].

1.5.4 Methods for assessing efficacy and resistance to antimalarial drugs

The principal methodologies involved in detecting drug resistance are *in vivo* assessment of therapeutic efficacy, in *vitro* studies of parasite susceptibility to drugs in culture, and molecular genotyping.

1.5.4.1 In vivo assessment of therapeutic efficacy

This is the gold standard for assessing antimalarial drug efficacy, involves repeated assessment of clinical and parasitological outcomes of treatment in a group of symptomatic and parasitaemic individuals treated with a controlled dosage of the drug during a fixed period of follow-up. The success of treatment is measured by the ability of the drug to resolve patient's symptoms and signs and to kill all parasites to ensure that the infection does not return. The *in vivo* efficacy studies are relatively simple to conduct in the field with little equipment and training of staff. However, treatment outcomes may be confounded by a complex interaction between the parasites, the drugs, and the host compared to in *vitro* tests where only the interaction between the parasites and the drugs takes place. A patient with unsuccessful treatment outcome may not necessarily reflect "true resistance". Instead a number of factors may be responsible for treatment failure, including inter-individual pharmacokinetic variations influencing drug absorption, low compliance, incorrect dosing, vomiting, poor drug quality, and drug interactions [132, 133]. Therefore, measurement of drug concentrations in clinical trials could help to improve the assessment of therapeutic efficacy of antimalarial drugs. Also host immunity may influence treatment outcome, partially acquired immunity in high endemic areas helps to kill he parasites that have not been adequately tackled by the antimalarial drug, but reduced immunity also results

in higher parasite biomass and reduced efficacy of antimalarial drugs [26, 119, 123, 134, 135].

The WHO has been preparing standard protocols for assessment of antimalarial drug efficacy since 1965, which have been updated several times based on the feedback from countries with malaria and expert opinions on malaria chemotherapy. These protocols provide guidance to national malaria control programmes and researchers on how best to obtain the minimum necessary information about the therapeutic responses to an antimalarial drugs so as to allow for comparison of the results within and between regions, and to follow trends over time.

There have been recommended changes in the study subjects, periods of follow- up and classification of treatment responses over time [91, 115, 116]. The WHO 2003 standardized protocol[136] has further improved comparability of results between different transmission settings. However, WHO consultative meetings in 2005 and 2008 for development of treatment guidelines recommended a number of changes, which are included in new the document[137]. The following changes were recommended in the protocol: Inclusion and exclusion criteria (age, 6–59 months, i.e. under 5 years in areas of high transmission, and all patients over 6 months of age in areas of low-to-moderate transmission); administration of rescue treatment to patients with parasitological treatment failure at all levels of malaria transmission; duration of follow-up (requirement for 28 or 42 days of follow-up as a standard, depending on the medicine tested); classification of response to treatment (applications of the same definitions of treatment responses at all levels of malaria transmission); requirement for genotyping by polymerase chain reaction (PCR) to distinguish between recrudescence and reinfection; sample size calculation should be determined by using classical statistical methods and additional 20% should be added for losses due to attrition; and for data analysis the Kaplan-Meier method is recommended, even though per protocol method can be used in parallel[136-138]. The recommended four outcome categories for the assessment of response to treatment are defined below:

Early treatment failure (ETF)

Danger signs or severe malaria on day 1, 2 or 3, in the presence of parasitaemia;
Parasitaemia on day 2 higher than on day 0, irrespective of axillary temperature;
Parasitaemia on day 3 with axillary temperature $\geq$ 37.5 °c; and
Parasitaemia on day 3 $\geq$ 25% of count on day 0.

Late clinical failure (LCF)

Danger signs or severe malaria in the presence of parasitaemia on any day between day 4 and day 28 (day 42) in patients who did not previously meet any of the criteria of ETF;

Presence of parasitaemia on any day between day 4 and day 28 (day 42) with axillary temperature ≥ 37.5 °C in patients who did not previously meet any of the criteria of ETF.

Late parasitological failure (LPF)

Presence of parasitaemia on any day between day 7 and day 28 (day 42) with axillary temperature < 37.5 °C in patients who did not previously meet any of the criteria of ETF or LCF.

Adequate clinical and parasitological response (ACPR)

Absence of parasitaemia on day 28 (day 42) irrespective of axillary temperature in patients who did not previously meet any of the criteria of ETF, LCF, or LPF.

1.5.4.2 In Vitro Tests

In vitro tests are based on the inhibition of normal maturation of trophozoites to schizonts by different concentrations of a given drug[139]. These tests are not influenced by host factors like *in vivo* tests. The advantages of in *vitro* tests include ability to perform multiple tests of several drugs in parallel and testing new experimental drugs. However, the limitations of these tests include requirement of highly trained staff and expensive laboratory equipment. The correlation of results obtained with in *vitro* and in *vivo* tests may be difficult to interpret[140]. Moreover, some prodrugs like proguanil, which require host conversion into active metabolites in human host, cannot be tested.

1.5.4.3 Molecular markers of antimalarial drug resistance

Recent advances in technology have improved our understanding of the molecular basis of antimalarial resistance, and have made possible use of molecular markers to assess the levels of drug resistance [115, 116, 141]. Molecular tests use PCR to detect parasite point mutations or gene amplifications/duplications associated with tolerance/resistance to antimalarial drugs. A number of tolerance/resistance associated genotypes (molecular markers) have been identified. For example: Point mutations in

P. falciparum chloroquine-resistance transporter *(Pfcrt)* gene have been associated with CQ and amodiaquine(AQ) resistance, the essential mutation is replacement of a lysine with threonine at codon 76 [142-149]; Also the protein product of *P. falciparum* multidrug resistance gene1 (*Pfmdr1*), wild type PFMDR1, acts as ATP-driven export / import pump for hydrophobic drugs and metabolites.The protein is found on the digestive vacuole membrane and seems to play a role in regulating traffic across the membrane, including a variety of antimalarial drugs. *Pfmdr1* 86N, the chloroquine susceptible-allele, is selected in reinfections after AL treatment [124, 125, 150]. Resistance to mefloquine, lumefantrine, and other structurally related aryl-amino-alcohols in *P. falciparum* have been associated with amplifications (i.e. duplications) of *Pfmdr1* [151-153]. Resistance due to *Pfcrt* mutations can be enhanced by mutations in *Pfmdr1*. Mutations on both *Pfcrt* and *Pfmdr1* genes are or have been selected by extensive use of CQ and AQ in Africa [149, 154-157].

The advantages of the molecular methods include that they require relatively small amounts of blood (parasite DNA), collected from a finger prick, dried on filter paper; one sample can be used to study molecular targets of several drugs. These tests are far easier to run than *in vitro* tests and they can be used for surveillance of resistance at population level. Disadvantage include requirement of technical staff and laboratory facilities, and limited number of known genetic determinants of antimalarial drugs resistance. Immunity also affects our ability to assess drug resistance. For example mutations in *Pfcrt* are associated with treatment failure, but these mutations are often found in patients who respond adequately to treatment [121, 122, 143].

1.5.5 PCR genotyping for genetic markers of recrudescence and reinfection

The current WHO guidelines for monitoring of therapeutic efficacy of antimalarial drugs recommend extending follow-up period for least 28 days and use of PCR to distinguish between recrudescence (treatment failure) and reinfections (new infections) [137]. The PCR genotyping in malaria involves molecular analysis of highly polymorphic markers in the parasite genome. The merozoite surface proteins 1 and 2 (*msp-1* and *msp-2*) and glutamate-rich protein (*glurp*) are the most commonly used markers[158]. To harmonize laboratory protocols and interpretation of data, the WHO and Medicines for Malaria Venture (MMV) have recently published standard operating procedures [159]. In this document, a recrudescence is defined as an episode of

parasitaemia occurring subsequent to treatment in which at least one allele/genotype at each locus examined is common to both paired samples, i.e. pretreatment (Day 0) and recurrent infection (Day X). A reinfection is defined as parasitaemia occurring subsequent to treatment in which all the alleles in parasites from the Day X are different from those in Day 0, for one or more loci tested.

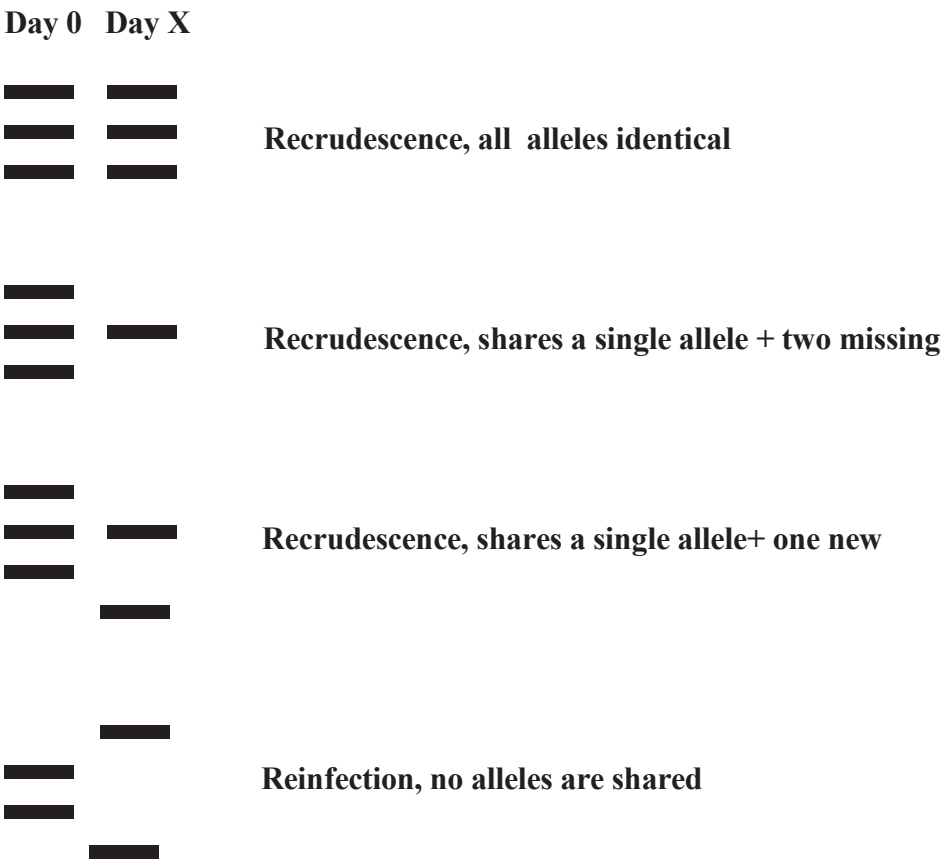


Figure 2. Illustration of different electrophoretic patterns on day of enrollment (Day 0) and day of parasite recurrence (Day X) during follow up in one genetic marker[159].

PCR genotyping still has limitations which include: Inability to detect/amplify minority clones (<10-20% of the parasite population) in polyclonal infections, which are common in areas of high or even moderate transmission[160-162]; A positive PCR result may represent gametocytes rather than asexual parasites in the blood sample; Limited discriminatory power, in case of coincidental reinfection by new parasite with same genotype as pretreatment parasites. This occurs particularly in areas with low transmission where the average complexity of infection is low and the genetic diversity of parasites in mosquitoes is limited, which may lead to misclassification of reinfection as recrudescence. Also studies conducted in high transmission areas have suggested an estimated 20-40% of PCR-corrected recrudescences to be actually reinfections [163, 164]; PCR adjustment protocols detect size polymorphisms, but not sequence polymorphisms; *P. falciparum* may be sequestrated in the deep vascular system, removing some genotypes from peripheral blood; and over-amplification of abundant DNA sequences especially with nested PCR techniques. These errors may lead to overestimation or underestimation of drug efficacy [165-168]

The use of other advanced methods such as capillary electrophoresis [169], heteroduplex tracking assays (HTAs) [164, 166, 170] or microsatellite methods[171] may improve detection of minority clones and small differences in size of amplified regions compared to visualization of nested PCR products on agarose gels.

1.6 ANTIMALARIAL DRUGS USED IN THIS THESIS

1.6.1 Artemether-lumefantrine

AL is the first fixed-dose ACT recommended and prequalified by the WHO. Artemether is derived from a naturally occurring substance artemisinin, extracted from the Chinese wormwood (*Artemisia annua*)[172]. Lumefantrine is a racemic fluorine derivative and belongs to the aryl-amino alcohol family, is structurally related to other antimalarial drugs (quinine, halofantrine, mefloquine). It was synthesized in the 1970s in China[173]. A fixed combination tablet (20 mg artemether/120 mg lumefantrine) was developed based on reported synergistic effects against *P. falciparum in vitro*[174]. The fixed combination therapy also improves compliance and has the advantage of carrying a lower risk of selecting drug resistant strains. Both compounds are effective as single agents [175].

Artemether generates reactive metabolites as a result of the interaction of its peroxide bridge and heam iron in the food vacuole of the malaria parasite, free radicals oxidize and alkylate proteins and lipids hence interfering with nucleic and protein syntheses within the parasite [176]. Also artemisinin and its derivatives abolish activity of PfATP6, a *P. falciparum* SERCA-type calcium-dependent ATPase in the endoplasmic reticulum [177]. The site of action of lumefantrine is also thought to be in the food vacuole of the parasite, it interferes with detoxification of heam to haemozoin. Also like artemether it is thought to interfere with the nucleic acid and protein synthesis within the malaria parasite[178].

The pharmacokinetic properties of artemether include, peak plasma concentrations which occur around 2–3 hours after oral administration and an elimination half-life of approximately 2 hours[179]. For lumefantrine absorption starts after 2 hours with peak plasma concentrations around 6-8 hours after intake. The oral bioavailability is variable and enhanced by intake of fatty foods and has a terminal elimination half-life of 4-6 days in patients with *P. falciparum* malaria [113, 179]. The day 7 level of plasma lumefantrine was found to be the main determinant of the efficacy of artemether-lumefantrine [113, 114, 153].

1.6.1.1 Clinical experience with AL

AL has been investigated in clinical trials for the treatment of *P. falciparum* malaria in more than 5000 patients (including more than 2000 patients aged <5 years) to date. Clinical experience with AL shows high efficacy, effectiveness and indicates a low frequency of adverse events after one treatment episode [180-184].

1.6.1.2 Dosing of AL

Most clinical studies conducted during the development of AL were using a 4-dose regimen [185, 186]. However, in Thailand, an area with multidrug-resistant malaria, cure rates with the 4-dose regimen showed to be suboptimal. Therefore, a 6-dose regimen given over 3 days was developed and found to be superior to the 4-dose regimen[187, 188]. No clinically relevant tolerability differences have been observed between the different dose-regimens tested so far. The registration of AL as a 6-dose regimen in Africa was approved mostly based on the data from Thai studies and a multi-center study in Africa [189]. The 6-dose regimen of AL is administered over

three days, i.e. at 0, 8, 24, 36, 48 and 60 hours after initiation of treatment, with children weighing 5-14 kg receiving 1 tablet/dose, 15-24 kg 2 tablets/ dose and 25-34 kg 3 tablets and adults >34 kg 4 tablets/dose. To improve adherence with treatment in infancy and children, Novartis in collaboration with MMV have developed a sweetened and cherry-flavored dispersible tablets. This formulation has been found to be highly efficacious (non-inferior to crushed AL) for treatment of children with uncomplicated malaria [190].

1.6.1.1 Tolerance/resistance to AL

AL treatment failure has been associated with *Pfmdr1* amplification in Asia[153] and in Africa with selection of specific alleles of genes documented or predicted to be associated to parasite drug tolerance/resistance (the N86Y, N184F and D1246Y SNPs in *Pfmdr1*)[125, 191, 192], (b) K76T in *Pfcrt* [126] and I876V in the *P. falciparum* multidrug resistance protein 1 gene (*Pfmrp1*) [193].

1.6.2 Sulphadoxine-pyrimethamine

SP is a synergistic combination of antifolate drugs; each tablet contains 500 mg sulphadoxine and 25 mg pyrimethamine. It is a blood schizonticidal drug. It is rapidly absorbed following oral administration, peak blood concentration occur after 4-6 hours after an oral dose and terminal half-life of sulphadoxine is 4-9 days and that of pyrimethamine is about 4 days[25]. It was indicated for treatment of uncomplicated malaria in Tanzania in 2001[28], now it is only used for IPTp.

1.6.2.1 Mechanism of SP action

Sulphadoxine inhibit the enzyme dihydropteroate synthase (DHPS), the enzyme responsible for the incorporation of p-aminobenzoic acid in the synthesis of folic acid and pyrimethamine antimalarial activity is by inhibiting plasmodial dihydrofolate reductase (DHFR), thus blocking the synthesis of nucleic acids in the malaria parasite[25, 194]. Resistance to SP developed rapidly in East-Africa and other parts of the world [195, 196]. Resistance to this drug and other antifolates is a result of accumulation of genetic mutations in *Pfdhfr* and *Pfdhps* [197-199].

1.6.2.2 Adverse events and dosing

Adverse events associated with SP treatment include skin reactions and in some cases in the severe form of Steven-Johnson Syndrome (erythma multiforme or toxic epidermal necrosis) [28, 200]. However, the latter is quite a rare event but can be fatal especially in patients with HIV/AIDS [201, 202]. Very rarely bone marrow suppression and hemolysis may occur in patients with G6PD-defieciency. SP is given as a single dose of 25mg/kg of the sulfa component[28]. The following simple schedule was recommended 5-7 kg, ¼ tablet; 7-11, ½ tablet; 11-19 kg, 1 tablet; 19-30kg, 1 ½ tablet; 30-45kg, 2 tablets; and over 45kg, 3 tablets[28].

1.7 RATIONALE FOR THE THESIS

1.7.1 Improving diagnosis

Confirmation of diagnosis, using either microscopy or RDTs is critical in the era of ACTs [1]. This strategy could improve rational use of antimalarial drugs and improve management for non-malaria cases. Also appropriate diagnosis may increase the useful therapeutic life of ACTs by decreasing the exposure of parasite populations to antimalarial drugs. However, quality of malaria microscopy in many PHC facilities in Tanzania is often of suboptimal standard (see section 1.4.3). This thesis addressed this issue by developing an intervention package (training in clinical algorithms and microscopy diagnosis of malaria) and evaluated its impact on prescription of antimalarial drugs and health outcome.

1.7.2 Assessment of efficacy, effectiveness and safety of AL

AL is the most widely adopted and deployed ACT as first-line treatment in Africa. However, several factors affect how efficacious the combination is, including potential parasite tolerance/resistance and the complex treatment regimen (efficacy/effectiveness is enhanced by 6-dose regimen). There is limited data on its efficacy, effectiveness and safety after repeated treatment during extended follow-up (> 28 days) at the health facility level.

Home management of malaria (HMM) strategy is an integral part of malaria case management strategy which aims to improve access to treatment for malaria in areas with limited access to health facilities[203]. However, there is limited data on effectiveness of AL when used at the community level.

2 AIMS OF THE THESIS

2.1 GENERAL AIMS

- To improve the quality of malaria case management in government health facilities and to assess the efficacy, effectiveness and safety of AL in Tanzania.

2.2 SPECIFIC OBJECTIVES

- To develop and institute an intervention package of malaria diagnosis, which reflects ministry of health guidelines for malaria case management with and without use of malaria microscopy.

- To assess the impact of training in microscopy-based versus clinical-based malaria diagnosis on antimalarial drug prescription and health outcome in childhood malaria at PHC level.

- To assess the need for blood sampling on consecutive days, both at study inclusion and reappearance of parasites during follow-up in antimalarial drug trials, to improve PCR correction of cure rates.

- To assess the efficacy and effectiveness of a 3-day treatment course with supervised and unsupervised intake of AL in children below 5 years of age with acute uncomplicated malaria, during extended follow-up periods.

- To assess the effectiveness of AL for the treatment of uncomplicated malaria in underfive children, when provided by community health workers (CHWs) and administered unsupervised by caregivers at home.

3 MATERIALS AND METHODS

3.1 STUDY SITES AND POPULATION

All four studies were conducted in Kibaha and/or Bagamoyo districts in the Coast Region, Mainland Tanzania. Kibaha District has an estimated population of about 132,045 and Bagamoyo 230,164, about 20% of the population in the districts is under the age of five (Tanzania National Census 2002). These districts are very similar in climate, environment, culture and economic activities. A majority of population are subsistence farmers. *P. falciparum* is the predominant malaria species and *Anopheles gambiae* complex and *Anopheles funestus* are the main vectors of malaria. Malaria transmission is high and occurs throughout the year with some accentuation during the rainy season (May-July) and to a lesser extent between December-January.

Study I was conducted at16 PHC facilities (Fukayosi, Kiwangwa, Yombo, Mbwewe, Msata, Lugoba, Matuli, Ubena estate and Chalinze villages) in Bagomoyo District and (Mbwawa, Mlandizi, Kikongo, Ngeta and Mwanabwito villages) in Kibaha District. Studies II and III were conducted at PCH facilities in Bagamoyo District: study II in Fukayosi village; and study III in Fukayosi and Yombo villages. Study IV was conducted at community level in Ngeta and Mwanabwito villages in Kibaha District. Studies I and II were conducted between 2003 and 2004, when SP and AQ were the first and second-line treatment for uncomplicated malaria, respectively. Studies III and IV were conducted between March 2007and April 2008; AL was the first line treatment for uncomplicated malaria by this time.

The study populations consisted of children with fever or uncomplicated *P. falciparum* malaria attending the health facilities or homes of CHWs.

3.2 STUDY I: IMPROVING MALARIA DIAGNOSIS AT PHC FACILITIES

The target of the intervention was on health workers at PHC facilities attending underfive children with fever in rural government health facilities. The health workers at facilities included assistant medical officers (AMO), clinical officers (CO), assistant clinical officers (ACO), mother and child health aides (MCHA), trained nurses, nurse auxiliaries and laboratory assistants.

The study was designed as a cluster randomized intervention trial. Health facilities were randomly allocated to either training of health staff in clinical algorithm plus microscopy (Arm-I, n = 5) or clinical algorithm only (Arm-II, n = 5) or no training (Arm-III, n = 6). Different teaching methods were applied to implement the intervention package, which included presentations of theoretical information, brain storming and group discussion. Clinical demonstrations were provided by the research team illustrating how to take history, physical examination, diagnosis, prescribing and counseling. Also there were clinical practice sessions by the health care workers assessing patients under supervision with individual feedback and practical sessions on malaria microscopy, which included preparation of thick blood smears, slide reading, parasite counting, and artifact identification. Health workers in Arms-I and II facilities received 5-day group training on malaria case management using clinical algorithms and the health workers in Arm-I received additional 5-day group training on malaria microscopy.

The impact of intervention was evaluated in management of fever among underfive children attending outpatient clinics in the selected facilities. Data were obtained from interviews with mothers/guardians of children, physical examination, blood sampling to check for malaria parasites and hemoglobin levels. The costs of drugs prescribed and actually taken were also calculated.

Primary endpoint: Proportion of study children receiving prescriptions of antimalarial drugs in the respective arms.

3.3 STUDIES II-IV: CLINICAL ANTIMALARIAL DRUG TRIALS

3.4 GENERAL METHODOLOGIES

Studies II and III were comparative clinical trials, patients were randomly allocated to treatment groups, whereas study IV was a single arm effectiveness study. Treatments were given orally in standard doses according to body weight either under supervision or unsupervised. Follow-up visits were extended up to day 42 in studies II and IV and to day 56 in study III after initial treatment. Clinical and laboratory evaluations for studies II and III (supervised group) were done on days 0, 1, 2, 3, 7 then once weekly up to day 42 or 56. For the unsupervised group in study III, patients were not required to come back on days 1, 2 and 3; other follow-up visits were similar to the supervised

group. For example, the standard follow-up schedule for study III during initial treatment is outlined in table 1. In study IV, treatments were only given to patients with positive RDT results. Only patients with confirmed parasitaemia by microscopy were followed-up once weekly up to day 42. In all trials, patients were encouraged to come back to the health facilities on any other day if they felt ill. In study III, patients with recurrent infections from day 14 to 56 were retreated with AL and remained in the same group; after that they were followed up for 42 days.

Giemsa-stained thick blood smears were collected and examined by experienced microscopists. Parasitaemia were quantified (parasites/μL) by a standard method (40 X number of parasites per 200 leucocytes on the thick film). Quality control was done according to WHO criteria[136]. For PCR analysis, two to three drops of blood were collected on filter paper (Whatman 3MM) on day 0 and each time blood smears were collected according to the protocol on and after day 7. In study III and IV, blood samples (100μl) were collected on day 7, for determining blood lumefantrine concentrations. For patients in study III (the supervised group), blood samples for full blood picture, liver and renal function tests were collected on days 0 and 7, samples were processed and analysed in the local laboratory at Bagamoyo District Hospital Research Unit.

The primary endpoints were PCR corrected cure rates on day 42 or 56. In studies II and III, treatment outcomes were also classified according to WHO standard protocol[136].

Table 1 Schedule of follow-up activities for initial treatment (Study III)

Visit Number	**1**			**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Any other**
Day of visit	**0**	**1** [A]	**2** [A]	**3** [A]	**7**	**14**	**21**	**28**	**35**	**42**	**49**	**56**	
Inclusion/exclusion	+												
Informed consent	+												
Medical history	+												
Physical examination	+	+	+	+	+	+	+	+	+	+	+	+	+
Body temperature and vital signs	+	+	+	+	+	+	+	+	+	+	+	+	+
Adverse events	+	+	+	+	+	+	+	+	+	+	+	+	+
Treatment	+	+	+										
Malaria (Thick film)	+	+	+	+	+	+	+	+	+	+	+	+	+
PCR filter paper	+				+ [C]	+ [C]	+ [C]	+ [C]	+ [C]	+ [C]	+ [C]	+ [C]	+
Full blood picture and haemoglobin [B]	+				+								
Serum biochemistry [B]	+				+								
Plasma lumefantrine concentration					+								

A = for supervised group only
B= If any results will be of clinical concern (clinically relevant deviations of laboratory test results), a repeat sample will be collected at the next scheduled visit.
C= will be done only when blood smears for malaria is positive.

3.4.1 Molecular analysis

DNA extraction

Each filter paper was dried and individually put in a plastic bag and stored at room temperature. All filter papers were subsequently transferred to the Karolinska Institutet, Stockholm, where genotyping was performed. DNA extraction was conducted using ABI Prism 6100 Nucleic Acid PrepStation^{TM} (Applied Biosystems, Fresno, CA, USA) as previously described [204]. Extracted DNA was frozen in aliquots at -20ºC until amplification by PCR.

PCR genotyping

Distinction between recrudescences and reinfections events were performed by the use of stepwise standard nested PCR protocols for *msp-2*, *msp-1*, and *glurp* [159, 205]. Recrudescent infections were defined as samples containing at least one matching allelic band in all markers, when compared with baseline (sample collected prior to treatment). A reinfection was defined as the absence of any matching allelic band at baseline and on the day of recurrent parasitaemia in at least one of the markers. Patients who had recurrent parasitaemia and no blood sample or negative PCR results were considered to have an uncertain PCR-corrected outcome. In study II only two markers were used (*msp-2 and msp-1*).

SNPs in *Pfcrt* and *Pfmdr1* previously associated with quinoline antimalarial drug resistance were analysed according to established ApoI restriction enzyme-based PCR-RFLP protocols [125]. The presence of *Pfmdr1* gene amplification was scrutinized through established Quantitative PCR (Q-PCR) protocols based TaqMan® (Applied Biosystems, Fresno, CA) fluorescent probes [152].

3.4.2 Determination of lumefantrine blood concentration

In studies III and IV, capillary blood samples (100 μL) were applied on filter papers pre-treated with 0.75 M tartaric acid. The samples were dried, zipped in small plastic bags and stored at -20°C the day after sampling before transportation to the Bioanalytics and Pharmacokinetics laboratory of Dalarna University, Sweden. Lumefantrine whole-blood concentrations were measured by solid-phase extraction and liquid chromatography[206].

3.4.3 Ethics

The studies included in this thesis were conducted in accordance with the Declaration of Helsinki and Good Clinical Practices guidelines set up by the International Conference on Harmonization[207, 208]. Before enrollment, the respective study objectives were appropriately explained to the participants, in the local language (Swahili), and written informed consent was thereafter obtained from the parents or legal guardians of the children. The study protocols were approved by two ethics committees: the National Institute for Medical Research in Tanzania and the Karolinska Institutet/Regional Ethics Committee (Stockholm, Sweden).

4 RESULTS

4.1 STUDY I: IMPROVING MALARIA DIAGNOSIS AT PHC LEVEL

This study assessed the impact of microscopy versus clinical algorithm on antimalarial prescriptions to febrile underfive children at 16 health facilities in Kibaha and Bagamoyo Districts.

Between July 2003 and March 2004, a total of 973, 1,058 and 1,100 children were enrolled in Arms-I (clinical algorithm+ microscopy), II (clinical algorithm only) and III (no training), respectively. Antimalarial prescriptions were significantly reduced in Arm-I (61.3%) compared to Arms-II (95.3%) and III (99.5%) (both P< 0.001), whereas antibiotic prescriptions did not vary significantly between the arms (49.9%, 54.8% and 34.2%, respectively). In Arm-I, 99.1% of children with positive blood smear readings received antimalarial prescriptions and so did 11.3% of children with negative readings. Patients with positive slide readings were less likely to be prescribed antibiotics than those with negative results (Relative Risk (RR) 0.66, 95% CI: 0.55, 0.72). On day 7 follow-up, more symptoms were reported in Arm-I compared to Arm-III, but fewer children had malaria parasitaemia (p=0.049). The overall diagnostic accuracy of microscopy did not improve: sensitivity of microscopy readings at PHC compared to reference level was 74.5% and the specificity was 59.0% but both varied widely between PHCs[209], also according to parasite densities (table 2)

Table 2. Sensitivity of microscopy by parasite density in Arm-I

Parasite density levels per μL blood	No of patients	Sensitivity (%)
< 1000	63	49.2 (CI: 41.3, 57.1)
1,000 – 4,999	119	66.4 (CI: 57.9, 74.9)
5,000 – 100,000	153	87.6 (CI: 82.4, 92.8)
> 100,000	26	96.2 (CI: 93.1, 99.2)
Total	361*	74.5 (CI: 67.6, 81.4)

CI = (95% confidence interval). *934 blood smears were reviewed at reference level, 361 positive blood smears

4.2 STUDY II: INFLUENCE OF CONSECUTIVE DAY BLOOD SAMPLING ON PCR-CORRECTED CURE RATES

This was an open-label explorative randomized study in children with uncomplicated *P. falciparum* malaria (AL n=50 and SP n=56). It was designed to assess the influence of consecutive day blood sampling both at study inclusion and parasite recurrence on PCR corrected cure rates. The primary endpoint was PCR corrected parasitological cure rates of the respective treatment arms using standard WHO protocol (single-day sampling, 1+1 day) or elaborated protocol(consecutive 4+2 days) for PCR genotyping at study inclusion and time of recurrent infection.

The 42-day PCR corrected parasitological cure rates were 98% or 94% in the AL group versus 70% or 66% in SP group (depending on whether the standard or elaborated protocol was used). In this area of high malaria transmission, a majority of patients experienced recurrent infections during follow-up: 38/50 (76%) in AL and 40/53 (75%) in SP group. Overall, PCR genotyping with *msp-2* from samples collected on day1-3 identified 32 additional parasite genotypes in 26/106 patients compared with day 0 samples. Most genotypes (21/32, [66%]) were detected on day 1. Initial *msp-2* genotyping identified 27 and 33 recrudescences using either standard or elaborated sampling. Additional *msp-1* genotyping confirmed 17 and 21 infections, respectively, to be recrudescences. The sensitivity of the standard PCR protocol was 27/33=82% [95% CI 68-96], and 17/21=81% [95% CI 63-99], in both genotyping steps. The elaborated protocol also improved ability to assess PCR corrected outcome in samples with non-conclusive results from paired blood sampling.

4.3 STUDY III: EFFICACY, EFFECTIVENESS AND SAFETY OF AL AFTER INITIAL AND RETREATMENT

This was a randomized trial that aimed to assess the efficacy, effectiveness, and safety of AL in children with uncomplicated *P. falciparum* malaria, during extended follow-up periods of 56 and 42 days after initial and retreatment, respectively.

A standard 3-day treatment of AL was given either supervised (all doses observed, n=180) or unsupervised (all doses unobserved outpatient treatment with nutritional advice, n=179). Patients with recurrent infections from day 14 to 56 were retreated within the same study group (supervised or unsupervised). The two primary endpoints were PCR-corrected parasitological cure rates by day 56 after initial treatment and by day 42 after retreatment, as estimated by Kaplan-Meier survival analysis.

After initial treatment, the PCR-corrected ACPR rates by day 56 were high and similar between the supervised and unsupervised groups: (98.1 % [95% CI 94.2-99.4%] versus 95.1% [95% CI 90.7-98.1%], p=0.29). The PCR corrected ACPR rates for the two groups were also comparable at day 14, 28 and 42. Some 77 supervised and 84 unsupervised patients were retreated with AL; PCR ACPR rates at day 42 were 92.9% (95% CI 81.8-97.3%) and 97.6% (95% CI 89.3-98.8%), respectively, p=0.58.

The risks of recurrent parasitaemia were high and similar between the two groups after both initial and retreatment: after initial treatment 46.9% (82 of 175) versus 50.9 % (86 of 169) (RR 0.92 [95% CI 0.74-1.14], p=0.46); and after retreatment 32.4% (24 of 74) versus 39.0% (32 of 82) (RR 0.83 [95% CI 0.54-1.27], p=0.39). The median blood lumefantrine concentrations on day 7 were significantly higher in the supervised compared with the unsupervised group; after initial treatment (304 versus 194 ng/mL; p<001) and after retreatment (253 versus 164 ng/mL; p=001). However, the lumefantrine concentrations did not differ according to outcomes (figure 3a & b). After initial treatment, all patients with recrudescent infections had lumefantrine concentrations below 500 ng/mL, whereas after retreatment 3/5 (60%) of patients with recrudescences had concentrations over 600 ng/mL.

Parasite and fever clearance were rapid after both initial and retreatment (only assessed in the supervised groups). Repeated treatment with AL was safe and well tolerated. The

proportion of patients with adverse events during follow-up periods was significantly higher after initial treatment compared to the retreatment (235 of 355, 66.2% versus 56 of 161, 34.8%; RR 1.90 [95% CI 1.52-2.38], p=0<0001). However, most adverse were mild or moderate with symptoms and signs related to malaria and other concomitant infections. All severe adverse events were severe malaria occurring in 23 patients, (10 supervised and five unsupervised after initial treatment and in four patients each in the supervised and the unsupervised groups after retreatment). Vomiting was the most commonly reported drug resulted adverse event which occurred in about 1% of patients after initial and retreatment.

There was a significant selection of parasites carrying the *Pfmdr1* N86 allele (in pure form or mixed with 86Y) from a baseline prevalence of 66.9% to 80.0% and 90.3% after initial and retreatment, respectively (p<0.002). One sample with a *Pfmdr1* copy numbers of approximately 1.5 was detected among recurrent infection after retreatment.

Figure 3a: Day 7 blood lumefantrine concentration by endpoint after initial treatment with AL

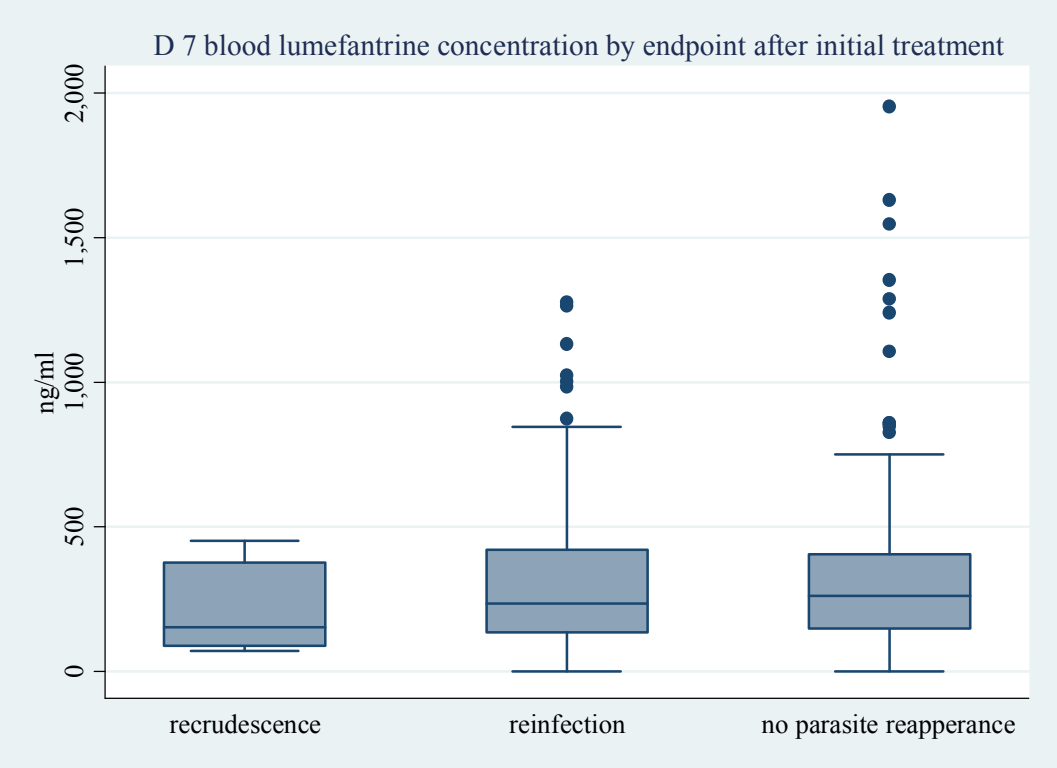
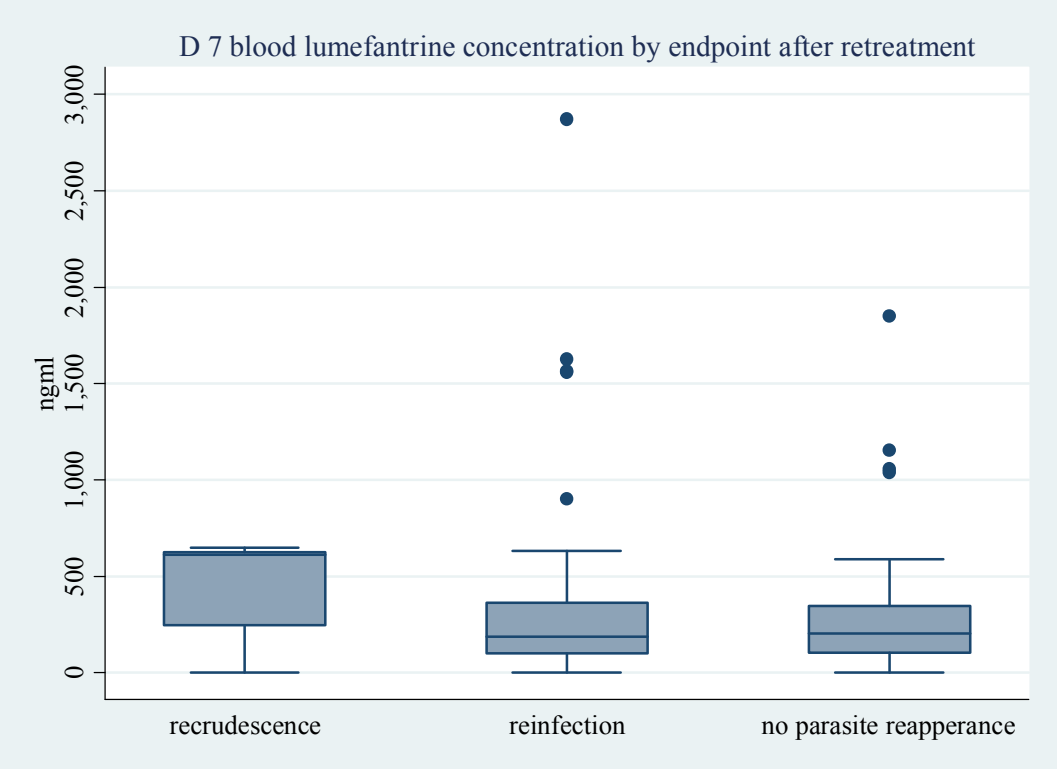


Figure 3b: Day 7 blood lumefantrine concentration by endpoint after retreatment with AL

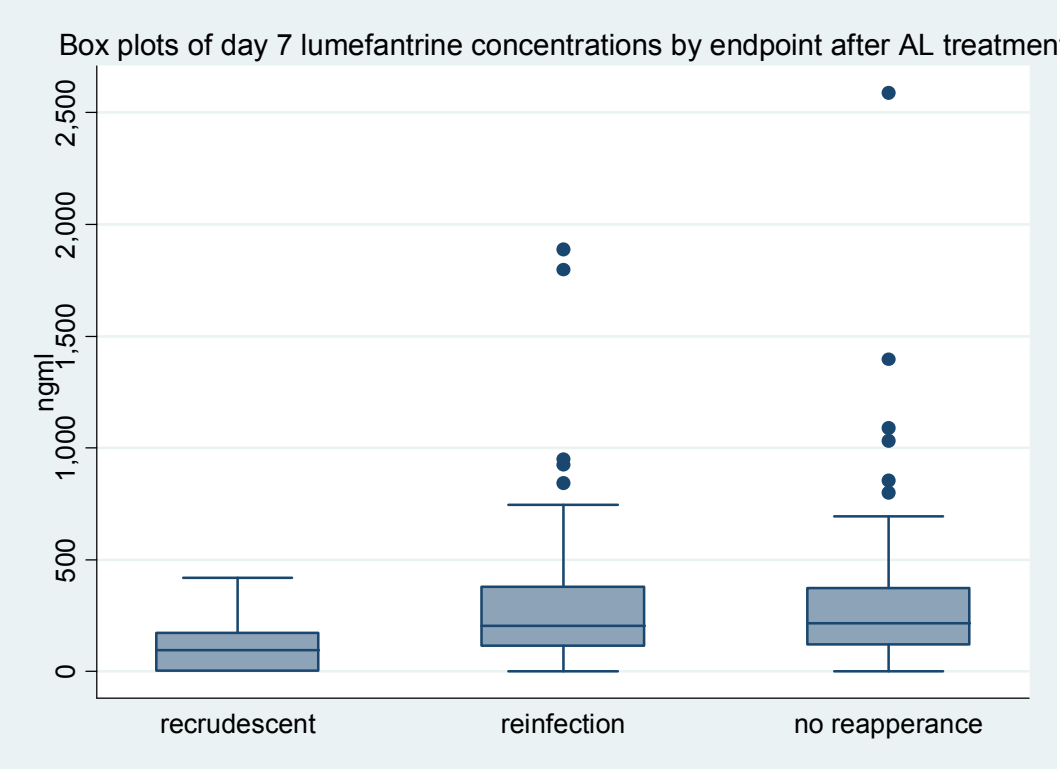


4.4 STUDY IV: EFFECTIVENESS OF AL IN HMM STRATEGY

This was a non-comparative study designed to assess the effectiveness of AL in 244 children below five years of age with uncomplicated *P. falciparum* malaria at Ngeta and Mwanabwito villages in Kibaha District. The first AL dose was administered supervised by CHWs followed by home treatment with nutritional advice. The primary endpoint was PCR corrected cure rate by day 42.

No episode of ETF was reported, but seven patients seen on day 7 were still parasitaemic. The PCR corrected cure rate by day 42 was 93.0 % (95% CI 88.3-95.9%). The median lumefantrine concentration was significantly lower in patients with recrudescences (97 ng/mL [IQR 0-234]; n=10) compared with reinfection (205 ng/mL [114-390]; n=92), or no parasite reappearance (217 [121-374] ng/mL; n=70; p≤0.046), (figure 4). Similar to study III, the risk of recurrent infections was high during follow-up. Some 141/241 (58.5%) patients had recurrent infections during follow-up, PCR genotyping confirmed majority of infections to be reinfections. There was a significant selection of parasites carrying the *Pfmdr1* N86 allele from73.1% at baseline to 93.5% after AL treatment, (p<0.001).

Figure 4: Day 7 lumefantrine concentrations by endpoint after treatment with AL



5 DISCUSSION

5.1 IMPROVED MALARIA DIAGNOSIS

Although there have been several interventions aimed at improving rational use of antimicrobials in developing countries, few studies have focused on rational use of antimalarial drugs[210, 211]. Improved targeting of antimalarial treatments has become critical in the era of ACTs, because ACTs are more expensive, limited in supply and potentially more toxic than previously used monotherapies. There is more evidence on changes in epidemiology of malaria especially in sub-Saharan African countries which scaled up malaria control interventions[1, 82-85], and on decline of prevalence of malaria parasitaemia among children aged two to ten years in sub-Saharan Africa[86]. Improved targeting of antimalarial treatment will reduce drug pressure; improve adherence and management of non-malaria febrile illnesses [25-27, 47]. Recently WHO recommended that parasite based diagnosis, with either microscopy or RDTs, should be used for all patients including underfive children in high transmission areas [25, 27].

In study I, we have shown that the use of microscopy based diagnosis at PHC facilities in Tanzania significantly reduces prescriptions of antimalarial drugs compared to clinical algorithms. Health workers adhered to tests results, prescribed antimalarial drugs to most patients with positive blood smear results and few patients with negative results. Our results are similar to the findings from a recent study in Uganda, which showed in-service training of health workers in malaria microscopy had impact on malaria case management [212]. However, our findings are in contrast to previous studies which showed the practice of prescribing antimalarial drugs to patients with negative results was common[53, 55, 74, 75, 213].

Also use of microscopy improved management of patients with other febrile illnesses, as health workers were more likely to prescribe antibiotics to patients with negative malaria tests results

Training in clinical algorithm did not improve rational use of antimalarial drugs; as almost all febrile patients in the clinical algorithm arm and control arm were prescribed

antimalarial drugs. This finding confirms the results from previous studies, which have shown limited usefulness of clinical algorithms in malaria diagnosis [45].

In this study diagnosis accuracy of microscopy was low because of several reasons: a majority of health workers had no formal training in microscopy, only two health workers had attended a one year course in microscopy, short period of training, and increased workload to clinical staff in PHC facilities which had no laboratory staff.

In summary, although the quality of microscopy was suboptimal, microscopy-based diagnosis and active follow of patients improved quality of malaria case management, as few patients were parasitaemic in the intervention arm compared to the control arm.

5.2 INFLUENCE OF CONSECUTIVE DAY BLOOD SAMPLING ON PCR CORRECTED CURE RATES

In study II, we have shown that single blood sample may not provide a complete picture of all parasite populations present in children with uncomplicated *P. falciparum* malaria in a high transmission area. Failure to detect all parasite sub-populations in a single blood sample can be due to parasite population dynamics or that some sub-populations were present at levels below the detection limit of PCR at a given time [158, 214]. Consecutive day blood sampling improved detection of minority populations; and most additional genotypes were detected on day 1. The use of microsatellites, and other advanced methods such as heteroduplex tracking assays may improve detection of minority populations [164, 166, 170, 171, 215]. However, these methods also have limitations and require more expensive equipment. More research is needed before WHO can recommend collection of samples on consecutive days[159].

5.3 EFICACY, EFFECTIVENESS AND SAFETY OF AL

In studies II, III and IV we have consistently shown that AL is highly effective in children with uncomplicated malaria. Our findings support previous findings from studies conducted by the manufacturer and independent researchers that AL is highly efficacious (cure rates of 90% to 95%)[175, 180, 182-184]. Our findings also confirm previous findings in East Africa [216-218] and in other parts of Africa [219, 220], which have shown AL to be highly effective even when administered unsupervised at home.

In study III, we have shown that AL was also highly efficacious in retreatment of recurrent infections, similar to findings from longitudinal studies in Africa [221-223]. This is a very important finding for policy makers who consider using AL or other ACTs as second line treatment instead of quinine, which is associated with poor treatment outcomes because of adherence problems and adverse events[216].

AL was also safe and well tolerated after repeated use in children with malaria. This finding supports three longitudinal studies, which assessed safety of repeated administrations of AL [222-224].

The findings in study IV are consistent with the findings of multi-center study sponsored by WHO/UNICEF, which showed that AL was highly effective when used in the HMM strategy [225]. These findings support scaling-up use of AL in HMM strategy. Recent findings show early access to ACTs at community level significantly reduces malaria related mortality and morbidity[226].

However, unsupervised treatment in studies III and IV were associated with lower blood lumefantrine concentrations compared to supervised treatment, similar to previous studies [217, 220, 227]. Low lumefantrine concentrations may be due to inadequate intake of fatty food and poor adherence in some patients. Fatty food intake significantly enhances bioavailability of both lumefantrine and artemether [113, 114, 228]. A recent review by Premji *et al* has shown that breast milk and standard African food contain adequate amount of fat for optimal efficacy of AL [229]. However, inadequate intake of fatty food with unsupervised AL treatment has been recently reported in a rural community in Tanzania[230]. Supervised intake did not guarantee adequate lumefantrine concentrations on day 7, similar to findings from previous studies in Uganda [217, 227]. A recent pharmacokinetic study has compared lumefantrine concentrations in children with *P. falciparum* malaria to previously published data for adults and showed that the level of exposure to lumefantrine is lower in children than in adults[231]. In study IV, we have shown that low lumefantrine concentrations were associated with recrudescence, but in study III this association was not very clear especially after retreatment, similar to findings from a recent effectiveness study in Benin [220].

In studies II, III and IV we have shown that the post-treatment prophylactic effect of AL in a high endemic area was limited. A longer post-treatment prophylactic effect provides time for hematological recovery and therefore reduces anaemia in patients. After AL treatment most patients had recurrent infections mostly due to reinfections as confirmed by PCR analysis. In high transmission areas in sub-Saharan Africa, high rates of reinfections are expected. Similar high risk of recurrent infections after AL treatment has been reported in other high transmission areas in sub-Africa [165, 232-234]. In this ACT, lumefantrine, the slowly eliminated partner drug has a shorter terminal elimination half life of 3-6 days compared to other partner drugs in other effective ACTs like piperaquine and mefloquine[235]. This can explain why comparative clinical trials have shown DHA+PPQ exerts a greater post-treatment prophylactic effect against recurrent infection than AL [233, 234, 236, 237]. However, a recent longitudinal comparative study of AL and DHA+PPQ in a high transmission area in Uganda has shown that there was no significant difference in the mean number of malaria episodes between patients randomized to AL or DHA+PPQ treatment [223]. Moreover, AL has shown longer post-treatment prophylactic effect compared to AS+AQ [218, 232, 238], these findings may be due to cross resistance of AQ with CQ.

Low lumefantrine concentrations may enhance selective transmission of tolerant/resistant genotypes especially in high transmission areas [127, 128]. In studies III and IV we have shown significant selection of *Pfmdr1* 86N associated with AL tolerance/resistance. There was also high prevalence of *Pfcrt* 76K before and after treatment with AL. These findings support previous *in vivo* and *in vitro* studies in Africa [124-126, 150, 191, 192, 239]. These findings suggest the urgent need for close future monitoring of emergency of resistance to ACTs in Africa.

6 CONCLUSIONS

1. Microscopy based diagnosis of malaria at PHC facilities reduces prescription of antimalarial drugs, as health workers respected malaria smear results.

2. Major variations were found in accuracy of microscopy, lack of qualified technicians and short in-service training period may have contributed.

3. PCR analysis of consecutive day blood samples both at enrollment and recurrent infections showed an increase in the number of recrudescent infections by improving detection of new genotypes and interpretation of non-conclusive results obtained with standard protocol.

4. AL was a highly efficacious treatment with >90% cure rates even with extended follow-up assessments of 42 and 56 days.

5. Although the median lumefantrine concentrations on day 7 were significantly lower in the unsupervised compared to supervised group, cures rates were high for both treatment groups.

6. The post-prophylactic effect of AL was limited; a majority of patients had recurrent infections during follow-ups.

7. In study IV, low lumefantrine concentrations were associated with recrudescences; whereas in study III, lumefantrine concentrations did not differ according to treatment outcomes.

8. AL was safe and well tolerated after both initial and retreatment.

9. AL selected for *Pfmdr1 86N* genotypes among post-treatment reinfections

Overall conclusion

Training in microscopy-based diagnosis improves malaria case management and reduces overprescription of antimalarial drugs. AL is highly efficacious treatment, even with unsupervised administration and well tolerated treatment in children with uncomplicated malaria in Tanzania.

7 FUTURE

In this thesis we have shown that microscopy-based diagnosis of malaria reduces antimalarial drug prescriptions compared to clinical-based diagnosis. However, malaria microscopy requires well trained technicians, and microscopes which are well maintained. RDTs are quicker and easy to use even for CHWs, have a potential to be deployed into rural health facilities where laboratories and qualified staff are not available. But issues still remain about how to maintain quality of RDTs in the PHC facilities and HMM strategy. We also need more evidence on feasibility, acceptability and safety of the use of RDTs particularly in HMM strategy. Therefore, we plan to expand our operational research into innovative ways of improving malaria diagnosis using RDTs, prescribers compliance to national guidelines, in-service training and continue to monitor quality of malaria case management in Tanzania. We also plan to continue to conduct clinical trials in order to assess the safety and effectiveness of AL and new ACT candidates over time as well as to longitudinally monitor evolution and spread of drug-resistance malaria.

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