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Impact of laboratory diagnosis for improving the management of uncomplicated malaria at peripheral health care settings in Coast region, Tanzania

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ABSTRACT

Malaria is a disease caused by parasites of the genus *Plasmodium*. Five species cause human disease, but the most common in tropical areas, and the cause of severe disease is *Plasmodium falciparum*. Control of morbidity and mortality is mainly achieved through appropriate malaria case management, which includes prompt diagnosis and treatment with effective antimalarial drugs. While definitive diagnosis of malaria is made by demonstration of parasites in the patient blood through microscopic examination of giemsa stained blood smears, in most clinical settings in Africa, diagnosis is limited by lack of facilities and personnel. The availability of malaria rapid diagnostic tests (RDTs) offers an opportunity to extend diagnostic services to areas previously not covered by conventional microscopy services.

Two intervention trials were conducted, one at primary health care (PHC) facilities using microscopy, and the other at community level, through community health workers (CHWs), using rapid diagnostic tests (RDTs) for malaria diagnosis, and the impact of the interventions on antimalarial drugs prescription practices, antibiotic prescriptions and health outcome was investigated (Study I and II). A descriptive, cross sectional study was conducted to assess health workers diagnostic and prescription practices following introduction of RDTs for universal testing of malaria at PHC level in Tanzania (Study III). An exploratory study was also carried out to assess the usefulness of Histidine rich protein 2 (HRP2) and lactate dehydrogenase (LDH) based RDTs for treatment monitoring and detection of recurrent infection following artemisinin-based combination therapy (ACT) during a 42 day follow up period (Study IV).

The use of parasite-based diagnostics significantly reduced antimalarial prescriptions at health facility and community level without affecting the health outcome of patients not treated with antimalarials (study I and II). The prescriptions of antimalarial drugs were 61% at intervention health facilities, whereas in the clinical and control arms the prescription rates remained high, 95% and 99%, respectively (study I). Similarly, 53% of patients tested with RDT at community level were provided antimalarial drugs compared to 96% among patients treated based on clinical diagnosis only (Study II). The availability of parasite-based diagnostics and antimalarial drugs within the community allowed early access to treatment as 67% of patients consulted CHWs within 24 hours of onset of fever (Study II). The rate of non adherence to test results was low in both study I and II.

Study III observed low use of parasite-based diagnostics among fever patients (63%), low non adherence to test results (14%), substantial prescription of antimalarial drugs to non-tested patients (28%) and high prescriptions of antibiotics among patients with negative RDT results (81%), as well as frequent stock outs of both RDTs and ACTs.

HRP2 based tests performed poorly when compared to LDH based tests for treatment monitoring, with median clearance times of 28 (7-42) and 7 (2-14) days respectively (Study IV). HRP2 based tests were unable to detect 8/10 recurrent infections during follow up compared to only two recurrent infections missed by LDH based tests.

These studies lead to a conclusion that the availability of parasite-based diagnostics helps to restrict treatment with antimalarial drugs to patients with malaria. However, non adherence to malaria test results could undermine the potential of RDTs, and in-depth studies should be conducted to identify its causes. As the relative contribution of malaria as a cause of fever is declining in Tanzania, there is need to improve the overall management of non-malarial fevers. The longer persistence of HRP2 antigen in blood makes HRP2 based tests not suitable for treatment monitoring and detection of recurrent infection calling for alternative diagnostic strategies for this purpose.

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 M. G., Massele A. Y., Tomson G., Premji Z. & Björkman A (2008). Impact of training in clinical and microscopy diagnosis of malaria on antimalarial drug prescription and health outcome at primary health care level in Tanzania: a randomized controlled trial. *Malaria Journal*. **7**:199

II. **Mubi M.**, Janson A., Warsame M., Mårtensson A., Källander K., Petzold M., Ngasala B., Maganga G., Gustafsson L., Massele A., Tomson G., Premji Z. & Björkman A (2011). Malaria rapid testing by community health workers is effective and safe for targeting malaria treatment: Randomised cross-over trial in Tanzania. *PLoS ONE* **6**(7)

III. **Mubi M.**, Kakoko D., Ngasala B., Premji Z., Peterson S., Björkman A., & Mårtensson A (2013). Malaria diagnosis and treatment practices following introduction of rapid diagnostic tests in Kibaha District, Coast Region, Tanzania. *Malaria Journal.* **12**:293

IV. Aydin-Schmidt B., **Mubi M.,** Morris U., Petzold M., Ngasala B., Premji Z., Björkman A. & and Mårtensson A. (2013) Usefulness of Plasmodium falciparum-specific rapid diagnostic tests for assessment of parasite clearance and detection of recurrent infections after artemisinin-based combination therapy. *Malaria Journal* **12:**349.

CONTENTS

LIST OF ABBREVIATIONS

ACT	Artemisinin-based combination therapy
CHW	Community health worker
CQ	Chloroquine
DNA	Deoxyribonucleic acid
HRP2	Histidine rich protein 2
IPTp	Intermittent Presumptive Treatment in pregnancy
IRS	Indoor residual spraying
ITN	Insecticide treated nets
LAMP	Loop-mediated isothermal amplification
LDH	Lactate dehydrogenase
LLNs	Long lasting insecticide treated nets
MUHAS	Muhimbili University of health and Allied Sciences
NIMR	National Institute for Medical Research
ORS	Oral rehydration solution
PCR	Polymerase chain reaction
PHC	Peripheral health care
pLDH	Parasite Lactate dehydrogenase
rbc	Red blood cell
RBM	Roll Back Malaria
RDT	Rapid diagnostic test
SIDA	Swedish International Development Cooperation Agency
SAREC	Swedish Agency for Research Cooperation
SP	Sulfadoxine-pyrimethamine
SPHSS	School of Public Health and Social Sciences
QN	Quinine

1 INTRODUCTION

1.1 General introduction to malaria

The global health burden from malaria has been declining in recent years (1,2). Globally, mortality due to malaria has declined by 45% between 2000 and 2012; with an estimated 207 million cases and 627,000 deaths in 2012 (1). The majority of these deaths (91%) occur in Africa, and 86% of them in children under five years of age (3,4). The decline in the burden of malaria has been attributed to the use of artemisinin-based combination therapy (ACT), and the scale up of other malaria control measures mainly the use of long lasting insecticide treated nets (LLNs) and indoor residual spraying (IRS).

1.1.1 Malaria parasites

Malaria is a disease caused by protozoan parasites of the genus *Plasmodium,* first described by Charles Laveran in 1880 (5). There are over 120 species of plasmodia infecting the blood of mammals, reptiles and birds. Four species are the most common and important cause of disease in humans, namely *Plasmodium falciparum, P. malariae, P. ovale* and *P. vivax* (6). A fifth species, *Plasmodium knowlesi*, a parasite of monkeys is now considered a human parasite as it is naturally transmitted to humans (7,8). *Plasmodium falciparum, P. malariae, P. ovale* and *P. vivax* are widely distributed in the tropics and subtropics, while *P. knowlesi* is confined to Malaysia and Borneo in South East Asia. *Plasmodium falciparum* is common in the tropics and subtropics, and is the chief infection in endemic areas of Africa. However, mixed species infections are also common in endemic areas, with *P. falciparum* and *P. malariae* or *P. ovale* in tropical Africa and *P. falciparum* and *P. vivax* in subtropical areas.

1.1.2 Malaria transmission

Natural transmission of malaria occurs through the vector, mosquitoes of the genus *Anopheles*. The discovery that mosquitoes are involved in the transmission of malaria was made by Ronald Ross in 1897 (5).There are about 430 species of *Anopheles*, 70 of which transmit malaria and only 40 are of importance (9). The most important vector of malaria in Africa belongs to the *Anopheles gambiae sensu lato* (*s.l.*) complex (*A. gambiae sensu stricto [s.s.]), A. arabiensis, A. melas and A. merus*) and *Anopheles funestus*. *Anopheles gambiae s.s.* and *A. funestus* are more widespread, while *A. melas and A. merus* are confined to coastal areas of West and East Africa, respectively. Only

female anopheles mosquitoes are vectors of human malaria, taking a blood meal to provide for nutrients and maturation of eggs, and hence facilitating transmission from one host to another. The feeding and resting behaviours of mosquitoes determine their efficiency as vectors of malaria (9).

Transmission is also possible through blood transfusion from an infected donor, from mother to foetus through the placenta, and through accidental puncture with an infected needle.

1.1.3 Life cycle of malaria parasites

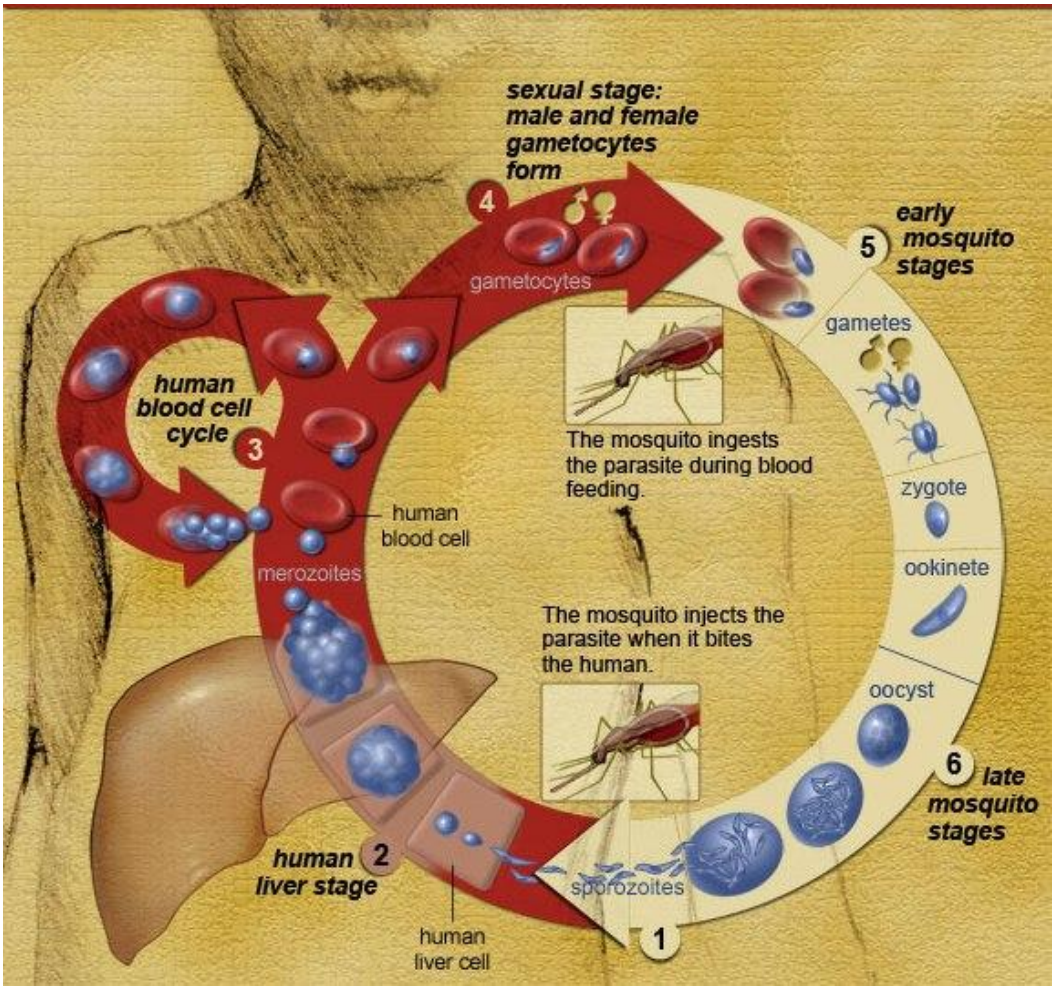
Plasmodium is a two host parasite, with female *Anopheles* mosquitoes as definitive hosts and humans as intermediate hosts (6). The life cycle of malaria parasites is described in Figure 1. Briefly, sporozoites are the infective stage to humans, inoculated through the bite of an infected female anopheles. Within an hour, they disappear from circulation and enter parenchyma cells of the liver where they divide and multiply (schizogony) to form exoerythrocytic schizonts. The liver schizont, with thousands of merozoites eventually bursts to release free individual merozoites, which are shed into peripheral blood. The duration of the exoerythrocytic cycle varies between 5 and 16 days, depending on species of parasite (6).

With *P. vivax* and *P. ovale*, some of the sporozoites which invade parenchyma cells of the liver differentiate into hypnozoites, which remain dormant for long periods and resume growth at a later time with subsequent release of merozoites into circulation and continuation of the erythrocytic cycle (6). Hypnozoites are the true cause of relapses.

Merozoites invade erythrocytes to start the erythrocytic cycle. Within the erythrocytes, the parasite is transformed into a ring form, the trophozoite, with a nucleus and cytoplasm surrounding a vacuole. The trophozoite feeds on haemoglobin and enlarges to form the erythrocytic schizont. It undergoes division (Schizogony) to form daughter parasites (merozoites). The infected erythrocyte eventually ruptures to release merozoites, which invade other erythrocytes to start the cycle all over. When infected erythrocytes rupture, in addition to merozoites, they also release residual debris, which is toxic to the host. The toxins stimulate the release of cytokines, the cause of fever. The duration of the erythocytic schizogony cycle is 48 hours in *P. falciparum, P. ovale* and *P. vivax*; 72 hours in *P. malariae and 24 hours for P. knowlesi* (6,8,10). This

duration is linked to the fever pattern observed with infection of the different species, referred to as tertian for *P. falciparum, P. ovale* and *P. vivax*, quartan for *P. malariae* and quotidian for *P. knowlesi*.

Figure 1: The life cycle of malaria parasites.



Source: http://www.niaid.nih.gov/topics/malaria/pages/lifecycle.aspx

Some of the merozoites develop into sexual forms, the male and female gametocytes, which do not divide in the human host. When ingested by a suitable female *Anopheles* mosquito with a blood meal, the male gametocyte undergoes exflagellation to produce eight flagellated gametes. The female gametocyte frees itself from the erythrocyte membrane to become a female gamete. Fertilization takes place to form a zygote, which enlarges and becomes motile, the ookinete. The ookinete penetrate an epithelial cell lining the stomach wall and starts to develop and divide to form the oocyst on the outer wall of the stomach. Divisions within the oocyst (sporogony) continue to produce slender pointed forms called Sporozoites. The oocyst eventually raptures to release

sporozoites, some of which find their way into salivary gland and hence the saliva. Sporozoites are injected into the next human host when the mosquito takes a blood meal. Development in the mosquito takes 10 to 15 days depending on species, with *P. malariae* taking the longest time (6).

The number of merozoites produced by the exoerythrocytic and erythrocytic schizonts differ between species. Morphological characteristics of the erythrocytic stages also differ, and are used in species identification through microscopic examination of thin blood smears.

1.1.4 Clinical presentation of malaria

The first physician to describe the clinical features of malaria was Hippocrates in the 5th century BC, and later on, the name 'malaria' was given by Italians due to its association with foul air in marsh areas around Rome (5). At that time, the causative agent of the disease had not been discovered.

Malaria is an acute febrile illness whose features can vary from mild to severe, also referred to as uncomplicated and severe malaria. The severity of the illness depends on the parasite species involved, the patient's immune status, the intensity of the infection, and the presence of accompanying conditions, with *P. falciparum* and *P. knowlesi* being able to cause fatal disease.

The most common feature of malaria is fever. Patients may experience flu-like symptoms before the fever sets in. The fever paroxysm starts with a high temperature, followed by chills, shivering and eventually sweating. Other symptoms are non specific and include headache, joint pain, malaise, body weakness, vomiting, diarrhoea, chest pain, poor appetite, anaemia and spleenomegally (11).

According to the WHO, a patient is considered to have severe malaria if there is *P. falciparum* asexual parasitemia and no other cause of symptoms, and the presence of one or more of the following symptoms: impaired consciousness, severe anaemia, multiple convulsions, circulatory collapse, abnormal bleeding, haemoglobinuria, jaundice, renal dysfunction, pulmonary oedema, prostration, and respiratory distress (12,13). Severe malaria, especially that due to *P. falciparum* is life threatening, and if there is delay in treatment, it can lead to serious complications and death (12,14). While

everybody is potentially at risk of infection due to malaria parasites, non immunes such as visitors to endemic areas and young children and pregnant women in endemic areas are at a higher risk of developing severe disease.

P. falciparum is the most pathogenic, causing the most severe disease and is responsible for almost all deaths attributed to malaria. This is mainly due to production of a large number of merozoites per liver schizont, ability to infect young and mature red blood cells and produce a large number of erythrocytic merozoites, destruction of infected and uninfected red blood cells by the spleen causing anaemia, the tendency to clump together around infected erythrocytes and sequestration of parasitized cells in blood vessels and adherence to internal organs such as the placenta and brain (6,15).

1.2 MALARIA DIAGNOSIS AND TREATMENT

Since malaria is an illness that may rapidly progress to severe disease and even death, early diagnosis and prompt treatment of acute malaria episodes is an important strategy to reduce morbidity and mortality (11). The first and most important role of diagnosis is in patient management. Diagnosis helps to identify the causative agent of the illness so that the patient may receive appropriate treatment. The early identification of malaria negative patients allows for investigation and appropriate treatment of other causes of fever (16). By identifying malaria cases and limiting treatment to infected patients only, diagnosis also helps in rational use of antimalarials drugs (17,18). Diagnosis also plays a role in treatment monitoring and identification of drug resistance. In some settings, it might be necessary to identify asymptomatic infections that may need to be treated. Diagnosis is also useful in surveillance, to monitor the impact of interventions (19). When used to guide patient management, a diagnostic strategy must have high sensitivity to ensure that all true cases are detected and treated, as well as high specificity to avoid false positives and unnecessary antimalarial treatment with a possibility of overlooking the true cause of the illness (16,20). A description of the different diagnostic strategies used for malaria diagnosis is presented below.

1.2.1 Clinical diagnosis

The diagnosis of malaria may be suggested by clinical features described by the patient, the most common of which is fever. In many parts of Africa where malaria is endemic, absence of laboratory services lead to treatment of malaria based on clinical symptoms,

especially the presence or history of fever. However, there are similarities in the presentation of malaria and other causes of fever such as pneumonia, flue, typhoid, etc. such that clinical features alone cannot predict a diagnosis of malaria and often lead to over-prescription with antimalarials (21). Even the WHO/UNICEF Integrated Management of Childhood Illnesses Algorithm (IMCI) developed to improve the accuracy of clinical diagnosis has been found inadequate in diagnosing malaria (22–24). Although the IMCI algorithm was aimed at identifying as many cases of potentially fatal illness as possible using simple diagnostic criteria, it had the disadvantage of leading to over-treatment with both antimalarials and antibiotics. Based on clinical symptoms and the IMCI algorithm, a large proportion of patients were diagnosed as having malaria and treated with antimalarial drugs while only a small proportion were actually parasite positive (25–27).

A review of published studies on algorithms for diagnosing malaria concluded that in areas where malaria is highly prevalent, the algorithms are highly sensitive but not specific enough to direct treatment, and they are also area specific (28). However, treatment of all febrile children in highly endemic areas with antimalarial drugs lacking laboratory support was considered appropriate, and the algorithms could also be used as a screening tool to reduce the number of patients requiring microscopy.

Malaria diagnosis requires a criteria or test that is highly sensitive and specific, to reduce the chance of missed and untreated infections which could progress to severe disease and death while also reducing over-diagnosis and over treatment with antimalarial drugs. It was therefore suggested that in order to prevent over-prescription and inappropriate treatment, and the rapid development of resistance to new first line anti-malarial drugs, clinical diagnosis should be supplemented by microscopy (28).

1.2.2 Laboratory diagnosis

Since clinical diagnosis is not specific, not being able to tell if the illness is due to malaria parasites or non malaria causes, definitive malaria diagnosis is usually achieved by demonstrating the presence of parasites in the blood of patients.

1.2.2.1 Microscopy

While the use of the microscope in biology was first reported during the 17 century (29), the first successful attempt to identify malaria parasites in blood of infected

patients was made by Charles Laveran in 1880 (CDC, 2012). Through careful observation while examining fresh unstained blood, he was able to identify malaria parasites. Further studies of the parasite were facilitated by the development of better staining methods.

The Giemsa staining method

The technique carries the name of Gustav Giemsa, who developed the stains during the late 1880s (30). The giemsa stain is a mixture of acidic and basic dyes and its principle components include Azure B, methylene blue and eosin. The stain is able to achieve differential staining of the parasite, with the nucleus staining red and cytoplasm blue. Under most circumstances, the stain is purchased as powder or stock solution. The procedure for preparation and staining of blood films is explained in detail elsewhere (31) and is briefly described here. A dilute working solution (5 or 10%) prepared from the stock solution is used for staining of blood smears and this has to be changed daily. The duration of staining depends on the concentration of the working solution. Thick and thin blood smears prepared from capillary or venous blood are air dried. The thin film is fixed prior to staining to preserve the morphology of parasites and red blood cells, an important aspect in species identification. The thick film, which uses a larger volume of blood has higher sensitivity for detecting parasitemia, while the thin film is for confirming the infecting species (18,32). Quantification of parasites can also be done using both types of smears. Microscopic examination of Giemsa stained blood smears is still used as a yardstick against which other tests are measured (30,32).

The performance of blood slide microscopy requires the availability of a good quality microscope, good quality consumables, a light source and well-trained personnel. In the hands of a well-trained and experienced technician, blood slide microscopy has a high sensitivity and specificity (32). Expert microscopy can detect as low as 5-10 parasites/µL of blood (17), while under routine conditions the detection level is 50-100 parasites/µL. It is also informative, being able to identify the infecting species, the circulating stages and detect morphological changes induced by drug treatment. In addition to that, parasite densities can be quantified and used to indicate severity or assess parasitological response to treatment. It is also relatively inexpensive with low operational costs, and the microscope can be shared with other disease control programmes such as tuberculosis, sexually transmitted infections and intestinal infections.

The disadvantages of blood slide microscopy include the fact that it is labour intensive and time consuming, taking about an hour to produce results, and its dependence on well-trained and supervised technicians and availability of electricity (33–35). Its accuracy depends on parasite density, being unable to detect very low parasitemia levels. The standard procedure uses a 5% giemsa solution and staining for 20-30 minutes. However, this may be reduced to 10 minutes by increasing the stain concentration to 10%.

Modifications to the standard giemsa staining of blood slides have been developed, to make parasite identification easy, reduce the processing time to produce results, and make it possible for people with minimal training to read the slides. These include the field staining technique, which utilizes a water based Romanovsky stain composed of two solutions and is much faster than the conventional giemsa stain. Others are the modified giemsa staining, and fluorescent techniques such as acridine orange and the Quantitative Buffy coat (QBC) techniques.

Fast thick smear (FTS)

A modified giemsa staining technique, the FTS method has been described (36). This method, which involves rapid drying, an isotonic fixative and a haemolysing solution containing saponin, was evaluated and advantages included reduced drying times (using an incubator at 37°C) and better smear readability. Parasite detection threshold was found to be 5 parasites/μL, a level comparable to that achieved with conventional thick smear examination by experienced microscopists. Although both the sensitivity and specificity of this method were found to be high, the use of saponin and the requirement for incubator or microwave for drying renders this test less likely to be used in resource poor settings in endemic areas.

Fluorescent techniques

Acridine orange (AO)

Staining of thin blood films with acridine orange and examination using fluorescent microscopes or light microscope fitted with an interference filter is among the modifications evaluated and in use in some places (37,38). The technique uses a fluorescent dye with affinity to the nucleic acid in the parasite. The method has

shown a sensitivity between 41-93%, with lower sensitivities at low parasite densities (35,39).

The advantages were the short processing times necessary to produce results (10 minutes), and that even less experienced observers were able to produce good quality results. Examination of slides is done at x600. Where fluorescent microscopes are used, cost considerations become important. Otherwise ordinary light microscopes fitted with an interference filter are adequate and cost effective.

Quantitative buffy coat (QBC) technique

Another technique is the QBC method, which is also based on acridine orange staining in which blood examination is done using a microscope fitted with a UV adapter. In this technique, blood is collected into commercially supplied capillary tubes coated with the dye, centrifuged for 5 minutes and the buffy coat examined under a microscope. A study in Tanzania found the test to have good performance, being faster in producing results (in 10 minutes) with a sensitivity of 81.3-100% and specificity of 86.4-100% (37). In a study conducted in an outpatient clinic in Kenya comparing different diagnostic techniques, a few problems were identified with this method, including the breakage of tubes during centrifugation, the difficulty in recognising parasites and the fact that quantification of parasites is not possible (40). Other structures that fluoresce (Howell Jolly bodies) were also confusing.

1.2.2.2 Molecular techniques

Molecular techniques are used to detect parasite DNA and RNA in patients' blood, using the principle of nucleic acid hybridization or amplification methods. Earlier methods used the hybridization techniques, in which DNA probes directed at repetitive sequences of parasite genes were used to detect parasites from a patients' blood on filter paper. With availability of PCR amplification, the number of copies of any target sequence may be multiplied, providing a way of studying various aspects of the parasite, such as genes responsible for antimalarial drug resistance, and for differentiating between infecting species. Several modifications of the PCR technique have been done such as automation of the thermal recycling used for amplification process, separation of the amplified material by agarose electrophoresis and other improvements to make the PCR user friendly.

The tests are highly sensitive and specific, with threshold of detection of <1 parasites/μL providing positive results from sub-patent infections (41,42). They are also useful in detecting mixed infections. They are now playing an important role in studies on drug resistance mutations (43), in clinical trials to assess cure rates with antimalarial drugs (44,45), as well as in differentiating recurrences from reinfections (46,47). They are also aiding in studying genetic mutations such as deletions of HRP2 and HRP3 genes which could affect the performance of RDTs (48–50). They are also used for quality control of microscopy and for monitoring disease control programmes to assess the impact of malaria control interventions. Due to the use of sophisticated and often expensive equipment requiring considerable expertise, and taking much longer time to produce results, at the moment they are mainly used in research laboratories and hospitals in developed countries.

Recent advances in PCR technology are geared at adapting the technique to a variety of conditions, including areas lacking sophisticated equipment. One such modification is the loop-mediated isothermal amplification (LAMP) which can amplify DNA under isothermal conditions (51). The technique is sensitive, fast (taking less than 1 hour) and simple; using heat treated blood samples as a template, requiring just a water bath or a heat block for carrying out the reaction and inspection with the naked eye or turbidity meter, features that makes it suitable for use in clinical settings (52–56).

1.2.2.3 Rapid immunochromatographic tests

The use of blood slide microscopy has been limited by concerns of the time taken to make a diagnosis and the need for well-trained technicians, which makes it difficult to maintain quality in poor resource settings (17). Rapid diagnostic tests (RDTs), which are immunochromatographic, based on detection of parasite antigen in blood have addressed these concerns. There are three main antigens targeted by available RDTs, the histidine rich protein 2 (HRP2), parasite specific lactate dehydrogenase (pLDH) and *Plasmodium* aldolase (33,35).

HRP2 is a water soluble antigen present in *P. falciparum* only and is produced by the parasite throughout the erythrocytic life cycle and young gametocytes. The antigen is secreted by the parasite in the blood, and is detectable in erythrocytes and different body fluids including serum, plasma, cerebrospinal fluid and urine. Its main drawback

is its persistence in blood for a longer period after clearance of parasites following successful therapy (35).

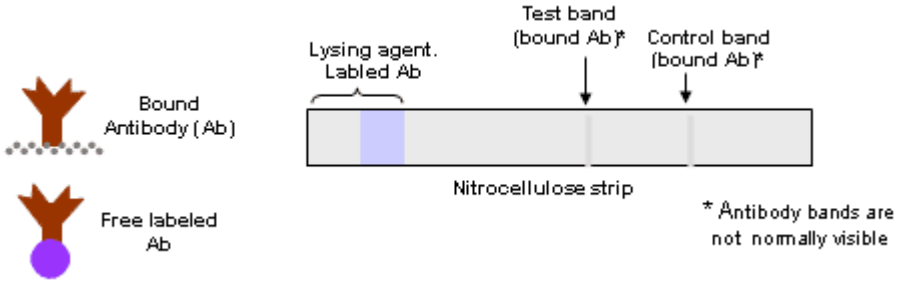
The LDH antigen is produced by all human species during their growth in the RBC. LDH is either species specific such as *Pf*LDH or *Pv*LDH or as Pan-*Plasmodium* LDH, detecting all 5 human malaria species (57). It is cleared from blood at about the same time as parasites following successful therapy. Previously, the LDH-based RDTs were less sensitive than HRP2-based tests in detection of *P. falciparum,* and were more fragile to high temperature and humidity during storage (58,59). The last generation of LDH *P. falciparum* specific tests based on monoclonal antibodies have shown equal sensitivity as the HRP2 based RDTs (60) and are less fragile to exposure to high temperature and humidity (61). The third pan-malaria antigen (PMA) targeted in RDTs is Aldolase, which is often used in combination with the *Pf*-specific HRP2.

RDTs are available in different formats such as dipsticks, cassettes or cards. Despite differences in the target antigen and the format, the tests have the same mechanism of action. They are lateral flow devices relying on detection of antigen-antibody complex migrating across a nitrocellulose membrane to produce a visible coloured line at the immobile phase (Figure 2). Tests detecting one species of parasites will have two lines, one test and one control band; while those detecting multiple species will have three lines, two test and one control band.

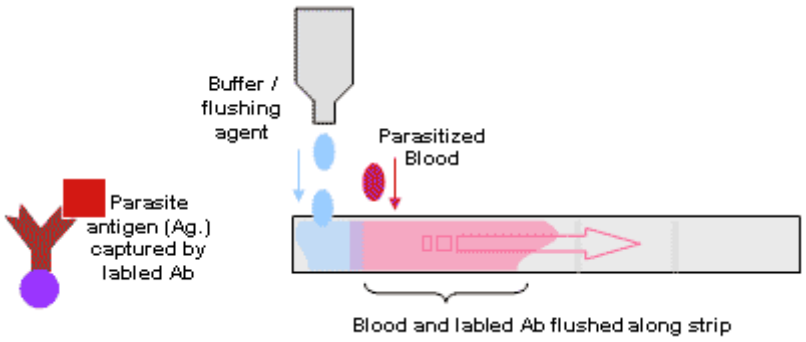
Evaluation of RDTs have been carried out in different settings, with different patient populations, with different malaria species, in environments with differing temperature and humidity, in outpatient departments in developed countries as well as rural areas with untrained personnel. They have been found to take a shorter time to produce results (5-20 minutes), and can be performed by personnel with minimal training (62–64). They also show high sensitivity and specificity and are seen as a cost effective approach to reduce over-treatment (33,58,65). They indicate presence or absence of parasite antigens, and whether the infecting species is *Plasmodium falciparum* or non-falciparum. Their ease of use, requiring neither a technician nor equipment or electricity, makes them a promising tool even in resource poor settings.

Figure 2: Mechanism of action of Rapid diagnostic tests

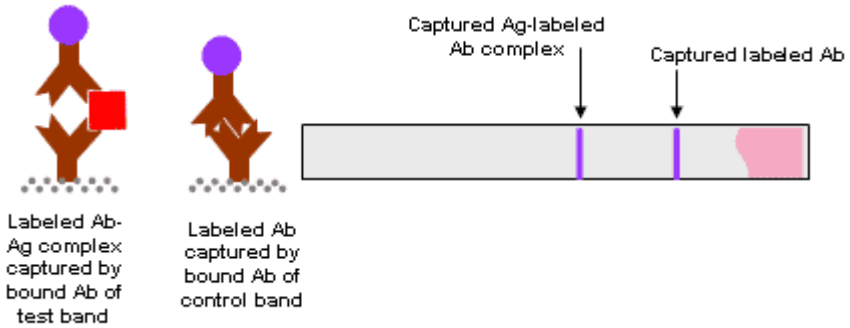
1. Dye-labeled antibody, specific for target antigen, is present on the lower end of nitrocellulose strip or in a plastic well provided with the strip. Antibody, also specific for the target antigen, is bound to the strip in a thin (test) line, and either antibody specific for the labeled antibody, or antigen, is bound at the control line



2. Blood and buffer, which have been placed on strip or in the well, are mixed with labeled antibody and are drawn up strip across the lines of bound antibody



3. If antigen is present, some labeled antibody will be trapped on the test line. Excess-labeled antibody is trapped on the control line



Source: WHO/FIND/CDC/TDR (2011) Malaria Rapid Diagnostic Test Performance Results of WHO product testing of malaria RDTs: Round 3 (2010-2011)

However, RDTs are not very informative and do not indicate parasite density. They are also less sensitive at low parasite densities (<100 parasites/µL of blood) when compared to microscopy and molecular tests. Other problems include the prozone phenomenon, deletion of the HRP2 gene and HRP2 antigen persistence. A prozone effect which is due to too much antigen has been demonstrated in some cases, leading to production of false negative results in patients with very high parasitemia (66,67). Finding of parasites with HRP2 deletions and genetic variability of the HRP2 antigen are also raising concern on their potential to produce false negative results especially in endemic Africa where *P. falciparum* is the dominant species and HRP2 based tests are used for diagnosis (48,68). Also some of the tests especially those based on detection of HRP2 remain persistently positive even after clearance of parasitemia, which has been attributed to persistence of antigens and presence of gametocytes, making these tests unsuitable for use in treatment monitoring. (62,69–71). Their performance may be affected by storage conditions such as temperature and humidity (72). Despite these problems, RDTs still provide a better chance of extending diagnostic services to areas where microscopy is not available (19).

Previously, RDTs were relatively more expensive, and they were not considered cost effective for widespread use during the era of chloroquine and sulfadoxine-pyrimethamine (17,35,73). However, costs of RDTs have gone down, expensive antimalarial drugs in the form of ACTs are in use, and the contribution of malaria as a cause of fever has gone down, making RDTs to be seen as cost effective tools for directing treatment.

Summary: Characteristics of malaria parasite based tests

The following table (Table 1) summarises some of the characteristics of laboratory based tests discussed.

Table 1: Performance characteristics of malaria diagnostic tests

VARIABLE	GSM	AO	PCR	LAMP	HRP2	LDH
Equipment	Simple	Simple	Sophisticated	Moderate	None	None
Expertise	Moderate	Moderate	High	Moderate	Low	Low
Cost	Low	Moderate	high	Moderate	Moderate	Moderate
Lab requirement	Yes	Yes	Yes, hi tech	Yes	No	No
Detection level	50-100/µL	100p/µL	<1-5p/µL	<1-5p/µL	100p/µL	100p/ µL
Time to results	45min-1h	20min	>2hr	<1hr	10-20min	10-20min
Sensitivity	High	High	Very high	Very high	Moderate	Moderate
Specificity	High	Low	Vey high	Very high	Low	High
Species detection	Yes	Poor	Yes	Yes	Possible	Possible
Quantification	Yes	Poor	No	No	No	No
Treatment monitoring	Yes	No	Yes	Yes	No	Yes

GSM = Giemsa stained microscopy AO = Acridine orange PCR = Polymerase chain reaction LAMP = loop-mediated isothermal amplification HRP2 = Histidine rich protein LDH = lactate dehydrogenase

1.2.3 Malaria diagnosis and treatment guidelines

Antimalarial drugs

Antimalarial drugs have different points of action on the life cycle of the parasite. The most commonly used drugs act on the erythrocytic stage of the parasite, preventing a clinical disease or curing an established one. They are usually referred to as blood schizontocidal drugs and include chloroquine (CQ), quinine (QN), amodiaquine (AQ), mefloquine, lumefantrine and artemisinin drugs (74,75). Chloroquine was the most widely used drug for treatment of uncomplicated malaria up to the 1980s, as is still effective against non-*falciparum* infections. In those areas where *P. falciparum* parasite developed resistance to chloroquine, which include all of sub Saharan Africa, the drug has been replaced with artemisinin combination therapies (ACT) (1). Quinine is usually used for treatment of severe or complicated malaria. Other drugs act on pre-erythrocytic stages, gametocytes and hypnozoites. These drugs prevent invasion of erythrocytes, transmission and relapses.

Since malaria is an acute and potentially fatal disease, its treatment should be based on drugs that are effective at killing the parasites and are affordable. Chloroquine was an effective, safe and cheap drug, and the development of resistance to the drug raised

global concern about antimalarial drug use. One of the strategies being advocated is the use of combination therapies, especially those based on artemisinins (ACT) (11). The principle behind combination therapy is that the chance of parasite resistant mutations simultaneously developing against two drugs with different mechanisms of action is very low (11,75).

Malaria diagnosis and treatment guidelines

According to the WHO, the objective of producing malaria treatment guidelines is to provide global, evidence-based recommendations on the treatment of malaria (11). The guidelines provide a framework for development of country specific guidelines. The first edition of the WHO malaria treatment guidelines was produced in 2006, at a time when resistance to chloroquine and sulfadoxine-pyrimethamine had reached very high rates, and its consequences on morbidity and mortality recognised (11). The main recommendation was the use of ACT for treatment of uncomplicated malaria. Following the release of these guidelines, malaria endemic countries started changing treatment policies to ACT. However, diagnostic approaches remained the same, based mainly on clinical symptoms and clinical algorithms.

As increasing evidence became available of a reduction in the contribution of malaria as a cause of fever and the safety of withholding treatment to parasite negative patients including children, the second edition of the guidelines was produced in 2010. The guidelines recommend that all patients suspected to have malaria should be tested, and treatment with ACT be confined to parasite positive patients (16,19). It is recommended that RDTs be used as diagnostic tools in areas where microscopy in not available or its quality guaranteed. It is also emphasized that ACTs should be used for treatment of uncomplicated malaria, and artemisinins should not be used as monotherapies.

1.3 THE TANZANIAN CONTEXT

In Tanzania, 90% of the population is at risk of malaria, and there are 11 million clinical episodes annually. Malaria is still one of the leading causes of morbidity and mortality. *P. falciparum* is responsible for over 90% of all malaria incidence in Tanzania, where the main vectors are *Anopheles gambiae s.l*, *A. arabiensis* and *A. funestus.* The coastal areas of Tanzania have the highest endemicity of malaria. Recent publications are showing a decline in the burden of malaria in Tanzania (76,77). Data from the Tanzania Demographic and Health Survey (DHS) is also showing a decline in the burden of malaria in some areas, e.g. in Ifakara where the prevalence of malaria has declined from 35% in 2000 to 10% in 2008 (78).

1.3.1 The health care system

The health care system in Tanzania is categorized into four levels: (1) the home or community, (2) the dispensary, (3) the health centre and (4) the hospital. At the community level there is a village health post supposed to be run by Community health workers (CHW). Many of them function on an ad hoc basis. There are two CHWs per village, one male and another female, although additional CHWs may be recruited depending on need. CHWs are not normally involved in curative services. Their main task is in preventive services, health education, child growth monitoring and follow-up of patients at home (HIV/AIDS, TB/Leprosy and other chronic conditions).

At the ward level there is a dispensary that caters for about 3-5 villages with a catchment population of about 10,000 people. A dispensary is normally run by one clinical officer and one public health nurse. At the level of division, there is a health centre that serves a catchment population of about 50,000 people. In addition to outpatient services offered at the dispensary level, the health centres also offer laboratory and in-patient services. The health centre staff includes several assistant medical officers (AMO)/clinical officers (CO), enrolled nurses and medical attendants.

On the top of the hierarchy are the hospitals - district hospitals, regional hospitals and referral hospitals, each serving as a referral facility for the lower level health facilities. The staffs at district hospitals include medical officers, and the facilities have larger inpatient and laboratory capacities. They offer a broad range of services including diagnostic services, blood transfusion and surgery. Regional hospitals are higher up with specialists in each department and more diagnostic and treatment capacity. Referral hospitals receive patients from regional and district hospitals that need specialist diagnostic and treatment

services not available at lower levels. They also serve as teaching hospitals. The referral system is however flexible such that patients from lower levels can be referred to higher levels where appropriate treatment is available, e.g. from dispensary to district level for blood transfusion services.

1.3.2 Malaria control in Tanzania

Malaria control in Tanzania is based on four strategies, namely malaria case management based on the use of RDTs and ACT, and the use of LLNS, IRS and Intermittent preventive treatment in pregnancy (IPTp). The case management strategy is explained in the next section. The use of treated mosquito nets has been advocated for a long time. A subsidized voucher system for treated nets was established in 2004 for pregnant women and in 2006 for infants. More recently, a large campaign to distribute free LLNs to all children under five years of age was implemented in 2010, followed by universal coverage of all households in 2011 mainly implemented through donor funds. Another strategy, IRS is undertaken in high malaria transmission areas, especially around Lake Victoria and other epidemic prone areas. According to the HIV/Malaria Indicator survey of 2012, 91% of households own at least 1 ITN, an increase from 23% in 2004/2005; and 14% of households had been sprayed in the last 12 months prior to the survey with higher rates of between 40% and 92% in high transmission and epidemic prone areas (79).

Pregnant women are at increased risk of getting infected due to lowered immunity during pregnancy. When malaria parasites infect the placenta, they cause effects on the pregnancy outcome and growth of the foetus, which may end up in abortion, still birth, low birth weight and maternal anaemia (80). In order to reduce serious consequences of malaria during pregnancy, in addition to the LLNs, IPTp is given to pregnant women during routine antenatal clinic visits. It involves the administration of at least two therapeutic doses of an antimalarial drug; in this case sulfadoxine-pyrimethamine (SP) at least one month apart, starting in the second trimester. The first dose is given between 20-24 weeks and the second at 28-32 weeks of pregnancy. A recent report indicated that only 33% of women took two or more doses of SP during their last pregnancy and were considered protected (79), and stock outs of SP are reported to be common.

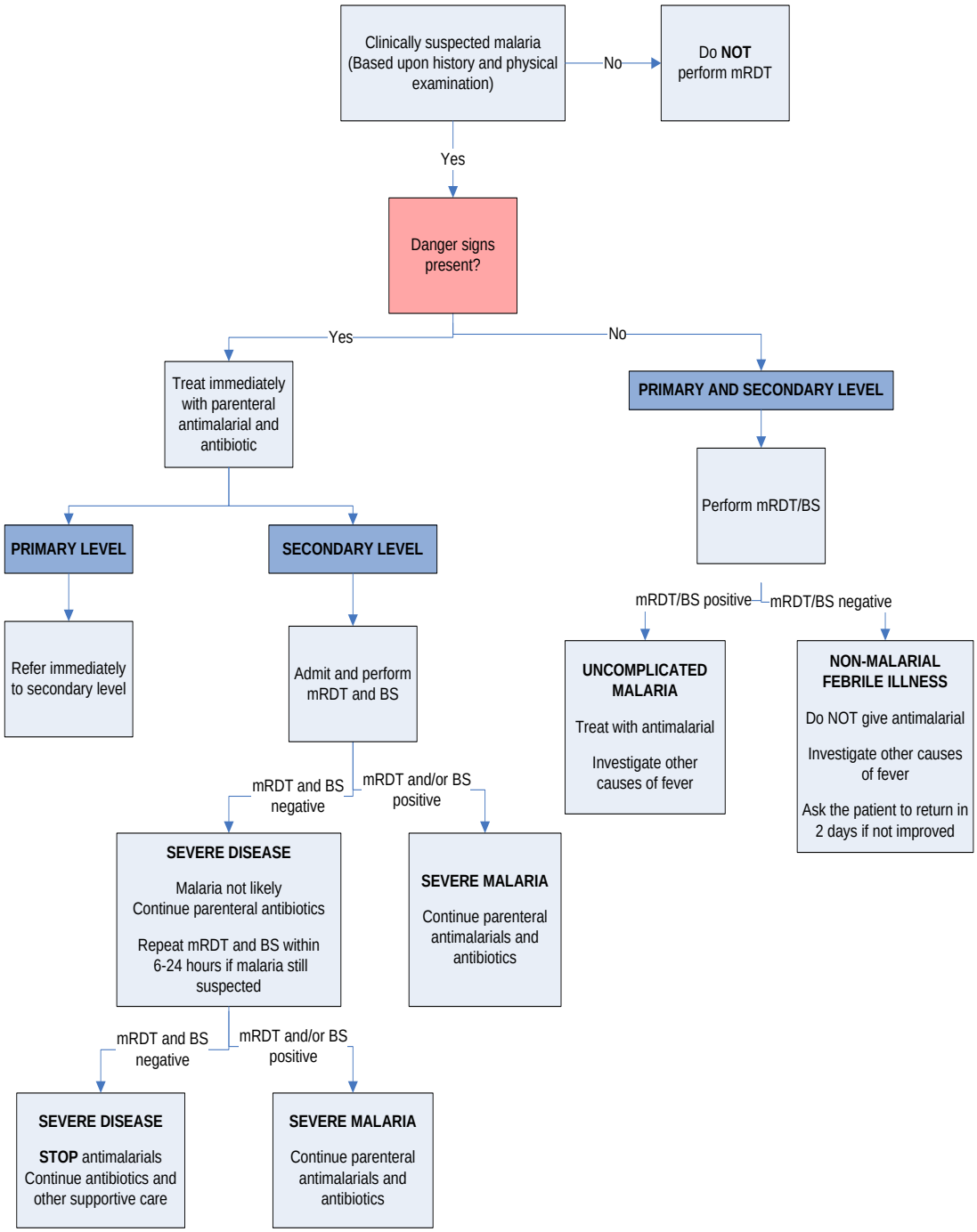
1.3.3 Malaria diagnosis and treatment guidelines

The malaria situation in Tanzania has undergone rapid changes in recent years. Prior to the year 2000, malaria diagnosis was mainly based on clinical features, and chloroquine was the drug of choice for over 40 years (81). Widespread resistance of parasites to CQ lead to resurgence of malaria, with an increase in morbidity and mortality due to malaria. Malaria endemic countries were eventually forced to change treatment policies in favour of more effective antimalarial drugs. Tanzania is one of the countries that within a short time span revised its malaria diagnosis and treatment guidelines, changing the first line drug from CQ to SP in 2001 when resistance to CQ had reached 60% (81), and eventually to ACT in 2007 when resistance to SP reached an average of 25% (82).

The guiding principle of the new national malaria treatment guideline is to provide safe, effective, good quality and affordable drugs to those who need them, yet at the same time encourage rational drug use, which will minimize the development of drug resistance. Early and correct diagnosis is therefore essential in the effort to encourage rational use of antimalarials and in ensuring that other causes of fever are properly managed to reduce severe morbidity and mortality.

Up to early 2009, 83% of health facilities in Tanzania had no laboratory capacity for malaria diagnosis. In other places microscopes were available but results inaccurate, leading to misdiagnosis and overuse of ACT. The country started introducing RDTs at all health facility levels in 2009 in line with the WHO recommendation, directing that all patients suspected to have malaria be tested before treatment (16,19). The RDT roll out was completed in 2012. The algorithm for the use of RDTs in Tanzania (Figure 3) shows steps by step instructions in deciding when to test and action following results of the test at different levels of health care (83). Emphasis is also placed on investigating for other causes of fever even when the test for malaria is positive so that other causes of fever or co-morbidities can also be properly managed.

Figure 3: Algorithm for the use of malaria RDT.



Source: Learners' manual for Malaria rapid diagnostic test. Final draft (83).

1.4 CHALLENGES IN MANAGING MALARIA

1.4.1 Prompt access to treatment and rational use of drugs

During the last years there has been a decrease in both morbidity and mortality due to malaria in many African countries following combined interventions with ACT for treatment, insecticide treated nets (ITN) and IRS (2,84,85). In areas where these interventions have been successful, surveillance is becoming increasingly important. Early and prompt parasite based diagnosis is essential in order to target ACT to patients with confirmed malaria infection, prevent overuse of ACTs, reduce costs and to minimize development and spread of antimalarial drug resistance (86). Furthermore, in areas where elimination of malaria is considered feasible, an optimal surveillance is critical to assure effective malaria control, to detect upcoming epidemics and to identify and contain remaining cases.

Early diagnosis and prompt treatment with effective drugs is an important strategy to reduce morbidity and mortality due to malaria. In view of the fact that malaria recognition and treatment occurs at home and is often inadequate, Heads of State of African countries made a commitment to ensure at least 60% of patients suffering from malaria should have prompt access to treatment within 24 hours of the onset of symptoms by the year 2005 (87), later raised to 80% by 2010. In settings with limited access to health care, strategies to improve access at community level are needed.

Home management of malaria is recommended by the WHO and has been used on a large scale in some countries (88,89). Previous studies on home management of malaria were based on clinical diagnosis, which meant that all children with fever were treated with antimalarial drugs. With the use of ACT for home management of malaria, the challenge is to increase access to effective drugs while targeting treatment to malaria patients only and achieving high compliance (90).

ACTs are highly efficacious, with very high cure rates. The combination of two drugs with different modes of action helps to achieve high cure rates and delay the development of parasite resistance. In this way, the therapeutic life of the drug is prolonged. However, ACTs are more expensive than previously used drugs (CQ and SP). The introduction of ACT brought challenges to the health care system. Overuse of the drug will impose a heavy burden on the health budget of poor developing countries and may facilitate development of parasite resistance to the drugs. There are already indications of selection of parasites during treatment with ACT (91,92) and decreased parasite drug susceptibility to ACT in South East Asia, which calls for strategies to improve diagnosis at all health care levels to restrict treatment to confirmed malaria patients.

Apart from its role in limiting resistance development, appropriate malaria diagnosis is part of good clinical practice as it helps in the overall management of the patient, by identifying patients with malaria to receive antimalarial drugs, and those without malaria who also deserve to be appropriately managed (19).

While microscopy of giemsa stained blood smears is the gold standard method for diagnosis of malaria, maintaining quality has been a challenge as it requires qualified and experienced personnel, equipment and supplies, which are often lacking in health facilities in endemic areas. The availability of RDTs malaria offers a suitable alternative especially in areas where microscopy is not available, or its quality cannot be guaranteed. They are an important tool to ensure that malaria diagnosis is available even in peripheral areas with limited resources.

Non adherence to test results have been reported in several studies, with up to 50% of RDT negative patients were still given antimalarial drugs (93–95). Low testing rates have also been reported, meaning underutilisation of testing facilities (96,97). Non adherence to test results is not limited to RDT, but was also seen with microscopy (98,99). However, other studies have shown opposite results with high adherence to test results and reduction in prescriptions of ACTs (100–103). In these studies, overall health outcome of fever patients improved after RDT use, probably because of improved management of non malarial fevers.

Increased efforts in malaria control which includes widespread use of ITN, IRS and ACT, are leading to a reduction in the proportion of malaria among fevers, making the extent of over-diagnosis and overtreatment even worse. Reduction in the malaria burden, with lower prevalence has been reported in several countries including Tanzania (2,76). With a decline in the malaria burden it becomes even more important to use parasitological diagnosis to restrict treatment to malaria positive cases only. With the availability of evidence of the safety of withholding treatment to parasite negative children (104), the WHO is now recommending universal testing of all fever cases before prescription with antimalarial drugs (16).

1.4.2 Use of RDTs in endemic areas

The National Malaria Control Program (NMCP) in Tanzania has rolled out RDTs as diagnostic tools in health facilities in the country with funding from the Global Fund. Rollout started in three pilot regions in June 2009 and by December 2012, all regions had been covered. According to the NMCP, test kits are available in all government health facilities and used as routine diagnostic tests. HRP2 based tests were introduced following the recommended by WHO that where more than 95% of the infections are due to *P. falciparum* infections, HRP2 based tests should be used.

An issue of concern with the HRP2-based tests is the finding of persistent antigen, causing false positive results for up to several weeks after successful treatment (71,105). In endemic areas where the possibility of new infections within a short time period exist, when patients return with symptoms after a recent antimalarial treatment it is not possible to differentiate between RDT positivity due to persisting antigens from a previous infection and actual parasitemia caused by treatment failure or re-infection. The low specificity of HRP2-based tests is a problem especially in high transmission areas (106) as it could lead to antimalarial treatment in non malarial patients and affects health workers adherence to results and reliance in new diagnostic tools. On the other hand the LDH-based RDTs are more specific since LDH is only produced by live parasites and does not persist after parasite clearance (107). It has therefore been suggested that LDH based tests would be more useful for follow-up after treatment to assess cure and clearance of malaria infection. Beside the problem with persisting antigens, presence of gametocytes can also give rise to false positive result, both for HRP2 and LDH based tests. Since ACTs are also effective against gametocytes, false positivity due to gametocytes has become less of an issue (108).

No previous study has comprehensively investigated the correlations between detection and clearance of whole parasites, parasite DNA and antigens using two different microscopic techniques (Giemsa stained thick films and acridine-orange stained thin films), PCR for DNA detection and two different RDTs (HRP2 and LDH based) during 42 days follow up after initiation of ACT treatment in children with uncomplicated *P. falciparum* malaria residing in a high endemic area.

1.5 RATIONALE FOR THE STUDY

Increased efforts in malaria control interventions have lead to the reduction in the contribution of malaria as a cause of fever. With this reduction, continued reliance on clinical symptoms as a basis for treatment of malaria in endemic countries will lead to overtreatment & irrational use of antimalarial drugs, increasing drug pressure and hence resistance development to the available drugs, and negatively affecting the health of the patient as the actual cause of fever will not be identified and treated

The availability and use of diagnostics is therefore an important aspect in the management of patients with fever. Not only is it important in rational use of antimalarial drugs, but is essential for overall patient management as it allows for other causes of fever to be investigated and appropriately treated.

The studies described in this thesis were aimed at assessing the impact of improving malaria diagnosis at health facility and community level on antimalarial prescriptions and health outcome, and the utilization of malaria diagnostics for patient follow up after treatment. Findings from these studies have policy implications by providing information on the impact of diagnostics on antimalarial prescription practices, safety of using CHWs and RDTs for malaria management at community level, on the role of RDTs for patient follow-up and other operational issues of RDT/diagnostics implementation.

2 AIMS AND OBJECTIVES

2.1 General aim

To assess the impact of laboratory diagnosis towards improving the management of uncomplicated malaria at peripheral health care (PHC) settings in Coast region, Tanzania.

2.2 Specific objectives

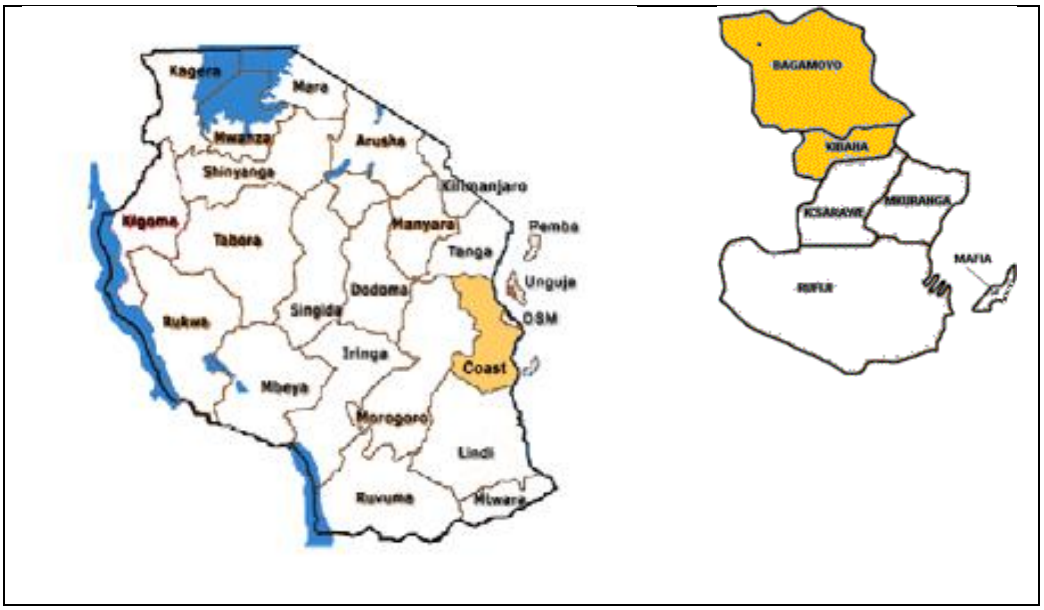
1. To assess the impact of malaria diagnosis training at health facility level on antimalarial drugs prescription and health outcome (Study I).
2. To assess the impact of CHWs using RDTs for uncomplicated malaria at community level on antimalarial drugs prescriptions and health outcome (Study II).
3. To assess current malaria diagnostic and prescription practices following the introduction of malaria RDTs in peripheral health care facilities (Study III).
4. To assess initial clearance and detection of recurrent *P. falciparum* as determined by microscopy, HRP2 and LDH-based RDTs, and PCR following treatment with ACT in Tanzanian children with uncomplicated malaria (Study IV).

3 MATERIAL AND METHODS

3.1 Study sites and study population

All the studies were conducted in Bagamoyo and Kibaha districts, Coast Region, Tanzania. Coast region is found along the coastal belt, which is a high malaria transmission area. The area experiences two rain seasons, the long rains in March-June and short rains in October-December. Transmission follows the rainfall pattern, with peak in May-July and December-January. According to the National Census of 2002, Bagamoyo and Kibaha districts had a population of 230,164 and 132,045, respectively, with 20% of the population under the age of five years (109). Malaria was the leading cause of outpatient attendance, hospital admissions and deaths.

Figure 4: Map showing location of Coast region and study districts in Tanzania



Study I was conducted in 2004 at sixteen PHC facilities in the two districts, at a time when SP was the first line drug for treatment of uncomplicated malaria. Study II was conducted in 2006 at community level in five villages in Kibaha district, when the home management strategy was advocated to facilitate early treatment, just before the replacement of SP with ACT as first line treatment for uncomplicated malaria. Study III was conducted in 2011 at ten PHC facilities, one year after introduction of RDT in Kibaha district. Study IV was conducted from 2009 to 2011 at two PHC facilities, one in Bagamoyo and the other one in Kibaha district. The study population for study I and

IV was children under five years of age, while for study II and III it was patients of all ages.

3.2 STUDY I: THE IMPACT OF IMPROVING MALARIA DIAGNOSIS AT PHC FACILITIES

This was a cluster randomised intervention trial testing two intervention packages. Sixteen PHC facilities were involved, 10 of them were in Bagamoyo district (Chalinze, Fukayosi, Hondogo, Kiwangwa, Lugoba, Matuli, Mbwewe, Msata, Msoga and Msata) and six in Kibaha district (Kikongo, Mbwawa, Mlandizi, Mwanabwito, Ngeta and Visiga). The facilities were stratified by previous exposure to IMCI training and randomly allocated to either training on clinical algorithm and microscopy (arm I), clinical algorithm only (arm II) or no training (control, arm III). Arms I and II had 5 PHC facilities each while arm III had 6. The intervention package included group training of all cadres of health workers on clinical and/or laboratory diagnosis with theoretical and practical demonstration depending on intervention arm. The training programme was developed and implemented by researchers from the Muhimbili University of Health and Allied Sciences (MUHAS) malaria project.

Children under five years of age with fever or history of fever, without symptoms of severe disease, making the first consultation for the current illness were included in the study after getting consent from parents or guardians. Patients records, including symptoms, tests performed and results as well as treatment prescribed were recorded in the case record forms (CRF). All patients were requested to attend a day 7 follow-up. They were also encouraged to come back any time if the fever persisted or the condition worsened. During these visits, the patients' health status was assessed, additional medication prescribed if indicated and blood smears collected on day 7.

In the microscopy intervention arm, blood smears were collected from all patients on day 0 and 7. They were stained with giemsa and examined by health workers at the facility, with a second reading done at MUHAS. From the other intervention arms, blood slides were only collected on day 7, and these were examined at MUHAS.

The impact of the intervention was evaluated by assessing the prescription rate of antimalarial drugs, antibiotics and health outcome on day 7 across the different arms of the intervention.

3.3 STUDY II: THE USE OF MALARIA RDTS BY COMMUNITY HEALTH WORKERS TO GUIDE TREATMENT AT COMMUNITY LEVEL

In view of the introduction of ACT in Tanzania, and the drive towards home management of malaria, we evaluated the possibility of using CHWs with the aid of RDTs for diagnosis to increase access to antimalarials at community level.

This was an intervention study with a cross over design. The study was performed in five villages (Kikongo, Mbwawa, Miswe, Mwanabwito and Ngeta) in Kibaha District, Twenty two Community Health Workers (CHWs) were involved in the study, and the number of CHWs selected per village ranged from 2-5, depending on the village geography and population size, each CHW being responsible for between 300 and 500 people. The intervention included training of CHWs on malaria diagnosis with or without the use of RDTs, together with the provision of the test kits for malaria diagnosis and ACT for treatment of uncomplicated malaria in the community. The training package included general knowledge on malaria, symptoms of severe disease, and symptoms of other diseases presenting with fever. They were instructed to refer patients with symptoms of severe disease or non malarial illnesses to health facilities for further management. They were also oriented to practical issues of testing and data handling including patient follow up planned for day 3 and 7.

The inclusion criteria were fever or history of fever within the last 24 hours, without symptoms of severe disease; while patients aged less than 3 months old and pregnant women were excluded from the study.

During implementation, CHWs changed the diagnosis method alternating between RDT and clinical diagnosis from one week to another. RDTs were performed according to manufacturer's instructions (Paracheck Pf, Orchid Biomedical Systems, India). Included patients were to be treated with ACT if RDT results were positive during RDT weeks, or if they had symptoms suggestive of malaria during CD weeks. The ACT (artemether-lumefatrine, Coartem) was administered at doses recommended by the manufacturer according to age (Novartis, Switzerland). Only the first dose of ACT

was given under supervision. If malaria was not diagnosed or suspected, or patients had symptoms suggestive of another cause of fever they were referred to health facilities for further management.

The effect of RDTs on prescription of ACT and health outcome was assessed. The main outcome variable was ACT prescriptions. Secondary outcome variables included health outcome by day 7, the proportion of patients getting treated within 24 hours of onset of fever, and compliance to treatment.

3.4 STUDY III: DIAGNOSIS AND PRESCRIBING PRACTICES FOLLOWING INTRODUCTION OF RDTS

The study was conducted in Kibaha district, one of the first districts in Tanzania to introduce RDTs for malaria diagnosis in 2009. Ten out of 25 eligible PHC facilities were randomly selected and included in the cross-sectional study. These were Kikongo, Kongowe, Mbwawa, Misugusugu, Miswe, Mwanabwito, Mwendapole, Ngeta and Ruvu station dispensaries, and Mlandizi health centre. The aim of this study was to identify malaria diagnosis and treatment practices after introduction of RDT as diagnostic tools at PHC facilities. The study also looked at health workers attitudes and perceptions about testing and their influence on practice.

Questionnaires were administered as interviews to health care providers attending patients with fever. These were used to collect information on their knowledge, attitudes and prescription practices in relation to malaria diagnostics. Exit interviews were also conducted with fever patients, and in the case of children with their caretakers, and information collected included their presenting symptoms, tests performed, test results, prescribed drugs and their knowledge about malaria testing. A health facility inventory was also undertaken and included interview with the person in charge of the facility and review of routine health facility records. The aim of the inventory was to collect information on staffing, availability of diagnostics (RDT and microscopy), supplies and essential drugs; and the malaria situation over the last one year.

3.5 STUDY IV: CLEARANCE AND DETECTION OF RECURRENT *PLASMODIUM FALCIPARUM* FOLLOWING TREATMENT WITH ACT

This was a descriptive, exploratory study conducted at two health facilities, Fukayosi dispensary in Bagamoyo district and Mlandizi health centre in Kibaha district, to assess the performance of different malaria diagnostic tests in the follow up of patients with uncomplicated malaria treated with ACT. Children under five years of age (6-59 months) with uncomplicated malaria, *P. falciparum* mono-infection and 50-6,250 asexual parasites/200 white blood cells (wbc) equivalent to a parasite density of 2000-250,000/uL attending the PHC facilities were enrolled into the study. Treatment with ACT was initiated on day of enrolment.

Patients were followed up over a 42 day period (day 1, 2, 3, 7, 14, 21 28, 35 and 42) and we assessed the clearance of malaria parasites, antigen and DNA and detection of recurrent infection using RDTs (HRP2 and pLDH based), microscopy (giemsa stain and acridine orange) and PCR using blood samples collected on filter paper. Patients were also followed up clinically for re-appearance of fever. All clinical and demographic information, including age, sex, temperature and symptoms at presentation (day 0) and on follow up dates, prescribed drugs (day 0 and afterwards) and information on use of ITNs was recorded in a CRF.

During follow up visits, a blood smear was examined at the health centre and results used to guide treatment if the patient complained of fever. The Giemsa stained thick blood smears and acridine orange stained smears from enrolment and follow up visits were examined at MUHAS and Muhimbili National Hospital (MNH) experienced microscopists. Quality control of slide reading was done at Karolinska Institutet (KI), Sweden. RDTs were performed according to manufacturer's instructions at the health facilities.

DNA was extracted from blood spots collected on filter papers (and if necessary on used RDTs) at the Malaria research unit of Karolinska Institute (KI), Sweden. The PCR analysis was performed using a *P. falciparum* specific method targeting the 18S rRNA gene as previously described (110). A subset of samples with a fixed volume on the filter paper were analyzed with quantitative PCR (111,112). In case of recurrent parasitemia during follow up stepwise genotyping of three highly polymorphic genetic

markers, i.e. the *merozoite surface protein* 1 and 2 as well as *glutamine rich protein*, was performed according to standard protocols to differentiate re-infections from recrudescence (44).

Table 2: Summary of methodologies for the studies included in this thesis

	Study I	**Study II**	**Study III**	**Study IV**
Study design	Randomised controlled intervention trial (3 arms)	Cross over intervention study (2 arms)	Cross-sectional descriptive study	Exploratory descriptive study
Intervention package	Training of PHC facility staff on clinical algorithm and microscopy	Training of CHW on malaria diagnosis using clinical and RDTs	NA	NA
Study population	Children <5 years of age with fever	Patients of all ages with fever	Patients of all ages with fever	Children <5 years of age with fever and *Pf* mono-infection
Sample size	3131 patients	2930 patients	195 patients, 20 PHC workers, 10 PHC facilities	53 patients
Data collection	Recording of signs and symptoms by PHC staff, Interview with mother or guardian, Microscopy of blood smear for malaria parasites	Recording of signs & symptoms by CHW, Interview with patient, mother or guardian, Testing for malaria by RDT	Exit interviews with patients, Interviews with PHC facility workers, PHC facility inventory	Recording of signs and symptoms, Laboratory tests at enrolment and follow up – microscopy, RDT, PCR (sample collected on filter paper)
Follow up	Up to day 7	Up to day 7	None	On 9 occasions up to day 42
Outcome variables	Positivity rate, Prescriptions of antimalarials and antibiotics, and health outcome by day 7	Positivity rate, Prescriptions of antimalarials & health outcome by day 7	Testing rates, Positivity rate, Prescriptions of antimalarials & antibiotics	Clearance times for parasites, antigen and DNA, Detection of recurrent infection

3.6 ETHICAL CONSIDERATIONS

These studies received ethical approval from the National Institute for Medical Research (NIMR) (study I) or the MUHAS Research and Publication Committee in Tanzania (studies II-IV), and from Karolinska Institutet/Regional Ethics Committee, Stockholm, Sweden for studies I, II and IV).

In addition to that, permission to conduct the study was obtained from district authorities, health facilities and village administration. Informed consent was obtained from health workers and patients and caretakers/guardians in case of children who participated in the studies.

4 RESULTS

4.1 STUDY I: THE IMPACT OF IMPROVING MALARIA DIAGNOSIS AT PHC FACILITIES

A total of 3131 children were included in the three arms of the study. In the microscopy intervention arm (arm I), 531/973 (54.6%) of children tested positive for malaria by PHC microscopy. Antimalarial drugs were prescribed to 576 children (61%) in arm I, 1008/1058 children (95.3%) in arm II (clinical) and to 1091/1100 children (99.5%) in arm III (control). Non compliance to test results was observed in arm I, with 11.3% of patients with negative results being given antimalarial drugs.

Overall, antibiotics were prescribed to 44.8% of all patients, and the rate did not differ significantly between the three arms with 45.9%, 54.8% and 34.2% of patients getting antibiotics in arms I, II and III respectively. Patients with positive malaria test results were less likely to be prescribed antibiotics than those with negative results.

The sensitivity and specificity of PHC microscopy when compared to expert microscopy were 74.5% and 59.0% respectively. The accuracy of microscopy varied between PHC facilities, with sensitivity increasing with parasite density.

When looking at health outcome by day 7, there was no difference in the proportion of patients reporting full recovery by day 7, with rates of 74.4%, 78.6% and 67.6% for arms I, II and III respectively.

Expert microscopy reading of day 0 slides from arm I revealed that 76 patients with positive results did not receive antimalarial drugs. Fourteen of the patients came back with symptoms between days 1 and 3 and were successfully treated. In the remaining patients, the fever resolved but parasitemia was cleared after getting antimalarial drugs.

4.2 STUDY II: THE USE OF MALARIA RDTS BY COMMUNITY HEALTH WORKERS TO GUIDE TREATMENT AT COMMUNITY LEVEL

Twenty two CHWs from 5 villages were involved in the study. A total 2930 patients (760 children under five years) were included in the study during the 5 months study period. Attendance to the CHW was not influenced by presence of RDT as only 1457 (49.7 %) patients came to the CHW on a week when RDT was used for diagnosing malaria. The proportion of patients consulting the CHW within 24 hours of onset of fever was similar for the RDT and CD groups, with 67.3% and 68.4% respectively.

When using RDTs for malaria diagnosis, 50.3% (733/1457) of patients tested positive for malaria. Positivity rate was highest in the 5-9 year age group and lowest in those above 15 years. With clinical diagnosis 91.6% (1415/1473) were classified as malaria and there was no difference by age.

ACT (artemether-lumefantrine) were given to 53.2% (775) of patients during the RDT weeks compared to 96.5% (1422) of patients during the CD week, OR= 0.04 ($p < 0.001$). The reduction in ACT drug prescriptions was most prominent in patients ≥15 years (57.3 % reduction) and lowest in the age range 5-9 years. Forty four (6.1%) RDT-negative patients were also given malaria treatment.

The health status on day 7 was evaluated for 2869/2930 (97.9%) patients. Full recovery was reported by 2735 (93.3%) of all patients. The proportion reporting full recovery among patients seen during the RDT week was 93.3% as compared to 97.3% from the CD week. There were no reports of severe disease or death among patients not treated with ACT during RDT weeks.

The number of patients given referral to the health facility from day 0-7 was 250 (17.2%) from the RDT group compared to 73 (5%) from the CD group. In both groups, referrals were more common among patients who did not receive ACT.

Deaths among patients within 28 days of inclusion were actively investigated. Three children died, all within 3 days of their inclusion into the study. Two of them (1 RDT positive, 1 CD, both BS positive) were treated with ACT and referred to PHC facilities for management of other conditions. The third child with both RDT and BS negative was not treated with antimalarials but referred. One adult also RDT and BS negative

died within 7 days of inclusion. The patient was referred to the PHC facility for management of other conditions.

RDT positivity was higher than BS positivity, at 50.3% vs. 22.6%. The quality of the slides was poor. Agreement between the 1st (field) and 2nd reader (expert, MUHAS) was 0.85 and the kappa coefficient was 0.59.

4.3 STUDY III: DIAGNOSIS AND PRESCRIBING PRACTICES FOLLOWING INTRODUCTION OF RDTS

Ten health facilities were involved in the study, one of which was a health centre and the rest were dispensaries. A health facility inventory revealed stock outs of both RDTs and ACT in 5 and 4 out of the 10 facilities respectively. Three of the facilities without RDTs were using microscopy, leaving two facilities without any malaria testing.

One hundred and ninety five patients of all ages (and guardians in case of children) were involved in the exit interview. Among them, 45% were children under five years of age and 55% were aged above 5 years. The testing rate in facilities with diagnostics was low as only 105/168 (63%) of patients were tested with either RDT or BS. The testing rate was significantly higher among children under five years of age than among older children and adults. Among tested patients, 31 (30%) were positive, and the positivity rate did not differ by age. Lack of diagnostic facilities as evidenced by stock outs of RDTs was the main reason for the low testing rate.

Antimalarial drugs were given to all patients with positive results, and to 10/74 (14%) of patients with negative results, and there was no difference in antimalarial prescription by age. The overall prescription rate among non tested patients was 28% (25/90), with a higher rate among patients in facilities without diagnostics (70%, 19/27). Antibiotics were prescribed to 76% of patients, and the rate was higher among patients with negative malaria tests results compared to those with positive results (81 vs. 69%, $p<0.01$) and among patients not tested compared to those tested for malaria (84 vs. 69%, $p=0.01$). Due to stock outs of ACT, some 18% of patients were prescribed non ACT drugs, mainly SP and QN.

Twenty health workers were interviewed, among them 12 had clinical training background and the rest were non-clinical. The majority of health workers (18/20) had

been trained on RDT use. Reasons given by health workers for prescribing antimalarial drugs to patients with negative results included not trusting RDT results ("false negative") and the presence of clinical symptoms suggestive of malaria, and patients with negative results responding to antimalarial drugs. A few patients did not trust negative RDT results, mainly because they were given positive results at private dispensaries, and they did not get an alternative diagnosis.

The utility of routine HF records was limited by poor record keeping, as data was often incomplete and was not compiled on a regular basis.

Table 3: Summary of results from studies on diagnosis and treatment practices (Studies I-III)

	Study I			**Study II**		**Study III**	
	Arm I[a]	**Arm II[b]**	**Arm III[c]**	**RDT**	**CD**	**With diagnostics**	**Without diagnostics**
Total no. of patients	973	1058	1100	1457	1473	168	27
Malaria testing							
Tested for malaria	973			1457		105 (63%)	
With positive results	531 (55%)			733 (50%)		31 (30%)	
Antimalarial drugs							
All patients	571 (61%)	1008 (95%)	1091 (99%)	775 (53%)	1422 (97%)	47 (28%)	19 (70%)
Test negative patients	50/442 (11%)			44/682 (6%)		10/74 (14%)	
Antibiotic drugs							
All patients	434 (46%)	593 (55%)	375 (34%)			127/168 (76%)	21/27 (78%)
Tested patients						72/105 (69%)	
Positive malaria test	36%					12/31 (39%)	
Negative malaria test	58%					60/74 (81%)	
Non tested						55/63 (87%)	

Arm I[a]: clinical algorithm and microscopy

Arm II[b]: clinical algorithm only

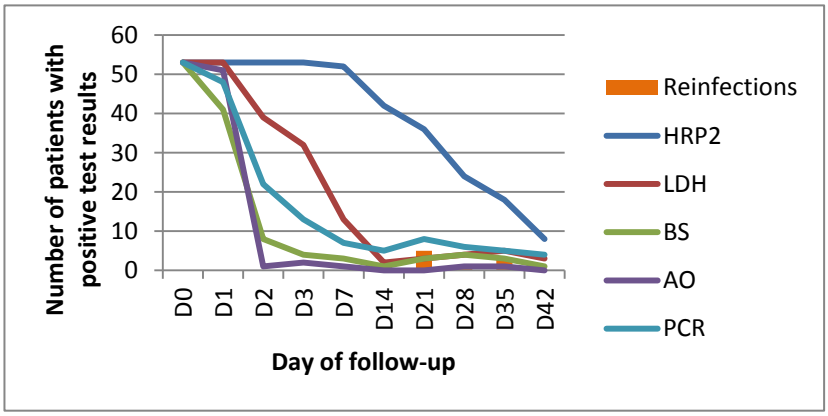
Arm III[c]: no training (control)

4.4 STUDY IV: CLEARANCE AND DETECTION OF RECURRENT *PLASMODIUM FALCIPARUM* FOLLOWING TREATMENT WITH ACT

Fifty three children completed the 42 day follow-up period and were included in the analysis. There were 35 (66%) males and 18 (34%) females, with mean age 42 months (range 10 – 59). All the children had a fever history of 1 to 4 days duration (mean 2.4 days). At the time of enrolment, 44 children (83%) had a fever in the range of 37.5°C to 39°C. Apart from fever, other symptoms reported were vomiting (42%), cough (26.4%), abdominal pain (17%) and diarrhea (7.5%). All the children were prescribed artemether-lumefantrine, the first line antimalarial drug. Other medication prescribed were antipyretics (94%), antibiotics (11%) and oral rehydration solution (ORS) (7%).

Median clearance times for all tests are based on the 53 children, except for HRP2 which is based on 43 children without re-infections. The majority of children cleared their parasitemia within 2 days as assessed by PCR and microscopy. Median clearance times for pLDH and HRP2 based tests was 7 (2-14) and 28 (7-42) days respectively. There were 10 re-infections during the follow-up period; 1 on day 14, 4 on day 21, 2 on day 28 and 3 on day 35. At the time of the recurrent infection, the HRP2 test was still positive in 8 of these patients. Due to longer clearance times, the HRP2 based test was able to identify recurrent malaria infection in only 2 of the cases, and these were in patients who had cleared their initial antigenemia. On the other hand, the LDH based test identified 8 of the recurrent infections on the day of occurrence and the 9th at the next follow up visit. PCR was able to identify all ten recurrent infections while microscopy identified 8 of the cases. Figure 5 is showing positivity for malaria parasites, antigen and DNA at follow-up days.

Figure 5: Comparison of different tests positivity on follow-up days



5 DISCUSSION

In order for malaria patients to be appropriately treated, there is need for parasite-based diagnostics to be made as early as possible after onset of symptoms, preferably in the community or at the lower levels of health care, and results be used to guide treatment.

Findings from our study at community level (study II) show that malaria can be safely managed at community level by CHWs equipped with easy to use diagnostics in the form of RDTs and ACTs (113). RDT based diagnosis reduced the prescription of antimalarial drugs by 45%. With more than 60% of patients seeking treatment within 24 hours of onset of fever, progression to severe disease may be arrested. There was no indication of severe malaria in patients with negative results and not treated with ACT, which shows that the use of RDT results to direct treatment did not affect the health of patients with negative results similar to findings in other studies (104,114). Findings from this study are supported by a recent review which shows that community based programmes with free or subsidized antimalarial drugs are likely to improve access to antimalarial drugs, and that RDTs can be safely used to reduce overuse of antimalarial drugs (115).

As malaria symptoms resemble those of other diseases with fever as the cardinal symptom, and with the possibility of co-infections, it is important to emphasize the need for thorough assessment of the patient to identify other causes of fever or co-morbidities. When health workers focus on results of malaria testing and forget about other causes of fever, there is a possibility of missing and not treating potentially serious infections with dire consequences on the health of the patient (20,116,117). In our study (study II), CHWs were trained to identify and refer other causes of fever, even among ACT treated patients. In this way, other diseases requiring antibiotic treatment (e.g. pneumonia) will be attended at the health facility and avoid the danger of complete reliance on malaria test results.

Improved diagnosis through training and use of microscopes at health facilities is also important (study I), and was found to reduce antimalarial treatment from 96.5% to 61% (118). The poor quality of health facility microscopy which was partly associated with the short training period in our study is a common finding in resource poor settings (119). Due to the possibility of missed infections where the quality of microscopy

cannot be guaranteed or false negative RDT results, close follow-up of patients should be part of the management plan for patients with negative results.

In order for malaria testing to have an impact on antimalarial prescriptions and treatment outcome, health workers must adherence to test results by restricting malaria treatment to patients with positive results only. In our studies, non adherence was also observed with 11%, 6% and 14% of patients with negative results treated with antimalarial drugs in the first three studies (113,118,120). These results were similar to findings from other recent studies which in addition to reduction in antimalarial prescriptions also showed improved health outcome (100–102), and were much lower than previous studies reporting up to 50% of patients with negative results treated with antimalarials (93,94,121,122).

Among factors identified as cause for non adherence to treatment guidelines are prior training, previous guidelines, interaction with others, experience and patient demand for antimalarials (123–126). A recent qualitative study in Ghana showed that patients expected RDTs to diagnose not only malaria, but any cause of their illness (127), and were disappointed when told they were negative for malaria without an alternative diagnosis. Health workers in the study area (study III) had used RDTs for more than a year, and they may have acquired experience and confidence with test results (120). Lack of trust by some of the health workers as well as lack of trust and demand for antimalarials by patients was reported. Under such circumstances, and in the absence of alternative diagnosis for the cause of fever, antimalarial drugs will continue to be prescribed, with delays in the treatment of other causes of fever. The availability of diagnostics, training and drugs for the management of other causes of fever help will to eliminate the problem (19,128). Training should also emphasize the actions in the algorithm which allow treatment of negative patients only in case the patient is a child under five with no possibility for follow up. The low testing rates in facilities with diagnostics has also been observed elsewhere (96,97), and in our study it was attributed to limited supplies of test kits and staff shortage.

Poor record keeping observed in most of the facilities means that data on prevalence of malaria and consumption of diagnostics and drugs is not available or of poor quality. Such data cannot be used for planning and forecasting of supplies and surveillance; and

could be an important factor in the stock outs observed. The importance of correct and timely record keeping should be emphasized during training and supervision.

High prescriptions of antibiotics were found in two of our studies (113,118). A study in Dar es Salaam also found that antibiotic prescriptions increased from 49 to 72% after introduction of RDTs especially among patients with negative malaria test results (D'Acremont et al. 2011). It was not in the scope of our studies to identify other causes of fever, and for that reason we cannot say if the antibiotic prescriptions were justified or not.

The higher sensitivity of HRP2 based tests in detecting *P. falciparum* infections and lower cost were the main reason for choice of these tests for diagnosis of malaria in Africa. However, persistence of antigen after treatment has also been observed in several studies (71,105,107,129). This is a problem in endemic areas where the possibility of re-infections within a short period exists and there is a need to differentiate positivity due to persisting antigens and that due to recurrent infections (71,130). In our study on antigen clearance (study IV) we also found a high re-infection rate with 10 out of 53 turning positive within a 42 day follow up period, and because of prolonged antigenemia, the HRP2 test could not detect 8 of the infections. On the other hand, the LDH based test performed better and could be useful for patient follow up after treatment. The logistics of managing two types of RDTs, HRP2 based for initial testing due to high sensitivity, and LDH test for follow-up after treatment due to high specificity needs to be considered. Microscopy of giemsa stained blood smears performed well in this respect, and strengthening this service at health facilities could provide a good tool for patient monitoring after treatment and for quality control of RDTs.

6 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Overall conclusion

Training and provision of diagnostics at community and health facility level reduces unnecessary use of antimalarial drugs and allows for early diagnosis and management of non malarial fevers. Due to longer persistence of HRP2 antigen in blood, HRP2 based tests performed poorly in patient follow up and detection of re-infections when compared to LDH based tests.

Study specific conclusions:

- Training of health care workers and use of microscopy for malaria diagnosis at health facilities reduced the prescription of antimalarial drugs by restricting treatment to test positive patients (study I).

- RDTs in the hands of CHWs can help to direct treatment with antimalarial drugs within the community, increasing prompt access to the drugs while limiting unnecessary use (study II).

- The use of malaria RDTs at community level was safe as no fatal or severe disease occurred among patients who did not get antimalarial drugs because of negative test results (study II).

- Even after adoption of the universal testing policy of all fever patients, low testing rates were observed, caused by irregular supply or non-availability of testing facilities (RDT and microscopy) and staff shortage (study III).

- Although non adherence to negative test results is still practiced, the rates observed in our studies (Studies I – III) were low, ranging from 6 to 14%. The main reasons for the practice were health workers not trusting RDT results and limited ability to diagnose and treat other causes of fever.

- Persistence of the HRP2 antigen makes tests based on this antigen unsuitable for patient follow-up after treatment and detection of reinfections, whereas LDH based tests are more suited for this purpose (study IV).

6.2 Recommendations

- The implementation of the universal testing policy is hampered by RDT stock-outs. There is need to strengthen forecasting and procurement to ensure constant availability of test kits.

- Non adherence to test results still exists although at a lower rate when compared to previous studies, and several factors were identified as being responsible for this practice in the cross sectional survey. Equipping health workers with knowledge, skills and facilities to be able to diagnose and manage other causes of fever will help to build their confidence in RDT results and increase their compliance. In-depth studies should also be conducted on health system and other factors affecting health worker prescription practice.

- HRP2 based RDTs are highly sensitive for diagnosis of *P. falciparum* infections. These advantages are set back by antigen persistence, which makes the tests unsuitable for patient follow up. Other tests, such as LDH based RDTs should be considered for this purpose. However, the logistics of handling two types of tests from procurement, training and supervision of health workers should be taken into account.

- The quality of microscopy at PHC facilities was low. Good quality microscopy is essential for correct diagnosis and post treatment evaluation of patients with malaria. There is need to strengthen microscopy services, which can be used for treatment monitoring in severe cases, patient follow-up after treatment and quality control of RDTs.

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