Assessment of the therapeutic efficacy of artemether-lumefantrine in the treatment of uncomplicated *Plasmodium falciparum* malaria in northern KwaZulu-Natal

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Abstract

Background
Recent malaria epidemics in KwaZulu-Natal indicate that effective anti-malarial therapy is essential for malaria control. Although artemether-lumefantrine has been used as first-line treatment for uncomplicated Plasmodium falciparum malaria in northern KwaZulu-Natal since 2001, its efficacy has not been assessed since 2002. The objectives of this study were to quantify the proportion of patients treated for uncomplicated P. falciparum malaria with artemether-lumefantrine who failed treatment after 28 days, and to determine the prevalence of molecular markers associated with artemether-lumefantrine and chloroquine resistance.

Methods
An observational cohort of 49 symptomatic patients, diagnosed with uncomplicated P. falciparum malaria by rapid diagnostic test, had blood taken for malaria blood films and P. falciparum DNA polymerase chain reaction (PCR). Following diagnosis, patients were treated with artemether-lumefantrine (Coartem®) and invited to return to the health facility after 28 days for repeat blood film and PCR. All PCR P. falciparum positive samples were analysed for molecular markers of lumefantrine and chloroquine resistance.

Results
Of 49 patients recruited on the basis of a positive rapid diagnostic test, only 16 were confirmed to have P. falciparum by PCR. At follow-up, 14 were PCR-negative for malaria, one was lost to follow-up and one blood specimen had insufficient blood for a PCR analysis. All 16 with PCR-confirmed malaria carried a single copy of the multi-drug resistant (mdr1) gene, and the wild type asparagine allele mdr1 codon 86 (mdr1 86N). Ten of the 16 samples carried the wild type haplotype (CVMNK) at codons 72-76 of the chloroquine resistance transporter gene (pfcrt); three samples carried the resistant CVIET allele; one carried both the resistant and wild type, and in two samples the allele could not be analysed.
Conclusions

The absence of *mdrl* gene copy number variation detected in this study suggests lumefantrine resistance has yet to emerge in KwaZulu-Natal. In addition, data from this investigation implies the possible re-emergence of chloroquine-sensitive parasites. Results from this study must be viewed with caution, given the extremely small sample size.

Recommendations

A larger study is needed to accurately determine therapeutic efficacy of artemether-lumefantrine and resistance marker prevalence. The high proportion of rapid diagnostic test false-positive results requires further investigation.
Declaration

I. Charles Hervey Vaughan-Williams declare that

I. The research reported in this dissertation, except where otherwise indicated, is my original research.

II. This dissertation has not been submitted for any degree or examination at any other university.

III. This dissertation does not contain other persons’ data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

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Presentations arising from research

22 August 2012: Malaria meeting with WHO representatives, Ghost Mountain Inn, Mkuze, KZN. Presentation: ‘Assessment of the therapeutic efficacy of artemether-lumefantrine in the treatment of uncomplicated *Plasmodium falciparum* malaria in northern KwaZulu-Natal’


Manuscript in press

### Acronyms and Abbreviations

<table>
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<tr>
<th>Acronym</th>
<th>Definition</th>
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<tbody>
<tr>
<td>ACT</td>
<td>Artemisinin-based combination therapy</td>
</tr>
<tr>
<td>AL</td>
<td>Artemether-lumefantrine</td>
</tr>
<tr>
<td><em>crt</em> gene</td>
<td>Chloroquine resistance transporter gene</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>HRP</td>
<td>Histidine-rich protein</td>
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<tr>
<td>kg</td>
<td>Kilogram</td>
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<tr>
<td><em>mdr</em> gene</td>
<td>Multi-drug resistance gene</td>
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<tr>
<td><em>P. falciparum</em></td>
<td><em>Plasmodium falciparum</em></td>
</tr>
<tr>
<td><em>pfcrt</em> gene</td>
<td><em>Plasmodium falciparum</em> chloroquine resistance transporter gene</td>
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<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>qPCR</td>
<td>Quantitative real-time polymerase chain reaction</td>
</tr>
<tr>
<td>RDT</td>
<td>Rapid diagnostic test</td>
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<tr>
<td>SP</td>
<td>Sulfadoxine-pyrimethamine</td>
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<tr>
<td>UKZN</td>
<td>University of KwaZulu-Natal</td>
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<td>WHO</td>
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CHAPTER I: INTRODUCTION

Background

Artemether-lumefantrine (AL) has been used as the first line treatment of uncomplicated *Plasmodium falciparum* malaria in northern KwaZulu-Natal since 2001 [1, 2] and is recommended for this purpose by the South African Department of Health [3]. The recent history of malaria epidemics in KwaZulu-Natal indicates that the therapeutic effectiveness of the anti-malarial medication used to treat patients suffering malaria is vital for malaria control and must be regularly assessed. Failure to do so risks the undetected emergence of antimalarial drug resistance, contributing to a malaria epidemic, which has major public health and economic consequences for the area, province and even country. A study of the effectiveness of AL should either confirm the continuing efficacy of the medicine, or provide a warning as to the acquisition of resistance by malaria parasites and the need to seek an alternative therapy before an epidemic occurs.

**Recent history of *P. falciparum* antimalarial drug resistance in KwaZulu-Natal**

South Africa, along with neighbouring Namibia, Botswana, Zimbabwe and Swaziland, is classified by the World Health Organisation (WHO) as a low malaria transmission country [4]. Most of South Africa’s population is not at risk from malaria, however malaria remains endemic mainly along the north-eastern border with Mozambique and Swaziland [4]. Mozambique is classified as a high malaria transmission country, with most of the population at high risk of malaria [4]. The Province of KwaZulu-Natal borders on both Mozambique and Swaziland, and malaria risk areas may be seen in figure 1 [5].
Chloroquine resistance was first detected in South Africa in 1985 [6]. In 1988, due to the emergence and spread of chloroquine-resistant *P. falciparum* malaria, sulfadoxine-pyrimethamine (SP) replaced chloroquine as the first-line treatment for uncomplicated *P. falciparum* malaria in KwaZulu-Natal [1, 7, 8]. This drug remained effective until 1996 when malaria incidence increased dramatically. Between 1996 and 2000, northern KwaZulu-Natal suffered increasingly severe malaria epidemics with more than 40,000 malaria notifications in 2000 [9]. Only in 2000 did a clinical study show that *P. falciparum* parasites in the region had developed resistance to sulfadoxine-pyrimethamine, the then recommended first-line treatment for malaria, rendering it largely ineffective in northern KwaZulu-Natal [7]. Subsequently the change of first-line medication to artemether-lumefantrine (AL), together with change of insecticide used for residual house spraying to DDT, dramatically reduced malaria incidence in northern KwaZulu-Natal [1, 2]. It has been estimated that the delay in changing first-line
treatment for malaria between 1996 and 2000 was responsible for substantial morbidity and mortality, as well as contributing to the size of the epidemic [10]. Artemisinin based combination therapy (ACT) is recommended for the treatment of uncomplicated *P. falciparum* malaria by the World Health Organization, and artemether-lumefantrine is one recommended combination [4, 8]. Artemether-lumefantrine is recommended for the treatment of uncomplicated *P. falciparum* by the South African Department of Health [3]. Studies of the therapeutic efficacy of artemether-lumefantrine in 2001 and 2002 indicated that AL was effective for treating uncomplicated malaria in northern KwaZulu-Natal [1, 11, 12].

**Statement of problem**

Since 2002 there have been no further studies in KwaZulu-Natal, or South Africa, of the continuing therapeutic effectiveness of AL in the treatment of uncomplicated malaria.

**What needs to be known?**

The WHO recommends routinely monitoring antimalarial resistance at least every three years, and a change in antimalarial medicine if the treatment failure proportion \( \geq 10\% \) after follow-up for at least 28 days [4, 8].

**Purpose of research**

For these reasons it was considered that an assessment of the efficacy of AL in KwaZulu-Natal was overdue and required in the interests of public health.

**Specific objectives**

The specific objectives of the study are: to quantify the proportion of patients treated with artemether-lumefantrine, who fail to clear *P. falciparum* from their blood 28 days after treatment; test for molecular markers of malarial drug resistance to AL and chloroquine; publish the results, and use the results to inform policy as to the best choice of drug for the treatment for uncomplicated malaria in Umkhanyakude Health District.
CHAPTER II: LITERATURE REVIEW

Purpose of literature review

The purpose of this literature review is to provide an understanding of the present situation with regards to malaria drug resistance in KwaZulu-Natal and the need for drug efficacy testing. For this the author felt that the history of antimalarial medication and malaria control should be included, as well as an outline of the biology of malaria which is necessary to understand some of the challenges faced in controlling the disease. Any discussion which involves malaria control must include reference to vector control as well as antimalarial medication.

Introduction

According to the WHO World Malaria Report, in 2010 there were 216 million episodes of malaria and 655 000 deaths from malaria worldwide [4]. Of these, 81% of the malaria cases and 91% of the deaths were in Africa [4]. The Institute for Health Metrics and Evaluation estimates a higher global mortality of 1,2 million of which 1,1 million were in Africa [13]. Resistance of Plasmodium falciparum malaria, the most virulent form of the disease, to antimalarial drugs has been one of the main obstacles to malaria control in the world and South Africa [6, 14].

Biology of malaria

Malaria is caused by a single-cell protozoan parasite called Plasmodium [15]. There are now five kinds of malaria described that infect humans: Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, Plasmodium ovale, and Plasmodium knowlesi, which was recently described in Borneo in 2009 [16, 17].

Most deaths are caused by Plasmodium falciparum, the other types rarely causing a fatal illness [15].
Malaria has a complicated life-cycle involving humans and the female *Anopheles* mosquito [18]. Malaria is transmitted to humans through the bite of an infected female *Anopheles* mosquito. The malaria parasites are first transported to the liver, where they multiply, and then to the blood stream where they multiply further and destroy blood cells. If an infected human is then bitten by an uninfected mosquito, the ingested parasites then develop in the mosquito, taking 10-18 days to mature and appear in the mosquito’s salivary glands, and are then able to infect a new human subject bitten by the mosquito [18]. The lifespan of a mosquito is similar to the time taken for malaria parasites to mature in the mosquito. In colder weather the lifespan is shortened preventing maturation of the malaria parasites, hence malaria transmission occurs mainly in warm climates [19].

**Brief history of malaria – World**

Symptoms of malaria have been described for thousands of years [18]. There have been a number of important events contributing to the understanding of the biology of malaria. In 1880 Laveran discovered the malaria parasite. In 1886 Golgi discovered different forms of the disease, with different periodicity of fevers; in 1890 Grassi and Filetti named *Plasmodium vivax* and *Plasmodium malariae* parasites; in 1897 Welch named the *Plasmodium falciparum* parasite; and in 1897 Ross demonstrated that malaria is transmitted by mosquitoes [18].

**Important steps in the treatment and prevention of malaria**

**Treatment**

There have been a number of significant historical steps in the treatment and prevention of malaria. In China in 340 AD, the Qinghao plant or *Artemesia annua* was recognized as a treatment for fever [18]. In 1971 the antimalarial drug artemesinin was isolated by Chinese scientists, and today derivatives of artemesinin are used in many antimalarial medications throughout the world, often in combination with other drugs [18].
In Peru in 17th Century the bark of the Chinchona or quina tree was introduced to Jesuit missionaries as a treatment for fevers by the indigenous people [18]. In 1820 quinine was isolated from the bark by Pierre Pelletier and Joseph Caventou and became the standard treatment for malaria, until the development of chloroquine in 1946, which then became the preferred drug for treatment of malaria [18, 20, 21].

From 1946, chloroquine became increasingly used as a cheap effective antimalarial with few side effects. By the 1960s it was widely available over the counter throughout Africa [22].

**Insecticide**

Dichloro-diphenyl-trichloroethane (DDT) was first synthesised in 1874, however the insecticidal properties of the compound were only discovered in 1939. DDT was first used for malaria control during the Second World War by the Allies [18].

The Global Eradication Campaign was launched by the WHO in 1955 using insecticide house spraying, antimalarial treatment and surveillance [18, 23]. It was successful in eradicating malaria from the more temperate countries with seasonal malaria, but less successful in most of the tropical endemic malarious countries. Often, an initial reduction in malaria cases was followed by a rebound epidemic due to reduction of population immunity to malaria [22].

**History of antimalarial drug resistance - World**

Malaria parasite resistance to antimalarial drugs, as well as mosquito insecticide resistance, has been a major obstacle to malaria control worldwide, and remains so to this day [4, 6, 23].

Pyrimethamine parasite resistance was noted in Kenya in the 1950s following regular administration of the drug to villagers as part on an eradication programme, after which use of the drug was stopped [23]. Between 1960 and 1962, the first reports of chloroquine resistant malaria parasites came from South America and South East Asia [23]. By 1973 chloroquine resistance had been reported more widely. The first
chloroquine-resistant parasites from Africa were reported in Kenya and Tanzania in 1978 [21]. In subsequent years chloroquine resistance spread throughout Africa and the world, such that by 1989 almost all malarious countries had chloroquine-resistant *P. falciparum* [21, 23].

Sulfadoxine-pyrimethamine (SP) replaced chloroquine as first-line malaria treatment in a number of countries – starting with Thailand in 1973, and including KwaZulu-Natal, South Africa in 1988, Malawi in 1993, and Kenya and Botswana in 1997; however resistance to SP quickly emerged [21].

Since 1999, artesiminin combination therapy has been recommended as a means of reducing emergence of antimalarial resistance [24]. The mechanism of action of the combination is through a rapid reduction in parasite numbers by artesiminin, and clearance of the remaining parasites by a longer acting combination drug [24]. These combinations are now recommended by the WHO [8]. Artemether-lumefantrine is the recommended treatment for uncomplicated *P. falciparum* malaria by the South African Department of Health [3].

Unfortunately resistance to artesiminin, characterised by slow clearing of the parasite has now been reported from South-East Asia [25], and suggested in Kenya [26].

**History of malaria control and antimalarial drug resistance in South Africa**

**Vector control**

In 1932, the first malaria control measures in South Africa were stimulated by the adverse effect of the disease on sugar production [6]. The first malaria control measures in South Africa were the use of Pyagra (liquid pyrethrum and kerosene) for indoor spraying, which proved effective [6]. Pyagra was replaced by DDT in 1946 [6]. DDT remained the insecticide used the house spraying in northern KwaZulu-Natal until 1996, when DDT was replaced by the pyrethroid insecticide deltamethrin [27]. This change in insecticide was followed by a dramatic increase in the area between 1996 and 2000 [1,
In 2000 deltamethrin resistant, but DDT sensitive *Anopheles funestus* mosquitoes were identified [27]. This discovery led to the reintroduction of DDT house-spraying in northern KwaZulu-Natal [1], which was followed by a dramatic increase in the incidence of malaria in the region [1].

**Antimalarial drug resistance**

Chloroquine resistance was first detected in KwaZulu-Natal in 1985 [6, 28]. This resistance was shown to have increased by 1988 [29] which led to sulfadoxine-pyrimethamine (SP) replacing chloroquine as the first line treatment for uncomplicated *P. falciparum* malaria in KwaZulu-Natal [1, 6]. This drug remained effective until 1996 when malaria incidence sharply increased. Between 1996 and 2000 northern KwaZulu-Natal suffered increasingly severe malaria epidemics with more than 40,000 malaria cases notified in 2000 [1, 2, 9]. Analysis of health institution statistics in northern KwaZulu-Natal indicated more than 60,000 malaria cases treated [1]. Only in 2000 did a clinical study show that *P. falciparum* parasites in the region had developed resistance to sulfadoxine-pyrimethamine, the then recommended first line treatment for malaria, rendering it largely ineffective in northern KwaZulu-Natal [7]. Subsequently the change of first-line medication to artemether-lumefantrine, together with change of insecticide used for residual house spraying to DDT, dramatically reduced malaria incidence in northern KwaZulu-Natal [1, 2]. It has been estimated that the delay in changing first-line treatment for malaria between 1996 and 2000 was responsible for substantial morbidity and mortality, as well as contributing to the size of the epidemic [10]. Artemisinin based combination therapy is recommended for the treatment of uncomplicated *P. falciparum* malaria by the World Health Organization [4], and artemether-lumefantrine (AL) is one of the recommended combinations [8]. Studies of the therapeutic efficacy of artemether-lumefantrine in 2001 and 2002 indicated that AL was effective for treating uncomplicated malaria in northern KwaZulu-Natal [1, 11, 12]. Since 2002, however, there have been no further studies in KwaZulu-Natal, or South Africa, of the continuing therapeutic effectiveness of AL in the treatment of uncomplicated malaria. The WHO recommends routinely monitoring antimalarial resistance at least every three years, and a change in antimalarial medicine if the
treatment failure proportion ≥10% by day 28, or the last day of follow-up if longer than 28 days [8, 30].

**Pharmacology of artemether-lumefantrine**

Artemether-lumefantrine is a combination of artemether and lumefantrine manufactured as Coartem® by Novartis: 20mg artemether and 120mg lumefantrine in tablet form [31]. The two drugs act in a complementary manner [32]. Artemether and its active metabolite, dihydroartemisin, rapidly kill most malaria parasites, while lumefantrine clears the remainder more slowly [32, 33]. As most parasites are killed by artemether, the likelihood of selecting resistant parasites to the partner drug, lumefantrine, is much reduced [32, 33]. Artemether is rapidly absorbed and metabolized, with a half-life of about two hours, whereas lumefantrine is absorbed more slowly and has a half-life of 3-4 days in malaria patients [32, 33].

Coartem® is taken as a six-dose oral regimen over 3 days [31]. The dosage depends upon the weight and age of the patient [31]. The adult dosage for persons aged 12 years or more, or children weighing 35kg and above, is four tablets as a single dose at the time of initial diagnosis, 4 tablets after 8 hours, and then 4 tablets twice daily on each of the following two days [31]. It is recommended that the tablets are taken with fatty food or milk to improve absorption [31-33].

**Recommended protocol and follow-up period for antimalarial efficacy testing**

The WHO protocol for assessment of clinical and parasitological response to antimalarial medication recommends patient assessment on days 0 (enrolment), 2, 3, 7, 14, 21, and 28. A 28 day follow-up is adequate for drugs with an elimination half-life of less than 7 days (such as lumefantrine) [30]. A longer follow-up is needed for drugs with a longer elimination half-life, such as mefloquine [34] and piperaquine [35] which require 42 days of follow-up [30, 36].

Inadequate responses to antimalarial treatment are classified as: ‘Early Treatment
Failure (ETF)’ in which there are danger signs or failure to reduce parasitaemia levels by day 3; ‘Late Clinical Failure (LCF)’ in which there are danger signs or parasitaemia and fever between days 4 and 28, and ‘Late Parasitological Failure (LPF)’ in which there is parasitaemia without fever between days 4 and 28 [30]. ‘Adequate Clinical and Parasitological Response (ACPR)’ is defined as absence of parasitaemia on day 28 in patients who did not suffer any of the other inadequate responses to treatment [30]. It is noted that a longer follow-up period increases the losses, reducing a study’s validity; however no similar comment is made about the number of return visits already required of patients with the present WHO protocols [30]. From these definitions it may be inferred that clinically assessing and testing a subject on day 28 should detect Late Clinical Failure and Late Parasitological Failure.

**Molecular markers of malaria resistance**

According to the 2002 WHO report, molecular markers may assist in clarifying the resistance situation [37]. Markers of resistance have been validated for a number of monotherapies including chloroquine [38] and lumefantrine [39]. The storing of blood samples on filter paper for future testing as new molecular markers become available is recommended [37]. Molecular markers may provide warning of developing resistance to an antimalarial drug in use in an area and give an indication of the persistence of resistance to a drug that has been withdrawn from an area [37].

At present the genetic basis for artemisinin tolerance or resistance has not been elucidated, leaving delayed parasite clearance times as the only clear determinant of artemisinin tolerance and resistance [40], and molecular markers of lumefantrine resistance as the only markers for resistance for AL.

Certain molecular markers are linked with resistance to lumefantrine, the partner drug in AL, specifically the *pfmdr1* copy number [39], and the *mdr1*86N allele at *pfmdr1* codon 86 [41, 42].
Use of molecular markers to distinguish between re-infection and recrudescence

A polymerase chain reaction (PCR) test is recommended as mandatory by the WHO to distinguish between *P. falciparum* recrudescence and re-infection 7 days or more after treatment in areas of both low to moderate, and high, transmission [30].

Genotyping of *P. falciparum* DNA extracted from dried blood spots based on variations in the genes coding for the glutamine-rich protein and merozoite surface protein 1 and 2 (msp-1, msp-2), may be used to distinguish between different strains of *P. falciparum* [43, 44]. The banding patterns of the three molecular markers may be compared before and after treatment, and if *P. falciparum* markers are still present, determine if treatment failure is due to a re-infection or recrudescence of the original infection.
CHAPTER III: PAPER Accepted FOR PUBLICATION IN ‘MALARIA JOURNAL’

The following research paper has been submitted to the journal: ‘Malaria Journal’ to be considered for publication.

Title
Assessment of the therapeutic efficacy of artemether-lumefantrine in the treatment of uncomplicated Plasmodium falciparum malaria in northern KwaZulu-Natal: an observational cohort study

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Abstract

Background
Recent malaria epidemics in KwaZulu-Natal indicate that effective anti-malarial therapy is essential for malaria control. Although artemether-lumefantrine has been used as first-line treatment for uncomplicated Plasmodium falciparum malaria in northern KwaZulu-Natal since 2001, its efficacy has not been assessed since 2002. The objectives of this study were to quantify the proportion of patients treated for uncomplicated P. falciparum malaria with artemether-lumefantrine who failed treatment after 28 days, and to determine the prevalence of molecular markers associated with artemether-lumefantrine and chloroquine resistance.

Methods
An observational cohort of 49 symptomatic patients, diagnosed with uncomplicated P. falciparum malaria by rapid diagnostic test, had blood taken for malaria blood films and P. falciparum DNA polymerase chain reaction (PCR). Following diagnosis, patients were treated with artemether-lumefantrine (Coartem®) and invited to return to the health facility after 28 days for repeat blood film and PCR. All PCR P. falciparum positive samples were analysed for molecular markers of lumefantrine and chloroquine resistance.

Results
Of 49 patients recruited on the basis of a positive rapid diagnostic test, only 16 were confirmed to have P. falciparum by PCR. At follow-up, 14 were PCR-negative for malaria, one was lost to follow-up and one blood specimen had insufficient blood for a PCR analysis. All 16 with PCR-confirmed malaria carried a single copy of the multi-drug resistant (mdr1) gene, and the wild type asparagine allele mdr1 codon 86 (mdr1 86N). Ten of the 16 samples carried the wild type haplotype (CVMNK) at codons 72-76 of the chloroquine resistance transporter gene (pfcrt): three samples carried the resistant CVIET allele; one carried both the resistant and wild type, and in two samples the allele could not be analysed.
Conclusions

The absence of \textit{mdr1} gene copy number variation detected in this study suggests lumefantrine resistance has yet to emerge in KwaZulu-Natal. In addition, data from this investigation implies the possible re-emergence of chloroquine-sensitive parasites. Results from this study must be viewed with caution, given the extremely small sample size. A larger study is needed to accurately determine therapeutic efficacy of artemether-lumefantrine and resistance marker prevalence. The high proportion of rapid diagnostic test false-positive results requires further investigation.

Key words: \textit{Plasmodium falciparum} malaria, artemether, lumefantrine, therapeutic efficacy, resistance markers, KwaZulu-Natal
Background

The World Health Organization (WHO) has recommended that drug efficacy be regularly assessed [1, 2]. Failure to detect the emergence of anti-malarial drug resistance, could lead to a drug-resistant malaria epidemic, which would have major public health and economic consequences for an area, province and country. The most recent malaria epidemics in KwaZulu-Natal, one of three provinces in South Africa with endemic malaria, were partially attributed to unrecognized resistance to the anti-malarial therapy being used at the time [3]. Artemether-lumefantrine (AL) has been first-line treatment of uncomplicated Plasmodium falciparum malaria in northern KwaZulu-Natal since it was introduced in response to these drug-resistant epidemics in 2001 [4, 5]. Studies should be performed to confirm the continued efficacy of AL, or provide a warning of emerging resistance, and the need to seek alternative therapy before a malaria epidemic occurs.

Recent history of Plasmodium falciparum anti-malarial drug resistance in KwaZulu-Natal

Chloroquine resistance was first detected in KwaZulu-Natal in 1985 [6], and had increased by 1988 [7], leading to sulfadoxine-pyrimethamine (SP) replacing chloroquine as the first-line treatment for uncomplicated P. falciparum malaria in KwaZulu-Natal [4, 8, 9]. SP remained effective until 1996 when malaria incidence increased sharply in KwaZulu-Natal. Between 1996 and 2000 northern KwaZulu-Natal suffered increasingly severe malaria epidemics, with more than 40,000 cases reported in 2000 [4, 5, 10].

Only in 2000, were P. falciparum parasites in the region shown to have developed at least 61% (and as high as 89%, excluding those lost to follow-up) resistance to SP in a clinical efficacy study, rendering the drug ineffective in northern KwaZulu-Natal [8]. The introduction of AL as the first-line medication for uncomplicated P. falciparum malaria, together with the reintroduction of DDT insecticide for indoor residual house spraying in 2001, dramatically reduced malaria incidence in the area [4, 5]. It has been estimated that the delay in changing first-line treatment for malaria between 1996 and 2000 was responsible for substantial morbidity and mortality, as well as contributing to
the size of the epidemic [3]. Malaria notifications in KwaZulu-Natal between 1991 and 2011 are shown (Figure 1).

![Graph showing malaria notifications in KwaZulu-Natal from 1991 to 2011.](image)

**Figure 2:** Malaria notifications in KwaZulu-Natal from 1991 to 2011.


It has been estimated that in 2000, at the height of the epidemic, the malaria incidence amongst the exposed population in northern KwaZulu-Natal was 5,972 per 100,000 [3]. It should be noted that the malaria notification system became overloaded during these epidemics, and that the notifications were incomplete. For example during the year 2000 one clinic, Ndumo Clinic, in northern KwaZulu-Natal, saw 30,885 cases based on laboratory results, a 50-fold increase compared to 1995 [5], and equivalent to 73% of the total provincial notifications of 42,248 [10].

Artemisinin-based combination therapy (ACT) is advocated for the treatment of uncomplicated *P. falciparum* malaria because of the rapid reduction in parasite load caused by artemisinin or its derivative; the consequent reduced likelihood of resistance
emerging to the partner drug; the reduction in gametocyte carriage, and rapid clinical response [11]. ACT is recommended by WHO for the treatment of *P. falciparum* malaria [12], and AL is one of the recommended combinations [2, 12]. Studies of AL therapeutic efficacy in northern KwaZulu-Natal during 2001 and 2002 indicated that AL was effective for treating uncomplicated malaria in the area [4, 13, 14]. Since 2002, there have been no further studies of the continuing therapeutic efficacy of AL in KwaZulu-Natal, or South Africa. The WHO recommends routinely monitoring anti-malarial resistance at least every three years, and a change in anti-malarial medicine if the treatment failure proportion is equal to or greater than 10% by day 28, or the last day of follow-up, if longer than 28 days [1, 12].

**Pharmacology of artemether-lumefantrine**

Artemether-lumefantrine is a combination of two drugs, artemether and lumefantrine, manufactured in tablet form as Coartem® by Novartis. Each tablet contains 20 mg artemether and 120 mg lumefantrine [15]. The two drugs act in an independent but complementary manner at different stages of the parasite life cycle [16]. Artemether and its active metabolite, dihydroartemisinin, rapidly kill most circulating malaria parasites, while lumefantrine clears the remainder more slowly [16, 17]. The probability of selecting parasites resistant to the partner drug, lumefantrine, is theoretically reduced due to the small parasite load remaining following activity of artemether [16, 17]. Artemether is rapidly absorbed and metabolized, with a half-life of about two hours, whereas lumefantrine is absorbed more slowly and has a half-life of 3-4 days in malaria patients [16, 17].

Coartem® is taken as a six-dose oral regimen over three days. The dosage depends mainly upon the weight of the patient. The dosage for persons aged 12 years or more, or younger children weighing 35 kg and above, is four tablets as a single dose at the time of initial diagnosis, four tablets after eight hours, and then four tablets twice daily on each of the following two days [15]. It is recommended that the tablets are taken with fatty food or milk to improve absorption [15, 17, 18].
Choice of follow-up period for anti-malarial efficacy testing

In the 2009, WHO anti-malarial drug efficacy testing guide [1], inadequate responses to anti-malarial treatment are classified as: ‘early treatment failure’ in which there are danger signs or failure to reduce parasitaemia levels by day 3; ‘late clinical failure’ in which there are danger signs or parasitaemia and fever occurring between days 4 and 28, and ‘late parasitological failure’ in which there is parasitaemia without fever between days 4 and 28. ‘adequate clinical and parasitological response’ is the absence of parasitaemia on day 28 (or day 42 for longer acting drugs), irrespective of axillary temperature, in patients who did not previously meet any of the criteria of early treatment failure, late clinical failure or late parasitological failure. For drugs with a half-life of less than seven days, such as artemisinin and lumefantrine, evaluation of clinical and parasitological response up to 28 days is recommended [1]. For those with longer half-lives such as mefloquine (three weeks [19]) and piperaquine (two to three weeks [20]), a follow-up of 42 days is recommended [1, 12, 21].

Although there has been no anecdotal evidence of resistance to AL in KwaZulu-Natal since its implementation, artemisinin resistance, characterised by slow clearing of parasite has been confirmed in South East Asia [22], and suggested in Kenya [23]. Previous research by Roper and colleagues demonstrated that SP resistance spread to southern Africa from East Africa [24]. In neighbouring Mozambique increase in prevalence of molecular markers associated with lumefantrine resistance since initial use of AL suggest the need for continued surveillance for the emergence of resistance to the drug [25]. The primary objective of this study was to screen for late AL clinical and parasitological failure, the first indication of emerging resistance to AL in South Africa.

Requiring patients to return a clinic six or seven times in one month for assessment requires considerable resources and the risk of drop-out from the study is high. A single follow-up assessment at 28 days was therefore chosen which required a patient to return only once. PCR is recommended by the WHO to distinguish between P. falciparum recrudescence and re-infection seven days or more after treatment in areas of both low to moderate, and high, transmission [1].
Molecular markers of malaria resistance

According to the 2002 WHO report [26], molecular markers may assist in determining resistance and provide an early warning of developing drug resistance before it becomes clinically apparent. Markers of resistance have been validated for a number of monotherapies, including chloroquine [27] and lumefantrine [28]. As the genetic basis for artemisinin resistance is not known, efficacy of AL is assessed by determining the molecular markers of resistance for the partner drug, lumefantrine. Certain molecular markers have been linked with resistance to lumefantrine, the partner drug in AL, namely the *P. falciparum* multidrug resistant (mdr)1 gene copy number [28], and the mdr186N allele [29, 30]. Multiple copies of the mdr1 gene has been linked with lumefantrine resistance in Southeast Asia [28], while mutations at the codon 86 of the mdr1 gene modulate lumefantrine efficacy [31].

Storage of blood samples on filter paper for future testing as new molecular markers become available is recommended [26].

Ethical issues

The study was approved by the University of KwaZulu-Natal Biomedical Research Ethics Committee, and by the Health Research Committee of the KwaZulu-Natal Department of Health.

Methods

The study population included symptomatic persons presenting to health facilities, diagnosed with uncomplicated *P. falciparum* malaria in Umkhanyakude Health District, northern KwaZulu-Natal, using the *P. falciparum* malaria rapid diagnostic test (First Response, malaria antigen *P. falciparum* (HRP2) detection rapid card test manufactured by Premier Medical Corporation Limited, Kachigam, Daman (UT) 396215, India).

Inclusion criteria

Symptomatic patients aged from five years to 69 years, self presenting to health facilities, diagnosed with uncomplicated malaria in Umkhanyakude Health District between January and May 2012, were invited to participate in the study.
Exclusion criteria

Patients with the following danger signs or symptoms of severe malaria: unable to drink; vomiting everything; a convulsion during previous seven days; lethargic or decreased level of consciousness; unable to stand or sit [32], were excluded. Pregnant women, patients aged less than five years and more than 69 years, and patients treated for malaria during the previous two weeks were also excluded.

Information provided

At recruitment patients were provided with an information sheet in English and isiZulu detailing the purpose of the study, which was also explained verbally. Patient queries were answered after which they were invited to provide written consent.

Investigations

Finger-prick blood spots blotted on to Guthrie 903 filter paper cards (Munktell GmbH, Barenstien, Germany), and blood samples were collected from all participants for molecular analysis and malaria microscopy. The patient was then asked to return in four weeks for repeat malaria film microscopy and blood spot collection, with the offer of ZAR50 (US$5.79) in travelling expenses upon return. RDT was not performed at follow-up due to persistence of histidine-rich protein, HRP-2, in patients for as long as 28 days after parasite clearance [14, 33]. Blood spots taken by nurses were sent to the investigator at the local hospital. These were then collected by the Principal Investigator, usually twice per month, and posted to the researcher performing the molecular analysis more than 400 km away.

Thick and thin blood films were prepared according to the National Health Laboratory Service standard operating procedure for processing specimens for malaria parasites [34]. Slides were stained using the rapid modified Wright-Giemsa stain (Rapidiff); thin films being fixed with methanol before staining. Parasitaemia was calculated from the percentage of red cells containing malaria parasites observed in 10 microscope fields using the 100x lens.

Parasite DNA was extracted from all blood spots using the QIAamp DNA Mini Kit (QIAGEN, Whitehead Scientific). The extracted DNA was then subjected to
quantitative real-time PCR (qPCR) and nested PCR analysis to confirm the presence of *P. falciparum* parasites [35, 36].

All samples confirmed as *P. falciparum* positive by PCR, were subjected to mutational analysis to detect the prevalence of molecular markers linked with resistance to lumefantrine, (*mdr1* gene copy number amplification) and chloroquine (mutations at *mdr1* codon 86, [27] and codons 72 to 76 of the chloroquine resistance transporter (*crt*) gene ) [37]. At the follow-up visit, the patient was clinically assessed, and a further finger prick blood sample taken for *P. falciparum* PCR and blood film.

**Results**

A total of 49 patients with a diagnosis of malaria based on a rapid diagnostic test were enrolled in the study. Two patients did not have their age recorded. The age range of the remaining 47 patients was 2 – 69 years; median 15 years, and mean 21.1 years. The largest group comprised those less than 10 years of age (Figure 2). Four patients were less than minimum age of five years stipulated in the study protocol. Their treatment, however, was identical to that in the National Treatment Guidelines [38], and they were included in the analysis.
Figure 3: Age and gender of patients with malaria RDT-positive recruited from Umkhanyakude Health District, January to May 2012 (N=47)
Confirmation of *Plasmodium falciparum* malaria by PCR

Only 33% (16/49) patients were confirmed to have *P. falciparum* malaria by PCR (Figure 3).

Closer inspection of the RDTs revealed that frequently too much blood had been used, rendering the test virtually impossible to interpret. The age range of those confirmed with *P. falciparum* was 2 - 40 years with median age 14.5 years and mean 16.8 years. Nearly half (7/16) of those with PCR-confirmed malaria were aged less than 10 years. Results for the *P. falciparum* PCR positive patients are summarized in Table 1.
Table 1: Results for patients with PCR confirmed *Plasmodium falciparum* malaria at enrolment

<table>
<thead>
<tr>
<th>Age</th>
<th>Gender</th>
<th>Travel 60 days prior to illness</th>
<th>Temp</th>
<th>mdr1 copy number (lumefantrine sensitivity)</th>
<th>mdr1N86Y gene (chloroquine sensitivity)</th>
<th>Chloroquine resistance transporter gene crt K76T codons 72-76</th>
<th>Follow-up day</th>
<th>Follow-up <em>P. falciparum</em> malaria PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>Female</td>
<td>Mozambique</td>
<td>36.5</td>
<td>1 (sensitive) Asparagine (mdr1 86N - sensitive)</td>
<td>CVMNK(sensitive)</td>
<td></td>
<td>28</td>
<td>Negative</td>
</tr>
<tr>
<td>7</td>
<td>Male</td>
<td>Not recorded</td>
<td>39.4</td>
<td>1 asparagine</td>
<td>CVMNK and CVIET(resistant)</td>
<td></td>
<td>191</td>
<td>Negative</td>
</tr>
<tr>
<td>32</td>
<td>Female</td>
<td>None</td>
<td>36.4</td>
<td>1 asparagine</td>
<td>CVIET</td>
<td></td>
<td>168</td>
<td>Insufficient sample</td>
</tr>
<tr>
<td>24</td>
<td>Male</td>
<td>None</td>
<td>39.3</td>
<td>1 asparagine</td>
<td>CVMNK</td>
<td></td>
<td>49</td>
<td>Negative</td>
</tr>
<tr>
<td>9</td>
<td>Female</td>
<td>Not recorded</td>
<td>Not recorded</td>
<td>1 asparagine</td>
<td>CVMNK</td>
<td></td>
<td>29</td>
<td>Negative</td>
</tr>
<tr>
<td>31</td>
<td>Male</td>
<td>Mozambique</td>
<td>38.3</td>
<td>1 asparagine</td>
<td>CVMNK</td>
<td></td>
<td>32</td>
<td>Negative</td>
</tr>
<tr>
<td>8</td>
<td>Female</td>
<td>None</td>
<td>36.7</td>
<td>1 asparagine</td>
<td>CVIET</td>
<td></td>
<td>29</td>
<td>Negative</td>
</tr>
<tr>
<td>15</td>
<td>Male</td>
<td>Mozambique</td>
<td>38.0</td>
<td>1 asparagine</td>
<td>CVIET</td>
<td></td>
<td>106</td>
<td>Negative</td>
</tr>
<tr>
<td>20</td>
<td>Male</td>
<td>Mozambique</td>
<td>41.0</td>
<td>1 asparagine</td>
<td>CVMNK</td>
<td></td>
<td>127</td>
<td>Negative</td>
</tr>
<tr>
<td>37</td>
<td>Female</td>
<td>Not recorded</td>
<td>38.7</td>
<td>1 asparagine</td>
<td>CVMNK</td>
<td></td>
<td>86</td>
<td>Negative</td>
</tr>
<tr>
<td>6</td>
<td>Female</td>
<td>None</td>
<td>39.0</td>
<td>1 asparagine</td>
<td>CVMNK</td>
<td></td>
<td>28</td>
<td>Negative</td>
</tr>
<tr>
<td>2</td>
<td>Female</td>
<td>Mozambique</td>
<td>Not recorded</td>
<td>1 asparagine</td>
<td>CVMNK</td>
<td>NA</td>
<td>Lost to follow-up; returned to Mozambique</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Male</td>
<td>None</td>
<td>38.2</td>
<td>1 asparagine</td>
<td>CVMNK</td>
<td></td>
<td>69</td>
<td>Negative</td>
</tr>
<tr>
<td>14</td>
<td>Female</td>
<td>None</td>
<td>38.0</td>
<td>1 asparagine</td>
<td>Not obtained</td>
<td></td>
<td>92</td>
<td>Negative</td>
</tr>
<tr>
<td>4</td>
<td>Female</td>
<td>Mozambique</td>
<td>38.0</td>
<td>1 asparagine</td>
<td>CVMNK</td>
<td></td>
<td>59</td>
<td>Negative</td>
</tr>
<tr>
<td>3</td>
<td>Male</td>
<td>Not recorded</td>
<td>38.0</td>
<td>1 asparagine</td>
<td>Not obtained</td>
<td></td>
<td>57</td>
<td>Negative</td>
</tr>
</tbody>
</table>

**Presence of fever**

Fever (auxiliary temperature ≥37.5°C) was recorded in 64% (27/42) of all patients who were initially diagnosed with malaria and 77% (11/14) of the PCR malaria confirmed cases, while 57% (16/28) of those PCR negative had a fever. Temperature was not recorded for two malaria PCR positive patients.
Recent travel
Six of the 16 PCR malaria-confirmed cases reported having travelled to Mozambique within the previous month.

Blood films
Due to liaison difficulties with a local laboratory, blood films were only obtained in 15/49 recruited patients, examined by the hospital laboratory technicians. Of those, only four were PCR-confirmed malaria samples, and of those four, one was *P. falciparum* positive by microscopy with a parasitaemia of 0.25%. All other 14 blood films were microscopy-negative for *P. falciparum*.

Molecular markers
All 16 PCR *P. falciparum* positive samples collected at enrolment had a single copy of the *mdr1* gene and carried the wild type asparagine allele at codon 86 of the *mdr1* gene (*mdr1* 86N) (Table 1). Results for 14 of the 16 samples were *crt* 72-76 genotyped. Ten samples carried the wild chloroquine sensitive haplotype (CVMNK), three the pure mutant haplotype (CVIET) associated with chloroquine treatment failure, while one carried both wild and mutant alleles. Two samples proved inadequate for *crt* genotyping.

Follow-up
Only 14% (7/49) of the patients returned for follow-up, of which six were *P. falciparum* PCR positive at enrolment. Malaria control personnel tracked down and obtained blood specimens for follow-up PCR from a further nine non-returning PCR-confirmed patients, at varying intervals from four weeks following recruitment. Of the initial PCR positive cohort, 14 were found to be negative at follow-up, one sample contained insufficient blood for testing and one patient was lost to follow-up.
Discussion

The primary aim of this study was to assess the therapeutic efficacy of AL, the current first-line treatment of uncomplicated *P. falciparum* malaria in northern KwaZulu-Natal. The study was hampered by a scarcity of diagnosed malaria cases. Of 49 patients enrolled in the study on the basis of a positive *P. falciparum* rapid diagnostic test, only 16 were subsequently confirmed to have *P. falciparum* malaria by PCR analysis.

Amplification of *mdr1* gene copy number associated with lumefantrine resistance in South East Asia was not detected in this study [39]. This result confirms the research findings in neighbouring Mozambique [25], where no variation in *mdr1* copy number was observed following two years of AL deployment, and supports the hypothesis that *mdr1* amplification is rare in Africa [31].

The *mdr1*N86Y mutation associated with chloroquine resistance [27] was completely absent in this study. This finding together with the high prevalence of the *crt 72-76* wild type haplotype (CVMNK) in the study area suggests AL deployment removed chloroquine drug pressure, allowing chloroquine sensitive parasites to re-emerge as seen in Malawi [40] and Mozambique [25]. This return of parasite sensitivity to chloroquine could result in the re-introduction of chloroquine in combination with a partner drug as an anti-malarial.

All follow-up samples were PCR negative for *P. falciparum*, implying sustained AL efficacy, 11 years after it was initially rolled out in KwaZulu-Natal. On a cautionary note, the high *mdr186N* allele prevalence is a cause for some concern. It has been suggested that increases in *mdr186N* prevalence is the first step towards lumefantrine tolerance [30]. Sustained lumefantrine drug pressure is probably driving the selection of the *mdr186N* allele in KwaZulu-Natal. In contrast, the removal of chloroquine drug pressure probably selected for this allele in Mozambique [25]. Given the wide use of AL in southern Africa and the high prevalence of resistance markers associated with lumefantrine resistance, close monitoring of AL efficacy and lumefantrine resistance markers is recommended to ensure effective first-line treatments are available.
Positive study outcomes

Despite the extremely small sample, some valuable data was produced by this study.

Blood spots for molecular analysis

In a low malaria-incidence setting, blood spot samples proved to be a good source for molecular analysis. Collection of the filter paper samples was relatively easy and inexpensive. Once collected and correctly stored the samples were resilient to delays in collection and transport. PCR is capable of detecting malaria parasites at a density of five parasites per microlitre, whereas thick film microscopy is only reliable at a density of 50 parasites per microlitre, meaning PCR is more than ten times more sensitive than microscopy for the diagnosis of malaria parasitaemia [41, 42]. PCR also has a specificity of nearly 100% for both *P. falciparum* and *P. vivax* [43].

Monitoring molecular markers of drug resistance, while a less rigorous method of assessing drug efficacy than *in vivo* sensitivity studies, is much less expensive and time consuming, and is a reasonable method of surveillance for emerging drug resistance [25]. The WHO recommends that in countries with very low levels of transmission, such as South Africa, studies of molecular markers of resistance should be conducted every year [1]. Molecular markers in 10 of the 14 available blood specimens indicated sensitivity to chloroquine, suggesting that chloroquine resistance may have decreased following removal of the selection pressure from using chloroquine as first-line therapy. Similar findings have been demonstrated in neighbouring Mozambique and been attributed to the withdrawal of chloroquine [25].

Study limitations

Enrolment procedure and administration

The incidence of malaria in the study area remained low during the study period and cases were geographically scattered, presenting to several different clinics and hospitals in Umkhanyakude District. Management and control of the patient records and specimens was difficult. The cooperation of healthcare workers from several health facilities was required and consistency of the enrolment procedure was difficult to
achieve. Blood was sent by clinic nurses to the local hospital laboratory which declined to perform blood films on many patients.

Of the 49 recruited patients only 16 were PCR confirmed malaria. The high proportion of false positive RDT results was probably mainly due to incorrect use of the test, indicating a lack of familiarity, and the need for more training. It is possible that false positive RDT results have erroneously inflated the notified malaria cases in the district for some time, and is deserving of further investigation.

**Sample size**

The study aimed to obtain a sample size of 50, which is the minimum recommended by the WHO regardless of rates of failure anticipated, in order to be representative [1]. However, this study could only include the malaria cases available. In an area which suffered severe malaria epidemics within the past 12 years, partly attributable to a lack of parasite resistance data required for upgrading antimalarial treatment policy [3], it is important to undertake regular drug resistance monitoring, or risk repeating the mistakes of the past. The last published malaria resistance studies in KwaZulu-Natal took place in 2002 [4, 14], and the data in this study could be used to inform a larger study.

**Follow-up**

Despite the financial incentive offered to recompense for travelling expenses, most patients had to be tracked down by malaria control personnel at varying time intervals after treatment.

**Use of single follow-up visit**

Use of a single follow-up visit on day 28, rather than follow-up visits on days 1, 2, 3, 7, 14 and 28, as recommended by the WHO [1, 26, 32], meant that in the event of persistence of *P. falciparum* parasitaemia by day 28, it would not be possible to distinguish between early treatment failure, late clinical failure, and late parasitological failure. The finding of persistence of parasitaemia by day 28 would provide a motivation for a further study following the WHO protocol [1] to distinguish the degree of resistance. However, as already mentioned, persuading patients to return for six follow-up visits is not easy, evidenced by the difficulty faced in this study of obtaining even a single follow-up from
patients. The single 28 day follow-up should detect most clinical and treatment failures, and seems particularly suitable for screening for late clinical failure and late parasitological failure.

**Conclusions**

Determining drug efficacy, particularly as malaria transmission approaches zero, is critical, as the last remaining parasites are most likely the most resistant [44]. Since therapeutic efficacy of AL in KwaZulu-Natal had not been assessed recently, this study attempted to address the issue. Unfortunately the extremely low incidence of malaria in northern KwaZulu-Natal impacted negatively on patient recruitment. As drug efficacy data is essential to inform policy, particularly as South Africa embarks on an elimination agenda [45], every attempt to obtain robust valid resistance data must be made. Future options include larger studies across multiple sites, and the follow-up of all malaria cases at 28 days with annual molecular marker studies [1].

Although 49 patients were recruited into the study based on RDT results, only 16 were confirmed *P. falciparum* positive by PCR. Preliminary investigations appear to indicate that incorrect use of RDT was the principal reason for the high proportion of false-positive results. Since definitive diagnosis is a fundamental tenet of the elimination agenda, further investigation into the cause of the false-positive RDT results is indicated, and corrective measures put in place to prevent misdiagnosis.

Despite the small sample size, all samples were malaria negative at Day 28, or longer, suggesting sustained AL efficacy in KwaZulu-Natal. Support for this is provided by the absence of *mdr1* copy number amplification found in this study. However rigorous regular lumefantrine resistance monitoring is recommended given the high prevalence of the *mdr1*86N allele associated with lumefantrine tolerance and widespread use of AL in southern Africa.
Abbreviations

ACT  Artemisinin-based combination therapy
AL  Artemether-lumefantrine
crt gene  Chloroquine resistance transporter gene
DNA  Deoxyribonucleic acid
HRP  Histidine rich protein
mdr gene  multidrug resistance gene
pfcrt gene  Plasmodium falciparum chloroquine resistance transporter gene
PCR  Polymerase chain reaction
RDT  Rapid diagnostic test
SP  Sulfadoxine-pyrimethamine
WHO  World Health Organization

Competing interests

The authors declare that they have no competing interests.

Authors’ contributions

All authors have reviewed the final draft and agreed to its submission. CHVW initiated the research project, wrote the protocol, co-ordinated the research, collated the data, and composed most of the research report. JR contributed to study design, performed the PCR and molecular analysis, and assisted with editing of the manuscript. ER participated in the study design and supervised Malaria Control Personnel for the follow-up of non-returning patients. EI co-ordinated the recruitment of patients at local clinics, the collection of samples, and was responsible for overseeing clinical care of recruited patients. HR co-ordinated local recruitment of patients, collection of samples, and was responsible for overseeing clinical care of recruited patients. KG reviewed the
study protocol, co-ordinated local recruitment of patients, and was responsible for overseeing clinical care of recruited patients. SK assisted with the development of the protocol and the write-up of the report of the research.

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References

5. Vaughan Williams CH: **Success of insecticide spraying in controlling malaria.** *S Afr Med J* 2003, **93:**160.
9. Sharp BL, le Sueur D: **Malaria in South Africa--the past, the present and selected implications for the future.** *S Afr Med J* 1996, **86:**83-89.


40. Kublin JG, Cortese JF, Njunju EM, Mukadam RA, Wirima JJ, Kazembe PN, Djimde AA, Kouriba B, Taylor TE, Plowe CV: Reemergence of chloroquine-


CHAPTER IV: DISCUSSION, CONCLUSION AND RECOMMENDATIONS

Introduction

In this chapter the lessons learned from the research will be discussed. Some issues have already been mentioned in the paper submitted for publication in the previous chapter; however in addition the practical challenges and lessons learned will be expanded upon.

Therapeutic efficacy of artemether-lumefantrine

The study found no definite evidence, either clinically or from molecular marker analysis, of resistance to artemether-lumefantrine, providing some reassurance that the drug is still efficacious, and that there is, as yet, no evidence of a need to change first line therapy for the treatment of uncomplicated malaria in northern KwaZulu-Natal. None of the 14 confirmed *P. falciparum* patients followed up were *P. falciparum* PCR positive at a follow-up. Nor did any of the 16 confirmed *P. falciparum* PCR-positive patients show an increase in the *pfmdr1* copy number associated with lumefantrine resistance [45]. A slight concern is that all 16 samples carried the *mdr1*86N (asparagine) allele. Although this means that there was no *mdr1*86Y allele associated with chloroquine resistance [38], an increase in the frequency of the *mdr1*86N allele has been suggested as a step towards lumefantrine tolerance [42]. As mentioned already, the final sample was very small, meaning that the results must be treated with caution.

Use of blood spots for PCR analysis

The study demonstrated the robustness of PCR analysis of blood spots as an investigative tool, in a rural area with staff unfamiliar with research. Blood spots for PCR are routinely used for the detection of HIV in exposed infants [46]. The study blood spots were easy to obtain, and resilient to delay in transport to the laboratory more than 400 km away. PCR is capable of detecting malaria parasites at a density of five parasites per microlitre or less [47, 48], whereas thick film microscopy is only reliable at a density of 50 parasites per micro litre or more [48]. PCR has also been shown to
have a specificity approaching 100% for both *P. falciparum* and *P. vivax* [49]. Ideally blood spots should be obtained from all *P. falciparum* cases in KwaZulu-Natal, while the present low malaria incidence persists so that a continuous molecular marker surveillance may be carried out [30].

**Challenges**

The study encountered a number of challenges:

**Malaria incidence**

The incidence of malaria in the study area remained low during the study period, which severely hampered the research project. Too low a malaria incidence to research was a gamble taken when the study was embarked upon. However, in 2011 there were circumstances which suggested that malaria incidence in 2012 could be sufficient to undertake the research.

District Hospitals in northern Umkhanyakude District had reported a surge in cases in early 2011. Malaria Control Programme statistics showed 84 ‘passive’ (reported by health institutions) cases for Jozini and Umhlabuyalingana Districts in northern KwaZulu-Natal for the period January to June 2011. Total active (cases actively sought out by the Malaria Control Programme) and passive cases in the two sub-districts for 2011 were 153, compared to 72 in 2010.

In addition to this apparent increase in malaria cases in 2011 compared to 2010, it appeared that malaria control measures in neighbouring southern Mozambique were deteriorating. Insecticide spraying in southern Mozambique has been one of the highly successful malaria control measures undertaken by the Lubombo Spatial Development Initiative implemented since 2000 [50]. However, it was reported at a meeting on 28 September 2011 between a Mozambiquan delegation from Maputo Province and a delegation from the KwaZulu-Natal Department of Health, that there was no funding to continue the insecticide spraying. A resulting resurgence of malaria in southern Mozambique would be likely to spill over into northern KwaZulu-Natal.
In the event, and revealed partly as a result of the research project, it appears that many of the reported ‘passive cases’ may have been incorrectly diagnosed. Active cases are diagnosed by microscopy and blood films obtained by Malaria Control Programme personnel. The field workers in Northern KwaZulu-Natal (Jozini and Umhlabuyalingana Sub-districts) obtain approximately 2000 blood films per month in the high risk Mozambiquan border area, targeting symptomatic persons and contacts of confirmed cases on which malaria microscopy is performed. For the period January to May in 2011, four positive *P. falciparum* cases were identified between the two sub districts, and in 2012, nine cases were diagnosed. In contrast, ‘passive cases’ are those cases presenting to health facilities and diagnosed by the health facility, usually by rapid diagnostic test (RDT). For the period January to May 2011, 87 passive cases were reported, and for the same period in 2012, 96 passive cases were reported. These statistics show many more ‘passive’ cases are reported, based on RDT diagnosis, than ‘active’ cases diagnosed by microscopy. From the large number of false positive rapid test results found during the study, it would appear that RDT- diagnosed passive cases may contain many false positive results, supported by the persistently low number of cases diagnosed by active surveillance. From the Malaria Control Programme statistics it seems unlikely that there were a substantial number of cases missed by the study, and that the incidence of malaria really was low.

**False positive rapid diagnostic test results**

The finding that 33/49 patients were diagnosed falsely positive by RDT was a major hindrance and disappointment in the conduct of the study. At one stage of the study a particular clinic was enrolling a surprisingly large number of patients, which prompted Malaria Control Personnel to visit the clinic to investigate a possible outbreak. It turned out that one nurse at the clinic was putting far too much blood onto the rapid tests such as to make them uninterpretable. Some of these tests are shown in figure 4, lined up below correctly used tests for comparison. It should be noted that not all false positive RDTs came from the one clinic, which indicates that they were not all due to misuse by one nurse.

The product leaflet of the RDT used claims 100% sensitivity and specificity compared to an in house group of samples [51]. The WHO rates the First Response® RDT as
100% sensitive at 200 parasites per μl, with only 3% false positive results, equivalent to 97% specificity [52]. With low malaria incidence a poor predictive value of a positive RDT is quite possible, even with a specificity as high as 97%. It would never-the-less appear that there is a need for further education and training regarding the use of malaria RDTs in the District.

**Figure 5: Rapid Diagnostic Tests from Study**

![Image of RDTs](image)

**Enrolment and administration**

This low malaria incidence resulted in cases occurring in a scattered distribution and patients presenting to several different clinics and hospitals within the district. Neither the Principal Investigator, nor even any of his co-authors were able to personally enrol patients and prepare blood films for review by Malaria Control Laboratory staff, as originally envisaged. To assist with patient enrolment, nurses from several health clinics were recruited to assist the study; however consistency of the enrolment procedure was difficult to achieve, with several different staff involved, each enrolling only a small number of cases.
Most blood samples for malaria film and blood counts were sent by clinic nurses to one local hospital laboratory with whom co-operation was not fully achieved, meaning that blood counts and blood films on many patients were not performed. One reason for the difficulty with the laboratory seems to have been that instead of simply filling in the laboratory request form in the usual way, the nurses were writing headings such as ‘Malaria Research’ on the forms, which seems to have contributed to the samples being disowned by the laboratory staff. This was a difficulty not anticipated, as the Head of District National Health Laboratory Services had been consulted and given approval, and the tests requested were nothing more than usual for a case of malaria. Also, the onsite Medical Manager was a co-investigator. It had been hoped that it would be possible for one of the investigators to personally make the blood films and bring them back for microscopy by the Malaria Research Programme, however the scarcity and scattering of cases prevented this. In hindsight greater consultation should have taken place with the individual hospital laboratory managers, especially when it became apparent that cases would be scattered and sporadic, and that blood films could only be done on samples sent from clinics or hospitals. This unforeseen difficulty also illustrates that once incidence of malaria incidence decreases to a low level, interest in malaria amongst healthcare workers and public may also decrease. Laboratories may become less likely to have the reagents necessary to prepare malaria films, and instead rely entirely on rapid diagnostic tests. It should be noted that the National Guidelines for the Treatment of Malaria [3], recommend either a rapid diagnostic test or microscopy of blood smear.

A number of other administrative difficulties occurred. Initially clinic nurses were advised to keep completed recruitment forms at the clinic, sending only the blood spots, and submitting all forms together upon the return of the patient for follow up. This system was quickly found to be ineffective, as most patients did not return for follow-up. After the first few patients, nurses were requested to submit the enrolment form with the enrolment blood spots taken for PCR and molecular markers. This lessened the likelihood of the enrolment form being lost, and through the information on place of residence, patients who did not return for follow-up could be tracked down by Malaria Control personnel.
Four children were recruited into the study below the stipulated minimum age of 5 years. The author had decided to set this age limit for a combination of reasons, including possible difficulty in swallowing tablets with younger children, which might affect the efficacy results. Malaria is an unpredictable, potentially lethal disease, even if treated correctly at an early stage, and although the study involved no change to the standard treatment, the author was reluctant to include very young children in the study. However the WHO specifically recommends efficacy trials to include children younger than 5 years [37, 53]. In the event younger children were included due to misunderstanding of the recruiting nurses. Once recruited, and treated, and with the small number of proven cases, there appeared to be no disadvantage to the children involved, not to include their results, and their data was valuable. The follow-up, which would be less likely to occur outside the study, could be to their advantage.

The study could only include the malaria cases available, and it should also be noted that had a sample of even this small size been used to audit therapeutic efficacy of sulfadoxine-pyrimethamine in 1998 or 1999 in KwaZulu-Natal, it would probably have demonstrated the need to change first line medication earlier than in 2000.

Monitoring molecular markers of drug resistance, while a less rigorous method of assessing drug efficacy than in vivo sensitivity studies, is much less expensive and time consuming, and is a reasonable method of surveillance for emerging drug resistance [54]. The WHO recommends that in countries with very low transmission, such as South Africa, studies of molecular markers of resistance should be conducted every year. Most of the specimens (10/14) from molecular markers appeared to be sensitive to chloroquine. This suggests that chloroquine resistance may have decreased following removal of selection pressure from chloroquine, as has been demonstrated in neighbouring Mozambique [54].

**Single follow-up visit**

The standard WHO protocol for assessment of antimalarial drug efficacy demands at least 6 follow-up visits [30]. Use of one follow-up at 28 days, or longer for drugs with longer half-lives, means that it is not possible to distinguish between the different classifications of failed response to treatment (early treatment failure, late clinical
failure, and late parasitological failure [30]). However none-the-less it would be expected that all the types of treatment failure would show parasites at the final follow-up, so a 28 day follow-up should be an effective tool for screening for parasite resistance, especially in the early stages of development of resistance. The difficulty of achieving multiple follow-up visits without a large drop-out rate seems to be underplayed in the protocol literature, although the large amounts of time, labour and money required have been noted [54]. In this study only 7/49 subjects returned for follow-up. They were mostly recruited from a rural area covered by a District Hospital more than 100km from the District Office where the author and Malaria Control Programme were based. Blood spots had to be first sent to the local hospital, and then usually collected by the author every two weeks, who would post them to Durban for analysis. This meant that with delay in samples being sent to the local hospital, combined with the collection interval from the hospital, it could take a month before it could be definitely ascertained that a patient had not returned. Malaria Control Programme personnel were then asked to trace the subject, which could also take time, as the subject was not always at home. It had not originally been envisaged that it would be practical to conduct any study with only the few scattered cases that finally presented.

In the opinion of the author the arduous protocol for the surveillance of the therapeutic efficacy of antimalarial medicines [30] is one factor in the lack of resistance data available in South Africa, and probably many other malarious areas of the world. There appears to be a need for a much simpler WHO-approved antimalarial resistance screening tool, such as the method used in this study.

**Conclusions**

This study found no definite evidence, either from follow-up after treatment, or molecular markers, of acquired *P. falciparum* resistance to artemether-lumefantrine in northern KwaZulu-Natal. Molecular markers also suggested that most cases of *P. falciparum* malaria would be sensitive to chloroquine.
**Recommendations**

South Africa is committed to the goal of malaria elimination [55]. With very low malaria incidence, as is presently the situation in northern KwaZulu-Natal, with an average of only one microscopically confirmed active surveillance case per month, according to KZN Malaria Control Programme statistics, drug resistance marker studies should be undertaken annually, and all cases of *P. falciparum* malaria should be followed up at 28 days, as recommended by the WHO [30]. Another option is to pool data from several sites to obtain a sample large enough to be statistically significant, although this method could be at the expense of being able to determine resistance patterns in specific localities. Use of PCR to confirm diagnosis and for follow-up monitoring provides an effective alternative to microscopy which may be easier to use in a rural setting, but requires adequate technical expertise and resources.

**Further research**

As well as continuing ongoing surveillance of antimalarial drug efficacy, further research appears to be needed into the reliability of ‘passive’ malaria cases reported by health institutions in northern KwaZulu-Natal, mainly on the basis of rapid tests. The study found a high false-positive proportion of cases reported by clinics, at least partly due to incorrect use of rapid tests. The difference in magnitude of ‘passive’ cases diagnosed by rapid tests and ‘active’ cases diagnosed by microscopy since at least 2010 in northern KwaZulu-Natal, suggests persistent over-diagnosis by health institutions for some time, and a possible chronic over-estimate of malaria incidence.
REFERENCES


20. Chichona - a short history [http://www.lib.cam.ac.uk/deptserv/rcs/cinchona.html#extraction](http://www.lib.cam.ac.uk/deptserv/rcs/cinchona.html#extraction)


ADDENDA

Research Project Protocol

Assessment of the therapeutic efficacy of artemether-lumefantrine in the treatment of uncomplicated Plasmodium falciparum malaria in northern KwaZulu-Natal

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1. Dr Jaishree Raman
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   Signature…………………………………

2. Mr Eric Raswiswi
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   Role: Supervise blood film microscopy, and assist with specimen and data collection
   Signature…………………………………

3. Dr Etienne Immelman
   Medical Manager
   Manguzi Hospital
   KwaNgwanase
   Role: Recruitment and care of subjects and data collection
   Signature…………………………………

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4. Dr Holger Reichel  
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   Mosvold Hospital  
   Ingwavuma  
   Role: Recruitment and care of subjects and data collection  
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5. Dr Kelly Gate  
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   Signature……………………………………………………

**Purpose of Protocol:**

Protocol for research dissertation contributing towards Master of Public Health degree  
with the University of KwaZulu-Natal
Summary

Background

The recent history of malaria epidemics in northern KwaZulu-Natal shows that resistance to the standard malaria treatment may arise quickly and contribute to epidemics. Artemether-lumefantrine has now been used for 10 years as the first line treatment for uncomplicated *P. falciparum* malaria, but has not been assessed for therapeutic efficacy for 9 years.

Purpose

The purpose of the study is to assess the therapeutic efficacy of artemether-lumefantrine for patients with uncomplicated malaria in Umkhanyakude Health District in 2011-12, and test for molecular markers of antimalarial drug resistance.

Objectives

The main objective is to quantify the failure proportion after 28 days of a sample of patients treated for uncomplicated malaria with artemether-lumefantrine. Molecular analysis of blood samples will search for molecular markers of resistance to artemether-lumefantrine and chloroquine.

Study design

The study will be an observational cohort.

Settings

The study will take place in 3 hospitals, and 28 attached clinics in Umkhanyakude Health District.

Study population

The study population will be patients with uncomplicated *P. falciparum* malaria presenting to 3 hospitals and their 28 attached clinics in Umkhanyakude Health District.

Study sample

A sample of between 50 and 100 patients aged between 5 and 69 years with uncomplicated *P.*
*falciparum* malaria will be recruited between September 2011 and May 2012.

**Data collection**

Patients will be tested for malaria using blood films and blood spots for molecular analysis at diagnosis and after 28 days. Molecular analysis will also be conducted for markers of chloroquine and artemether-lumefantrine resistance.

**Statistical methods**

The failure proportion will be calculated, with 95% confidence intervals. In the event of a substantial number of failures, association will be sought between failure and parasitaemia count, age and gender. The proportion of patients with markers for malarial drug resistance will be calculated with 95% confidence intervals.
Introduction /background

Artemether-lumefantrine (AL) has been used as the first line treatment of uncomplicated *Plasmodium falciparum* malaria in northern KwaZulu-Natal since 2001 [1, 2].

The recent history of malaria epidemics in KwaZulu-Natal indicates that the therapeutic effectiveness of the anti-malarial medication used to treat patients suffering malaria, together with the effectiveness at killing mosquitoes of the insecticide used for residual house-spraying, are vital for malaria control and must be regularly assessed. Failure to do so risks a recurrence of malaria epidemics, which has major public health and economic consequences for the area and province as a whole.

A study of the effectiveness of AL should either confirm its’ continuing efficacy or provide a warning as to the acquisition of resistance by malaria parasites and the need to seek an alternative therapy before an epidemic occurs.

Literature Review

In 1988, due to the emergence and spread of chloroquine – resistant *P. falciparum* malaria, sulfadoxine-pyrimethamine (SP) replaced chloroquine as the first line treatment for uncomplicated *P. falciparum* malaria in KwaZulu-Natal [1, 3]. This drug remained effective until 1996 when malaria incidence began to increase. Between 1996 and 2000 northern KwaZulu-Natal suffered increasingly severe malaria epidemics with more than 60,000 cases reported in 2000 [1, 2]. Only in 2000 did a clinical trial, in conjunction with genetic studies, show that *P. falciparum* parasites in the region had developed resistance to sulfadoxine-pyrimethamine, the then recommended first line treatment for malaria, rendering it largely ineffective in northern KwaZulu-Natal [3]. Subsequently the change of first-line medication to artemether-lumefantrine, together with change of insecticide used for residual house spraying to DDT, dramatically reduced malaria incidence in northern KwaZulu-Natal [1, 2]. It has been estimated that the delay in changing first-line treatment for malaria between 1996 and 2000 was responsible for substantial morbidity and mortality, as well as contributing to the size of the epidemic [4]. Artemether combined with lumefantrine is one of the artemisinin-based combination therapies (ACT) recommended for the treatment of uncomplicated *P. falciparum* malaria by the World Health Organization (WHO) [5]. Studies of the therapeutic efficacy of artemether-lumefantrine in 2001 and 2002 indicated that AL was effective for treating uncomplicated malaria in northern KwaZulu-Natal [1, 6, 7]. Since 2002, however, there have
been no further studies in KwaZulu-Natal, or South Africa, of the continuing therapeutic effectiveness of AL in the treatment of uncomplicated malaria. The WHO recommends routinely monitoring antimalarial resistance [8], and a change in antimalarial medicine if the treatment failure proportion \( \geq 10\% \) [5].

**Pharmacology**

Artemether-lumefantrine is a combination of artemether and lumefantrine manufactured as Coartem\textsuperscript{®} by Novartis: 20mg artemether and 120mg lumefantrine in tablet form [9]. The two drugs act synergistically. Artemether and its active metabolite, dihydroartemesin, rapidly kill most malaria parasites, while lumefantrine mops up the remainder more slowly [10]. Artemether is rapidly absorbed and metabolized, with a half-life of about two hours, whereas lumefantrine is absorbed more slowly and has a half-life of 3-4 days in malaria patients [10].

Coartem\textsuperscript{®} is taken as a 6 dose oral regimen over 3 days. The dosage depends upon the weight and age of the patient. The adult dosage for persons aged 12 years or more, or children weighing 35kg and above is four tablets as a single dose at the time of initial diagnosis, 4 tablets after 8 hours, and then 4 tablets twice daily on each of the following two days [9]. It is recommended that the tablets are taken with fatty food or milk to improve absorption [9].

**Choice of follow-up period**

A WHO protocol for the assessment of therapeutic efficacy of antimalarial drugs recommends assessment of patients on days 0, 1, 2, 3, 7 and 14, and defines three categories of therapeutic response:

- Adequate clinical response
- Early treatment failure – occurring up to day 3
- Late treatment failure, defined as: ‘Development of danger signs or severe malaria in the presence of parasitaemia on any day from day 4 to 14 without previously meeting any of the criteria for early treatment failure’ [11].

A more recent WHO report recommends a change in the definition of late treatment failure in areas of low transmission to:

Presence of parasitaemia on any day from day 4 to day 28 and a measured axillary temperature of \( \geq 37.5^\circ\text{C} \), without previously meeting any of the criteria of early treatment failure [12].
Professor NJ White points out that for rapidly eliminated drugs a 28 day follow-up is needed, and longer for more slowly eliminated drugs [13]. The 2010 WHO Malaria Treatment Guidelines recommend at least 28 days follow-up to assess parasitological cure, and 42 days for regimes containing mefloquine and piperaquine [5].

As yet there has been no anecdotal evidence of resistance to AL in KwaZulu-Natal. Follow-up malaria films of patients with malaria treated with AL performed by the Malaria Control Program in 2011 in KwaZulu-Natal have been predominantly negative. The primary objective of the study is to seek evidence of late clinical failure, which would be the first sign of resistance to AL. Requiring patients to return for assessment several times requires considerable resources, and the risk of drop-outs is high.

Considering:

- The 2002 WHO report on monitoring antimalarial drug resistance [12],
- KwaZulu-Natal is presently an area of low malaria transmission;
- The analysis of Professor White [13],
- Lumefantrine, the more slowly eliminated drug in AL has a half-life of a few days, and not weeks like mefloquine [10], and
- The fact that requiring a patient to return once four weeks later is a fairly simple request,

a single follow-up assessment at 28 days is chosen.

**Purpose of the study**

- The purpose of the study is to assess the therapeutic efficacy of artemether-lumefantrine (AL) for the treatment of uncomplicated *P. falciparum* malaria in Umkhanyakude District, northern KwaZulu-Natal in 2011, and to search for molecular markers of drug resistance.

**Specific objectives**

The specific objectives are:

- To quantify the proportion of patients treated with artemether-lumefantrine, who fail to clear *P. falciparum* from their blood 28 days after treatment
- Test for molecular markers of malarial drug resistance to AL and chloroquine
• Publish the results
• Use the results to inform policy as to the best choice of drug for the treatment for uncomplicated malaria in Umkhanyakude District.

**Type of research**

The research project will be a clinical epidemiological study

**Definitions**

**Early Treatment Failure (ETF)** [11]

Development of danger signs or severe malaria on Day 1, Day 2, or Day 3 in the presence of parasitaemia;

Axillary temperature $\geq 37.5^\circ C$ on Day 2 with parasitaemia $> Day 0$ counts;

Axillary temperature $\geq 37.5^\circ C$ on Day 3 in the presence of parasitaemia, and

Parasitaemia on day 3 $\geq 25\%$ of count on Day 0.

**Late Treatment Failure (LTF)** [12]

Presence of parasitaemia on any day from day 4 to day 28 and a measured axillary temperature of $\geq 37.5^\circ C$, without previously meeting any of the criteria of early treatment failure

**Late Parasitological Failure** [12]

Presence of parasitaemia on any day from Day 7 to Day 28 and axillary temperature $<37.5^\circ C$ without previously meeting any of the criteria of early treatment failure or late clinical failure

**Danger Signs** include

• Not able to drink or breastfeed;

• Vomiting everything;

• Recent history of convulsion;

• Lethargic or unconscious state; and
Unable to sit or stand up [11].

**Uncomplicated malaria:**

Symptomatic infection with malaria parasitaemia without signs of severity and/or evidence of vital organ dysfunction [5].

**Research Methods**

**Study setting**

The study will be set in 3 hospitals and 28 attached clinics in Umkhanyakude District.

**Study design**

An observational descriptive cohort study design will be used.

**Target population**

The target population will be persons diagnosed as suffering from uncomplicated *P. falciparum* malaria treated with artemether-lumefantrine in KwaZulu-Natal. As malaria drug resistance can vary considerably between countries and even provinces, the results will only be generalisable to KwaZulu-Natal.

**Study population**

The study population will include persons, aged between 5 and 69 years, diagnosed with uncomplicated *P. falciparum* malaria in Umkhanyakude Health District, presenting to 3 hospitals or their 28 attached clinics in northern KwaZulu-Natal.

**Inclusion / Exclusion criteria**

**Inclusion criteria:**

Patients diagnosed with malaria rapid diagnostic test (RDT) as suffering *P. falciparum* malaria, with diagnosis subsequently confirmed by blood film.

**Exclusions**

The following categories of patients will not be included in the study:
- Patients with severe or complicated malaria;
- Pregnant women;
- Patients aged less than 5 years and more than 69 years; and
- Patients treated for malaria during the previous two weeks.

**Study Sample**

**Method of selecting sample**

As far as possible, all patients, aged 5 years to 69 years, diagnosed with uncomplicated malaria in Umkhanyakude District between September 2011 and May 2012, will be invited to participate in the study. Recruitment will cease if 100 patients are recruited. Patients will be recruited who present to 3 hospitals or the 28 attached clinics. Patients will be attended by either a doctor or a primary healthcare trained nurse.

**Size of sample**

An attempt will be made to recruit 100 patients; however the feasibility of recruiting this number will depend upon the incidence of malaria in Umkhanyakude District. Taking

A 10% failure rate or higher as unacceptable;

Setting the probability of \( \alpha \), a type I error, at 0.05;

Setting the probability of \( \beta \), a type II error at 0.2;

A minimum of 49 patients will be needed.

**Data sources**

**Measurement instruments / data collection techniques**

Patient data will be collected according to a proforma at recruitment.

Blood will be taken at recruitment and follow-up for

- malaria thick and
- thin films, and
o molecular analysis.

Genotyping of *P. falciparum* DNA extracted from dried blood spots based on variations in the genes coding for the glutamine-rich protein and meroziote surface protein 1 and 2, will be used to determine if treatment failure is due to a re-infection or recrudescence of the original infection [14, 15]. Infections will be classified as recrudescent if PCR products for all three markers from Day 0 and Day of failure parasites are identical. If the banding patterns for any marker differ between Day 0 and Day of failure parasites, then the infection will be classed as a re-infection.

This study will assess the prevalence of molecular markers linked with resistance in the partner drug lumefantrine, specifically the *mdr1* copy number [16], and mutations at *mdr1* codon 86 [17], since no verified marker for artemisinin resistance exists.

**Measures to ensure validity**

**Internal**

All patients will have a blood film made which will be analysed by an experienced microscopist, and confirmed by another microscopist. Blood spots will also be taken and sent for malaria polymerase chain reaction (PCR).

A patient will be considered to be infected with *P. falciparum* malaria if:

- The blood film is definitely positive for *P. falciparum*, or
- The PCR test is positive.

A borderline blood film with negative PCR will be considered negative.

In the unexpected event of the blood film being clearly positive, but the PCR being negative, the result will be investigated.

**Reduction of bias**

**Selection bias**

**Attrition bias**

Effort will be made to reduce the number of follow-up drop outs. Patients will be offered R50 to assist with travelling expenses when they return for follow-up.
Selection bias

All eligible persons diagnosed with malaria will be invited to participate in the study

Information bias

Reporting bias:

There will be no discussion of results between the microscopists reporting on blood films and the technician performing the PCR test.

Detection bias:

Molecular analysis, combined with microscopy, should minimise the number of false negative results.

External Validity / Generalisability

Pilot study

There will be no pilot study, due to the low number of patients with malaria in the district.

List of Variables

- Age
- Gender
- Weight
- Duration of illness
- Other medications
- Travel history within past 60 days
- HIV status (if already known)
- Home address
- Artemether-lumefantrine dosage given
- The following at both enrolment and 28-day follow-up:
  - Temperature
- Pulse rate
- Blood pressure
- *P. falciparum* malaria rapid diagnostic test result
- Thick blood film for malaria diagnosis
- Thin blood film for malaria diagnosis
- Polymerase chain reaction analysis for *P. falciparum*
- Reinfection/recrudescence assessment using nested PCR
- Assessment of mdr1 copy number (as an indicator of AL resistance)
- Assessment of Chloroquine sensitivity

**Plan for Data collection**

- A data collection sheet will be completed at enrolment and samples taken for
- 28-day follow-up data collection sheet
- Data to be collated in excel spreadsheet

**Plan for Data handling/processing**

Data to be collated in a Microsoft excel spreadsheet

**Statistical methods**

**Descriptive statistics**

The proportion of patients with confirmed uncomplicated *P. falciparum* malaria, treated with artemether-lumefantrine, and cleared of parasites by 28 days will be calculated.

The proportion of patients demonstrating molecular markers of resistance to antimalarial drugs will also be calculated.

**Analytic statistics**

The 95% confidence interval for failure ratio will be calculated and compared to the proposed maximum 10% acceptable failures.

The 95% confidence interval for the proportion of patients testing positive for markers of
resistance to antimalarial drugs will also be calculated.

**List of possible confounders**

The study is not an analytical study with a control group

**List of associations to be measured**

In the event of sufficient failures to compare with other variables, failure to clear parasites would be compared to parasitaemia count, age, and gender.

**Ethical considerations**

**Institutional Ethical Review Board**

The study will be submitted to the University of KwaZulu-Natal Biomedical Research Ethics Committee for approval.

**Permissions**

Permission will be obtained from the District Manager, Umkhanyakude Health District Office, Jozini, and approval obtained from the KwaZulu-Natal Department of Health Provincial Health Research Committee.

**Informed consent and participant information**

All patients aged 18 years or over will be asked to sign a consent form written in English and Zulu. For children aged less than 18 years consent will be obtained from a parent or legal guardian.

**Work plan**

**Budget**

Travelling expenses for subjects

R50

Molecular cost per sample with test run in duplicate:

Smear/RDT confirmation using qPCR:

R35
Reinfection/recrudescence assessment using nested PCR  
Assessment of mdr1 copy number  
Assessment of Chloroquine sensitivity  
Sample transport costs to Medical Research Council laboratory:  
Total cost per subject:  
Total budget if 100 patients recruited:  

**Study period / Time lines**

It is hoped that recruitment of patients may begin in September 2011. The study will continue until 100 patients are recruited or until May 2012, whichever is sooner.
References


Acronyms & abbreviations

- AL: artemether-lumefantrine
- ACT: artemesinin-based combination therapy
- ETF: early treatment failure
- LFT: late treatment failure
- PCR: polymerase chain reaction
- RDT: rapid diagnostic test

Addenda

**Enrolment Data collection sheet**

<table>
<thead>
<tr>
<th></th>
<th>Yes / No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Today’s date</td>
<td></td>
</tr>
<tr>
<td>Patient information sheet given?</td>
<td>Yes / No</td>
</tr>
<tr>
<td>Patient consent form signed?</td>
<td>Yes / No</td>
</tr>
<tr>
<td>Method of positive malaria diagnosis</td>
<td>Rapid Test (RDT) / Blood film</td>
</tr>
<tr>
<td>Active surveillance case?</td>
<td>Yes / No</td>
</tr>
<tr>
<td>Hospital or Clinic name</td>
<td></td>
</tr>
<tr>
<td>Hospital/Clinic card number:</td>
<td></td>
</tr>
<tr>
<td>Name</td>
<td></td>
</tr>
<tr>
<td>Date of birth</td>
<td></td>
</tr>
<tr>
<td>Age (Must be ≥ 5 yrs and &lt; 70 yrs)</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>Male / Female</td>
</tr>
<tr>
<td>Date became ill</td>
<td></td>
</tr>
<tr>
<td>Other medication</td>
<td></td>
</tr>
<tr>
<td>Travel 60 days prior to illness</td>
<td></td>
</tr>
<tr>
<td>HIV status, if known</td>
<td></td>
</tr>
</tbody>
</table>

**Symptoms of complicated malaria:** If any answer: ‘Yes’, do not enroll in study, but refer to hospital.

- Unable to Drink: Yes / No
- Vomiting everything: Yes / No
- Convulsion during past 7 days: Yes / No
- Lethargic or decreased consciousness: Yes / No
- Unable to stand or sit: Yes / No
- Pregnant: Yes / No

**If presenting to Clinic or Hospital:**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td></td>
</tr>
<tr>
<td>Pulse rate</td>
<td></td>
</tr>
<tr>
<td>Blood Pressure</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td></td>
</tr>
<tr>
<td>Dose Coartem (tablets bd)</td>
<td></td>
</tr>
<tr>
<td><strong>Address</strong></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Area</td>
<td></td>
</tr>
<tr>
<td>Chief / Induna</td>
<td></td>
</tr>
<tr>
<td>Homestead Head</td>
<td></td>
</tr>
<tr>
<td>Nearest adjacent homestead</td>
<td></td>
</tr>
<tr>
<td>River</td>
<td></td>
</tr>
<tr>
<td>School</td>
<td></td>
</tr>
<tr>
<td>Store</td>
<td></td>
</tr>
<tr>
<td>Magisterial District</td>
<td></td>
</tr>
<tr>
<td>Country of origin</td>
<td></td>
</tr>
<tr>
<td>Cellphone number/telephone number</td>
<td></td>
</tr>
</tbody>
</table>

**Study samples taken:**

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Yes / No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thick film</td>
<td>Yes / No</td>
</tr>
<tr>
<td>Thin film</td>
<td>Yes / No</td>
</tr>
<tr>
<td>Blood spot for molecular analysis</td>
<td>Yes / No</td>
</tr>
<tr>
<td>Return date (28 days)</td>
<td>Yes / No</td>
</tr>
</tbody>
</table>

**Compiled by**

SA / Moz / Swaz / Other
# 28 Day follow-up data collection sheet

<table>
<thead>
<tr>
<th>28 Day follow-up data collection sheet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Today's date</td>
</tr>
<tr>
<td>Malaria Rapid Test Result today</td>
</tr>
<tr>
<td>Hospital or Clinic name</td>
</tr>
<tr>
<td>Hospital/Clinic card number:</td>
</tr>
<tr>
<td>Name</td>
</tr>
<tr>
<td>Date of birth</td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>Gender</td>
</tr>
<tr>
<td>Date enrolled in study</td>
</tr>
</tbody>
</table>

## Symptoms of complicated malaria: If any answer ‘Yes’, please refer to hospital.

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Yes / No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unable to Drink</td>
<td></td>
</tr>
<tr>
<td>Vomiting everything</td>
<td></td>
</tr>
<tr>
<td>Convulsion during past 7 days</td>
<td></td>
</tr>
<tr>
<td>Lethargic or decreased consciousness</td>
<td></td>
</tr>
<tr>
<td>Unable to stand or sit</td>
<td></td>
</tr>
<tr>
<td>Pregnant</td>
<td></td>
</tr>
</tbody>
</table>

## Observations

<table>
<thead>
<tr>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
</tr>
<tr>
<td>Pulse rate</td>
</tr>
<tr>
<td>Blood Pressure</td>
</tr>
<tr>
<td>Weight (kg)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dose Coartem (tablets bd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Were all tablets taken as instructed?</td>
</tr>
<tr>
<td>If not, which tablets were taken?</td>
</tr>
</tbody>
</table>

## Study samples taken:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Yes / No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thick film</td>
<td></td>
</tr>
<tr>
<td>Thin film</td>
<td></td>
</tr>
<tr>
<td>Sample for Malaria PCR</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>R50 given for travelling expenses, and signed for?</th>
<th>Yes / No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Payment of R50 received for travelling expenses</td>
<td>Patient signature</td>
</tr>
</tbody>
</table>

Compiled by
Dear Sir/Madam,

I am Dr CHV Williams, and work at

Umkhanyakude Health District Office
Jozini
3969

Telephone: 035 5721357
Cellphone: 072 584 3472
Email: hervey.williams@kznhealth.gov.za

A blood test shows that you have malaria which can be a serious illness.

Since the year 2001 coartem™ has been used to treat uncomplicated (less severe) malaria in KwaZulu-Natal. It seems to work well, but we want to check that it is still curing malaria. We wish to follow-up 100 patients with uncomplicated malaria in Umkhanyakude District.

We are inviting you help us by letting us take an extra drop of your blood today, to study the malaria, and to come back for a check-up after 4 weeks, when we will ask for another drop of blood to confirm the malaria has disappeared.

Your blood will only be used to test for malaria. In this study your treatment will be the same as for anyone else with malaria. We will just observing you more closely than usual.

This study has been ethically reviewed and approved by the UKZN Biomedical Research Ethics Committee (approved number:………….)

In the event of any problems or concerns you may contact Dr CHV Williams at the above address, or the
Biomedical Research Ethics Committee at

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
Research Office, Westville Campus Govan Mbeki Building
Private Bag X 54001, Durban 4000
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604769 - Fax: 27 31 2604609; Email: BREC@ukzn.ac.za

You are not obliged to participate in this study, and are free to change your mind and withdraw at any time. You will still receive the usual treatment for malaria. If you return after 4 weeks, we will offer you R50 for traveling expenses.

It is intended to publish the results of the study, however no patient identities will be revealed. No personal information obtained in the study will be shared with anyone not involved in the research. An anonymous dried frozen spot of blood will be kept at the Malaria Research Programme laboratory of the Medical Research Council in Durban for future malaria research.
Mnumzane/Nkosikazi
Igama lami ngingu Dr CHV Williams, ngisebenza e-Mkhanyakude emnyangweni wezempiwo. Iminingwane yekheli ikanje:
Umkhanyakude Health District Office
Jozini 3969
IZinombolo zocingo: 035 572 1357; 072 584 3472
I-Email: hervey.williams@kznhealth.gov.za

Ukuhlolwa kwegazi lakho kuxombise ukuthi unomalaleveva okungaba isifo esinobungozi kakhulu.

Kusukela ngonyaka ka-2001 i-CoartemTM yasetshenziswa ukwelapha umalaleveva KwaZulu Natal. Ibonakale isebenza kahle kakhulu, kodwa sisafuna ukuhlola ukuthi isaqhubeka nokusebenza kahle ekwelapheni kwalesisifo.

Ebesikucela kuwena ukuba usinikeze imvumo yokuthi sithathe elinye futhi iconsi legazi lakho namuhlha ukuze sizokwazi ukuqhuba lolucwane ukuqhubeka elisediso, labho-ke sizophinde sikucela futhi ukuba usinikeze enye imvumo yokuthatha elinye iconsi legazi ukuze siqinisekise ukuthi welapheke ngempela kumalaleveva.

Igazi lakho lizosetshenziswa ukhuhlolelwa umalaleveva nje kuhlela, hhayi okunye. Kulokuhlolelwana amaphilisi owatholayo azaqhubeka afane nabononde abanye abaphethwe umalaleveva umehluko ukuthi nje wena sizobe sikubheke eduze kunabanye ngokulokhu sikucela ukuba ubuye njalo emtholampilo uma kudingekile.

Lolu cwaningo luvunyelwe labhekwa ngokwesisekelo sezinkolelo yiUKZN Biomedical research ethics committee (izningaba ezikhathi ezimake)

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
Research office, Westville campus Govan Mbeki Building
Private Bag X54001, Durban 4000
KwaZulu-Natal, South Africa
Tel: 27 31 2604769; Fax: 27 31 2604609; Email: BREC@ukzn.ac.za

Sifisa ukukwazisa ukuthi awuphoqwanga ukuba yingxenye yalolucwane nje ukuhlolelwana nokuthatha elisediso, kodwa ayikho iminingwane yomuntu
ezovezwa. Iminingwane yakho etholakale kulolucwalingo iyimfihlo, angeke ivezelwe abantu abangangekho emphathini walolucwalingo.

I consi legazi eliqandisiwe lanomawubani lizo bekwa eMalaria Research Programme laboratory ye Medical research council eThekwini lapho izosetshenziselwa eminye imicwaningo esikhathini esiphambilini.


Uma uzingwa ungabingcono, noma ukugula kuqhubeleka phambilile emva kwezinsuku ezimbili, kumele ubuye masinyane empholampilo noma esibhedlela.

Abakhulelwe, izingane ezingaphansi kweminyaka emihlanu (5) nabadala abangaphezu kweminyaka engamashumi ayisikhombisa (70) abavumeleki ukuba yingxenye yalolucwalingo.

Uyacelwa ukuba ubuye ngomhlaka..........................................................
Dear Sir/Madam,

I am Dr CHV Williams, and work at

Umkhanyakude Health District Office
Jozini
3969

Telephone: 035 5721357
Cellphone: 072 584 3472
Email: hervey.williams@kznhealth.gov.za

A blood test shows that your child has malaria which can be a serious illness.

Since the year 2001 coartem™ has been used to treat uncomplicated (less severe) malaria in KwaZulu-Natal. It seems to work well, but we want to check that it is still curing malaria. We wish to follow-up 100 patients with uncomplicated malaria in Umkhanyakude District.

We are inviting you to help us by letting us take an extra drop of your child’s blood today, to study the malaria, and to come back for a check-up after 4 weeks, when we will ask for another drop of blood to confirm the malaria has disappeared.

Your child’s blood will only be used to test for malaria. In this study your child’s treatment will be the same as for anyone else with malaria. We will just observing your child more closely than usual.

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You are not obliged to have your child participate in this study, and are free to change your mind and withdraw at any time. Your child will still receive the usual treatment for malaria.
If you return after 4 weeks, we will offer you R50 for traveling expenses.

It is intended to publish the results of the study, however no patient identities will be revealed. No personal information obtained in the study will be shared with anyone not involved in the research.
An anonymous dried frozen spot of blood will be kept at the Malaria Research Programme laboratory of the Medical Research Council in Durban for future malaria research.
Your child should take coartem™ tablets, 2 times per day for 3 days, with milk or a meal, until the pills are finished. Otherwise the malaria is more likely to come back.

If you feel that your child is not getting better within two days, or are getting worse, you must come back to the clinic or hospital at once.

We do not want to include in this study: pregnant women, children who are less than 5 years, or people who are more than 70 years.

Please Return on……………………………………………………………………(Date)
Patient information sheet - children - Zulu translation
Iphepha lesaziso seziguli ngokuhlolowa kwezinsuku eziwu-28 emva kokuthatha amaphilisi i-coartemTM eyelapha umalaleveva

Mnumzane/Nkosikazi

Igama lami ngingu Dr CHV Williams, ngisebenza e-Mkhanyakude emnyangweni wezempilo. Imininingwane yekheli ikanje:
Umkhanyakude Health District Office
Jozini 3969
IZinombolo zocingo: 035 572 1357; 072 584 3472
I-Email: hervey.williams@kznhealth.gov.za

Ukuhlolwa kwegazi lengane yakho kukhombise ukuthi inomalaleveva isifo esinobungozi kakhulu.

Kusukela ngonyaka ka-2001 i-CoaratemTM yasetshenziswa ukwelapha umalaleveva KwaZulu-Natal. Ibonakale isebenza kahleakkahu, kodwa sisafuna ukuhlola ukuthi isaqhubeka nokusebenza kahle ekwelapheni kwalesisiso.

Ebesikucela kuwena ukuba usinikeze imvumo yokuthi sithathe elinye futhi iconzi legazi lengane yakho namhla ukuze sizokwazi ukughuba lolucwankingo lukamalaleveva eqazini lakah. Uyacelwa futhi ukuba uphinde ubuye emva kwamasondo amane ukuzozhubeka nokuhlolelwisa lisisifo, lapho-ke sizophinde sikucelwe futhi ukuba usinikeze enye imvumo yokuthatha elinye iconzi legazi lengane ukuthi isiqinisekise ukuthi welaphhe ngempela kumalaleveva.

Igazi lengane yakho lizosetshenziswa ukuhlolelwisa umalaleveva nje kuphela, hhayi okunye. Kulokhukuhlolewa amaphilisi owatholayo azoqhubeka afane nabobonke abanye abaphethwe umalaleveva umehluko ukuthi nje sizobe simbheke umtwana eduze kunabanye ngokulokhu sikucela ukuba ubuye njalo emtholampilo uma kudingekile.

Lolu cwaningo lvunyelwe labhekwa ngokwesisekelo sezinkolelo yiUKZN Biomedical research ethics committee (inombolo esemtethweni………)

Uma uneminye imibuzo/izinkinga ungaxhumana noDr CHV Williams kulekheli elilandelayo:

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
Research office, Westville campus Govan Mbeki Building
Private Bag X54001, Durban 4000
KwaZulu-Natal, South Africa
Tel: 27 31 2604769-Fax:27 31 2604609; Email: BREC@ukzn.ac.za

Sifisa ukukwazisa ukuthi awuphoqwanga ukuba yingxene yalolucwaningco lokuhlolelwisa umalaleveva nokuthi ulokhu ubuya njalo emva kwamasondo amane ukuzohlolelwisa emtholampilo. Uma ungathandi ukuba yingxene yalolucwaningco uvumekilele ukwenqaba. Noma wenqabile uzoqhubeka ukunikwa amaphilisi okwelapha umalaleveva njengokwejwayelekile.
Uma kumele ubuye emva kwamason to amane uzonikeza imali yokugibela engango R50.

Imiphumela yalolu cwan ingo izoshicilelwa, kodwa ayikho imin ingwane yomuntu ezovezwa. Imin ingwane yakho etholakale kulolucw aningyo iyimfi hiho, angeke ivezelwe abantu abangange kho em phathini waloluc w aningyo.

I consi legazi eliqandisiwe lanomawubani lizo bekwa eMalaria Research Programme laboratory ye Medical research council eThekwini lapho izosetshenziselwa eminye imicwaning yo esikhathini esiphambili.

- Ingane yakho kumele ithathe amaphilisi awu___ eCoartemTM, kabili ngosuku izinsuku ezintathu. Lamaphilisi kumele iwathathe nobisi noma emvakokudla aze aphele. Uma ingawathathanga njengoba uyaliwe man ingi amathuba okuthi iphinde ipathwe umalaleveva.

Uma uzizwa ungabingcono, noma ukugula kuqhubekela phambili emva kwezinsuku ezimbili, kumele ubuye masinyane emtholampilo noma esibhedlela.

Abakhulelwe, izingane ezingaphansi kweminyaka emihlanu (5) nabadala abangaphezu kweminyaka engamashumi ayisikhombisa (70) abavumelekile ukuba yingxenye yalolucw aningyo.

Uyacelwa ukuba ubuye ngomhlaka.................................................................
Consent Form (English-Adults)

Consent form for 28 day follow-up after treatment with coartem™ to treat P. falciparum malaria

I agree to return on……………………………..for a blood test see if the malaria has gone away.

I,………………………………………………………….., have been informed about the study entitled:
‘Assessment of the therapeutic efficacy of artemether-lumefantrine in the treatment of uncomplicated plasmodium falciparum malaria in northern KwaZulu-Natal’
by (…………………………………………………………….).

- I understand the purpose and procedures of the study.
- I have been given an opportunity to answer questions about the study and have had answers to my satisfaction.
- I declare that my participation in this study is entirely voluntary and that I may withdraw at any time without affecting any treatment or care that I would usually be entitled to.

If I have any further questions/concerns or queries related to the study I understand that I may contact the researcher,
Dr CHV Williams,
Umkhanyakude Health District Office, Jozini, 3969
Telephone: 035 5721357; Cellphone: 072 584 3472,
Email: hervey.williams@kznhealth.gov.za

If I have any questions or concerns about my rights as a study participant, or if I am concerned about an aspect of the study or the researchers then I may contact:

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
Research Office, Westville CampusGovan Mbeki Building
Private Bag X 54001, Durban 4000
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604769 - Fax: 27 31 2604609; Email: BREC@ukzn.ac.za

______________________________    ______________________
Signature of Participant                     Date

______________________________    ______________________
Signature of Witness                          Date
(Where applicable)

______________________________    ______________________
Signature of Translator                       Date
(Where applicable)
**Consent form adults – Zulu translation**

**Imvume yokuhlolwa emva kwezinsuku eziwu-28 uthathe amaphilisi i-coartem™ eyelapha umalaleveva**

Mina (igama nesibongo) ________________________ Ngiyavuma ukubuya ngomhlaka (usuku) __________________ ngizohlolwa igazi, ukuze kubhekwe ukuthi welapheke ngempela umalaleveva.

Ungazisile u – (igama lomuntu okwazise ngalolucwaningo) __________________ ngalolucwaningo lukamalaleveva lokubheka ukusebenza kwamaphilisi i-coartem asentshenziswa ekwelapheni lesisifo lapha KwaZulu Natal

- Ngiyasizwa isizathu nendlela ezosentshenziswa kulolucwaningo.
- Nginikiwe ithuba lokubuza imibuzo ngalolucwaningo futhi izimpendulo engizinikeziwe zingenelisile.
- Ngiyaqinisekisa ukuthi angiphoqwanga ukuba yingxenye yalolucwaningo nokuthi ngazisiwe ukuthi ngingaphuma noma yinini uma ngingasathandi ukuba yingxenye ngaphandle kokuphazamiseka kokwelashelwa kwami lesisifo.
- Ngiyazi futhi ukuthi uma ngineminye imibuzo/izinking namganoma yini engingayiqondi kaulelela nalolucwaningo ngingaxhumana nomhloli omkhulu walolucwaningo okungu -Dr CHV Williams, kulelikheli elilandelayo:

Umkhanyakude Health District office, Jozini, 3969
Izinombolo zocingo: 0355721357; 0725843472, I-Email: hervey.williams@kznhealth.gov.za

Ngiyazi futhi ukuthi uma ngineminye imibuzo ngamalungelo ami okuba yingxenye yalolucwaningo, nanoma ngabe yini engingayiqondi kaulelela nalolucwaningo nangomcwaniyi ngingaxhumana namahhovisi ezocwaningo kulelikheli eilandelayo: BIOMEDICAL RESEARCH ETHICS ADMINISTRATION, Research Office, Westville Campus Govan Mbeki Building Private Bag X54001, Durban 4000 KwaZulu-Natal, SOUTH AFRICA
Inombolo yocingo: 27312604769 - Fax: 27312604609; Email: BREC@ukzn.ac.za

___________________________                                        ________________________
Isiginesha yami                                               usuku

___________________________                                        ________________________
Isiginesha kafakazi (lapho edingekile)                         usuku

___________________________                                        ________________________
Isiginesha yomhumushi (lapho edingekile)                       usuku
Consent Form (English-Children)
Consent form for 28 day follow-up after treatment with coartem™ to treat P. falciparum malaria

I ………………………agree to return with my child…………………on……………………………..for a blood test see if the malaria has gone away.

I,………………………………………………………….. have been informed about the study entitled: ‘Assessment of the therapeutic efficacy of artemether-lumefantrine in the treatment of uncomplicated plasmodium falciparum malaria in northern KwaZulu-Natal’ by (…………………………………………………………….).

- I understand the purpose and procedures of the study.
- I have been given an opportunity to answer questions about the study and have had answers to my satisfaction.
- I declare that the participation of my child in this study is entirely voluntary and that I may withdraw my child at any time without affecting any treatment or care that my child would usually be entitled to.

If I have any further questions/concerns or queries related to the study I understand that I may contact the researcher,
Dr CHV Williams,
Umkhanyakude Health District Office, Jozini, 3969
Telephone: 035 5721357; Cell phone: 072 584 3472,
Email: hervey.williams@kznhealth.gov.za

If I have any questions or concerns about the rights of my child as a study participant, or if I am concerned about an aspect of the study or the researchers then I may contact:

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
Research Office, Westville Campus Govan Mbeki Building
Private Bag X 54001, Durban 4000
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604769 - Fax: 27 31 2604609; Email: BREC@ukzn.ac.za

___________________________
Signature of Parent or Guardian
___________________________
Date

___________________________
Signature of Witness
(Where applicable)
___________________________
Date

___________________________
Signature of Translator
(Where applicable)
___________________________
Date
Consent form children – Zulu translation

Imvume yokuhlolwa emva kwezinsuku eziwu-28 uthathe amaphilisi i-coartem™ eyelapha umalaleveva

Mina ______________________(igama nesibongo) ngiyavuma ukuletha ingane yami ongu (igama nesibongo somntwana)_________________________ ngomhlaka (usuku)________________________ ukuze ahlwwe igazi kubhekwe ukuthi welaphheke ngempela kumalaleveva.

Mina ngaziwe u–(igama lomuntu okwazise ngalolucwaningo)_________________________ ngalolucwaningo lukamalaleveva lokubheka

• Ngiyasizwa isizathu nendlela ezosentshenziswa kulolucwaningo.

• Nginikiwe ithuba lokubuza imibuzo ngalolucwaningo futhi izimpendo engzinikeziwe zingenelisile.

• Ngiyaqinisekisa ukuthi angiphoqwanga ukuba yingxenye yalolucwaningo nokuthi ngaziwe ukuthi ngingaphumuma nominyini uma ngingasathandi ukuba yingxenye ngaphandle kokuphazamiseka kokwelashelwa kwami leseisifo.

• Ngiyazi futhi ukuthi uma ngingeminaye imibuzo/izinkinga nangamandla yini engingayiqondi kahle mayelana nalolucwaningo ngingaxhumana nomhloli omkhulu walolucwaningo okungu -Dr CHV Williams, kulelikheli elilandelayo:

Umkhanyakude Health District office, Jozini, 3969
Izinombolo zocingo: 0355721357; 0725843472, I-Email: hervey.williams@kznhealth.gov.za

Ngiyazi futhi ukuthi uma ngingeminaye imibuzo ngamalungelo ami okuba yingxenye yalolucwaningo, nanoma ngabe yini engingayiqondi kahle ngalolucwaningo ngamocwaningi ngingaxhumana namahhovisi ezocwaningo kulelikheli elilandelayo: BIOMEDICAL RESEARCH ETHICS ADMINISTRATION, Research Office, Westville Campus Govan Mbeki Building Private Bag X54001, Durban 4000
KwaZulu-Natal, SOUTH AFRICA
Inombolo yocingo: 27312604769 Fax: 27312604609; Email: BREC@ukzn.ac.za

___________________________ _______________________
Isiginesha yami usuku

___________________________ _______________________
Isiginesha kafakazi (lapho edingekile) usuku

___________________________ _______________________
Isiginesha yomhumushi (lapho edingekile) usuku
Letter of permission from District Office

To Whom It May Concern:

Re: Proposed research project entitled: 'Assessment of the therapeutic efficacy of artemether-lumefantrine for the treatment of uncomplicated Plasmodium falciparum malaria in northern KwaZulu-Natal'

This office supports the above-titled research project, with Dr CH Vaughan-Williams as principal investigator, being conducted in Ukhanyakude Health District in 2011 and 2012, subject to the necessary ethical approval being granted by the UKZN Biomedical Research Ethics Committee.

Signed,

Ms MP Themba
District Manager
Umkhanyakude Health District Office

uMnyango Wizempilo. Departement van Gesondheid
Fighting Disease, Fighting Poverty, Giving Hope
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Data Analysis Sheet (Part B)

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**Malaria Journal Instructions for authors**

*Malaria Journal* is aimed at the scientific community interested in malaria in its broadest sense. It is the only journal that publishes exclusively articles on malaria and, as such, it aims to bring together knowledge from the different specialties involved in this very broad discipline, from the bench to the bedside and to the field. *Malaria Journal* offers a fast publication schedule while maintaining rigorous peer-review; this is achieved by managing the whole of the publication process electronically, from submission to peer-review.

Each article type published by *Malaria Journal* follows a specific format, as detailed in the corresponding instructions for authors; please choose an article type from the list on the left to view the instructions for authors.

The instructions for authors includes information about preparing a manuscript for submission to *Malaria Journal*, criteria for publication and the online submission process. Other relevant information about the journal's policies, the refereeing process and so on can be found in 'About this journal'.

*Malaria Journal* publishes the following article types:

- Research Articles
- Case reports
- Case studies
- Commentaries
- Meeting reports
- Methodologies
- Opinions
- Reviews

**Instructions for authors**

**Research Articles**

Submission process | Preparing main manuscript text | Preparing illustrations and figures | Preparing tables | Preparing additional files | Style and language

See 'About this journal' for descriptions of different article types and information about policies and the refereeing process.

**Submission process**

Manuscripts must be submitted by one of the authors of the manuscript, and should not be submitted by anyone on their behalf. The submitting author takes responsibility for the article during submission and peer review.

Please note that *Malaria Journal* levies an article-processing charge on all accepted Research Articles; if the submitting author's institution is a BioMed Central member the cost of the article-processing charge may be covered by the membership (see About page for detail). Please note that the membership is only automatically recognised on submission if the submitting author is based at the member institution.
To facilitate rapid publication and to minimize administrative costs, *Malaria Journal* accepts only *online submission*.

Files can be submitted as a batch, or one by one. The submission process can be interrupted at any time; when users return to the site, they can carry on where they left off.

See below for examples of *word processor* and *graphics file formats* that can be accepted for the main manuscript document by the online submission system. Additional files of any type, such as *movies*, animations, or *original data files*, can also be submitted as part of the manuscript.

During submission you will be asked to provide a cover letter. Use this to explain why your manuscript should be published in the journal, to elaborate on any issues relating to our editorial policies in the *'About Malaria Journal'* page, and to declare any potential competing interests. You will be also asked to provide the contact details (including email addresses) of potential peer reviewers for your manuscript. These should be experts in their field, who will be able to provide an objective assessment of the manuscript. Any suggested peer reviewers should not have published with any of the authors of the manuscript within the past five years, should not be current collaborators, and should not be members of the same research institution. Suggested reviewers will be considered alongside potential reviewers recommended by Editorial Board members or other advisers.

Assistance with the process of manuscript preparation and submission is available from *BioMed Central customer support team*.

We also provide a collection of links to useful tools and resources for scientific authors on our *Useful Tools* page.

**File formats**

The following word processor file formats are acceptable for the main manuscript document:

- Microsoft word (DOC, DOCX)
- Rich text format (RTF)
- Portable document format (PDF)
- TeX/LaTeX (use *BioMed Central*’s TeX template)
- DeVice Independent format (DVI)

Users of other word processing packages should save or convert their files to RTF before uploading. Many free tools are available which ease this process.

TeX/LaTeX users: We recommend using *BioMed Central*’s TeX template and BibTeX stylefile. If you use this standard format, you can submit your manuscript in TeX format. If you have used another template for your manuscript, or if you do not wish to use BibTeX, then please submit your manuscript as a DVI file. We do not recommend converting to RTF.

Note that *figures* must be submitted as separate image files, not as part of the submitted manuscript file.

**Preparing main manuscript text**

General guidelines of the journal’s style and language are given below.

**Overview of manuscript sections for Research Articles**
Manuscripts for Research Articles submitted to *Malaria Journal* should be divided into the following sections (in this order):

**Title page**

**Abstract**

**Keywords**

**Background**

**Methods**

**Results and discussion**

**Conclusions**

**List of abbreviations used** (if any)

**Competing interests**

**Authors' contributions**

**Authors' information**

**Acknowledgements**

**Endnotes**

**References**

**Illustrations and figures** (if any)

**Tables and captions**

**Preparing additional files**

The **Accession Numbers** of any nucleic acid sequences, protein sequences or atomic coordinates cited in the manuscript should be provided, in square brackets and include the corresponding database name; for example, [EMBL:AB026295, EMBL:AC157000, DDBJ:AE000812, GenBank:U49845, PDB:1BFM, Swiss-Prot:Q96KQ7, PIR:S66116].

The databases for which we can provide direct links are: EMBL Nucleotide Sequence Database (EMBL), DNA Data Bank of Japan (DDBJ), GenBank at the NCBi (GenBank), Protein Data Bank (PDB), Protein Information Resource (PIR) and the Swiss-Prot Protein Database (Swiss-Prot).

You can download a template (Mac and Windows compatible; Microsoft Word 98/2000) for your article.

For reporting standards please see the information in the **About** section.

**Title page**

The title page should:

- provide the title of the article
- list the full names, institutional addresses and email addresses for all authors
- indicate the corresponding author

Please note:

- the title should include the study design, for example "A versus B in the treatment of C: a randomized controlled trial X is a risk factor for Y: a case control study"
- abbreviations within the title should be avoided

**Abstract**

The Abstract of the manuscript should not exceed 350 words and must be structured into separate sections: **Background**, the context and purpose of the study; **Methods**, how the study was performed and statistical tests used; **Results**, the main findings; **Conclusions**, brief summary and potential implications. Please minimize the use of abbreviations and do not cite references in the abstract. **Trial registration**, if your research reports the results of a controlled health care intervention, please list your trial registry, along with the unique identifying number (e.g. **Trial registration**: Current Controlled Trials ISRCTN73824458). Please note that there should be no space between the letters and numbers of your
Keywords
Three to ten keywords representing the main content of the article.

Background
The Background section should be written in a way that is accessible to researchers without specialist knowledge in that area and must clearly state - and, if helpful, illustrate - the background to the research and its aims. Reports of clinical research should, where appropriate, include a summary of a search of the literature to indicate why this study was necessary and what it aimed to contribute to the field. The section should end with a brief statement of what is being reported in the article.

Methods
The methods section should include the design of the study, the setting, the type of participants or materials involved, a clear description of all interventions and comparisons, and the type of analysis used, including a power calculation if appropriate. Generic drug names should generally be used. When proprietary brands are used in research, include the brand names in parentheses in the Methods section.

For studies involving human participants a statement detailing ethical approval and consent should be included in the methods section. For further details of the journal's editorial policies and ethical guidelines see 'About this journal'.

For further details of the journal's data-release policy, see the policy section in 'About this journal'.

Results and discussion
The Results and discussion may be combined into a single section or presented separately. Results of statistical analysis should include, where appropriate, relative and absolute risks or risk reductions, and confidence intervals. The Results and discussion sections may also be broken into subsections with short, informative headings.

Conclusions
This should state clearly the main conclusions of the research and give a clear explanation of their importance and relevance. Summary illustrations may be included.

List of abbreviations
If abbreviations are used in the text they should be defined in the text at first use, and a list of abbreviations can be provided, which should precede the competing interests and authors’ contributions.

Competing interests
A competing interest exists when your interpretation of data or presentation of information may be influenced by your personal or financial relationship with other people or organizations. Authors must disclose any financial competing interests; they should also reveal any non-financial competing interests that may cause them embarrassment were they to become public after the publication of the manuscript.

Authors are required to complete a declaration of competing interests. All competing interests that are declared will be listed at the end of published articles. Where an author gives no competing interests, the listing will read 'The author(s) declare that they have no competing interests'.
When completing your declaration, please consider the following questions:

**Financial competing interests**

In the past five years have you received reimbursements, fees, funding, or salary from an organization that may in any way gain or lose financially from the publication of this manuscript, either now or in the future? Is such an organization financing this manuscript (including the article-processing charge)? If so, please specify.

Do you hold any stocks or shares in an organization that may in any way gain or lose financially from the publication of this manuscript, either now or in the future? If so, please specify.

Do you hold or are you currently applying for any patents relating to the content of the manuscript? Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript? If so, please specify.

Do you have any other financial competing interests? If so, please specify.

**Non-financial competing interests**

Are there any non-financial competing interests (political, personal, religious, ideological, academic, intellectual, commercial or any other) to declare in relation to this manuscript? If so, please specify.

If you are unsure as to whether you, or one your co-authors, has a competing interest please discuss it with the editorial office.

**Authors' contributions**

In order to give appropriate credit to each author of a paper, the individual contributions of authors to the manuscript should be specified in this section.

An 'author' is generally considered to be someone who has made substantive intellectual contributions to a published study. To qualify as an author one should 1) have made substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; 2) have been involved in drafting the manuscript or revising it critically for important intellectual content; and 3) have given final approval of the version to be published. Each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the content. Acquisition of funding, collection of data, or general supervision of the research group, alone, does not justify authorship.

We suggest the following kind of format (please use initials to refer to each author's contribution): AB carried out the molecular genetic studies, participated in the sequence alignment and drafted the manuscript. JY carried out the immunooassays. MT participated in the sequence alignment. ES participated in the design of the study and performed the statistical analysis. FG conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

All contributors who do not meet the criteria for authorship should be listed in an acknowledgements section. Examples of those who might be acknowledged include a person who provided purely technical help, writing assistance, or a department chair who provided only general support.

**Authors' information**

You may choose to use this section to include any relevant information about the author(s) that may aid the reader's interpretation of the article, and understand the standpoint of the author(s). This may include details about the authors' qualifications, current positions they hold at institutions or societies, or any other relevant background information. Please refer to authors using their initials. Note this section should not be used to describe any competing interests.
Acknowledgements

Please acknowledge anyone who contributed towards the article by making substantial contributions to conception, design, acquisition of data, or analysis and interpretation of data, or who was involved in drafting the manuscript or revising it critically for important intellectual content, but who does not meet the criteria for authorship. Please also include the source(s) of funding for each author, and for the manuscript preparation. Authors must describe the role of the funding body, if any, in design, in the collection, analysis, and interpretation of data; in the writing of the manuscript; and in the decision to submit the manuscript for publication. Please also acknowledge anyone who contributed materials essential for the study. If a language editor has made significant revision of the manuscript, we recommend that you acknowledge the editor by name, where possible.

The role of a scientific (medical) writer must be included in the acknowledgements section, including their source(s) of funding. We suggest wording such as ‘We thank Jane Doe who provided medical writing services on behalf of XYZ Pharmaceuticals Ltd.’

Authors should obtain permission to acknowledge from all those mentioned in the Acknowledgements section.

Endnotes

Endnotes should be designated within the text using a superscript lowercase letter and all notes (along with their corresponding letter) should be included in the Endnotes section. Please format this section in a paragraph rather than a list.

References

All references, including URLs, must be numbered consecutively, in square brackets, in the order in which they are cited in the text, followed by any in tables or legends. Each reference must have an individual reference number. Please avoid excessive referencing. If automatic numbering systems are used, the reference numbers must be finalized and the bibliography must be fully formatted before submission.

Only articles, datasets and abstracts that have been published or are in press, or are available through public e-print/preprint servers, may be cited; unpublished abstracts, unpublished data and personal communications should not be included in the reference list, but may be included in the text and referred to as “unpublished observations” or “personal communications” giving the names of the involved researchers. Obtaining permission to quote personal communications and unpublished data from the cited colleagues is the responsibility of the author. Footnotes are not allowed, but endnotes are permitted. Journal abbreviations follow Index Medicus/MEDLINE. Citations in the reference list should include all named authors, up to the first 30 before adding ‘et al.’.

Any in press articles cited within the references and necessary for the reviewers’ assessment of the manuscript should be made available if requested by the editorial office.

Style files are available for use with popular bibliographic management software:

BibTeX

EndNote style file

Reference Manager

Zotero

Examples of the Malaria Journal reference style are shown below. Please ensure that the reference style is followed precisely; if the references are not in the correct style they may have to be retyped and carefully proofread.
All web links and URLs, including links to the authors’ own websites, should be given a reference number and included in the reference list rather than within the text of the manuscript. They should be provided in full, including both the title of the site and the URL, in the following format: The Mouse Tumor Biology Database [http://tumor.informatics.jax.org/mtbwi/index.do]. If an author or group of authors can clearly be associated with a web link, such as for weblogs, then they should be included in the reference.

Examples of the Malaria Journal reference style

Article within a journal

Article within a journal supplement

In press article

Published abstract

Article within conference proceedings

Book chapter, or article within a book

Whole issue of journal

Whole conference proceedings

Complete book

Monograph or book in a series

Book with institutional author
**PhD thesis**

**Link / URL**

**Link / URL with author(s)**
Neylon C: Open Research Computation: an ordinary journal with extraordinary aims. [http://blogs.openaccesscentral.com/bmcblog/entry/open_research_computation_an_ordinary](http://blogs.openaccesscentral.com/bmcblog/entry/open_research_computation_an_ordinary)

**Dataset with persistent identifier**
Zheng, L-Y; Guo, X-S; He, B; Sun, L-J; Peng, Y; Dong, S-S; Liu, T-F; Jiang, S; Ramachandran, S; Liu, C-M; Jing, H-C (2011): Genome data from sweet and grain sorghum (Sorghum bicolor). *GigaScience*. [http://dx.doi.org/10.5524/100012](http://dx.doi.org/10.5524/100012)

**Preparing illustrations and figures**
Illustrations should be provided as separate files, not embedded in the text file. Each figure should include a single illustration and should fit on a single page in portrait format. If a figure consists of separate parts, it is important that a single composite illustration file be submitted which contains all parts of the figure. There is no charge for the use of color figures.

Please read our [figure preparation guidelines](#) for detailed instructions on maximising the quality of your *figures*.

**Formats**
The following file formats can be accepted:

- PDF (preferred format for diagrams)
- DOCX/DOC (single page only)
- PPTX/PPT (single slide only)
- EPS
- PNG (preferred format for photos or images)
- TIFF
- JPEG
- BMP

**Figure legends**
The legends should be included in the main manuscript text file at the end of the document, rather than being a part of the figure file. For each figure, the following information should be provided: Figure number (in sequence, using Arabic numerals - i.e. Figure 1, 2, 3 etc); short title of figure (maximum 15 words); detailed legend, up to 300 words.

Please note that it is the responsibility of the author(s) to obtain permission from the copyright holder to reproduce figures or tables that have previously been published elsewhere.
Preparing a personal cover page

If you wish to do so, you may submit an image which, in the event of publication, will be used to create a cover page for the PDF version of your article. The cover page will also display the journal logo, article title and citation details. The image may either be a figure from your manuscript or another relevant image. You must have permission from the copyright to reproduce the image. Images that do not meet our requirements will not be used.

Images must be 300dpi and 155mm square (1831 x 1831 pixels for a raster image).

Allowable formats - EPS, PDF (for line drawings), PNG, TIFF (for photographs and screen dumps), JPEG, BMP, DOC, PPT, CDX, TGF (ISIS/Draw).

Preparing tables

Each table should be numbered and cited in sequence using Arabic numerals (i.e. Table 1, 2, 3 etc.). Tables should also have a title (above the table) that summarizes the whole table; it should be no longer than 15 words. Detailed legends may then follow, but they should be concise. Tables should always be cited in text in consecutive numerical order.

Smaller tables considered to be integral to the manuscript can be pasted into the end of the document text file, in A4 portrait or landscape format. These will be typeset and displayed in the final published form of the article. Such tables should be formatted using the 'Table object' in a word processing program to ensure that columns of data are kept aligned when the file is sent electronically for review; this will not always be the case if columns are generated by simply using tabs to separate text. Columns and rows of data should be made visibly distinct by ensuring that the borders of each cell display as black lines. Commas should not be used to indicate numerical values. Color and shading may not be used; parts of the table can be highlighted using symbols or bold text, the meaning of which should be explained in a table legend. Tables should not be embedded as figures or spreadsheet files.

Larger datasets or tables too wide for a landscape page can be uploaded separately as additional files. Additional files will not be displayed in the final, laid-out PDF of the article, but a link will be provided to the files as supplied by the author.

Tabular data provided as additional files can be uploaded as an Excel spreadsheet (.xls) or comma separated values (.csv). As with all files, please use the standard file extensions.

Preparing additional files

Although Malaria Journal does not restrict the length and quantity of data included in an article, we encourage authors to provide datasets, tables, movies, or other information as additional files.

Please note: All Additional files will be published along with the article. Do not include files such as patient consent forms, certificates of language editing, or revised versions of the main manuscript document with tracked changes. Such files should be sent by email to malariajournal@biomedcentral.com, quoting the Manuscript ID number.

Results that would otherwise be indicated as “data not shown” can and should be included as additional files. Since many weblinks and URLs rapidly become broken, Malaria Journal requires that supporting data are included as additional files, or deposited in a recognized repository. Please do not link to data on a personal/departmental website. The maximum file size for additional files is 20 MB each, and files will be virus-scanned on submission.

Additional files can be in any format, and will be downloadable from the final published article as supplied by the author. We encourage authors to use formats which facilitate reuse. e.g. We recommend CSV rather than PDF for tabular data.
Certain supported file formats are recognized and can be displayed to the user in the browser. These include most movie formats (for users with the Quicktime plugin), mini-websites prepared according to our guidelines, chemical structure files (MOL, PDB), geographic data files (KML).

If additional material is provided, please list the following information in a separate section of the manuscript text:

File name (e.g. Additional file 1)

File format including the correct file extension for example .pdf, .xls, .txt, .pptx (including name and a URL of an appropriate viewer if format is unusual)

Title of data

Description of data

Additional files should be named "Additional file 1" and so on and should be referenced explicitly by file name within the body of the article, e.g. 'An additional movie file shows this in more detail [see Additional file 1]'.

**Additional file formats**

Ideally, file formats for additional files should not be platform-specific, and should be viewable using free or widely available tools. The following are examples of suitable formats.

**Additional documentation**

- **PDF (Adobe Acrobat)**

**Animations**

- **SWF (Shockwave Flash)**

**Movies**

- **MP4 (MPEG 4)**
- **MOV (Quicktime)**

**Tabular data**

- **XLS, XLSX (Excel Spreadsheet)**
- **CSV (Comma separated values)**

As with figure files, files should be given the standard file extensions.

**Mini-websites**

Small self-contained websites can be submitted as additional files, in such a way that they will be browsable from within the full text HTML version of the article. In order to do this, please follow these instructions:

Create a folder containing a starting file called index.html (or index.htm) in the root.
Put all files necessary for viewing the mini-website within the folder, or sub-folders.

Ensure that all links are relative (ie "images/picture.jpg" rather than "images/picture.jpg" or "http://yourdomain.net/images/picture.jpg" or "C:\Documents and Settings\username\My Documents\mini-website\images\picture.jpg") and no link is longer than 255 characters.

Access the index.html file and browse around the mini-website, to ensure that the most commonly used browsers (Internet Explorer and Firefox) are able to view all parts of the mini-website without problems, it is ideal to check this on a different machine.

Compress the folder into a ZIP, check the file size is under 20 MB, ensure that index.html is in the root of the ZIP, and that the file has .zip extension, then submit as an additional file with your article.

Style and language

General

Currently, Malaria Journal can only accept manuscripts written in English. Spelling should be US English or British English, but not a mixture.

There is no explicit limit on the length of articles submitted, but authors are encouraged to be concise. There is also no restriction on the number of figures, tables or additional files that can be included with each article online. Figures and tables should be numbered in the order in which they are referred to in the text. Authors should include all relevant supporting data with each article.

Malaria Journal will not edit submitted manuscripts for style or language; reviewers may advise rejection of a manuscript if it is compromised by grammatical errors. Authors are advised to write clearly and simply, and to have their article checked by colleagues before submission. In-house copyediting will be minimal. Non-native speakers of English may choose to make use of a copyediting service.

Help and advice on scientific writing

The abstract is one of the most important parts of a manuscript. For guidance, please visit our page on Writing titles and abstracts for scientific articles.

Tim Albert has produced for BioMed Central a list of tips for writing a scientific manuscript. American Scientist also provides a list of resources for science writing. For more detailed guidance on preparing a manuscript and writing in English, please visit the BioMed Central author academy.

Abbreviations

Abbreviations should be used as sparingly as possible. They should be defined when first used and a list of abbreviations can be provided following the main manuscript text.

Typography

Please use double line spacing.

Type the text unjustified, without hyphenating words at line breaks.

Use hard returns only to end headings and paragraphs, not to rearrange lines.

Capitalize only the first word, and proper nouns, in the title.
All pages should be numbered.

Use the *Malaria Journal* reference format.

Footnotes are not allowed, but endnotes are permitted.

Please do not format the text in multiple columns.

Greek and other special characters may be included. If you are unable to reproduce a particular special character, please type out the name of the symbol in full. **Please ensure that all special characters used are embedded in the text, otherwise they will be lost during conversion to PDF.**

**Units**

SI units should be used throughout (liter and molar are permitted, however).
30 October 2011

Dr. V Vaughan-Williams
Department of Public Health
Nelson R Mandela School of Medicine
University of KwaZulu-Natal

Dear Dr. Williams

REF: BE099/11

PROVISIONAL APPROVAL

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your response dated 14 September 2011 to queries raised on 29 August 2011.

The study is given PROVISIONAL APPROVAL pending receipt of:

1. Permission from Umkhanyakude Health District and Hospital Managers and Clinics
2. Postgraduate Education Committee Approval

Only when full ethical approval is given, may the study begin. Full ethics approval has not been given at this stage.

PLEASE NOTE: Provisional approval is valid for 6 months only - should we not hear from you during this time - the study will be closed and reapplication will need to be made.

BREC is registered with the South African National Health Research Ethics Council (REC: 290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

Yours sincerely

Mrs A Marimuthu
Senior Administrator: Biomedical Research Ethics
Dear Dr C H Vaughan-Williams

Subject: Approval of a Research Proposal

1. The research proposal titled ‘Assessment of the therapeutic efficacy of artemether-lumefantrine in the treatment of uncomplicated Plasmodium falciparum malaria in northern KwaZulu-Natal’ was reviewed by the KwaZulu-Natal Department of Health.

The proposal is hereby approved for research to be undertaken at Umkhanyakude.

2. You are requested to take note of the following:
   a. Make the necessary arrangement with the identified facility before commencing with your research project.
   b. Provide an interim progress report and final report (electronic and hard copies) when your research is complete.

3. Your final report must be posted to HEALTH RESEARCH AND KNOWLEDGE MANAGEMENT, 10-102, PRIVATE BAG X9051, PIETERMARITZBURG, 3200 and e-mail an electronic copy to hrkm@kznhealth.gov.za

For any additional information please contact Mrs G Khumalo on 033-3953189.

Yours Sincerely

[Signature]

Dr E Lutge
Chairperson, Health Research Committee
KwaZulu-Natal Department of Health

Date: 12/12/2011

uMnyango Wezempilo, Departement van Gesondheid
Fighting Disease, Fighting Poverty, Giving Hope
Dear Dr Knight


The Postgraduate Education Committee considered the abovementioned application and raised various queries. These have been addressed and the protocol is given provisional approval for the Master of Public Health degree. This decision will be ratified at a full sitting of the Committee scheduled for 6th December 2011.

Please note that the study may not begin without ethics approval.

Yours sincerely

Dr A Voce
Dean’s Assistant: Coursework Programmes
Postgraduate Education Committee

University of KwaZulu-Natal

http://www.ukzn-nrmsm.co.za
## Study Data Table, including data not published

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