Efficacy and safety of artesunate/mefloquine in the treatment of uncomplicated *P. falciparum* infections.

This dissertation has been written in part fulfillment of the degree of Masters of Medicine (Internal Medicine), University of Nairobi.

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AKNOWLEDGEMENT

I wish to express my sincere gratitude to all the following;

DECLARATION

I certify that this dissertation is my original work and that it has not been presented for a degree in any other university.

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DEDICATION

To my wife Pauline, children Alusa and Alex, and my parents.

ABBREVIATIONS

Na^+-sodium

K^+- potassium

ALT-alanine aminotransferase

AST-aspartate aminotransferase

ECG-echocardiogram

Hb-haemoglobin

RBC- red blood cells

WBC-white blood cells

B.P.-blood pressure

MOH-ministry of health

ACT-artemisinin combination therapy

SP-sulphadoxine/pyrimethamine

WHO-world health organization

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1 ABSTRACT

Introduction: The treatment of *plasmodium falciparum* malaria is facing significant challenges due to the rapidly emerging drug resistance patterns to the commonly used antimalarial monotherapies. Many countries are now changing/have changed their antimalarial drug policies in line with the current recommendation for the use of combination therapies.

Justification: Even though artesunate and mefloquine have used as monotherapies in the treatment of malaria in Kenya for a long time, there is insufficient data on the clinical outcome when used as a combination therapy . This study was conducted to derive data on the efficacy and safety profile of artesunate/mefloquine combination in the treatment of uncomplicated *p. falciparum* malaria in the Kenya.

Study area: Bungoma District of Kenya, an area with a stable endemic malaria transmission pattern.

Study design: This was an open-label single arm clinical trial on patients with uncomplicated *p. falciparum* malaria. 200 patients were enrolled in the study and treated with a three-day course of oral artesunate/mefloquine administered simultaneously, and followed up on out-patient basis upto the 28th day for clinical and parasitological responses. Adverse events, both drug-related and non drug-related were recorded during the treatment and follow-up period.

Results: In the evaluable patient population, day 28 cure rate was 98.4%, while days14 and 7 cure rates were 98.4% and 99.2% respectively. Day 28 cure rate in the Intention To Treat population using the last observation carried forward method (LOCF) was 92%. There was rapid relief of symptoms by 82%, 89% and 97% on days 1, 2 and 3 respectively. The median time to fever clearance was one day. The commonest drug related adverse events were mild to moderate degrees of headache, dizziness and asthenia occurring in 57% of the patients. Severe adverse events occurred in 6% of the patients. There was no significant derangement in the haematological, biochemical, and ECG parameters of the patients on treatment.

Conclusion: Artesunate/mefloquine combination was found to be highly effective in the treatment of uncomplicated *p.falciparum* malaria, with a significant though tolerable side effect profile.

Recommendation: Arteunate/mefloquine is recommended a viable option for use in the treatment of uncomplicated *p.falciparum* malaria in this region. Further studies on this drug combination need to be conducted in other parts of the country to determine if the obtained results are reproducible hence possible widescale use.

II. INTRODUCTION

Approximately 400 million people get infected with malaria every year and half of them succumb to it. (1). Whereas 90% of these cases of infections and deaths occur in Africa, more so in the Sub-Saharan region, every region of the world is affected by malaria (2). Malaria is prevalent in Africa, India, the Middle East, Southeast Asia, China, Oceania, Central and South America, Mexico and the Dominic Republic (3)

Although the disease affects both the young and the old, children below the age of 5 years are particularly at increased risk with mortality of up to 1 in every 20 infections. Pregnant women, particularly primigravidae, non-immune travellers and splenectomized patients are the other groups of individuals at higher risk for severe malaria infections.

Malaria endemicity in Kenya varies from region to region, with a holoendemic pattern in the coastal and Lake regions and malaria free areas on the high grounds of Aberdare ranges and Mt. Kenya (4). There is perrenial transmission of malaria in the lake region and the coastal areas of altitudes not exceeding 300 metres above sea level (stable transmission).

Intermittent transmission occurs annually, biannually or in variable epidemics in parts of Eastern and Rift valley provinces (unstable malaria transmission). Epidemic transmissions have been reported in the highlands, semi-arid areas and other areas bordering endemic zones with resultant high morbidity and mortality in all age groups (5).

Malaria as a disease has far reaching social, political and economic consequences on individuals, communities and nations.

In 1992, a ministerial conference on malaria held in Amsterdam endorsed a strategy to focus on local and national capabilities of countries to fight malaria while adapting to specific country circumstances. Emphasis was put on early diagnosis and treatment, implementing preventive measures, control of epidemics, and research.

In line with advances in development of new drugs to treat malaria, other approaches in the fight against malaria are also under way e.g.

i) Vaccine development to target sporozoites, asexual blood stage and block transmission

ii) Use of impregnated mosquito nets (e.g. rollback malaria 2000 programme)

iii) Genetic engineering of an uninfectable mosquito

iv) Use of insecticide sprays and other measures to control the vector.

Malaria is caused by the blood parasite plasmodium, which belongs to the family of plasmadiidae. The genus exists in an asexual form in the vertebrae host and a sexual form in the mosquito host. Four species are recognized to cause disease in man i.e. *p. falciparum*, *p. vivax*, *p. malaria* and *p. ovale*. *p. falciparum* species accounts for 98% of all malaria cases in Kenya and is associated with a high morbidity and mortality (4). Transmission to man occurs via the bite of female anopheles mosquitoes as the vector. Other modes of transmission include transfusion of infected blood and sharing of infected needles.

III.PARASITOLOGY

Life Cycle:

This is the same for all the species.

A. Sporogony (sexual phase)

Gametocytes ingested by an anopheles mosquito undergo fertilization to form a zygote which forms an ookinete and later an oocyst in the stomach of the mosquito. Sporozoites are released from the oocyst and reach the mosquito's salivary glands. During a blood meal, the sporozites are injected into the human bloodstream.

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B. Schizogony (asexual phase)

Inoculated sporozoites enter parenchymal cells of the liver where they undergo exoerythrocytic and erythrocytic multiplication before forming gametocytes. Sporozoites of *p. ovale* and *p. vivax* form hypnozoites which account for relapse of malaria infections months to years after the primary attack.

Parasites consume and degrade heamoglobin of an infected erythrocyte, and render the cell more antigenic and less deformable. Cytoadherence to venular and capillary endothelium by these cells is facilitated by formation of strain specific adhesive proteins on the surfaces of the parasitized cells. Infected cells also adhere to uninfected cells to form rossettes.

Sequesteration of parasitized and sometimes normal erythrocytes leads to many of the manifestations of malaria infection. Splenic sequestration, macrophage activation and release of cytokines are some of the defense mechanisms elicitated by the body faced with infection.

Individuals who live in malaria-endemic regions with frequent exposure to the disease develop partial immunity to the disease with no infection. This immunity is lost once the individual lives outside an endemic area for some months.

Early diagnosis and initiation of treatment, especially for *p.falciparum* malaria, is quite prudent to ameliorate malaria complications.

Diagnostic tests for malaria include;

- Thick and thin blood slide tests
- Serology for malaria antigens

Malaria causes an acute febrile illness with periods of febrile paroxysms whose fever pattern depends on the infecting species. Other features include malaise, fatigue, myalgia, arthralgia, lassitude, headaches, dizziness, nausea and vomiting. Malaria may present with no complications as above or complicated with features of prostration, respiratory distress, convulsions, severe anaemia, renal failure, hypoglycaemia and disseminated intravascular coagulation (DIC).

Treatment of malaria involves supportive measures and specific antimalarial drugs.

There are various drugs currently available for treatment of malaria. These exist as single or combination therapies.

IV. Available antimalarial drugs

1. 4 aminoquinolines– chloroquine, amodiaquine
2. 8 aminoquinolines – primaquine
3. Quinoline methanol – quinine, quinidine
4. Antibiotics – tetracycline, doxycycline, clindamycin
5. Halofantrine
6. Proguanil
7. Sulfadoxine/pyrimethamine and sulfalene/pyrimethamine
8. Mefloquine
9. Atovaquone
10. Artemisinin derivatives – artemether, arteether and artesunate

Currently there is no antimalarial drug available that acts on all stages of the malaria life cycle to inhibit or kill the parasites, rather they all have a selective action on the different phases of the parasite's life cycle. Some of these drugs are highly efficacious and rapidly acting e.g. chloroquine, quinine, quinidine, mefloquine, atovaquone and artemisinin derivatives, while other like proguanil, pyrimethamine, sulfonamides and the antibiotics are slow acting and less effective.

P. falciparum resistance to conventional anti malarial drugs is an emerging trend. Improper widescale use of anti malarial drugs, inadequate dosing and use of slowly eliminated drugs contribute to the selection of resistant mutants (6-9).

Resistance to all known antimalarial drugs with the exception of artemisinin derivatives has developed to various levels in several countries (10).

Resistance to chloroquine was first reported in Thailand in 1957. Cases were later reported in South America in 1960 and in Papua New Guinea in 1976. Chloroquine resistance was reported in Kenya in 1978 and later in the Comoros Island, Tanzania and Uganda (11) . Chloroquine-resistant *p.falciparum* is now widespread in all countries with *p.falciparum* malaria except Haiti, Mauritius, most of the Middle East and Egypt (12). Strains resistant to chloroquine have shown rapid leakage of the drug from the parasites' food vacuoles, a process that is reverted by calcium channel blockers eg verapamil in in-vitro studies (13). The gene for this resistance has been localized on a segment of chromosome 7 of *P. falciparum*. The role of multiple drug resistance, MDR,is not conclusive in chloroquin resistance.

Resistance to pyrimethamine – sulfonamide combinations is widespread in south East Asia, parts of East, central and southern Africa, and South America (14)

Resistance to pyrimethamine(dihydrofolate reductase –DHFR- inhibitor) has been explained by point mutation in the active site of DHFR enzymes i.e serine to Asparagine at position 108 reducing the binding of pyrimethamine to the active site.

Wellem and colleagues using PCR found 90% of isolates of resistance to Fansidar in the Brazilian Amazon to contain the DHFR Asn –108 mutation. Cross-resistance has been demonstrated between pyrimethamine and proguanil, chlorproguanil and cycloguanil.

There is progressive increase in Minimum Inhibitory Concentration (MIC) of quinine for *P. falciparum* in Thailand, with increase in R1 and R II resistance patterns unless it is combined with tetracycline or any other accessory drug (12).

A trial in this region demonstrated only a 60% cure rate for quinine monotherapy.

Mefloquine resistance has been reported in South East Asia and some African countries. More than 50% of strains in Thailand have demonstrated mefloquine resistance (15). Increased *p.falciparum* sensitivity to mefloquine was demonstrated in vitro following its combination with artesunate(16). Resistance of *p.falciparum* to mefloquine is associated with amplification of the MDR-like genes.

Halofantrine resistance has been reported too and cross-resistance between mefloquine, halofantrine and quinine can occur (2).

Resistance to clindamicin and atovaquone develops rapidly if these agents are used as monotherapy for *p. falciparum* infections. Resistance to atovaquone is via single point mutations in the cytochrome-b gene.

There has been no reported resistance to the artemisinin derivatives.

The use of antimalarial drug combinations has been advocated as the way forward in effective treatment of malaria (17). Drugs used in such combination therapy should have well matched pharmacokinetic and phamarmacodynamic properties with no additional toxicity (18)

Drugs with a short elimination half-life are less vulnerable to the development of drug resistance than those with a long half-life. Combination of a drug with a short half-life but high cure rates and one with a long half-life ensures that relatively fewer parasites are exposed to the latter drug while not exposing any single component of the combination to the whole mass of the parasites.

When an infection fails to be eradicated from the body, selection pressure occurs and encourages the development of resistance. Resistance potential is a function of parasite biomass, therefore the risk of resistance of isolates emerging from a primary infection are greatly reduced if the parasite biomass is reduced.

Combination of these pharmakinetic and pharmadynamic characteristics reduces the chance of a resistant mutant occurring and prolongs the life of individual components of the therapy.

As a response to the antimalarial drug resistance situation, the WHO recommends that treatment policies for falciparum malaria in all countries experiencing resistance to monotherapies should be combination therapies, preferably those containing an artemisinin derivative (ACT-artemisinin based combination therapy)

The current WHO recognized antimalarial combinations include;
Artemether/lumefantrine
Artesunate/amodiaquine
Artesunate/SP
Amodiaqine/SP
Artesunate/mefloquine

A study using a combination of artesunate and pyrimethamine/sulfadoxine for treatment of uncomplicated malaria in Gambian children has shown promising results (19). However in Kenya there is already substantial resistance to sulfadoxine/pyrimethamine and such a combination may not be very useful.

In other studies in Thailand and Africa, artesunate/mefloquine was found to be a highly effective combination therapy for the treatment of uncomplicated malaria (20,21). This high efficacy is also accompanied by protection of the individual drugs against resistance (22).

The combination of artesunate/mefloquine is available as Artequin in pre-packed blister packs to improve compliance hence achieve acceptable cure rates.

V. ARTEQUIN

Artequin is a free combination of two antimalarial drugs, artesunate and mefloquine. Artesunate is a water-soluble hemisuccinate ester of artemesinin, a substance derived from the herb Artemisia annua (sweet wormwood) which has been used for treatment of fevers in china for more than 1000 years. By 1972 Chinese scientists had extracted and crystalised the major antimalarial agent, quinghaosu, now known as artemisinin (23).

They synthesized derivatives with greater antimalarial activity than artemisinin itself, namely dihydroartemisinin (a reduced product), artemether (an oil-soluble methyl ester) and artesunate (a water-soluble hemisuccinate salt of dihydroartemisinin).

Fig.1:Chemical structure of artemisinin and its derivatives

ARTEMISININ

DIHYDROARTEMISININ

ARTEMETHER

ARTESUNATE
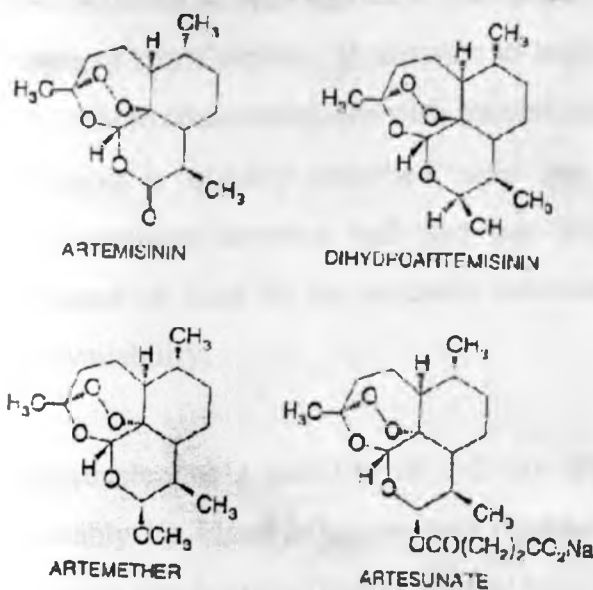
The antimalarial activity of artemesinin probably results from production of free radicals following iron – catalyzed cleavage of artemisinin endoperoxide bridge in the parasites' food vacuole (24). It is a potent blood schizonticide with activity against all human malaria parasites even those resistant to other antimalarial drugs, but no activity on hepatic stages. It has gametocidal activity. Artemesinin-resistant *p.falciparum* malaria has not yet been identified.

Mefloquine is a synthetic 4 quinoline methanol chemically related to quinine.

Fig.2:Chemical structure of mefloquine
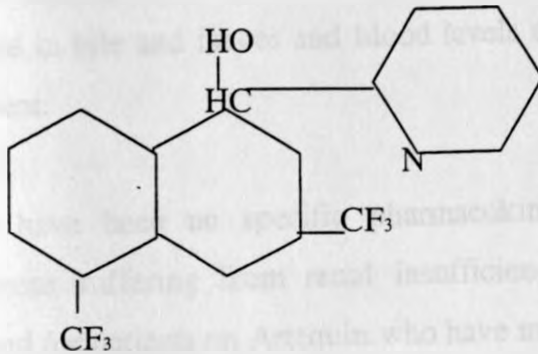
The mechanism of action of mefloquine is unknown. It however has strong blood schizonticidal activity against *P. falciparum* and *P. vivax* though not effective against hepatic stages or gametocytes. Resistance to mefloquine has been reported in regions of Southeast Asia where cross-resistance with quinine and halofantrine was noted.

Artequin is quickly absorbed after oral administration and reaches maximum plasma concentrations between half and one hour (artesunate 1-2hrs, mefloquine 6-24hrs). The presence of food in the stomach increases the rate of absorption and leads to increased bioavailability.

Artesunate has a half-life of 1-3 hrs after oral administration. It is hydrolysed in vivo probably by blood estarases and cytochrome P45O system to dihydroartemisinin (DHA) which is also highly effective against malaria.

The concentration of DHA in *p.falciparum* infected erythrocytes in vivo was found to be 300-fold the plasma concentration, and only 2-fold in uninfected cells, with plasma protein binding of 59% for artesunate and 43% for DHA.

Elimination half-life of artesunate is 0.5 hours. Elimination half-life of DHA is approximately 0.75 hrs and is cleared predominantly by hepatic bio-transformation to pharmacologically inactive metabolites.

The concentration of mefloquine in erythrocytes is 2.6 to 4 times that in plasma with more than 98% of the drug bound to plasma proteins. Several metabolites of mefloquine have been identified, the major one being the corresponding quinoline carboxylic acid which is inactive against *p.falciparum*. Elimination half- life of mefloquine is 21 days (15-33), is mainly secreted in bile and faeces and blood levels can be detected for months after completion of treatment.

There have been no specific pharmacokinetic studies for patients on mefloquine and artesunate suffering from renal insufficiency. No specific dose adjustment is however required for patients on Artequin who have minimal renal impairment since only a very small faction of the drug is eliminated via the kidneys.

Hepatic insufficiency has also been shown not to have an effect on bioavailability and clearance of oral artemisinin. The elimination of mefloquine in such patients may however be delayed with resultant high plasma concentrations. This calls for caution in patients with hepatic insufficiency receiving Artequin (25).

The transformation of artesunate to the DHA after an oral dose is so rapid that the pharmacokinetic properties and drug interactions of the two are expected to be the same .The pharmacokinetic properties of mefloquine have been shown to vary between healthy volunteers and patients, ethnic grouping and commercial preparations used (26).

Combination therapy using artesunate and mefloquine has been studied and used for many years in South East Asia (14, 15). There have been two other studies in Africa and Thailand with good results (20, 21)

Artemisinim and its derivatives are a group of antimalarials with very rapid onset of action and parasite clearance. DHA, an intermediate product of artesunate, arteether and artemether, is 4-5 times more potent than the parent compound though with high recrudescence rate if used for shorter than five days.

The use of artemisinin with a long half-life drug like mefloquine provides a solution to this problem. This combination has been shown to increase cure rates of *P. falciparum*, reduce transmission through its gametocidal activity and may slow development of resistance (22).

Moreover this combination has been shown to induce faster symptomatic response with shorter parasite clearance times than mefloquine alone, and less recrudescence as compared to artesunate monotherapy when used for less than 5 days (19).

In a multi-centre study in 104 African patients with uncomplicated *p.falciparum* malaria, Artequin showed 100% cure rate at day 14 with no recrudescence by day 28, and mean fever and parasite clearance times 32 and 45 hours respectively (21)

The use of artesunate/mefloquine given simultaneously for 3 days was shown to be as effective and well tolerated as a standard sequential regime treatment where mefloquine would be introduced on the second day and given for only 2 days, with the added benefit of improved compliance because of the prepacked combination blisters (20)

A study carried out in Thailand (25) showed that when given as a single dose of mefloquine simultaneously with DHA, the pharmacokinetics were similar except for the absorption rate of mefloquine which was faster in the presence of DHA. The combination of DHA and mefloquine resulted in synergistic effects on blood schizonticidal activity and was more effective than either mefloquine or artesunate given alone.

In another study testing four different combination regimens of Dihydroartemisin/mefloquine using single dose 300mg of dihydroartemisinin plus one 250mg two doses of mefloquine showed no difference in response in any of the treatment groups. There was no significan difference in the susceptibility to mefloquine between primary and recrudescent isolates Most patients tolerated the drug well and none had serious adverse effects or discontinue treatment due to adverse effects or laboratory abnormalities (26).

Indications of Artequin

- Treatment of non-complicated *P. falciparum* malaria in endemic areas
- Treatment of multidrug resistant strains of *p.falciparum.*
- Treatment of malaria due to mixed malaria parasites.

Dosage

A treatment course for adults weighing between 30 and 55kg comprises of 3 doses of one artesunate 200mg tablet and one mefloquine 250 mg tablet given simultaneously once daily for three consecutive days (Artequin 600/750). For patients with body weight more than 55kg a higher Artequin dose is recommended (Artequin 600/1500).

The tablets must be taken simultaneously without chewing them, with a large amount of liquid and if possible a meal.

Due to limited clinical experience Artequin is currently not recommended for use in patients who weigh less than 30kg.

Contraindications

- Known hypersensitivity to artesunate or mefloquine.
- Epileptics - mefloquine may increase the risk of seizures.
- Concomitant use with halofantrine, quinine, quinidine because of potential fatal prolongation of the QT interval.
- Pregnancy.
- Psychiatric disorders, arrhythmias, cardiac conduction defects.

Not recommended

- Prophylaxis of malaria.
- Driving vehicles, piloting aircraft, operating machinery because of mefloquir induced dizziness.

Side Effects

- Gastrointestinal disorders – abdominal pain, vomiting, diarrhoea.
- Nervous system – dizziness, insomnia.
- General disorder – asthenia, anorexia, incidents of sleep and behavioural disturbance, headache and rash have been associated with use of mefloquine. Similar symptoms often accompany an acute malarial attack hence may overlap with the side effects of Artequin.
- Cardiovascular system – transient first degree heart block.
- Rarely mild and transient reduction in reticulocytes and granulocytes, transient increase in transaminases and total bilirubin.

VI. JUSTIFICATION

Malaria remains a major course of morbidity and mortality in sub-Saharan Africa, including Kenya. This constitutes a major threat to health and a direct hindrance to economic development of the affected nations.

Antimalarial drug resistance continues to be a major problem due to unjudicious wide scale use of antimalarial drugs, inadequate dosing and use of drugs that are eliminated very slowly. Due to high-level resistance to the once recommended first line treatment for uncomplicated malaria in Kenya, sulfadoxine/pyrimethamine, this country's drug policy has shifted to adopt artemether/lumefantrine combination.

Another ACT currently recommended by the WHO is the artesunate/mefloquine combination. This combination has been tried in some African countries and in Thailand for the treatment of uncomplicated malaria with good results. Although this drug combination has been in clinical use in Kenya for some time, there is no data yet to show its efficacy and safety profile when used by our local population.

This study intends to derive data on the efficacy and safety of this drug when used in the treatment of uncomplicated *p.falciparum* infections in the country, with a view to making recommendations for its widespread use as a choice for first line treatment of uncomplicated *p.falciparum* malaria.

VII. HYPOTHESIS

That artesunate/mefloquine is efficacious and safe in the treatment of uncomplicated *p.falciparum* malaria infections.

VIII. OBJECTIVES

- To determine the efficacy of artesunate/mefloquine when used to treat uncomplicated *p.falciparum* malaria infections.
- To asses the side effects associated with the use of artesunate/mefloquine when used in the treatment of uncomplicated *p.falciparum* malaria infections.

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ACTIVITIES:

- Administration of drug to all patients eligible for the study
- Follow-up by symptomatology review and documentation of any new complains
- Parasite count from recruitment to the end of the study period
- Laboratory measures of baseline and follow-up haematology, biochemistry and ECG profile

IX. STUDY DESIGN

This was an open-label trial of patients with *p.falciparum* infections. It was conducted in Bungoma District of Western province, this being a stable malaria endemic region. The study was conducted between January 2004 and April 2004.

A hypothesized day 28 cure-rate of 90% as based on similar previous studies was used.

A sample size for estimating cure rate on day 28 of with a 90% power to detect a difference in the proportion of success of 10% or greater at a 5% level of significance was a minimum of 139 patients. 200 patients were enrolled in the study.

Sampling was done consecutively for all patients who me the inclusion criteria.

A complete follow-up period was 28 days.

Artesunate/mefloquine (Artequin) was administered in the dosage of approximately 4-5 mg/kg/day of artesunate and 8 mg/kg/day of mefloquine. Patients were categorized into two groups depending on weight i.e. group 1 for weights between 35 and 55 kg and group 2 for weights over 55 kg such that patients in group 1 received Artequin 600/750 while those in group 2 received Artequin 600/1500. The drug was administered as 2 tablets of Artequin (1x 200mg artesunate and 1x250 mg mefloquine) for group 1 and 3 tablets of Artequin (1x200 mg artesunate, 2 x 250 mg mefloquine) for group 2 each daily for 3 consecutive days.

Inclusion criteria

- Males and females weighing between 30kg and 55 kg for Artequin 600/750.
- Males and females weighing over 55kg for Artequin 600/1500.
- Uncomplicated *p.falciparum* malaria, i.e, no evidence of neurological involvement, haemolysis, renal impairment, or hypotension.
- Parasite count greater than 1000/mm^3.
- Haemoglobin greater than 10 grams per dl
- No history of ingestion of other antimalarials within the prior two weeks
- The ability to take oral medication.
- Informed consent to take part in the study.

Exclusion Criteria

- Other species of malaria parasites or mixed infections.
- Complicated malaria requiring parenteral treatment.
- Random blood sugar less than 2.5mmol/l.
- ECG QTc interval greater than 400 ms (>0.45).
- Severe underlying disease requiring specific treatment.
- Pregnancy.

The investigator reviewed patients suspected of having malaria infection seen in the casualty of the hospital and their symptomatology and vital parameters noted. A thick smear blood slide was then taken from a finger prick and examined. Those with a significant presence of *p.falciparum* parasites i.e. at least 3 mps/HPF were evaluated further for random blood sugar using a glucometer, electrocardiography using a portable ECG machine, and a urine pregnancy test for all women in the child-bearing age by a rapid test kit.

Patients who satisfied these criteria were counseled and enrolled upon signing a consent form (or signed by a parent/guardian in case of minor patients).

Blood was then drawn from the antecubital vein to determine baseline haematological and biochemistry profiles, and to prepare a thin film blood slide. Haematological tests done were counts for RBCs, WBCs, platelets, haemoglobin, and haematocrit. The blood chemistries done were BUN and creatinine, liver function tests (bilirubin, transaminases, alkaline phosphatase) and electrolytes (Na^+, K^+).

Patients then gave stool for examination of ova and cysts, and relevant medication for any demonstrable infection was given.

The first dose of Artequin was given at the time of recruitment (day 0) and each subsequent day's dose given during the follow-up period. Patients were asked to swallow the drugs in the presence of the investigator.

Patients who vomited their drugs within half an hour of swallowing were given repeat doses while those who skipped any day's drug(s) were continued from the omitted dose.

X. FOLLOW-UP

All patients enrolled on day 0 were followed upon days 1 to 5,7,14 and 28 to monitor their progress and side effects. During each visit there was a review of symptomatology and physical examination. Development of new complains were documented as adverse events and categorized as drug-related or non-drug related.

The laboratory investigations done during the follow-up were:

- Repeat thick and thin films on day 1,2,3,4,5,7,14 and 28
- Repeat blood chemistries on day 4
- Repeat blood counts on days 4, 7, 14 and 28
- Repeat ECG on day 4 .

All blood samples for hematology were refrigerated immediately after collection, and the samples for biochemistry centrifuged to get plasma for storage. The tests were then run every alternate day using standard methods. Haematology and biochemistry analysis was done at Nairobi Hospital laboratory Eldoret Annex, while thick and thin blood films for parasitology were stained and examined on a light microscope under oil immersion by the chief investigator and a senior laboratory technician from the department of Internal Medicine. Parasitological examination was done even on the patients whose treatment was changed or discontinued due to adverse events.

Efficacy was assessed by duration to relief of symptoms, defervescence and parasite clearance. Patients were considered cured if they were asymptomatic and with a negative blood slide at day 7.

Partial cure was considered if malaria parasite count dropped by at least 50% of baseline by day 7 with or without improvement of symptoms.

No cure was considered if the condition remained the same or got worse by day 3 as determined by:

- Increase in parasitaemia from the baseline
- Increase in temperature
- Onset of symptoms and signs indicative of complicated malaria (encephalitis, meningismus, drop in haemoglobin, vomiting, dehydration, hypotension, and renal involvement).

Recrudescence/reinfection was considered in patients who showed reappearance of parasitaemia after initial clearance with treatment.

The primary efficacy end-point was the 28- day cure rate defined as proportion of patients with clearance of parasites within seven days of initiation of treatment without subsequent reappearance of parasites within 28 days.

Secondary efficacy end-points were day 7 and 14 cure rates, rate of fall in parasite levels, fever clearance time, and rate of clearance of initial symptomatology recorded at presentation. Drug-related adverse events were assessed by the development of new features and categorized as:

   i. Mild- tolerable, patient up and about

  ii. Moderate- caused discomfort, but patient up and about

 iii. Severe- interfered with patient's activities.

     Worsening of haematology and biochemistry parameters, and ECG QTc values from baseline were also considered as adverse events.

The primary safety end-point was the incidence of headache, dizziness and abdominal discomfort.

Secondary safety end-points were deterioration of haematology, biochemical and ECG profiles.

QUALITY CONTROL

Every 100th sample for hematology and biochemistry would be split into two and sent for laboratory analysis as two independent samples to determine the reproducibility of the results. This figure accounted for approximately 1% of the investigated samples undergoing quality control check.

Readings of temperature, blood pressure, weights and random blood sugar were counter-checked against those recorded with other similar equipment of the hospital and any errors adjusted accordingly. This procedure was done every fortnight.

ECG readings were repeated on the same individual shortly after an initial test for every tenth patient, and the reproducibility determined.

Parasite counts were checked for accuracy by randomly subjecting a tenth of the slides prepared in a day to a repeat count by an independent person and where a large difference in count was found, a re-repeat count was done by the investigator and his assistant whereupon an average of the two would be used.

XI. STATISTICAL ANALYSIS

Data was collected for all patients who were enrolled in the study and took at least one dose of medication. Baseline characteristics were for all patients enrolled in the study were computed.

Data analysis was done both for those patients who reached definable end-points i.e. completed follow-up or had treatment failure while on follow-up (evaluable patients-WHO/HTM/RBM/2003.50) and for the intention to treat population (ITT analysis) using the last observation carried forward method (LOCF).

Symptoms, parasite and fever clearance times were analyzed by survival analysis using Kaplan-Meier survival curves.

Data was analyzed using SPSS 10.0 software.

Ethical approval was obtained from the Kenyatta National Hospital Scientific Ethics Committee.

A signed informed consent was obtained from each patient (or parent/guardian if patient was below 18 years) before recruitment into the study after a thorough explanation of the study details to the patient(s). Patients were assured that the study was safe and no harm would be visited upon them knowingly. A patient would do voluntary withdrawal from the study at any time without victimization or compromising on their medical care.

Any other incidental disease that would be discovered among the patients would be attended to or referred accordingly.

Patients were reimbursed travel expenses to and from the clinic during the entire follow up period.

The study was sponsored by Mepha pharmaceatical LTD.

XII. RESULTS

A total of 200 patients were enrolled in the study between January and April 2004. All were treated and followed up on an out-patient basis. Of these, 150 patients weighing 30 to 55 kg received Artequin 600/750 and were categorized in group 1 while 50 patients with weights over 55 kg received Artequin 600/1500 and were categorized in group 2.

The mean artesunate and mefloquine dose over the 3 days for patients in group 1 was 12.7 mg/kg (range 10.9 to 20) and 15.9 mg/kg (range 13.6 to 25) respectively, while that in group 2 was 8.6 mg/kg (range 6 to 10.7) and 21.7 mg/kg (range 15 to 26.7) for artesunate and mefloquine respectively.

Summary of the drug regimens used:

| Group	artequin	artesunate (mg)	mefloquine(mg)
1	600/750	12.7(10-20)	15.9(13.6-25)
2	600/1500	8.6(6-10)	21.7(16-26)

The male to female ratio was 1:1.6, with 78 males (39%) and 122 females (61%). Males constituted 29 % (43/150) of patients in group1 and 70 % (35/50) of patients in group 2. While 55% of all males were categorized in group1 and 45% in group 2, 88% of the females were in group 1and 12% in group 2.

Table 1: Patients' sex distribution

		Group-no(%)		Total
		1	**2**	
Sex	**Male**	43(29)	35(70)	78(39)
	Female	107(71)	15(30)	122(61)
Total		150(100)	50(100)	200(100)

Fig 3: Patients' group/sex percentages
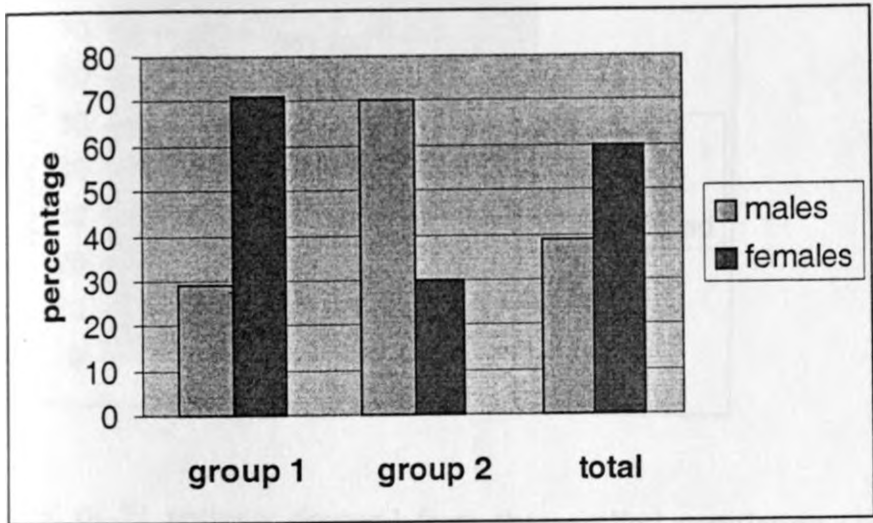
The mean age for the patients was 31.8 (S.D. ± 16.3) years with a range of 8 to 90 years. Patients in group 1 had a mean age of 31.3 (S.D. ± 17.7) years with a range from 8 to 90 years while patients in group 2 had a mean age of 33.6 (S.D. ± 11.2) years with a range of 16 to 65 years.

The mean weight for the patients was 52.8 (S.D.± 12.1) kg with a range of 30 to 100 kg. Patients in group 1 had a mean weight of 47.2 (S.D.± 6.5) kg while those in group 2 had a mean weight of 69.6 (S.D.± 9.3) kg.
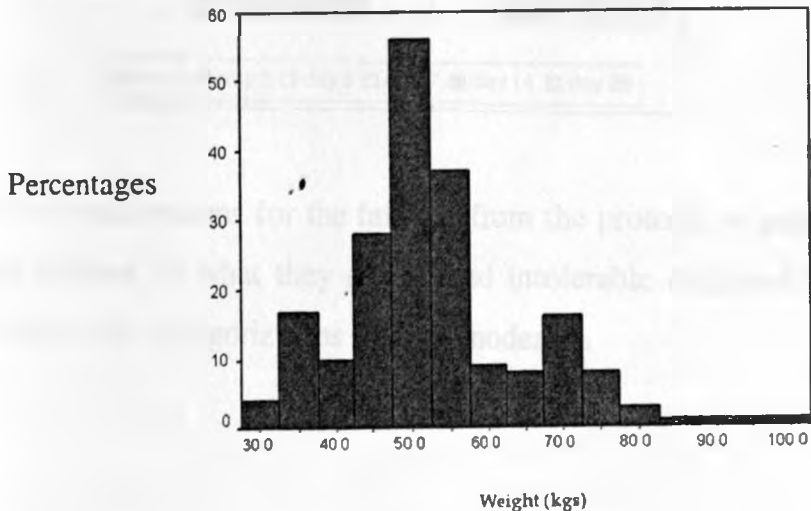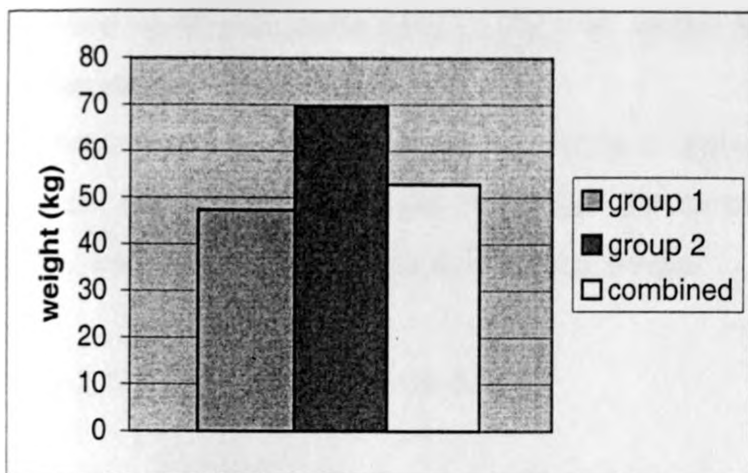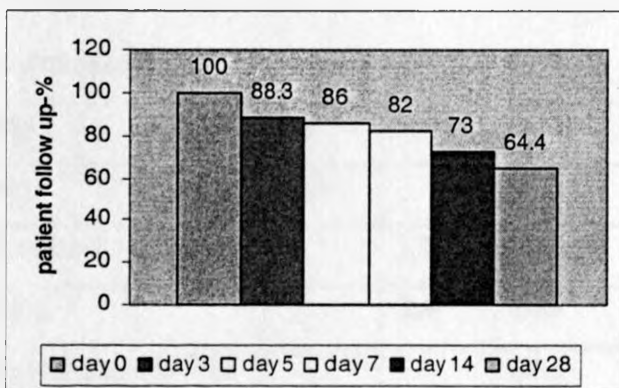
Fig 4: Patients' weight distribution

Fig 5: Patients' mean weights

A total of 71 patients dropped from the enrolled population (34.5% overall dropout rate-34.4% and 36% for group 1 and 2 respectively) and were therefore not included in the evaluable patient/ per protocal analysis population.

The daily follow- up of patients was as shown below.

Fig 6: Patient follow up

There were several reasons for the fall-out from the protocol. 4 patients refused to complete medication because of what they considered intolerable dizziness. These side effects were however objectively categorized as mild to moderate.

Six patients did not turn up for subsequent visits because of having temporarily changed residence i.e. 4 policemen went on duty to another district while 2 students were denied permission from their schools.

2 patients died from other illnesses during the follow-up, 1 from a road traffic accident and the other from severe pneumonia. 58 patients did not turn up for unexplained reasons while 1 patient withdrew voluntarily for unspecified reasons.

Table 2: Summary of reasons for dropout:

Patients' No	Reasons
4 (2%)	" Severe" side effects
6 (3%)	Changed residence
2 (1%)	Died from other causes
59 (29.5%)	Unknown
1 (0.5%)	Voluntary withdrawal

Table 3: Flow of patients

Patients	No	No	%
Screened	422		
Not recruited		222	
Recruited		200	100
Completed day 28 F/U			64.5
Treatment failure			1
Dropped out			29.5

Table 4: Summary of baseline characteristics

	Total	Patients included	Patients excluded
No	200	131	79
Male %	39	42	47
Mean age (yrs)	31.8	31.4	31.5
Mean wt (kg)	52.8	52.5	53.5
Mean body temp	37.5	37.8	37.6
Temp >37.2^{0}c %	58	53	51
Mean resp (/min)	22	21	22
Mean pulse (/min)	94	100	97
Mean parasite/mm^3	15672	15540	15760
Mean Hb (g/dl)	12.6	13.1	12.8
Mean platelet x 10^9	198	201	197
Mean WBC x10^3	5.4	6.1	5.7

There were a total of 519 entities of complains at the time of presentation as shown in the figure below:

Fig 7: Presenting complains
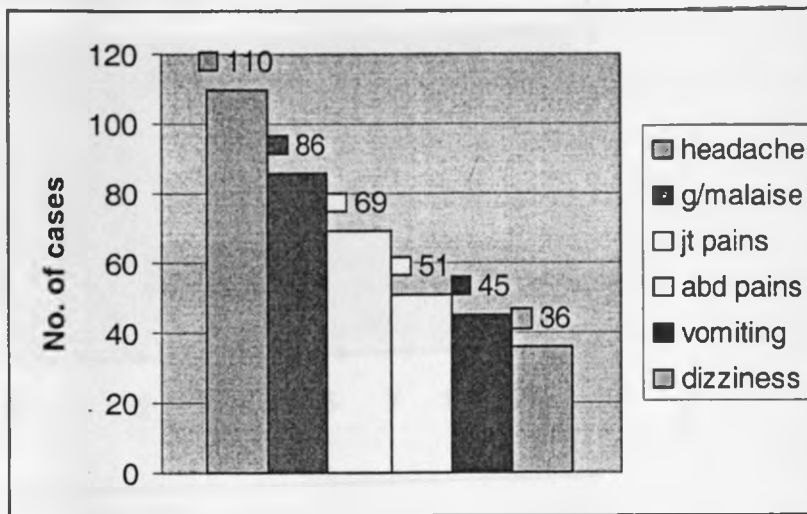
At the time of presentation the mean body temperature was 37.5°C (S.D. ± 1.07), mean respiratory rate 22.4 breaths per minute, mean pulse rate 94 per minute and no significant findings in physical examination.

The mean parasite count at the onset of treatment was 15672 with a range of 1920 to 35140. Group 1 patients had a mean parasite count of 14693 while those in group 2 had a mean parasite count of 17800. The mean entry parasite count for the evaluable population was 15476, 16603 and 15730 for patients in group 1, 2 and the total respectively.

Efficacy

The median time to parasite clearance for patients in both treatment groups was 2 days with a range of 1-3 days. The mean daily parasite clearance rate was 52.11%, 90.45% and 99.88% on days 1, 2 and 3 respectively.

One patient in group 1 deteriorated his clinical condition while still parasitaemic on day 1 and had to be treated with parenteral quinine. This patient was considered to have had early clinical failure, thus no cure. A second patient had recurrence of symptomatology of malaria and reappearance of parasites in his bloodstream on the 14th day of followup. This patient was considered to have had a recrudescence/reinfection and categorized as late clinical failure.

Fig 8:Mean daily parasite clearance
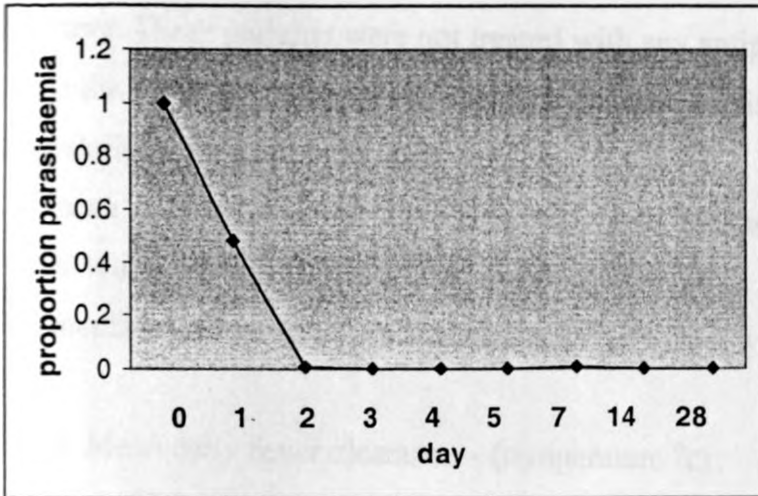
In the evaluable patient population, the cure rates at day 28, 14 and 7 were 98.4%, 98.4%, and 99.2% respectively while the day 28 cure rate for the intention to treat population was 92%.

Fig 9: Cure rates for the evaluable population
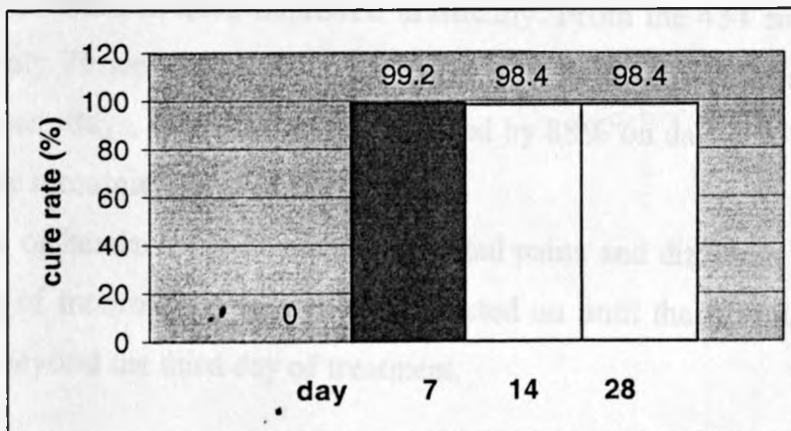
42% of the evaluable patients had fever (axillary temperature >37.2°C) at the start of treatment. These patients were not treated with any antipyretics (although there was provision to use the latter in the treatment schedule if fever persisted beyond the second day of single antimalarial drug adminstration).

The mean daily body temperature showed a rapid decline to no fever (<37.2°C) on the second day of treatment.

The median time to fever clearance time was 1 day, with a range of 1 to 2 days.

Fig 10: Mean daily fever clearance – (temperature °c)
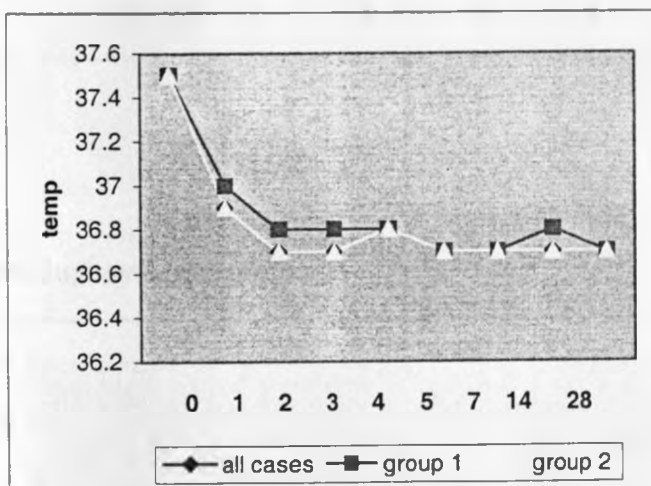
During the follow-up period, residual daily symptomatology as compared to that at presentation was noted to have improved drastically. From the 434 entities of complains at presentation, only 79 were reported on the day 1, this being an 82% reduction of complains. On the subsequent days, symptomatology reduced by 88% on day 2, 97% on day 3 and more than 99% on the remaining days (fig 9, 10).

The symptoms of headache, joint pains abdominal pains and dizziness were all cleared after the second day of treatment while malaise persisted on until the fifth day of visit. Vomiting did not persist beyond the third day of treatment.
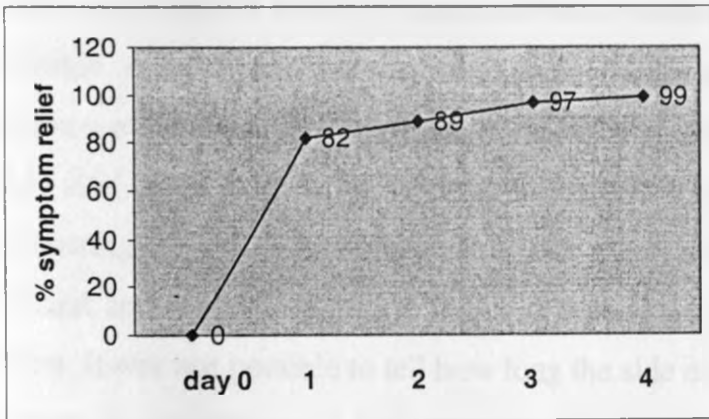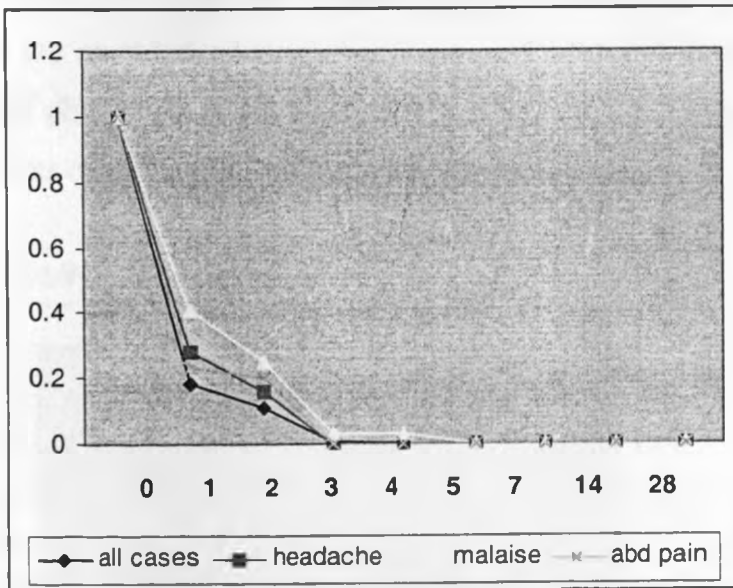
Fig 11: Cumulative symptom relief

Fig 12: Individual symptom relief

The mean daily blood pressure and pulse rate during the entire period of treatment remained normal.
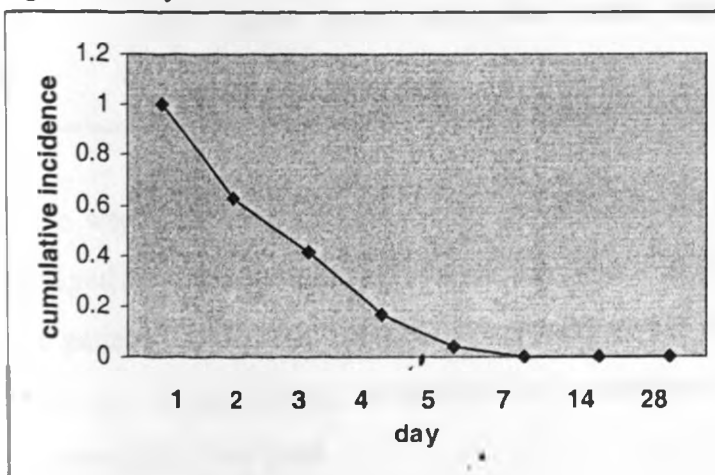
Side effects

There was a big overlap between the presenting symptomatology of patients and drug related adverse events that developed while patients were on treatment. Only new features that were not present on the day of initiating treatment were counted as drug related adverse events. By such a design, some events that were due to the illness may have been documented as drug related hence overestimating the recorded side effects while any event that was drug related but had also presented as a symptom before treatment was not recorded hence underestimating the symptomatology. Any side effect noted in a patient was recorded only once (at first appearance). Although this was a good way of avoiding overestimation of the side effects, it was not possible to tell how long the side effects would last.

57% of patients experienced at least one side effect during the entire study period. There was an overlap of side effects among individual patients.

The highest number of adverse events was recorded on the second day of treatment (day 1). There then followed progressive reduction in the events with each subsequent day of follow-up with less than 10 adverse events recorded for each day after the fourth day of followup.

94% of the recorded adverse events were of mild and moderate category such that patients were still able to go about their daily activities without any significant disability. 6% of the side effects occurring in 6 patients were severe in nature

Fig 13: Daily side effects

The commonest side effects were dizziness-35%, asthenia-26%, headache-23% and nausea-11%. Abdominal pain, vomiting, itching and skin rashes also developed but in smaller numbers.

Three patients from group 1 and two from group 2 developed moderate maculo-papular rashes between the second and fifth days of treatment varyingly. The conditions got better subsequently and had resolved by the fourteenth day of followup.

The proportion of adverse events of dizziness, headache, nausea, abdominal pain, vomiting and rashes was higher among patients in group 2 compared to those in group 1 while asthenia occurred more frequently in patients in group 1 compared to group 2.

This higher rate of each adverse event amongst patients in group 2 as shown in Fig.14 could have occurred because of the higher dosage of mefloquine used in their treatment.

Fig 14: Cumulative side effects (%) vs. group
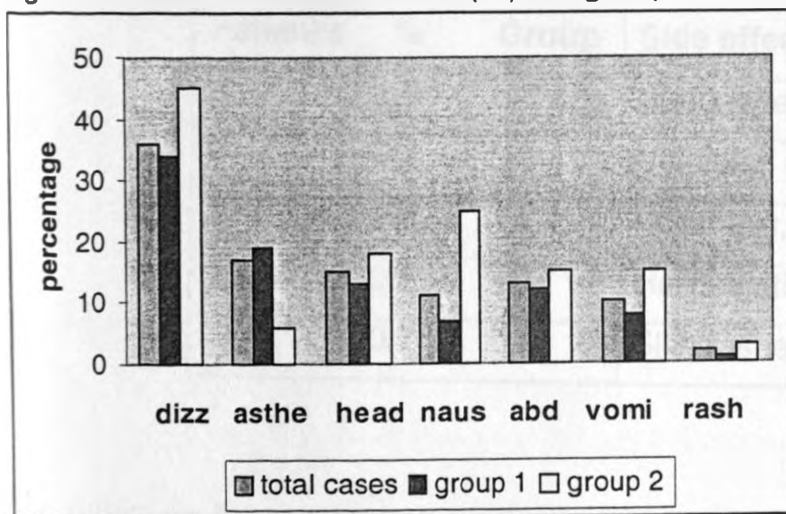
There were 2 cases (1%) of severe headache from group 2 patients on day 4, which were managed symptomatically to resolution by the fifth day.

One patient (0.5%) from group 1 developed severe dizziness and vomiting on the fifth day of follow-up, necessitating admission for symptomatic treatment. This patient's condition improved after two days.

One patient (1%) from group 1 discontinued medication prematurely on day 2 because of severe side effects of vomiting and malaise. This patient was still parasitaemic and deteroriating (either due to the side effects or the malaria infection) and was put on quinine and followed up with good recovery. Another patient from group 1 developed severe itching and skin rashes on day 3 and was managed symptomatically. One patient (0.7%) from group 1 developed episodes of hallucination, abnormal behavior and violence on the fourth day of follow-up, most likely due to mefloquine toxicity. This patient was admitted and treated with antipsychotic drugs and improved within 24 hours.

Table 5: Severe side effects

No of Patients	%	Group	Side effect(s)	Day of onset
2	1.5	1	Headache	4
1	0.7	1	Vomiting, asthenia	2
1	0.7	2	Dizziness, vomiting	5
1	0.7	1	Hallucinations	4
1	0.7	1	Skin rashes	3

The follow-up haematological profile showed some changes. Whereas all the subsequent values of haemoglobin, WBC and platelets had some variations, these changes did not attain any clinical or statistical significance when compared to the baseline values.

Table 6: Mean haematological profile

	Hb (g/dl)/ p value	Plt (x10^9/l)/ p value	Wbc(x10^9/l) p value
Day 0	12.6	198	5.4
Day 7	13.2(0.509)	204(0.142)	5.9(0.099)
Day 14	12.8(0.014)	218(0.087)	5.7(0.309)
Day 28	12.1(0.669)	204(0.140)	6.0(0.008)

Fig 15: Followup haemoglobin values for day 0, 7, 14 and 28
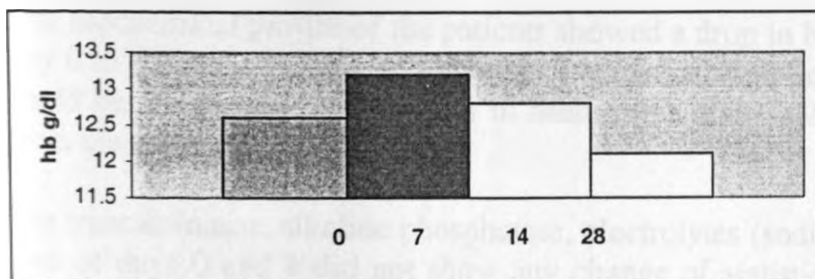
day 7 p=0.509   day 14 p=0.014   day 28 p=0.669

Fig 16:Followup platelet values for days 0, 7, 14 and 28
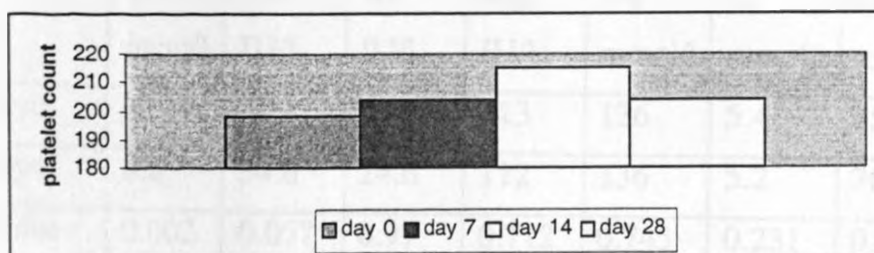
day   7 p value=0.142
day 14 p value=0.087
day 28 p value=0.140

Fig 17:Followup WBC values for days 0, 7, 14 and 28
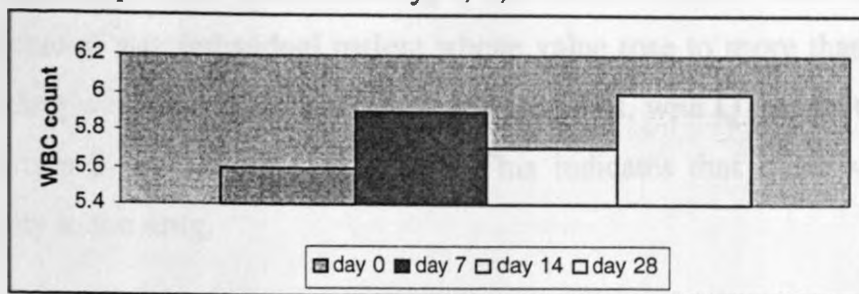
day 7 p value=0.009
day 14 p value=0.309
day 28 p value=0.008

The biochemical profile of the patients showed a drop in bilirubin levels from 15.5 mcm/l on day 0 to 8.6mcm/l on day 4, a change of statistical significance with a p value of 0.001. This could be attributed to a reduction in haemolysis once malaria parasites had been eliminated from the patients.

The transaminases, alkaline phosphatase, electrolytes (sodium and potassium), creatinine and urea of days 0 and 4 did not show any change of statistical significance. These parameters indicate a relatively good tolerability to the drug by the kidneys and the liver.

Table 7: Follow up biochemistry values

	Biliru mcm/l	Ast IU/l	Alt IU/l	Alp IU/l	Na$^+$ mmol/l	K$^+$ mmol/l	Cr mcm/l	Urea mmol/l
Day0	15.5	42.1	24.9	113	136	5.4	85.3	3.6
Day4	8.6	34.6	24.6	112	136	5.2	76.2	3.5
pvalue	0.002	0.057	0.97	0.712	0.745	0.231	0.077	0.475

The mean corrected QT interval on day 0 was 0.27 and repeat value on day 4 was 0.26. There was no case of any individual patient whose value rose to more than 0.35 during follow-up. This finding was similar for the two dosage regimes, with QT of 0.26 and 0.27 for the group 1 and group 2 respectively on day 4. This indicates that there was good cardiovascular tolerability to the drug.

Fig 18: Follow up QTc values

	1	2
Series1	0.27	0.26
	day 1	day 4

QT interval (y-axis): 0.25, 0.26, 0.27, 0.28
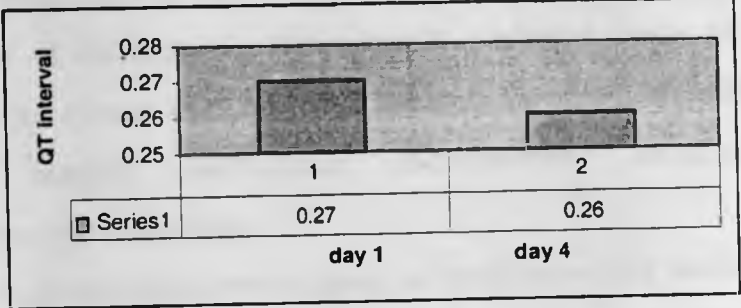
p value for Total QT =0.215

XIII. Discussion

Artemisinin derivatives are rapidly-acting antimalarial drugs, even against multi-drug resistant strains of *p.falciparum* with minimal toxicity but exhibit high recrudescence rates when used as monotherapy for less than 5 days. This problem is overcome by combining them with other antimalarial drugs with a relatively longer half-life. Such artemisinin-based combination therapies not only improves efficacy but also delay emergence of resistant mutants of parasites(22). The principle of combining drugs with different modes of action and mechanisms of resistance is the currently preferred mode of treating malaria infections. Drugs that have been used in clinic trials in combination with artemisinins with variable outcomes include mefloquine, lumefantrine, amodiaquine, chloroquine and sulphadoxine/pyrimethamine.

For the artemisinin-based combination to be effective and justifiable, the partner drug should have good efficacy and no demonstrated high failure rates (>25%) when used as monotherapy in the specified locality.

Whereas artesunate reduces the parasite biomass drastically, its nature of a short half-life leaves a certain fraction of parasites which are then exposed to the longer acting drug e.g.mefloquine, until complete clearance.

The use of artesunate/mefloquine combination administered concurrently for three days was used in this study to treat uncomplicated *p. falciparum* infections. The study was done in an area where no trials on mefloquine sensitivity had been carried out, though the locals used mefloquine on a low scale.

Using the per protocal patient population for analysis, this study demonstrated a primary efficacy end-point of day 28 cure rate of artesunate/mefloquine in treatment of uncomplicated *p. falciparum* malaria to be 98.4 % with 0.08 % recrudescence/reinfection rate and 0.08% failure rate, secondary efficacy end point of parasite clearance at day 7 and 14 of 99.2% and 98.4% respectively , median time to fever clearance of one day, relief of symptoms by 82% at day 1, 89% at day 2 and 97% at day.3.

Day 28 cure rate obtained by intention to treat analysis where patients lost to follow up were considered by their last observations was much lower at 92%. Unlike the earlier method of analysis where only patients who had definite end-points (completion of study, treatment failure or severe side effects) were considered hence the higher cure rates, the later method gave inferior results because many patients discontinued the study before they had obtained cure (82% at day 7, 73% at day 14 and 64.5% at day 28). The use of per protocol analysis has been advocated for use in single arm trials especially in the field of malaria (WHO/HTM/RBM/2003.50).

This outcome is similar to other studies that evaluated the efficacy of artesunate/mefloquine combination in treatment of uncomplicated malaria, i.e. Massougbodgi et al found a 14 day cure rate of 100% when a three day course of artesunate/mefloquine was used simultaneously and 98% when used sequentially and no recrudescence by day 28 in a study in West and Central Africa in 2001 (21). Krudsood et al showed a 28 day cure rate of 100% in patients on 3 day simultaneous dosage of artesunate/mefloquine and 99% for sequential dosing in a study in Thailand in 2001 (20). Price et al in a study in the Thai-Myanmar border found a cure rate of 86.1% and 87.7% for artesunete and artemether combinations with mefloquine respectively (22). The African studies were conducted on out-patient basis and experienced high drop out rates while the Asian studies had lower drop outs because of being in patient based. Statistical analysis of cure rates was however also based on per protocol evaluation hence comparable to this study.

The side effects reported in this study were predominantly headache, dizziness, vomiting and asthenia. These were mainly in the category of mild to moderate and tolerable by the patient except six cases that were severe in nature including one neuropsychiatric type. Only moderate changes were reported in the organ systems of the liver, kidney and the bone marrow. There was good cardiovascular tolerance to the drug with no fatal prolongation of QT interval.

In the other studies aforementioned, the incidence of side effects was similar with headache, dizziness and abdominal discomfort being commonest. No neuropsychiatric features were reported however.

The co-adminstration of artesunate/mefloquine in the treatment of uncomplicated *p. falciparum* malaria gave high cure rates and a tolerable safety profile. The choice of a combination regimen with such results in the wake of resistance to most traditionally used antimalarial drugs will help reduce the morbidity and mortality from malaria infections, especially in areas with high resistance to monotherapy treatment regimes.

In changing antimalarial treatment policies for countries, factors like rapid cure rates and low propensity to development of resistance to the adopted drugs in the short-term are to be considered. The effect of artesunate to reduce gametocyte carriage and thereby reduce transmissions is advantageous especially when initiating malaria control programmes.

Clinical evaluations of cure rates at day 28 have been questioned when used in areas where transmissions are high because the effects of artesunate may not be obvious. The use of Polymerase Chain Reaction (PCR) is handy in differentiating recrudescence of existing infection from reinfection by a new parasite.

The combination of artemether-lumefantrine available as a fixed dose formulation has recently been adopted in Kenya as the first line treatment of uncomplicated *p.falciparum* infections to replace sulfadoxine/pyrimethamine, an SP that had shown high levels of resistance. The good trial results of artesunate/mefloquine combination provide a reliable alternative to the earlier combination regime.

XIV Conclusion

The use of artesunate/mefloquine simultaneously in the treatment of uncomplicated *p.faciparum* malaria infections had a high efficacy with a day 28 cure rate of 98.4% (92% using the intention to treat population). There was rapid symptom relief, almost complete within the first three days and tolerable profile of adverse events, mainly within the first two days

Limitations

- This study experienced a high dropout rate because patients had to be seen on an out-patient basis for a long period even when the actual treatment time was only three days
- The overlapping nature of the symptoms of malaria and the side effects of the medication could cause errors in evaluating the safety profile of the drug hence overestimation of side effects cannot be excluded.
- Parasite genotyping was not done hence it was hard to differentiate recrudescence from reinfections for the patient who had late treatment failure.
- Adverse events could not be correlated with drug toxicity since drug levels were done.

XV Recommendations

- Artesunate/mefloquine combination has high cure rates and a tolerable safety profile and should be considered as an option for first line treatment of uncomplicated *p.falciparum* malaria infections in this locality
- More studies need to be done on larger patient populations and in different regions of the country to possibly replicate the clinical efficacy of artesunate/mefloquine combination shown in this study.
- There is need to conduct randomized controlled trials of this combination therapy with other currently recommended drugs to adequately advice on the best drugs to use for each given locality.

Appendix 1

PATIENT INFORMATION

Title of the study:

A study on the efficacy and side effects of artesunate/mefloquine (Artequin) in the treatment of uncomplicated *p. falciparum* malaria.

Participation Information

Introduction

I am performing a study to see if Artequin can treat and cure malaria which is not severe in nature. The use of this medicine to treat is not new. Studies in humans have shown Artequin to be 100% effective in treating uncomplicated Malaria in Thailand and Cameroon. So I would like to do a similar study in a small number of people infected with mild forms of malaria to see if the findings observed in other countries can be observed in Kenya. I want therefore to confirm whether Artequin works well in our population. Unpleasant experiences in patients who have taken Artequin include: abdominal pain, feeling like vomiting, diarrhoea, dizziness, lack of sleep and loss of appetite. Your participation in this study is voluntary, and you can withdraw anytime and no penalties will be preferred against you.

Procedures to be followed;

1. If you agree voluntarily to participate in this study, I shall perform a physical examination and laboratory investigations to determine whether you qualify to be in the study or not.

2. If you qualify to be in this study you will be given Artequin. The Artequin will be given once a day for three days (2 to 3 tablets daily for three days)

3. During the first day of treatment, I shall obtain small amounts of blood from your second finger using a needle prick to determine if you have malaria parasites. I shall also do an investigation on the heart called an echocardiogram, determine your blood sugar levels, and do a urine pregnancy test on all women of the child-bearing age.

4. If you are suitable for the study, I shall obtain about 1½ tea spoon of blood (7mls) from your antecubital veins to asses the working condition of your kidneys, bone marrow and liver, and also take stool for laboratory analysis

5   From the second to the seventh day, and fourteenth and twenty eigth days, I shall repeat the finger prick tests for malaria parasites. On day three and seven, I shall take blood again from your antecubital vein to repeat the tests of the first day, and repeat an echocardiogram on day three and seven. These repeat procedures will help me to know whether the Artequin is effective against Malaria or not and if it may be causing any harm on your body organs.

If you notice any unpleasant side effects, kindly let me know about them immediately or during your reviews.   I shall make sure that no harm is visited on purposely.

6   You will be re-embursed a total of Kshs.900 to cover for your travel expenses in the following order; days 1,2 and 3 at 70/=; days 4 to 7 at 100/=; and days 14 and 28 at 145/=.

7   If you do not respond to Artequin treatment, you will be given alternative antimalarial treatment that will be deemed appropriate.

8   I believe that the above information has cleared any fears or doubts you might have been having. However, during the entire period of the study, do not hesitate to ask me any question that might come up in your mind.

If you have any questions or complains kindly call me, Dr.Samia B.M. on Telephone number   **0722829620**

APPENDIX 2

INFORMED CONSENT FORM

I Mr/Mrs/Miss _____ being a person aged 18 years and over, do hereby give consent permission to Prof/Dr/Mr/Mrs/Miss _____ to include and carry out research on me/ my dependant in the intended research protocol as explained to me and understood by me. I understand that the research involves giving me/ my dependant Artequin for treating malaria from which I/my dependant is suffering.

I have also been made to understand the implications, risks and immediate benefit, if any, of the test and/or treatment. I accept the tests and/or treatment to be carried out and the risks hereto.

I understand that I/ my dependant have the right to withdraw from the research any time, for any reason without penalty or harm. If I/ he or she withdraws, I am made to understand that I/they will be cared for by the doctors like any other patient.

All the above conditions have been explained to me in _____ language of which I am fluent. Data/biological samples will be coded and remain confidential (name not disclosed), I have the right to obtain a copy of this informed consent.

Signature   _____

Investigator   _____

Date   _____

APPENDIX 3

DAY "1"

INFORMATION SUMMARY

TREATMENT EVALUATION FORM

1. Date: _____

2. Patient Names: _____ Study No._____

3. Sex: _____ Age: _____ Weight____

4. Hospital: _____

5. Any relevant medical history?   Yes: _____

                                                      No: _____

6. If YES, please summarize: _____

    _____

7. Any significant findings from physical examination?

    Yes: _____

    No: _____

    If "YES" Please summarize:

    _____

    _____

    Name of Doctor/Clinician: _____

APPENDIX 4
INVESTIGATIONS SCHEDULE

KEY: Y = TO BE CARRIED OUT   N =NOT TO BE DONE

NO	TEST	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 7	Day 14	Day 28
1.	Parasite Thick Smear	Y	Y	Y	Y	Y	Y	Y	Y	Y
2.	Parasite Thin Smear	Y	Y	Y	Y	Y	Y	Y	Y	Y
3.	Pregnancy test	Y	N	N	N	N	N	N	N	N
4.	Bilirubin	Y	N	N	Y	N	N	N	N	N
5.	Transaminase SGPT	Y	N	N	Y	N	N	N	N	N
6.	Transaminase SGOT	Y	N	N	Y	N	N	N	N	N
7.	Alkaline Phosphatase	Y	N	N	Y	N	N	N	N	N
8.	Serum Glucose	Y	N	N	N	N	N	N	N	N
9.	Potassium	Y	N	N	Y	N	N	N	N	N
10.	Sodium	Y	N	N	Y	N	N	N	N	N
11.	Creatinine	Y	N	N	Y	N	N	N	N	N
12.	Urea	Y	N	N	Y	N	N	N	N	N
13.	ECG	Y	N	N	Y	N	N	N	N	N
14.	Stool o/c/blood	Y	N	N	N	N	N	N	N	N
15.	Hb (g/dl)	Y	N	N	N	N	N	Y	Y	Y
16.	Haemotocrit	Y	N	N	N	N	N	Y	Y	Y
17.	RBC – x 10^3	Y	N	N	N	N	N	Y	Y	Y
18.	Platelet –x 10^3	Y	N	N	N	N	N	Y	Y	Y
19.	WBC - x10^3	Y	N	N	N	N	N	Y	Y	Y
20.	Temp.	Y	Y	Y	Y	Y	Y	Y	Y	Y
21.	Pulse rate	Y	Y	Y	Y	Y	Y	Y	Y	Y
22.	B.P.	Y	Y	Y	Y	Y	Y	Y	Y	Y
23.	Resp. rate	Y	Y	Y	Y	Y	Y	Y	Y	Y

APPENDIX 5

SIDE EFFECTS

NAME _____ **STUDY NO.** _____

Definitions.

1.Mild- tolerable, patient up and about,

2. Moderate- causes discomfort, but patient is up and about;

3.Severe- interferes with patient's activities.

D1 _____

D2 _____

D3 _____

D4 _____

D5 _____

D7_____

D14_____

D28_____

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