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TREATMENT OF UNCOMPLICATED MALARIA IN GUINEA-BISSAU.

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SUMMARY

Treatment of malaria has become increasingly difficult due to high levels of resistance to the cheap and commonly used antimalarials. In Guinea-Bissau, the first line drug is chloroquine (CQ), the second line drug is sulfadoxine-pyrimethamine (S/P), and the third line therapy is quinine (QU). During the last ten years we have studied these drugs in the Bandim area at the outskirts of the capital Bissau, to a large extent with the same methodology and the same staff.

Chloroquine given in the standard total dose of 25 mg/kg proved to be less efficient with an adequate clinical and parasitological response (ACPR) rate on day 28 of 68% in 1996/1997 and 76% in 2001/2004. A higher total dose of 50 mg given in two daily doses over three days showed an ACPR rate of 86% and 90%. No severe adverse effects were found. Artemisinin derivatives reduce the parasite load and improve thereby in theory the efficacy of the partner drug. However, treatment with artesunate and CQ was no better than with CQ alone. Artesunate monotherapy for three days gave high treatment failure rates of 50% on day 35. S/P was efficient both as first and second line treatment with 35 day cure rates of 86% and 90%, respectively. No decrease in efficacy was seen from 1996 till 2004. However, as a high rate of mutations associated with resistance to S/P has been found in Guinea-Bissau its use should be restricted in order to prolong its period of usefulness.

Quinine has traditionally been used during three days for treatment of uncomplicated malaria in Guinea-Bissau. However, treatment for 3 days gave high treatment failure rates. QU for three days followed by a standard dose of CQ did not add to the cure rate of CQ monotherapy and should therefore be abandoned and replaced by quinine for the full seven days.

Amodiaquine (AQ) has been proposed as first line treatment when CQ 25 mg/kg has to be abandoned due to the level of resistance. We compared treatment with AQ and CQ. High rates of ACPR on day 35 were found treating with AQ 15 and 30 mg/kg (88% and 92%, respectively) but this was not better than following treatment with CQ 50 mg/kg (88%). AQ can thus be saved as a future partner drug for a combination therapy with artemisinin.

To know whether a treatment benefits the child, effectiveness studies have to be performed. We compared supervised and unsupervised treatment with CQ. No differences were found, neither in the ACPR-rate nor in the drug concentrations on day 7. When good information is provided, mothers give the correct treatment to their children at home.

Conclusion: 1) A total dose of 50 mg CQ/kg is effective and safe and much cheaper than the new combination therapies. As an interim strategy, a change of the recommended first line treatment for uncomplicated malaria to 50 mg/kg bodyweight of CQ could be implemented easily and without delay. 2) The use of S/P should be limited, but it can be used as second line therapy. 3) AQ could be reserved as the partner drug in a future combination therapy.

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The present thesis is dedicated to Claudina Cabral, with whom we had the great pleasure to work since our first study. I learned a lot from the discussions and exchange of ideas with her and was impressed by her experience as a laboratory technician and by her knowledge of the study area. Taking a walk in Bandim together with Claudina gave the impression that she knew most of the inhabitants by name. And even if she had taken blood samples from many of the children they would always greet her with joy. It is a great loss to the malaria studies at the Bandim Health Project that Claudina fell seriously ill at the end of our last study and died just before Christmas 2005. She is seriously missed.

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ABBREVIATIONS

ACPR:	adequate clinical and parasitological response
ACT:	artemisinin combination therapy
CSB:	Centro de Saúde de Bandim (Bandim Health Centre)
dhfr:	dihydrofolate reductase
dhps:	dihydropteroate synthetase
ETF:	early treatment failure
GDP:	gross domestic product
GNP:	gross national product
LCF:	late clinical failure
LNSP:	Laboratório da Saúde Pública (National Health Laboratorie)
LPF:	late parasitological failure
MINSAP:	Ministerio da Saúde Pública (Ministry of Health)
Msp-2:	merozoite surface protein - 2
PCR:	polymerase chain reaction
pfATPase:	<i>P. falciparum</i> adenosine triphosphatase
pfcr1:	<i>P. falciparum</i> chloroquine resistance transporter gene
pfmdr 1:	<i>P. falciparum</i> multidrug resistance gene 1
PSB:	Projecto de Saúde de Bandim (Bandim Health Project)
RBM:	Roll Back Malaria
S/P:	sulfadoxine-pyrimethamine

PAPERS INCLUDED IN THE PRESENT THESIS

- I** Kofoed, P. E., Co, F., Johansson, P., Dias, F., Cabral, C., Hedegaard, K., Aaby, P. and Rombo, L. 2002. Treatment of uncomplicated malaria in children in Guinea-Bissau with chloroquine, quinine, and sulfadoxine-pyrimethamine. *Trans. R. Soc. Trop. Med. Hyg.* **96**:304-309.
- II** Kofoed, P. E., Lopez, F., Johansson, P., Sandstrom, A., Hedegaard, K., Aaby, P. and Rombo, L. 2002. Treatment of children with Plasmodium falciparum malaria with chloroquine in Guinea-Bissau. *Am. J. Trop. Med. Hyg.* **67**:28-31.
- III** Kofoed, P. E., Poulsen, A., Co, F., Hedegaard, K., Aaby, P. and Rombo, L. 2003. No benefits from combining chloroquine with artesunate for three days for treatment of Plasmodium falciparum in Guinea-Bissau. *Trans. R. Soc. Trop. Med. Hyg.* **97**:429-433.
- IV** Kofoed, P. E., Ursing, J., Poulsen, A., Rodrigues, A., Bergquist, Y., Aaby, P. and Rombo, L. Different doses of amodiaquine and chloroquine for treatment of uncomplicated malaria in children in Guinea-Bissau. Implications for future treatment recommendations. Submitted.
- V** Kofoed, P. E., Rodrigues, A., Aaby, P. and Rombo, L. Continued efficacy of sulfadoxine-pyrimethamine as second line treatment for malaria in children in Guinea-Bissau. Submitted.
- VI** Kofoed, P. E., Lopez, F., Aaby, P., Hedegaard, K. and Rombo, L. 2003. Can mothers be trusted to give malaria treatment to their children at home? *Acta Trop.* **86**:67-70.

INTRODUCTION

Malaria

The parasite, *Plasmodium falciparum*

Malaria is caused by protozoan parasites belonging to the genus *Plasmodium*. There are more than 120 species of *Plasmodia* out of which four account for almost all human infections. *P. falciparum* causes the majority of infections in sub-Saharan Africa and is responsible for the most severe disease and for the mortality. Out of the four species only *P. malariae* may infect other primates than humans. *P. ovale* and *P. vivax* form resting stages in the liver, hypnozoites, which can reactivate and cause clinical relapse months to years after the initial infection.

The life cycle of the malaria parasites comprises two phases. An exogenous sexual phase, sporogony, occurring in mosquitoes belonging to the genus *Anopheles*, and an endogenous asexual phase, schizogony, occurring in the human body. When a female *anopheline* mosquito infected with *P. falciparum* bites a human, sporozoites are injected into the blood stream and circulate to the liver, where hepatocytes are invaded, and an asymptomatic pre-erythrocytic phase of infection begins. It has been shown that one bite can introduce several genetically different parasites into the host (44). The sporozoites develop into liver schizonts from which up to 30,000 merozoites are released into the blood circulation, where they invade erythrocytes to begin the erythrocytic cycle of infection, with rapid, asexual multiplication of parasites. This phase elicits the clinical manifestations of malaria. In the erythrocytes they become trophozoites which, in the case of *P. falciparum*, develop into new merozoites within 48 hours. After being released they reinvade new erythrocytes. After a variable number of erythrocytic schizogony cycles, a minority of the merozoites develop into sexual blood stages of the parasite. The male and female gametocytes, when taken up by a female *anopheline* vector, begin the invertebrate phase of the parasite cycle.

Clinical malaria in children

Malaria remains one of the leading causes of morbidity and mortality in many tropical and subtropical countries, children and pregnant women being at special risk. It is estimated that malaria causes between 1 million and 3 million deaths per year, most of these in African children, Africa bearing over 90 % of the malaria burden of the world (5,67,203). More than 500 million attacks of malaria take place every year, including 2 – 3 million severe attacks (67). The situation is getting worse, the main reason being the spread of drug-resistant parasites which has led to increasing numbers of malaria-associated deaths, especially in east-Africa (98,204).

The severity of clinical attacks of malaria varies from an illness characterized by only a few hours of fever to one that kills within days. The incubation period is usually 10 – 14 days. None-immune children infected with *P. falciparum* present with high fever that may be accompanied by chills, rigors, sweating, and headache and often also generalised weakness, backache, myalgias, vomiting, and pallor (211). For children living in endemic areas, the severity of a malaria attack depends on the age, the state of immunity, the nutritional status, the general health, the genetic constitution of the patient, as well as strain of the infecting parasite. In semi-immune individuals, high parasite-counts can sometimes be detected in an asymptomatic person, whereas the same number of parasites will cause severe symptoms in another person. This is explained by differences in sequestration of the parasites during an attack of malaria and by different levels of antibodies in the patients.

There are no absolute diagnostic or clinical criteria for malaria, though the acute illness is nearly always accompanied by fever. Several algorithms attempting to differentiate malaria from other causes of febrile illness in children have been tested (28,122,144,169,179,180) including symptoms as headache (115), chills, sweating, shivering, joint pains, and intermittent fever (16,237); a rectal temperature above 37.6°C, a palpable spleen or nail-bed pallor (170). However, none of the algorithms using clinical features alone have proven sensitive or specific enough to predict malaria (29). This conclusion was supported by a recent study from Kenya, which evaluated a set of clinical signs and symptoms and found that 16 %

of children ≤ 5 years of age, and 44 % of those 6 to 14 years of age who had a history of fever and parasitaemia ≥ 5000 parasites/ μ l of blood would have been sent home without treatment when the algorithm was used (134). In most health facilities the diagnosis is therefore still mainly based on 1) fever or a history of fever, 2) the presence of malarial parasitaemia (if laboratory facilities are available), and 3) in some cases, the absence of other obvious causes for fever (66,134).

Acquired immunity

Individuals living in endemic areas and repeatedly exposed to *P. falciparum* infections acquire immunity to clinical disease. During the first months of life infants are partly protected against malaria by passively transferred IgG via the placenta. The risk of clinical malaria increases until six months of age after which it decreases (79). The acquisition of clinical protection leads to less probability of having a malaria attack, rather than the acquisition of complete protection (176). By school age, most children have developed a considerable degree of immunity with fewer clinical episodes, lower parasitaemias, and an enhanced immune response (118). However, the immunity is not total and they will continue to get re-infected with parasites (65). In children with an acquired immunity, there is a significant production of parasite stage-specific antibodies that react with a large number of antigens. The humoral response has an important role in the protection against the erythrocytic stage of the parasite (26).

Malaria control measures

Several control strategies reduce malaria associated morbidity and mortality. These include vector control as trials evaluating the effect of insecticide-treated bed nets have reduced the overall child mortality and the number of episodes of clinical malaria considerably (77,157). Long-lasting insecticidal nets, in which insecticides are incorporated into the net fibres, have now been developed. However, such an approach needs a major and sustained commitment from international donors as having to pay full price makes the insecticide treated bed nets unavailable to the most vulnerable groups (182).

Vaccines against *P. falciparum* are being developed, and research within this area has progressed rapidly over the past years. The decision of which antigens to include in a vaccine is difficult and should take into account which antigens serve a function critical to the parasite, and whether they are associated with naturally acquired immunity. However, it is likely to take at least a decade before an efficacious vaccine is available for wider use in malaria-endemic countries (67).

In children, chemoprophylaxis lowers mortality and morbidity from malaria substantially, but it is difficult to sustain over longer periods, it might promote drug resistance, and it could impede the development of natural immunity (64). However, in Mozambique, Høgh et al found that one year of chemoprophylaxis starting at six months of age did not impair the protective immune response in the following year (80). Studies have shown that the administration of S/P or amodiaquine at specific times during the first year of life lowered the incidence of malaria and severe anaemia without any rebound in clinical malaria the following year (120,188,189). It is currently being investigated whether this approach, called intermittent preventive therapy, should be recommended for routine implementation as it is now recommended for pregnant women.

Still, chemotherapy for proven or presumed malarial cases remains the fundamental basis of malaria control (145,177). In malaria-endemic countries, most people with fever receive treatment for malaria at some time during the course of their illness (121). In line with this, the majority of mothers in Bissau claimed that they treated their children with chloroquine whenever they had fever (97). Issues such as adherence, availability, cost, perceived value, and acceptability will influence how drugs are used and, therefore, also determine their ultimate effectiveness and usefulness (149).

Combination therapy

The aim of introducing combination treatments is to delay the spread of drug resistance and to prolong the therapeutic lifespan of the antimalarial drugs used (4,21,241). To extend the useful therapeutic life, a combination treatment must be introduced before either of the drugs in the combination is used as monotherapy (236). The principle of the artemisinin-containing

combination therapies (ACT) is to combine a rapidly acting, short-lived artemisinin compound (thus achieving rapid parasite and fever clearance) with a long-resident antimalarial, protecting either drug against resistance and preventing recrudescence after artemisinin therapy (146). Until now only one coformulated drug exists, i.e. artemether-lumefantrine (Coartem™, Riamet™). A dual-branding, dual-pricing strategy should make this drug available for developing countries at a preferential price (148). Both amodiaquine and mefloquine are in the process of being developed as fixed dose combinations with artemisinin derivatives, too (148). The coformulated drugs will diminish the actual complexity of the dosing of ACT.

The high cost of combination therapies, and especially of ACT, makes studies evaluating the user's willingness to pay the higher price crucial. A study in Nigeria concluded that combination therapy based on user fees may not be worthwhile, but that donors should be willing to commit funds to make the treatments affordable to the poor consumers (152). In Tanzania, in an area with high resistance to monotherapy, the families were willing to pay more for an efficient treatment, but nowhere near the real costs of delivering the new ACT (248).

Only few studies have evaluated the treatment practices after policy changes to ACT. In Zambia the first line treatment was changed to artemether-lumefantrine in December 2002. In February 2004 the effect on the treatment practices after the policy change was evaluated. It was found that most clinicians still prescribed S/P rather than artemether-lumefantrine for children with uncomplicated malaria, and that interventions such as in-service training did not seem to influence the choice of treatment (262). A study on the adherence to ACT has shown that as few as 39.4% were "probably adherent" to a treatment course of one dose of S/P and three doses of artesunate, of which the first dose of artesunate and the S/P was given under supervision (39). In Myanmar, the treatment failure rate was significantly higher in a group receiving artesunate-mefloquine without supervision as compared to a group receiving the medication under supervision (202). An effectiveness study in Uganda did not find any difference in the 28-day cure rate in children who received artemether-lumefantrine under supervision as compared to children who received the treatment without supervision.

However, the mean lumefantrine blood concentration on day 3 was significantly lower in the unsupervised group indicating a problem of adherence (158).

Safety data on the new combination therapies are still sparse. As an example artemether-lumefantrine is not recommended for use in patients less than 12 years and weighing less than 35 kg in Sweden as in other parts of Europe (105,116).

The increased demand of artemisinin derivatives has created a problem of availability (35,193), and a lag time of 2 to 3 years might be needed to meet an increase in demand (20). This should be taken into consideration when implementing a new drug policy including artemisinin derivatives.

National policies on malarial treatment

Most malaria-endemic countries have national programmes to fight malaria. One of the main tasks is to give recommendations for therapies to administer at different health care levels. Deciding on a policy for first line treatment (treatment of uncomplicated malaria), for second line therapy (therapy of treatment failures) and for third line therapy (treatment of severe and complicated malaria) in a setting like Guinea-Bissau, the following should be taken into consideration:

- The efficacy of the treatment: has it been tested in the country?
- Dosing complexity (and thereby assumed adherence to the treatment).
- The safety of the drug and possible adverse effects.
- The costs of the treatment and if subsidised, the sustainability of the funding.
- Availability of the drug.
- Is the first line treatment suitable for home-based treatment, and if not, what could be recommended instead?
- When a first line therapy has been decided, is there a suitable second line and an efficient third line antimalarial drug?

For a national malaria programme to design a strategy for treatment of malaria, efficacy studies are needed testing different schedules. Can the well known, cheap and readily available drugs still play a roll? Which doses and treatment schedules could be expected to give the highest adherence and still be efficient? The studies presented in the present thesis aim to provide this information for the National Malaria Programme, Programa de Luta contra o Paludismo, in Guinea-Bissau.

Antimalarial drugs

The bark of the Cinchona tree from the Andes and *Artemisia annua* from China has been used for centuries to treat fevers. As the supply of quinine was cut off during World War II, efforts were put into developing new and better synthetic antimalarials. The efforts led to the discovery of chloroquine, which then became the most important antimalarial. It was used by WHO in its global malaria eradication programme, both as chemoprophylaxis with tablets and as chloroquine-medicated salt. This practice was not stopped until emerging resistance to chloroquine in the beginning of the 1960s. Other compounds developed during the war served as prototypes for proguanil, pyrimethamine, mefloquine, and atovaquone.

Chloroquine

Chloroquine is a 4-aminoquinoline working exclusively against those stages of the intraerythrocytic cycle during which the parasite is actively degrading haemoglobin, suggesting that chloroquine interferes with the feeding process (222). Chloroquine is taken up only to a very limited extent by uninfected erythrocytes, by contrast it is concentrated several thousand fold inside the malaria parasite where it interferes with the polymerisation and detoxification of ferriprotoporphyrin IX, which is released during haemoglobin digestion (222). The drug is less accumulated in the food vacuoles of chloroquine-resistant parasites suggesting that the resistance is at least partly mediated by excluding chloroquine from the site of action rather than an alteration in the target of the drug (184).

Chloroquine is rapidly and almost completely absorbed from the gastrointestinal tract, and the peak plasma concentration is reached 1.5 to 3 hours after oral administration. In plasma, 50 to 65 % is bound to proteins. The apparent volume of distribution is very high and ranges from 116 to 285 l/kg. Chloroquine accumulates in high concentrations in tissues, particularly in liver, lungs, spleen, and kidney. Plasma kinetics show a multiexponential curve (101,243) with a long terminal half life of 75 to 136 hours in children. Chloroquine is eliminated very slowly from the body (84,101).

Chloroquine is usually well tolerated. Commonly reported adverse events include headache, malaise, dizziness, blurred vision, mild gastrointestinal upset, and itching (101,217). However, chloroquine has a narrow therapeutic interval. Following toxic doses of chloroquine, symptoms develop within 10 to 30 minutes of ingestion. Respiratory difficulties and cardiovascular symptoms followed by hypotension progressing to cardiogenic shock and cardiac arrest being the most severe event (84,206). The cases described in the literature almost all relate to the deliberate ingestion of chloroquine (12,55,123,136,154,167,214,231).

The acute toxicity of chloroquine is limited to transient but very high concentrations in the blood during an incomplete distribution phase, especially after parenteral administration (49,113). When 15 mg chloroquine per kg was infused over a four-hour period or as a single oral dose, no serious effects were observed (48,69). Peak concentrations of chloroquine after oral administration are obtained within 1 to 8 hours (233). Only 0.1% of the absorbed drug remains in the plasma (245). The major distribution of chloroquine occurs within hours and results in substantially lower trough concentrations at the time of the next dose, even when administered twice daily. Furthermore, orally administered chloroquine gives a peak plasma concentration that is less than 20% of the concentration obtained after parenteral administration (70,246).

Amodiaquine

Amodiaquine is also a 4-aminoquinoline, structurally similar to chloroquine. It is a more active inhibitor of the growth of *P. falciparum in vitro* than chloroquine. Amodiaquine is rapidly metabolized *in vivo* to its diethyl metabolite, which is less active (222). Amodiaquine

competitively inhibits chloroquine accumulation, suggesting that these compounds share a similar mechanism. Amodiaquine and other 4-aminoquinolines probably act in a manner similar to chloroquine (168,222).

Amodiaquine is well absorbed from the gastrointestinal tract and highly protein bound. Plasma peak concentrations are reached 4 hours after absorption. After oral administration, amodiaquine appears to be metabolised in the liver to a large extent by first-pass effect. The terminal half life of monodesethylamodiaquine is 15 days (84).

Amodiaquine prophylaxis was associated with hepatitis and agranulocytosis. Following treatment, drug-related symptoms are only reported in few children, mainly vomiting and itching. In clinical studies, no clinical or biochemical evidence of hepatic reactions has been detected, however a decline in the mean absolute neutrophil counts was reported (217). Data from studies in which amodiaquine has been combined with S/P or artemisinin have also demonstrated good tolerability (41,209). A review on amodiaquine for treating malaria concludes that “amodiaquine appears to be no more toxic than chloroquine or sulfadoxine-pyrimethamine, when administered in doses up to 35 mg/kg total dose over three days” (147). The tolerability data from the clinical trials are thus favourable, but very few safety data exist on the use of repeated amodiaquine treatments.

Quinine

The quinoline-methanol quinine acts primarily on the intraerythrocytic asexual stages of the parasite, in which it interferes with the polymerisation and detoxification of ferriprotoporphyrin IX. It competitively inhibits chloroquine accumulation suggesting that it shares a similar mechanism of accumulation (222).

Quinine is rapidly and almost completely absorbed when given orally. Plasma concentrations reach a maximum after 1 to 3 hours. The drug is mainly bound to plasma proteins. The apparent volume of distribution is approximately 1.8 l/kg and the terminal elimination half life 9 to 15 hours. In children, the elimination half lives are shorter and the volume of distribution

smaller than in adults. In patients with malaria, the elimination half life of quinine is increased and the volume of distribution reduced (84,101,107).

Oral quinine intake is associated with cinchonism, a constellation of minor but unpleasant adverse effects consisting of nausea, headache, tinnitus, high-tone impairment and blurred vision. More rarely, vomiting, abdominal pain, diarrhoea and vertigo are seen. When used in severe malaria, quinine might cause hyperinsulinaemic hypoglycaemia (217). Lethal hypotension can result from intravenous quinine injections. Therefore, the drug should be given as slow infusion or as intramuscular injections, whenever oral treatment is not feasible. However, intramuscular injections might cause sterile abscesses (217). Serious cardio-toxicity after quinine overdose is rare, but fatalities have been described following overdoses (84).

Quinine is recommended as the third line drug for treatment of malaria by the National Malaria Programme in Guinea-Bissau. It should “be reserved for severe or complicated cases, even if it can be used to treat uncomplicated malaria whenever justified (e.g. therapeutic failure of the second line treatment)”. Furthermore, it is recommended to give 10 mg/kg three times a day for seven days – initially intravenously, but to change to tablets in the absence of danger signs or complications (164). Taking tablets three times a day for a whole week provokes poor adherence (10). We have previously shown that quinine should be given for 7 days as both 3-day and 5-day courses produce high recrudescence rates whereas treatment for 7 days gave a high cure rate (96). However, both the total dose and the number of daily doses can be reduced to minimize the risk of side effects and to improve adherence (94,95).

Sulfadoxine-pyrimethamine

Sulfadoxine-pyrimethamine (S/P) is a fixed dose combination of the two antifolates sulfadoxine and pyrimethamine with the advantage of a single dose treatment. Sulfadoxine interferes with the de novo synthesis of folate by inhibiting the enzyme *dihydroopteroate synthetase* (*dhps*) early in the folate pathway. Trimethoprim inhibits the *dihydrofolate reductase* enzyme (*dhfr*). The two drugs are synergistic and have been used both for prophylaxis and therapy of falciparum malaria.

Both components are completely absorbed after oral administration, are highly protein bound, and reach peak plasma concentrations after 2 to 6 hours. The mean elimination half life for sulfadoxine ranges between 123 and 195 hours, and for pyrimethamine from 80 to 95 hours (84).

Adverse reactions are infrequent and mild during malaria treatment, mainly gastrointestinal upset, headache, and rarely itching. Severe adverse effects as erythema multiforme, Stevens Johnson syndrome and toxic epidermal necrolysis have been documented with S/P used as prophylaxis. The risk of severe skin reactions following treatment is unknown, but apparently substantially lower than during prophylaxis (217). Hepatic toxicity, haematological reactions and hypersensitivity reactions are reported. The list of the possible sulphonamide related adverse effects is long (217).

Artemisinin

Artemisinin is a sesquiterpene lactone with an endoperoxide bridge. Inside the cell, the peroxide bridge is cleaved by an iron-dependent mechanism. This results in the generation of short-lived radicals, which inactivate a parasite ATPase (168). This might explain why the drug is selectively toxic to malaria parasites (125). The drug shows a broad stage specificity, but is most effective against late ring to early trophozoite stages (218).

Lipophilic (artemether) and hydrophilic (artesunate) artemisinin derivatives have been developed. After oral administration, the time to plasma peak concentration is 2 hours (84). Artemisinin is rapidly hydrolysed to dihydroartemisinin, which in itself is quickly eliminated (138), with a half life of less than 60 minutes (168).

Few serious adverse effects have been reported in humans, mainly neutropenia, reduced reticulocyte count, and anaemia, and the artemisinin derivatives are well tolerated (62). However, audiometric changes associated with the treatment of uncomplicated malaria with artemether-lumefantrine have been reported recently (224). Animal studies have documented CNS toxicity with artemisinin (62). However, no convincing evidence has been provided that short course therapy with artemisinin either alone or in combination with mefloquine is

associated with neurotoxicity in humans (217), even if few case reports have been published (50,127).

Resistance in *P. falciparum* to antimalarial drugs

Resistance

Antimalarial drug resistance has been defined as “the ability of a parasite strain to survive and/or multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within tolerance of the subject” (253). It is not an all-or-none phenomenon, and the entire spectrum of response ranges from the anticipated norm to complete failure. As far back as in 1948 the first reports came from Calcutta confirming the resistance to proguanil, from Malaysia and India in 1953 on resistance to pyrimethamine, and the first report on chloroquine resistance from Venezuela in 1960 (155). In 1973, chloroquine resistance had been reported from several countries in South America and Asia, but not until the late 1970s from Africa (36). The resistance to chloroquine in Africa has spread from east to west. In the most affected countries, the first line treatment has been changed from chloroquine to S/P only to find resistance emerging to this drug as well (162,216,219). The efficacy of quinine has diminished in some areas, and low level resistance is now widespread in parts of Southeast Asia. Decreased sensitivity to quinine is present in more than 50 % of *P. falciparum* infections in areas of Southeast Asia (57).

The evolution of drug resistance in *P. falciparum* is a public health disaster (117) and the mechanism of the development of the resistance is being investigated intensively. Many theoretical models try to explain the mechanism and strategies to minimize the evolution of resistance have been published (72). Molecular analyses of the *chloroquine-resistance transporter gene (crt)* have established that chloroquine resistance has originated from four mutational events only – none of these occurred in Africa (239,249).

The genetic basis for chloroquine resistance is not known in detail, but requires several sequential mutations in the *pfcr* gene and probably in other genes as well, especially in the *multi-drug resistance gene 1 (pfmdr1)* (73,238). Certain mutations in the *crt* and *pfmdr1* genes might also be associated with resistance to amodiaquine (141). Thus, chloroquine resistance probably has a complex, multigenetic basis.

By contrast, the genetic basis of resistance in *P. falciparum* to S/P is quite well known. Resistance to pyrimethamine is associated with mutations in the *dihydrofolate reductase (dhfr)* gene, point mutations at codon 108 being the most important although additional mutations at positions 51 and 59 are associated with higher resistance (54,156). These three mutations combined with a point mutation at codon 164 confer high-level resistance not only to pyrimethamine but also to chlorcycloguanil (the active metabolite of chlorproguanil, which is combined with dapsone in the antimalarial drug LapDap™) (160). When used as monotherapy, resistance develops rapidly to both pyrimethamine and proguanil (159). Resistance to sulphadoxine has been linked to mutations in the *dihydropteroate synthetase (dhps)* gene at codon 437 with additional mutations at codons 540, 436, 581 and 613, increasing the level of resistance (25,92,103,226). Determination of these mutations has been suggested to be used as an indicator of the level of resistance *in vivo* against S/P, whereas the clinical importance in the individual patient is more debatable (88,103).

Reduced sensitivity to artesunate *in vitro* has been reported from an area in Yunnan Province in China, where the drug has been used for a long time (259). Several case reports of presumed resistance have been published (59,114,183,195,225), though clinical treatment failures might be explained by pharmacokinetic factors (125). On the other hand, it has recently been demonstrated that PfATPase-6 point mutations were associated with decreased *in-vitro* susceptibility to artemisinin in *P. falciparum* parasites isolated from areas with uncontrolled use of artemisinin derivatives (86). Genetic mutations which have been associated with resistance to chloroquine have been shown to affect *in vitro* sensitivity to artemisinin derivatives. Thus, mutations in position 76 of the *pfcr* gene, which decrease sensitivity to chloroquine, tend to increase sensitivity to artemisinin (125).

According to Hastings (72) there are several theoretical ways in which mutations can enter the population. They can be selected from mutations pre-existing in the population before the drug is deployed. These mutations are usually deleterious for the parasite and are therefore removed by natural selection, but they do exist at low frequencies. The second source of mutations arises from the merozoites emerging from the liver. Once in the blood stream they are susceptible to the antimalarial drug, and a selection could occur in a previously treated person with sub-therapeutic drug levels. Finally, the mutations could occur spontaneously in the cell lineages leading to a mutant sporozoite, which upon inoculation into a host will lead to an infection consisting entirely of resistant parasites, and if surviving this parasite might spread in the population.

To overcome the development of resistance, combination therapies, especially including artemisinin have been advocated (9,18,21). Theoretical arguments suggest that the use of antimalarial therapies in combination with artemisinin-derivatives will slow the rate at which resistance emerges (75,242). Combining mefloquine with artemisinin has halted further progression of mefloquine resistance in Southeast-Asia (139). If resistant mutations pre-exist within the huge number of asexual parasites in infected patients, or if the origin of mutation is primarily through drug selection of spontaneous mutations from within the asexual parasitaemia of treated patients, then addition of a second drug to form combination therapies will always reduce the probability of a resistant mutation surviving the treatment (72). If the origin is primarily through sub-therapeutic drug selection of emerging merozoites, then the relative half life of the drugs becomes crucial. If they are mismatched, one of them might be rapidly eliminated, which sooner or later leaves the other drug present with sub-therapeutic drug concentrations (72). In line with this, Greenwood advocates that combinations of drugs with similar half lives are desirable (67).

With the increasing resistance to most antimalarial drugs it has become crucial to evaluate the level of resistance for health planners to recommend both first and second line treatments. This can be done by *in-vivo* testing, by *in-vitro* assays, and by genetic analyses of the parasites.

Assessing the efficacy of anti-malarial drugs *in vivo*

WHO developed the first standard *in vivo* test system for assessing the sensitivity of *P. falciparum* to chloroquine in 1965. The recommended methods have been adjusted several times since then (250,257). In 1996, a new protocol was developed for routine assessment of the clinical efficacy of anti-malarial drugs which were administered for no more than three days in high transmission areas (255). Following the first 5 years of experience of this protocol, it was modified into a single, globally standardised protocol that outlined procedures for monitoring anti-malarial drug efficacy in all endemic areas (256,257). The 2005 WHO protocol recognised the importance of follow-up periods of at least 28 days and of performing survival analyses which is reflected in the revised outcome classification (250).

In the 1996 protocol it was recommended only to include children below 5 years of age and only children who at the time of appearance at the health centre had an axillary temperature of $\geq 37.5^{\circ}\text{C}$. In the revised protocol, also children above 5 years of age can be included. In low to moderate transmission areas, a history of fever during the past 24 hours is accepted. Only children with mono infection with *P. falciparum* and without other febrile illnesses than malaria should be assessed.

The WHO classification of the outcome of treatment has changed significantly during the last 10 years. The present classification is based on both clinical and parasitological parameters:

- *Early treatment failure (ETF)*: a) development of danger signs on day 1, day 2 or day 3 in the presence of parasitaemia, b) parasitaemia on day 2 higher than day 0, irrespective of the temperature, c) parasitaemia on day 3 with axillary temperature $\geq 37.5^{\circ}\text{C}$, or d) parasitaemia on day 3 $\geq 25\%$ of the parasite count on day 0.
- *Late clinical failure (LCF)*: a) development of danger signs or severe malaria on any day after day 3 in the presence of parasitaemia, b) parasitaemia and axillary temperature $\geq 37.5^{\circ}\text{C}$ (or in low to moderate transmission areas a history of fever during follow-up).
- *Adequate clinical response* (only in the 1996 protocol. In the new protocols it has been replaced by the two categories below): a) absence of parasitaemia on day 14 during

follow-up irrespective of axillary temperature, or b) axillary temperature < 37.5°C irrespective of presence of parasitaemia. This parameter was only found in the 1996 protocol. In the new protocols, it has been replaced by the following two categories:

- *Late parasitological failure (LPF)*: presence of parasitaemia and axillary temperature < 37.5°C during follow-up without previously meeting any of the criteria for clinical treatment failure.
- *Adequate clinical and parasitological response (ACPR)*: Absence of parasitaemia on day 28, irrespective of axillary temperature, without the patient previously meeting any of the criteria of early treatment failure, late clinical failure or late parasitological failure.

Children moving from the study area, being treated with antimalarials by a third party, or having a mixed malaria infection during follow-up are excluded, as are children not completing the treatment due to withdrawal of consent (including children excluded due to side effects of the drug), and children developing danger signs on the day of inclusion. Previously, data was recommended to be analysed as per protocol. This approach retained only data from patients with evaluable results for analysis, i.e. those patients with known efficacy end points: early treatment failure, late clinical failure, adequate clinical response (in the later protocols was divided into late parasitological failure and adequate clinical and parasitological response). Data from all other patients were thus excluded from the analysis and did not contribute to the denominator. The method recommended by the latest protocol is survival analysis utilising the data from all patients until the time of loss to follow-up. As the criteria specified by the WHO protocol are intended for routine monitoring of drug, which should be administered no more than once a day for three days, many studies report the results following modified classifications (2,11,13,32,63,119,133,142,181,186,192,200,260). As both the classification of the outcome and the recommended way of handling loss to follow-up has changed with the introduction of the newest protocol, direct comparisons over time are more difficult.

The data can be analysed by intention-to treat analysis or by evaluability analyses. The intention to treat is a strategy for the analysis of randomised, controlled trials, and its main

purpose is to maintain the integrity of the randomisation. It is most suitable for effectiveness investigations which answer the question whether the patient benefits from the treatment regimen (81). In an intention-to-treat analysis it is even more important to elucidate, whenever possible, the reasons for withdrawals and for loss to follow-up to ensure that these events are not related to the treatment received.

To evaluate the efficacy of a treatment when the correct dose of the anti-malarial has been given, an evaluability analysis can be performed, thus obtaining information on treatment failures (resistance) in the population studied (3). In this analysis patients not receiving the study treatment or receiving anti-malarials by third person or by self-medication during follow-up are excluded from the analysis, as recommended by the WHO protocols. Even if the clinical effectiveness may be overestimated in the evaluability analysis, more exact estimates of the efficacy are obtained.

It has been increasingly recommended to include polymerase chain reaction (PCR) analyses to distinguish between recrudescence parasitaemia and reinfections during follow-up, especially when doing follow-up for more than 14 days (250,256). The *merozoite surface protein-2* (*msh-2*) is supposed to be the best and easy marker for PCR correction (99,140). Still, there are technical issues limiting the benefits from the results obtained by PCR. Most techniques are insufficiently sensitive to pick up minority populations of parasites present at day 0, and it is therefore possible that “new” parasites identified during follow-up actually represent recrudescence of parasites from a resistant minority population that was present from the start rather than true reinfections. In Tanzania, it was shown that infected children had several genotypes of *P. falciparum* simultaneously, some could be identified one day, others the next day (53). Taking two specimens 24 hours apart has been suggested as a way to overcome this problem. The most important confounding factor is the presence of gametocytes at the time of recrudescence, as conventional techniques cannot distinguish DNA from asexual forms from DNA of gametocyte origin, thereby tending to overestimate the risk of recrudescence (250). The classification of recrudescence and reinfection also depends on the genotyping methods applied making comparisons of estimates from different studies difficult (200). It has therefore been suggested that the most useful comparison of the clinical outcome of different

treatment schedules should be based on results unadjusted by genotyping, including all retreatments for clinical malaria (260).

***In vitro* testing of sensitivity to anti-malarial drugs in the parasite**

In vitro sensitivity tests eliminate host factors such as acquired immunity. As in the case of molecular markers, *in vitro* tests might provide an early warning of impending resistance before it becomes clinically apparent, furthermore it can be used to assess the baseline susceptibility to drugs before introducing a new treatment (124,252). Nevertheless, as discordance exists between *in vitro* and *in vivo* tests, Ringward and Basco conclude that the *in vitro* tests cannot substitute *in vivo* data on therapeutic efficacy (173). Furthermore, *in vitro* tests have not been developed for all antimalarial drugs, and the technique is sometimes difficult, e.g. for testing S/P *in vitro*.

Drug resistance detection by molecular methods

Point mutations in the genome of *P. falciparum* are correlated to the resistance to some antimalarials. Molecular markers of resistance are only available for a few drugs. Resistance to pyrimethamine has been associated with point mutations in the *dihydrofolate reductase* (*dhfr*)-gene (54,156) and resistance to sulphadoxine to mutations in the *dihydropteroate synthetase* (*dhps*)-gene (6,25,92). Chloroquine resistance probably has a multigenetic basis. The chloroquine resistant phenotype has, however, been shown to be linked to the *P. falciparum* chloroquine resistance transporter gene (*pfcr1*), but also to mutations in the multidrug resistance gene (*pfmdr1*) (40,46,197,198,234).

With the improved methods of performing polymerase chain reaction, it has become technically possible to use PCR as a tool for monitoring the molecular markers of drug resistance in the malaria parasite. Determination of mutations in genes associated with drug resistance has been suggested as an indicator of the level of resistance against anti-malarials (102,103,161,250), whereas the clinical importance of these mutations in the individual patient is debatable in endemic areas (15,88,150). Molecular markers could thus provide an early warning system in an area where an antimalarial is still clinically efficient but where

genetic studies show an increase in the level of mutations coding for resistance to the drug. The technique could also be useful in monitoring changes in prevalence of molecular markers in places where a drug has been withdrawn or where a drug combination is in use (250,256).

Ethical considerations doing clinical studies in developing countries

Improving the health status in sub-Saharan Africa is done against a background of poverty, limited scientific, administrative, and political development, poor health, and reduced life expectancy – all a reflection of inequality. These countries need health care research addressing their burden of disease at many levels. The fifth version of ethical guidelines in the Declaration of Helsinki also includes problems related to clinical trails in resource poor settings (171). The declaration states that “every patient – including those of a control group, if any – should be assured of the best proven diagnostic and therapeutic method” (230). There is, however an ongoing debate on how to conduct research ethically in the midst of gross disparities in health care (1,24). Recently, researchers from both developed and undeveloped countries have been asked to state their position on these issues. There was “a broad consensus that a therapy known to be less effective than the best standard of care can be tested in resource poor settings where the best therapy is normally unavailable and difficult to implement” (91). The same group examined whether recent published trials conducted in sub-Saharan Africa met the best current clinical standards as stated by the Helsinki declaration. Only 16% of the studies reviewed within the areas of HIV, tuberculosis and prevention of malaria did so (91), but at the same time it was questioned whether it has any value comparing the effects of interventions intended for sub-Saharan Africa, where approximately 50% of the population live on less than one dollar a day, with the best current method from well-resourced settings. Still participants in research projects should be offered the best intervention currently available defined by the national public health system (24,221).

Ethical clearance should be obtained from both the donor country and the country in which the study is performed, if possible. In many sub-Saharan African countries ethical committees are only in the process of being established. In Guinea-Bissau a committee has only existed since

year 2000 and is therefore still not functioning smoothly. Before a formal committee existed, permissions to perform studies were obtained from the Ministry of Health.

It is clearly important to inform prospective participants in a research project on all aspects of the study, and that the person has the right to withdraw at any point and still receive the treatment usually given. The information provided “should be accurate, concise, clear, simple, specific to the proposed research and appropriate for the social and cultural context in which it is being given” (221). As many individuals are illiterate, unfamiliar with the concepts of medicine held by the investigators and unaware of the background for the disease to be studied it is very important to involve researchers who are familiar with the cultural constraints related to receiving information..

Informed consent should also always be obtained whether in writing or verbally. The Nuffield Council of Bioethics Working Party states that among illiterate people it “is not consistent with the duty of respect for persons to require a prospective participant to sign a written form that they are unable to read” (221), but where only verbal consent to research is contemplated an appropriate process for witnessing the consent should be included in the protocol.

THE STUDIES

Aim of the studies

Overall: To evaluate the efficacy of treatment regimens that are currently used or likely to be used for treatment of uncomplicated malaria in children in Guinea-Bissau.

A) Chloroquine:

- Evaluate the recommendation from the National Malaria Programme of chloroquine 25 mg/kg as first line therapy
- Monitor changes in *in vivo* sensitivity to chloroquine
- Examine whether a higher dose of chloroquine is effective
- Evaluate the efficacy of the commonly used treatment schedule of quinine for 3 days followed by chloroquine
- Evaluate the efficacy of chloroquine combined with artesunate

B) Sulfadoxine-pyrimethamine:

- Evaluate the efficacy of S/P as first and second line treatment
- Monitor changes in efficacy of S/P when used as second line treatment

C) Amodiaquine:

- Evaluate the efficacy of amodiaquine in two different doses
- Compare the efficacy of amodiaquine with chloroquine

D) Effectiveness:

- Study the adherence to chloroquine as recommended by the National Malaria Programme

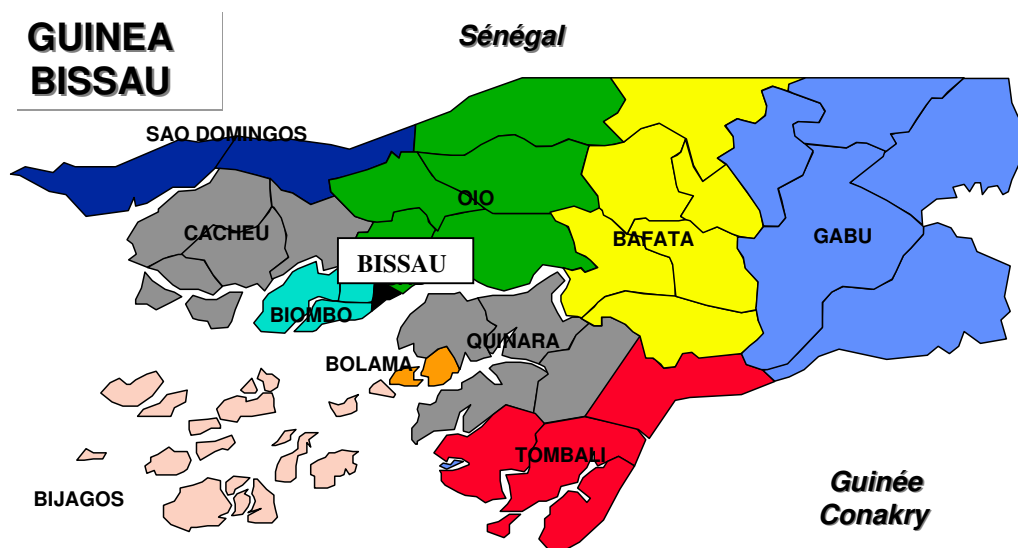
Study area

Guinea-Bissau

The Republic of Guinea-Bissau is a former Portuguese colony located in West Africa, bordering to Senegal in the north, the Republic of Guinea in the southeast, and the Atlantic Ocean in the west, covering an area of 36,000 sq. km (Figure 1). The population was in 2004 almost 1.5 million people out of which more than 20% live in the capital, Bissau. Since the independence in 1974, Guinea-Bissau has been one of the poorest countries in the World with a GNP per capita of 160 US\$. The under-5-mortality rate is 204 per 1000 live births and the total life expectancy is 45.5 years. Around 40% of the population is under 15 years of age. More than 30 ethnic-linguistic groups are presented in the country, but the lingua franca spoken by most people living in Bissau is Guinean Creole (227).

The total expenditure on health per capita in 2004 was 9 US\$, in total accounting for 6.3% percent of the GNP. The government expenditure accounts for 48%, the rest being private out-of-pocket expenditures. Approximately 90 % of the public health sector is financed by international donors. In 2004 the country had 203 physicians and 1340 nurses (251). It is estimated that only 40 % of the population in the country has access to health services (164).

Figure 1: The Republic of Guinea-Bissau:



Bandim and the Bandim Health Project

The Bandim Health Project, Projecto de Saúde de Bandim (PSB), has performed community studies in Guinea-Bissau since 1978. PSB now covers several suburban areas (bairros) of the capital. The bairros are subdivided into minor areas, zones, in which all houses are numbered. Detailed maps of the zones are continuously updated (Figure 2). The “addresses” are written on the children’s health cards. Through the project’s routine surveillance system, data on births, deaths and migration are collected and all children registered in the registration system. The studies included in the present thesis were performed in Bandim I and Bandim II which have been under demographic surveillance since 1978 and 1984, respectively. At the start of the studies in 1994, approximately 25,000 people lived in the areas, 50% being under 18 years and 19% under 5 years of age. Only approximately one third of the women have been to school for more than 3 years. Many ethnic groups are represented in the study area, the main being Pepel, Balante, Manjaco, Mancanha, and Fula (190). The houses are mainly clay huts or mud-brick houses, usually without internal ceilings, leaving a gap between the walls and the roof permitting mosquitoes to move freely from one room to another. Most houses are crowded dwellings consisting of four to eight rooms and are inhabited by several families. There is no sewage system, and the water supply is mainly a mixture of private wells and public stand pipes.

The population in Bandim is very mobile, often changing residence either inside or outside the study area. Furthermore, it is very common to travel to the home-village for shorter or longer periods of time, especially during harvesting seasons. Of special interest is the cashew-nut season, as many women earn cash by collecting cashew fruits during the season from March to June. Younger children often follow their mothers to the rural areas whereas older children, especially if they go to school, stay in Bissau. Even within Bissau most mothers bring their youngest children to the market or to work, particularly if they are still being breastfed. Likewise, it is common for relatives from rural areas to come to Bissau for visiting, often for extended periods of time. These visitors (hospedes) tend to use the health services of Bandim claiming to live in the area.

In 1993 a new health centre, Centro de Saúde de Bandim (CSB), was inaugurated. CSB is situated centrally in Bandim I. It is staffed with a varying number of physicians, nurses, and midwives. Most patients turn up in the morning, though the centre is also open in the afternoon and evening. Children are seen at the child clinic (consulta de crianças), primarily by a nurse. If any clinical problem arises, a physician is called. A small pharmacy, supplied with the most basic drugs, is part of the centre. Generally, the patients have to pay for the medicine and for consultations, except that the PSB pays for the consultations of children and pregnant women.

A minor laboratory is attached to the CSB. Blood samples are drawn here and a few analyses performed. However, the supply of material is inconsistent and the centre has had electricity only for shorter periods of time. Therefore, malaria films are examined by a light microscope (Optimus™). The staff and the quality of the services of the laboratory are supervised by the National Health Laboratory, Laboratório Nacional da Saúde Pública (LNSP) which is situated in Bissau. Materials for the studies included in the present thesis were supplied by the PSB to ensure the quality. Examination of malaria films from the first study (Paper I) and control readings of the malaria films has been performed at the LNSP.

The population in Bandim is served by the health centre in Bandim, but also has access to the health centre in the neighbouring bairro Belém, to a mother and child health clinic and to the paediatric ward at the national hospital, Hospital Nacional Simão Mendes, both situated in the centre of the city 1.5 to 5 km from the study area. Furthermore, a high number of private pharmacies are scattered all over Bissau playing an increasing role in treatment, including treatment of children suffering from malaria.

Malaria endemicity

Very few data are available on the true prevalence of malaria in different parts of Guinea-Bissau, but most rural areas are considered mesoendemic to holoendemic. In the Cacheu region in the north-western part of the country a study found parasite rates in children aged 2 to 9 years of 44 % - 79 %, suggesting malaria to be mesoendemic to hyperendemic (85). In a

village just outside Bissau the level of endemicity varied from hypo- to holoendemic with closeness to rice fields being one of the important determinants for parasitaemia (60).

In a study from Bissau, *P. falciparum* was found in 85% to 95% of the people infected with *Plasmodium* species, a minor proportion of these were mixed infections especially with *P. malariae*, whereas *P. ovale* was rarely identified (8). Malaria is stable in the study area, parasite rates in children aged 3 to 6 years found to be 59 % (112), and 26 % in children aged 1 to 2 years at the end of the rainy season (205).

The rainy season lasts from June to November, with rainfalls varying from 1250 mm to 2750 mm per year. Malaria transmission occurs in all seasons (60), but the disease burden from malaria is highest in the beginning of the rainy season and especially at the end of and shortly after the rainy season (17,164). This pattern could possibly be explained by the heavy rains initially in the season washing away the mosquito larvae thus limiting the transmission of malaria.

Organisation of the studies

The staff

The studies were all performed at the CSB, which serves the population of Bandim I and Bandim II. The staff was recruited at the health centre among the nurses and laboratory technicians employed by the Ministry of Health (MINSAP). Physicians from the CSB participated in the supervision of the clinical work of the staff. In most of the studies physician(s) from CSB were co-investigators as well.

One senior nurse has participated in all the studies, another in all but one (paper IV). The nurses were trained in assessing the clinical status of the children and in giving structured information to the parents/caretakers. They were furthermore trained in randomising the children to the different study groups according to the protocol and have extensive experience in performing interviews. As experienced nurses, they have knowledge of handling children

and of persuading them to swallow the medicine. At the start of each new study the procedures were reinforced and the differences between the new and the previous study were clarified. The nurses were supervised continuously during the studies and the procedures controlled.

Apart from the last few months of the last studies (papers IV and V), the same laboratory technician worked on the project. She ensured that the necessary laboratory material was available, did the first reading of the malaria films and performed the home visits. She was very well known by mothers and children in Bandim, as she visited a considerable number of houses and drew blood from many of the children. She knew the bairros and by knowing many of the inhabitants, she could almost always obtain the necessary information. This ensured as high a follow-up rate as possible

Having the same staff through almost the whole study period ensured that the methodologies of the different studies are very much alike, and that the variability between nurses and laboratory technicians was minimised.

Children included

The studies were conducted at the CSB and children attending the “consulta de crianças” were eligible. The same inclusion criteria were applied throughout all the studies. The inclusion criteria were:

- 1) Temperature $\geq 37.5^{\circ}\text{C}$ or a history of fever during the past 24 hours.
- 2) Not having a severe concurrent febrile illness.
- 3) No danger signs present (e.g. unconscious children, children with severe anaemia, jaundice, or severe vomiting) or considered by the doctor in charge to need the services of a hospital for other reasons.
- 4) Monoinfection with *P. falciparum* with ≥ 20 asexual parasites per 200 leucocytes.
- 5) Living in Bandim (to ensure follow-up).
- 6) The mother or other caretaker willing to give informed consent.
- 7) Stating not to have taken any antimalarial drug during the previous week.

- 8) In study III children with a bodyweight less than 9 kg were not included due to the size of the Arsumax™ tablets.

The age range of the children was from the start defined by the age group seen at “consulta de crianças”, which consists of children up to the age of approximately 15 years. Whenever children come to the Bandim Health Centre for medical attention, they are expected to bring their child health card (cartão de criança) containing date of birth and address. However, older children have often lost their card, and even mothers of young children might come to the health centre without bringing the card, either because they have forgotten it or because it has disappeared. Children visiting relatives in Bandim sometimes give the address of the house in which they stay while in Bandim. Some of these children were included in the studies, thus violating the inclusion criteria. They often returned to their homes, some even before terminating the treatment, others during follow-up. These children constitute a significant part of the losses to follow-up.

Mothers/caretakers of children fulfilling the inclusion criteria were informed of the study and if accepting, a malaria film was taken and examined immediately. All children included had a second malaria smear prepared just before receiving treatment for later confirmation of the parasite count.

Only children having at least 20 asexual parasites per 200 leukocytes were enrolled. In Guinea-Bissau, Lisse and co-workers found a leukocyte count of $7.7 \times 1000/\mu\text{l}$ in asymptomatic children both with and without parasitaemia, and $9.3 \times 1000/\mu\text{l}$ in children with clinical malaria in spite of a relative lymphocytopenia (111). These results correspond to what has been found in Nigeria where the leukocyte counts were $7.6 \times 1000/\mu\text{l}$ and $9.0 \times 1000/\mu\text{l}$ in children without parasitaemia and in children with parasitaemia, respectively (207). Previously we measured the total leukocyte count on inclusion and found values somewhat higher than reported by Lisse, $10.6 \times 1000/\mu\text{l}$ (data not reported). We therefore choose to report the parasitaemia per 200 leukocytes instead of recalculating into μl using an arbitrary leukocyte count.

In all the studies, children were allocated to the different treatment groups by block randomisation in order to ensure equal distribution during the different seasons of the year, as both the intensity of malaria transmission (17) and the immune status of the children (110) differ during the different seasons.

The medication

Studies I to V were designed to evaluate the efficacy of the treatments. Therefore, the medication was supervised by the nurses. In studies III and IV we decided to have one of the health workers visiting the child's house in case the child did not turn up as planned, to ensure that as many of the children as possible received the complete treatment.

Whenever feasible, tablets were used. Only when dosing was impossible due to the size of the tablets, syrup was available. If necessary due to the young age of a child, tablets were crushed mixed with a small amount of water and fed to the child on a teaspoon.

One of the most common symptoms in children suffering from malaria is vomiting. The children were observed for 30 minutes following the medication, and the dose was repeated if they vomited. If the child continued to vomit, the physician in charge, or if not available, the study nurse, re-evaluated the child. Mostly, the condition was assessed to be so severe that the child should be admitted to hospital for treatment. If not, the medication was repeated with Quinimax™ intramuscularly and the child continued in the study even if this modified the assigned treatment slightly.

Follow-up

In all studies the children were visited once a week in their homes. As previously described, far from all mothers brought the child's health card and several visitors from outside Bandim gave the address of the family in Bandim they were visiting. Therefore, some children not living in Bandim were included, thus violating the inclusion criteria. PSB continuously updates maps of the area, as houses collapse while others are constructed in a ceaseless

process (Figure 2). These maps have been of invaluable help in locating the houses of the children included.

To allow for days off, national holidays etc. the children could be visited one day prior to the day planned. If the child could not be found, the health worker had to return two more times. If the child was still not found, the health worker only returned for the next visit. Children not seen for more than two weeks were excluded from further follow-up, as the recall of medication given to the child was then considered too unreliable. By asking information from neighbours, the health worker often managed to find the mother and/or the child if they stayed in Bandim, e.g. working at the market or being at school. A considerable number of children left the study area follow-up. Family members and neighbours could then often provide information as to the whereabouts of the mother and child and as to when they were expected to return – or if they had left for good.

In 1998 a civil war broke out resulting in a higher loss to follow-up rate in study I. Since the end of the war in May 1999, there have been several episodes of political unrest, each time influencing the studies, though only to a small extent.

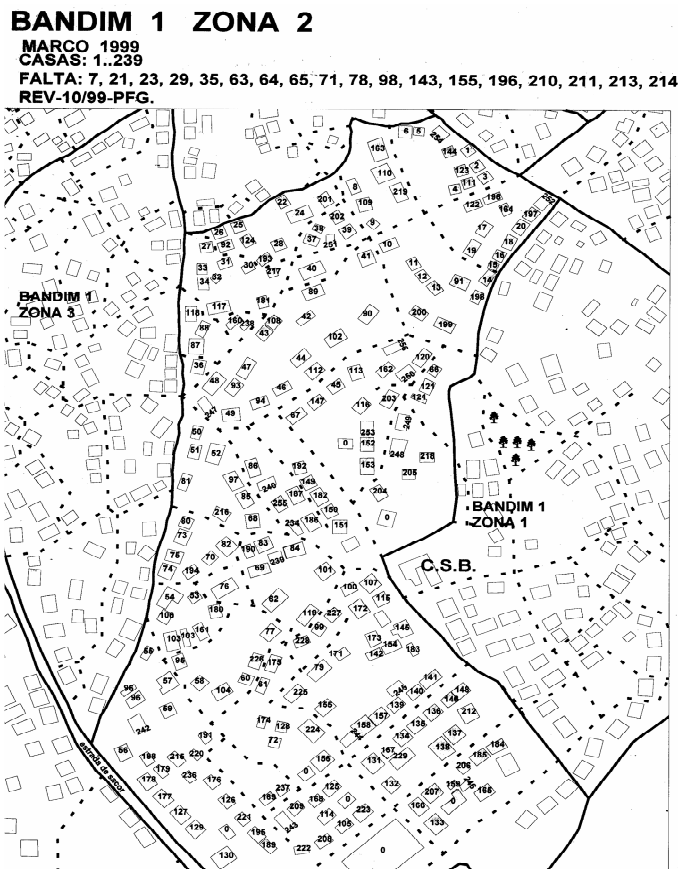


Figure 2:

A map of zona 2 in Bandim I, one of the areas in Bandim. The map was drawn by the Bandim Health Project using the soft-ware MapInfo™. Such maps are used by the health workers during follow-up to locate the houses of the children included in the study.

The length of follow-up

WHO has developed protocols for *in vivo* evaluation of the efficacy of antimalarial drugs which includes recommendations for 14-day and 28-day follow-up periods. In the 1996 protocol there were even suggestions for adaptations to a 7-day protocol (255). The 14-day protocol is intended for areas with intense malaria transmission. If longer follow-up is used, the most recent protocol suggests PCR assessment to distinguish between reinfections and recrudescence. In areas of low to moderate transmission, a 28-day follow-up is generally recommended (257).

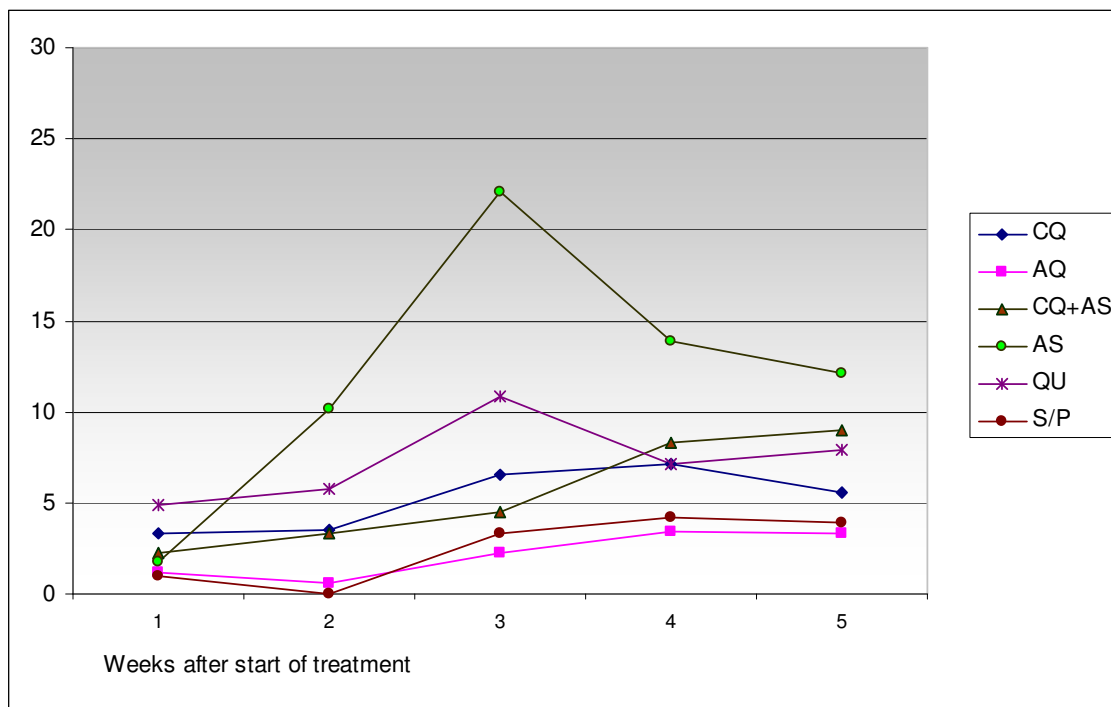
It is nonetheless questionable if the short follow-up periods are sufficient for evaluation of the effect of antimalarials even in areas with high transmission. In Uganda it was found that limiting follow-up to the 14-day WHO protocol would seriously have underestimated the risk of treatment failure, as most were found to occur between day 15 and day 28 after treatment with S/P combined with either chloroquine or amodiaquine (11). In a study from Zanzibar, in which the outcome of treatment with artesunate plus amodiaquine was compared to that of artemether-lumefantrine, 28% and 58% respectively for the two treatment schedules, of the total failures had been missed by a follow-up period of 28 days, prompting the authors to recommend at least 42 day follow-ups (119).

In study I, we followed the children until day 70. Pooling the data from the four groups revealed that the weekly risk of having recurrent parasitaemia gradually decreased from day 35 to day 70. This indicates that re-appearing occurs as long as until day 70. This could be due to the fact that most children treated with the ineffective schedules (quinine for three days and quinine for three days followed by chloroquine 25 mg/kg) got parasitaemia at an early stage and therefore weighed less at the end of the pooled analyses the longer the follow-up. However, only looking at the groups treated with S/P and chloroquine 50 mg/kg did not change the outcome.

Reappearance of parasitaemia occurs earlier following treatment with rapidly eliminated drugs than following treatment with antimalarials with a longer half life (244). As soon as the drug levels become sub-therapeutic surviving parasites are allowed to multiply and the patient

to be reinfected. When pooling the data from all our studies (Figure 3), the slowly eliminated drugs, S/P, chloroquine and amodiaquine, have the highest weekly risk of recurrence of parasitaemia around day 28 and day 35 after initiation of treatment.

Figure 3:



The weekly risk of getting parasitaemia during follow-up for children treated with quinine (QU) (study I plus 94-96), chloroquine (CQ) (studies I, II, III and IV), sulfadoxine-pyrimethamine (S/P) (studies I and V), amodiaquine (AQ) (study IV), chloroquine plus artesunate (CQ + AS) (study III) and artesunate monotherapy (AS) (study III).

Presumably, recrudescence occurs even after day 35 as indicated by the results found during the longer follow-up period in study I. The antimalarials with the shorter drug elimination time (artemisinin and quinine) have the highest weekly risk of recurrent parasitaemia two weeks after termination of treatment. The weekly risk of recurrent parasitaemia for the children treated with artesunate and chloroquine in combination being highest at a very late stage could be due to the fact that artesunate is very efficient in reducing the number of parasites, therefore requiring more parasite life cycles before surviving parasites reach a number above the detection level. Having had follow-up periods of only 2 weeks or even 4 weeks would have underestimated the failure rate considerably. For the treatment schedules

having the highest risk of recurrent parasitaemias very late (as e.g. artesunate combined with chloroquine), even 5 weeks of follow-up probably underestimates the real percentage of patients having recrudescence.

In the latest WHO protocols for *in vivo* drug evaluation, LPF has been defined as parasitaemia without fever after day 7 (257). This is an important category, as it has been shown that most recrudescence parasitaemias, which initially might be asymptomatic, eventually develop into clinical malaria (220). We defined LCF slightly different from WHO, as we included children with a positive malaria film with more than 20 parasites per 200 leucocytes during follow-up as treatment failures. This was done from previous experience: the vast majority of children with parasitaemia at this level developed clinical malaria, often before the next weekly visit. We therefore considered it unethical not to treat these children. During follow-up, approximately 75% of children with recurrent parasitaemia developed clinical malaria before or on day 35.

Only in study IV was PCR performed to distinguish between reinfections and recrudescence. We found that approximately 80 % of children with re-appearing parasitaemia had recrudescence, leaving only 20 % with reinfections, which would give a reinfection rate of approximately 1% per week. All the included children were, however, treated with long acting antimalarials, so a certain level of protection against new infections can be expected up to the end of follow-up. Therefore, the weekly risk of being reinfected for a child cannot be directly calculated from these results. Looking at the risk of getting re-appearing parasitaemia 4 to 5 weeks after treatment with an efficient dose of the rapidly eliminated antimalarial quinine, a higher rate of reinfections would be expected, probably in the order of 2 - 4% per week (94-96).

With the longer follow-up periods which are obviously needed, especially when evaluating the combination therapies with artemisinin, PCR adjusted cure rates could be an advantage when the level of resistance to a drug or drug combination is the main aim of the study. Still, the outcome of interest from the parent's point of view is whether the child will be cured and for

how long he/she remains healthy, so the most useful comparison of different treatments might after all be based on treatment outcome results unadjusted by genotyping (260).

We choose 5 weeks of follow-up and in study I even 10 weeks. We observed considerable differences between the treatment failure rates after 14 days, indicating that a significant number of recrudescence occur after this time. The failure rates would thus be greatly underestimated if we had used the shorter follow-up schedules. As we followed the children for a longer period of time than recommended and thus increased the number of blood samples taken, we decided not to include a malaria film on day three as recommended by WHO, unless the child had symptoms.

Forms and questionnaires

To the extent possible, we kept the forms and the questionnaires as alike as possible, primarily to facilitate the training and supervision of the staff when initiating a new study, but also to maximise the possibilities for comparing the results from one study to another. Forms for planning the medication and the follow-up were essential for the timing of the work. A special questionnaire was filled in at each follow-up visit.

When a child was included in the study, he/she received a card stating the period during which the child would be treated free of charge at the CSB when seeking medical attention for any reason. Showing this card prompted the completion of a special form providing information of the recent medical history, of the clinical condition of the child, and ensuring that a malaria film was examined and the result registered. The forms and questionnaires were important instruments in the supervision of the health personal involved in the studies. Furthermore, they were essential in the control of the data collected.

Laboratory facilities

Malaria films were examined at the CSB using a light microscope. The quality of the laboratory material used in the studies was ensured through the PSB. The laboratory technician working at the studies had a long experience reading malaria films using a light

microscope. In the studies I, III, and IV malaria films were re-examined at the LNSP using microscopes with light bulb, to ensure the quality of the readings performed at the CSB.

In studies II, IV, VI, and the first part of study V a capillary blood sample was collected for analyses of drug concentrations. This was performed in Sweden as described (51,108,109). In study IV, we performed PCR analyses on blood samples drawn on inclusion and at the time of treatment failures from children with LCF or LPF.

Ethical issues

The rationale of the studies included in the present thesis was to test antimalarial treatments already used in Bissau and to evaluate possible changes in dosing in order to give recommendations for improved treatment schedules. This was evaluated against the recommended treatment given by the National Malaria Programme. In study IV we discussed the ethics of including a group treated with chloroquine 25 mg/kg knowing that the failure rate would probably be high. Still, we decided to do so for three reasons: 1) the failure rates had decreased from 1995/1996 (study II) to 2000/2001 (study III); 2) chloroquine 25 mg/kg remained the recommended treatment for uncomplicated malaria in the country; and 3) to evaluate the efficacy of an elevated dose of chloroquine it would be important to compare the cure rates to that of the standard dose. In order not to continue a study or a treatment arm with a drug proving to be highly inefficient, treatment with any of the study drugs had to be terminated if more than 50% of at least 40 children enrolled in the study arm had treatment failure during follow-up. These rules came into use in study III.

All the studies were approved in Guinea-Bissau, initially by the Ministry of Health, later by the National Ethical Committee. Several studies were initiated following verbal permission while we only received written permission at a later stage. The studies were approved by the Central Ethical Committee in Denmark, and were approved or accepted by the Ethical Committee at Karolinska Institute in Stockholm.

In all the studies, the caretaker, most often the mother, was informed of the study by one of the project nurses. Doing so, the nurses followed written directions in order to standardise the

information. In studies I, III and IV, the mothers were offered the information in writing in Creole. Very few wanted that. Due to the high rate of illiteracy, the nurse providing information also signed a document stating that the information had been provided, the name and the relation to the child of the person having received the information and the name of the person giving the consent for participation in the study. Few mothers refrained from participating; one reason could have been the prospect to receive all treatments free of charge. When asked, they instead insisted that malaria was a serious condition, and that they wanted to participate if that could help us fight the disease. Withdrawal of children during treatment or follow-up happened by request of the father or a grandmother, indicating that the mothers in fact did want to help solve a problem they themselves considered severe.

Drawing blood from children should always be justified. Also, the higher the number of blood samples, the higher the risk of withdrawal from the study. For every study, we therefore weighed the scientific benefit of a certain result against the inconvenience for the child. In that process our Guinean colleagues were conservative wanting to minimize the number of samples. In some studies it can be argued that it would have been valuable to have more information, especially on the parasite-clearance rate. However, in a clinical study the rate of treatment failures is more important than the time it takes to clear the parasites from the blood.

Statistical analyses

In studies I and II, children not completing the treatment or during follow-up receiving treatment with an anti-malarial drug by third part or by self-treatment were considered withdrawals and therefore excluded in accordance with the WHO protocols. In studies III and IV the health worker visited the house with the medication, in case the child did not return to the health centre. In all the studies, children were excluded if their house could not be found by the health worker (considered not to live in Bandim and thus a violation of the inclusion criteria).

In studies I to V, the cumulative outcomes were calculated using the log rank test with the corresponding Mantel-Haenszel relative risk estimates, which is in accordance with the latest

recommendations from WHO (256,257). This method allows for inclusion of data from patients who are withdrawn or lost to follow-up without requiring that assumptions be made about unknown outcomes. As the history of medication was considered unreliable, when children were not seen for more than two weeks during follow-up, they were excluded for further follow-up, but included in the calculation until the last day of being seen (censored in the beginning of the time interval).

The WHO recommendations for reporting outcome of efficacy studies are valid for treatment schedules of no longer than three days and consisting of one daily dose only. We did not follow the WHO protocols in details. Admissions during treatment have not been handled in exactly the same way in all the studies. These differences in the reporting of data make direct comparisons imprecise. All results were therefore recalculated (Table 3 to 6) in accordance with the latest WHO recommendations (256).

RESULTS AND DISCUSSION

Description of the children included

The studies were performed over a period of ten years. The methodology was to a great extent the same for all the efficacy studies. The outcome of the treatments has, however, been presented slightly different. To be able to compare the cure rates for the different studies in the present thesis, the results have been recalculated in the following way (the criteria are slightly modified based on the guidelines of the WHO protocols for efficacy studies):

We excluded the following children: Children admitted to hospital on the day of inclusion (too ill to be included), children who did not complete treatment for a reason not related to the treatment given (withdrawal of consent), children who could not be found on the first day of follow-up (assumed not to live in Bandim), and children treated by a third person or by self treatment during follow-up without confirmed parasitaemia (withdrawal of consent).

1. Early treatment failure (ETF): a) development of danger signs on day 1, day 2, or day 3 in the presence of parasitaemia, b) parasitaemia on day 2 higher than day 0 (when available), c) children admitted to hospital or dying before day 7.
2. Late clinical failure (TCF) (only if not meeting the criteria of early treatment failure):
 - 1) fever and a positive malaria film, 2) a history of fever and clinical symptoms of malaria as well as a positive malaria film, 3) a malaria smear containing 20 or more parasites per 200 leukocytes during follow-up, 4) admitted to hospital or dying after day 7.
3. Late parasitological failure (LPF): reappearing parasitaemia on day 7 or later for children without ETF or LCF.
4. Adequate clinical and parasitological response (ACPR): absence of parasitaemia without the child meeting any of the criteria of ETF, LCF or LPF.

The results are calculated by survival analysis and given as cumulative ACPR rates until day 35. For all studies the ETF rate is given. For all but study I, the LCF and the LPF rates at day

35 are given. As the studies were performed in the same area, to a large extent with the same staff and using the same inclusion criteria, the results given in the tables can be used for comparison over time of the efficacy of the treatments.

Table 1: Description of the children included in the efficacy studies:

Study	Study group	Male	Female	Age	Weight	Parasites
I	QU 15mg x 2 x 3 days	58	56	3.5 (6/12 – 14.0)	14.0 (7.7 – 36.1)	890
	QU followed by CQ*	48	59	3.8 (8/12 – 13.0)	14.0 (8.1 – 14.0)	970
	CQ 50 mg/kg	59	50	4.0 (5/12 – 17.0)	14.8 (7.8 – 41.8)	670
	S/P	41	54	4.8 (7/12 – 15.0)	14.8 (8.0 – 49.6)	890
II	CQ 25 mg/kg	32	40	3.9 (0.4 – 13.0)	14.3 (7.6 – 39.5)	325
	CQ 50 mg/kg	30	38	3.2 (0.8 – 13.0)	14.1 (6.3 – 64.0)	456
III	Artesunate 8 mg/kg	63	52	5.4 (1.3 – 15.5)	17.0 (9.8 – 49.6)	430
	CQ 25 mg/kg	56	63	5.4 (1.2 – 15.0)	16.3 (10.0 – 47.4)	415
	AS followed by CQ**	53	67	5.8 (0.5 – 15.0)	17.1 (9.5 – 55.0)	470
	AS plus CQ***	58	61	5.2 (1.3 – 14.0)	16.0 (10.0 – 44.0)	480
IV	CQ 50 mg/kg	100	84	5.0 (0.2 – 14.3)	16.2 (5.1 – 43.0)	350
	Amodiaquine 15 mg/kg	91	90	5.3 (0.3 – 14.6)	16.5 (6.0 – 47.2)	360
	Amodiaquine 30 mg/kg	91	91	5.9 (0.3 – 14.7)	17.1 (5.6 – 48.3)	360
	CQ 25 mg/kg	91	91	4.9 (0.4 – 14.8)	16.2 (5.3 – 47.0)	360

* : Quinine 10 mg/kg twice a day for 3 days followed by chloroquine in a total dose of 25 mg/kg.

** : Artesunate 8 mg/kg given for 3 days followed by chloroquine in a total dose of 25 mg/kg.

***: Artesunate 8 mg/kg and chloroquine 25 mg/kg given concomitantly.

Table 1 presents the age, weight and parasite counts on inclusion of the children included in the different studies. Due to the randomization procedures these parameters for children from the different treatment groups within the studies are very similar. Children exposed to *P. falciparum* gradually become immune to malaria, even if they still develop clinical disease (78). Therefore, the response to treatment would be expected to be more favourable in older children. Pooling data from all the efficacy studies we found children aged 5 years or less to have a higher failure rate than older children (Table 2).

The parasite density at which fever develops was found to decrease with increasing age (175,201). In line with this, we found that a higher percentage of older children had a parasite count of less than 500 parasites per 200 leukocytes (0 to 35 months: 62% (316/508), 36 to 71 months: 57% (322/566), and over 71 months: 69% (501/730), the RR for children above 5 years of age to have less than 500 parasites/200 leucocytes: 0.92 (0.84 – 1.00), $p = 0.06$).

Table 2: Treatment failure during follow-up by age groups:

Age in months	Day 28 of follow-up		Day 35 of follow-up	
	Percentage ^a	Relative risk ^b	Percentage ^a	Relative risk ^b
0 – 35	27% (110/401)		33% (129/387)	
36 – 71	20% (90/455)	0.72 (0.56–0.92)**	27% (120/421)	0.82 (0.67-1.01)*
≥ 72	17% (99/598)	0.60 (0.47–0.77)**	23% (130/565)	0.69 (0.56-0.85)**

a) Percentage of children with treatment failure during follow-up (late clinical failure or late parasitological failure). The number of children with treatment failure/total number of children is given in parenthesis (children lost to follow-up not included).

b) Cumulative relative risk of having treatment failure for children 36 to 71 months old and children aged 72 months or more in relation to the children less than 36 months of age (Mantel-Haenszel relative risk estimate, 95% confidence interval in brackets). [The cumulative relative risk of treatment failure for children aged 72 months or more vs. children between 36 months and 71 months (=ref.), were on day 28: 0.80 (0.58 – 1.12)* and on day 35: 0.79 (0.59 – 1.06)*, respectively].

*: $p > 0.05$, **: $p < 0.05$.

The immunity of the child and the parasite density at the start of the treatment influences the outcome of the treatment given (244). The higher the parasite count, the higher the risk of recrudescence. We found a treatment failure rate (LCF and LPF) on day 28 of 17% (163/977) and 25% (126/510) for children with an initial parasite count of less than 500 parasites/200 leukocytes vs. children with a parasite count of 500 or more. On day 35 the percentages were 22% (208/939) and 32% (161/497), respectively.

Efficacy of chloroquine as an antimalarial drug in Guinea-Bissau

In spite of reports of increasing resistance, chloroquine in a total dose of 25 mg/kg bodyweight given over three days was still recommended by the national malaria programme in Guinea-Bissau as the first line therapy for treating uncomplicated malaria during the time of our studies. In other African countries, where chloroquine has been abandoned as the recommended treatment, the drug is still readily available from the private market (61).

Since chloroquine is continuously used in Guinea-Bissau, we have monitored the efficacy of this treatment regularly since 1995, both in the dose recommended by the malaria programme and in a higher dose. In many health centres, chloroquine is often prescribed in doses exceeding the 25 mg base/kg recommended by WHO (L.Rombo, unpublished observation), a practice which has also been reported from other African countries (194).

The efficacy of chloroquine monotherapy

During the past 20 years, a steady increase in failure rates following treatment with the standard dose of chloroquine has been reported (135). In sub-Saharan Africa, Baird found that the risk of parasitological treatment failure was almost uniformly greater than 40%, whereas the risk of clinical treatment failure tended to be higher in eastern and central parts of Africa than in West Africa, typically more than 30% vs. less than 20% (10). However, studies in West Africa have also demonstrated treatment failure rates at day 14 as high as 21% in Nigeria, 61% to 78% in Sierra Leone, 74% in Liberia, and 53% in Ghana (31,33,142,208); and on day 28 of 73% in the Gambia (213), and 31% in Nigeria (71). In Equatorial Guinea, the day 14 failure rate remained constant at a level between 40% and 55% from 1992 till 1999 in spite of chloroquine being the first line treatment in the whole period (174).

In Bissau we found considerably lower treatment failure rates treating with chloroquine 25 mg/kg than reported in most of the above studies. The highest rate was found in study V in 1995 to 1996, with a total treatment failure rate of 32% on day 28. The majority of these children had LCF (Table 3).

Table 3: Outcome following treatment with chloroquine from all studies:

	Study II (1995 – 1996)		Study I (1996 – 1999)			Study III (2000 – 2001)	Study IV (2001 – 2004)	
	25 mg*	50 mg**	50 mg**	15 mg x 2 #	Qu + CQ##	25 mg*	25 mg*	50 mg**
ETF	2 % (1/63)	2 % (1/62)	0 % (89/89)	3 % (3/97)	1 % (1/88)	0 % (0/116)	2 % (4/165)	1 % (1/164)
ACPR - D7	81 % (52/63)	98 % (61/62)	98 % (87/89)	96 % (93/97)	99 % (87/88)	99 % (115/116)	98 % (161/165)	98 % (161/164)
ACPR - d14	76 % (57/60)	98 % (57/57)	97 % (85/86)	88 % (83/90)	97 % (83/85)	92 % (105/113)	94 % (150/158)	97 % (157/159)
ACPR - d21	68 % (41/46)	93 % (53/56)	90 % (72/77)	73 % (62/75)	88 % (72/79)	85 % (107/105)	86 % (136/149)	94 % (147/151)
ACPR - d28	68 % (40/40)	86 % (48/52)	85 % (58/62)	68 % (54/58)	76 % (57/66)	79 % (86/93)	76 % (117/132)	90 % (134/141)
ACPR - d35	64 % (38/40)	81 % (45/48)	79 % (54/58)	52 % (40/52)	63 % (45/54)	71 % (71/79)	72 % (105/111)	88 % (127/130)
LCF- d35	32 %	16 %				21 %	18 %	8 %
LPF- d35	2 %	1 %				8 %	8 %	3 %

ETF and cumulative ACPR, and the cumulative LCF and LPF at day 35 from the different studies calculated by evaluability analyses. We excluded children admitted to hospital on the day of inclusion, children who did not complete treatment, children who could not be found on the first day of follow-up, and children treated by a third person or by self-treatment during follow-up without confirmed parasitaemia.

* : Treating with a total dose of 25 mg chloroquine phosphate per kg bodyweight given in one daily dose for 3 days.

** : Treating with a total dose of 50 mg chloroquine phosphate per kg divided into two daily doses for 3 days.

: Treating with quinine 15 mg/kg twice a day for 7 days.

: Treating with quinine 10 mg/kg twice a day for 3 days followed by chloroquine in a total dose of 25 mg/kg.

In contrast to the poor efficacy with chloroquine in the recommended total dose of 25 mg/kg, much better cure rates were obtained treating with the higher total dose of 50 mg/kg divided

into 2 daily doses for 3 days (Figure 4). On day 35, ACPR rates of 81%, 79% and 88% were found, and only between 16% and 8% of the included children experienced LCF (Table 3). Other studies have examined higher doses of chloroquine *in vivo* with varying results. In Gabon 35 mg/kg was found to increase the efficacy of treatment, and the authors conclude that “higher dose chloroquine might be a useful treatment for children in developing countries where partial immunity is likely” (247). In Burundi 50 mg/kg turned out to be better than 35 mg/kg and 40 mg/kg (34). However, other studies have found failure rates on day 14 of 67% (194), and on day 28 of 48% (38) and 68% (126) following treatment with 50 mg chloroquine per kg.

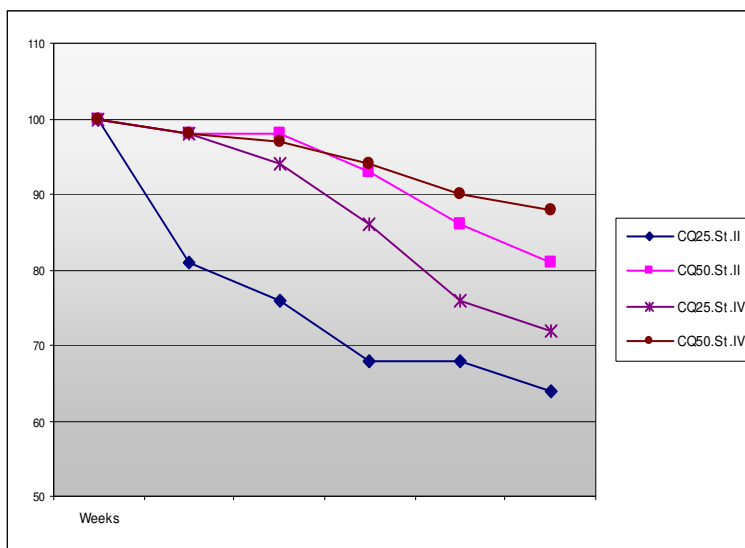


Figure 4:
The ACPR rates following treatment with chloroquine 25 mg/kg and 50 mg/kg in 1996/1997 (CQ25.St II and CQ50.St II) and in 2001/2004 (CQ25.St IV and CQ.St IV), respectively.

Schapiro discussed the optimal dosage of chloroquine (187). In 1985, good clinical and parasitological efficacy of chloroquine in a total dose of 10 mg/kg was documented in Togo. As resistance spread a few years later, this was overcome by increasing the dose to 25 mg/kg. In spite of 90 % of infections being *in vitro* resistant to chloroquine a mortality of nil in a population of 900 people followed over one year was found in Indonesia despite the fact that chloroquine was the only antimalarial available in the area. According to Schapiro the low mortality in spite of the chloroquine-resistant malaria was possibly related to the frequent use of high dose of chloroquine.

The results from the studies with chloroquine monotherapy performed in Bissau are interesting as the level of *in vivo* resistance has remained constant, or maybe even diminished, during the study period from 1995 to 2004 (Figure 4), and as treatment with chloroquine 50 mg/kg ensures a good cure rate in the study population.

It seems as if chloroquine resistance spread more rapidly in East Africa than in West Africa. Factors such as intensity of transmission, population immunity and population movements could be considered when explaining this difference (36,153). It has been suggested that the evolution of chloroquine resistance reaches a minimum at values of childhood prevalence of parasitaemia of around 60% to 80% (215), which corresponds to the prevalence found in Guinea-Bissau of 44% to 79% (85). This could thus in part explain the lack of increase in chloroquine resistance found.

It has been postulated that the evolution of drug resistance is a two-stage process, in which mutations encoding for drug tolerance precede those encoding for resistance, and that the evolution of tolerance is determined by the level of drug used in the community, whereas the evolution of resistance is a much more complex process (74). In the first phase, treatment is still clinically successful in spite of the parasites having only intermediate susceptibility to the drug, whereas in the next step resistant parasites are rapidly selected resulting in widespread clinical failures (76). However, the emergence of resistance can be slowed down by decreasing the use of drugs in the community (74). Furthermore, chloroquine-resistant parasites may lose their survival advantage by removal of the drug pressure (235). This was observed in Gabon where resistance to chloroquine declined from 1992 to 1998 following a change in the recommendation for treatment of malaria (191). In Malawi S/P replaced chloroquine as first line antimalarial drug in 1993, after which a marked decline in the prevalence of parasites containing the *pfcr76T* allele was reported (102), and an *in vivo* test found a parasitological treatment failure rate of 9% as compared to 58% before the change of policy (128). In Mali, the discontinuation of chloroquine prophylaxis for children below 9 years of age led to a decrease in parasitological resistance from 80% to 45% (185).

From June 1998 to May 1999 Guinea-Bissau experienced a civil war with great impact on the health system, probably also on the availability and consumption of chloroquine. This might partly explain why the resistance *in vivo* to chloroquine 25 mg/kg decreased, resulting in an increase of the ACPR rate on day 35 from 64% before the war to 71% and 72% after the war (study II, vs. studies III, and IV). The findings from the *in vivo* studies corroborate findings from *in vitro* studies performed for more than 10 years in an area just outside Bissau, in which the level of resistance has remained unchanged during the whole study period (Ursing, in preparation), and from genetic studies in Bissau which do not indicate a major selection of mutations coding for chloroquine resistance (Ursing, in preparation).

Studies evaluating the treatment with chloroquine 50 mg/kg have shown good cure rates. Similar to the studies using the standard dose, the level of *in vivo* resistance to the higher dose also declined from before the war till after the war (Figure 4) with ACPR rates on day 14 and day 35 of 98% and 81%, respectively in study II vs. 97% and 88% in study IV. This is well on the safe side of the 10% treatment failure rate on day 14 suggested by Björkman and Bhattarai as the level calling for a change in treatment policy (19).

The results of our *in vivo* studies corroborate a model for chloroquine chemotherapy which shows that increasing the dose of chloroquine will increase the efficacy of the treatment considerably, even if the effect might be even higher when spacing the dose by 10 days (82). The model predicts that doubling the dose of chloroquine administered is sufficient to give 7 orders of magnitude reduction in parasitaemia (82), a result consistent with the fact that doubling the dose of chloroquine to a patient with resistant parasites can be sufficient for radical cure (165). A recent study from Ghana found a higher success with chloroquine therapy in children who had been treated with chloroquine before admission to hospital (166), an indication of better cure rates with higher blood concentrations of the drug.

Quinine followed by chloroquine

Children diagnosed with malaria at a health centre in Guinea-Bissau will most likely be treated with Quinimax™ intramuscularly if vomiting. Likewise, the treatment of the majority of children admitted to a hospital with the clinical diagnosis of malaria will be initiated with

Quinimax™. When the children improve after a few days, the treatment with quinine/Quinimax™ will be terminated and followed by a full course of chloroquine, which is 25 mg/kg given over 3 days.

Treating adult Thais suffering from falciparum malaria with quinine and chloroquine concomitantly did not improve the clinical response. We are not aware of any study evaluating the effect of the two drugs given in sequence as is done routinely in Guinea-Bissau as well as in other West African countries. We therefore included a group being treated with quinine 10 mg/kg twice a day for three days followed by chloroquine for 3 days in a total dose of 25 mg base (study I). Low ACPR rates were found on day 28 and day 35 (Table 3), though better than previously seen for quinine mono-therapy with the same dose (96).

Chloroquine combined with artesunate

Artemisinin derivatives eliminate circulating parasites very rapidly with an estimated parasite reduction rate of 10^3 to 10^5 parasites per dose and per parasite cycle (244), but due to their very short half lives they have to be administered over at least 7 days if used as monotherapy (138,244), and even then a high rate of recrudescence is observed (56).

The introduction of ACT has been strongly advocated (9,100). Theoretically, the rapid reduction in the parasite biomass when treating with artemisinin derivatives protects the partner drug in a combination therapy against development of resistance, as one of the most important factors in this process is the number of parasites exposed to drug concentrations within the “selective concentration limits”. Thus, an important reason for introducing combination therapy would be to diminish the risk of development of resistance (229,242). Before an artemisinin combination therapy can be introduced, a partner drug has to be selected. A meta-analysis in 2004 found that addition of artemisinin to other antimalarial drugs improved efficacy, but not all combinations were equally efficient (4) and it is therefore not clear whether ACT is superior to other combination therapies in sub-Saharan Africa (45).

As chloroquine is cheap and well known, this would be an attractive candidate as partner drug for an artemisinin combination therapy, and the use of such a combination has been suggested

for treatment of malaria in Africa (21), especially in areas where the parasite is still sensitive to chloroquine (137). However, in vitro studies have shown that chloroquine is markedly antagonistic to artemisinin derivatives both in chloroquine-sensitive and chloroquine-resistant *P. falciparum* parasites (30,68,210).

Table 4: Outcome of treatment with chloroquine and artesunate in study III:

	Artesunate 8 mg/kg	Artesunate and chloroquine concomitantly	Artesunate and chloroquine in sequence	Chloroquine 25 mg/kg
ETF	0% (0/111)	0% (0/111)	2 % (2/116)	0 % (0/116)
ACPR-d7	98 % (109/111)	98 % (109/111)	97 % (113/116)	99 % (115/116)
ACPR-d14	88 % (97/108)	94 % (95/99)	95 % (109/112)	92 % (105/113)
ACPR-d21	79 % (74/95)	89 % (86/91)	91 % (103/107)	85 % (107/105)
ACPR-d28	59 % (62/72)	81 % (74/81)	84 % (91/99)	79 % (86/93)
ACPR-d35	52 % (51/58)	74 % (62/68)	76 % (79/87)	71 % (71/79)
LTF-d35	39 %	17 %	17 %	21 %
LPF-d35	9 %	9 %	5 %	8 %

ETF and cumulative ACPR, and the cumulative LCF and LPF at day 35 calculated by evaluability analyses. We excluded children admitted to hospital on the day of inclusion, children who did not complete treatment, children who could not be found on the first day of follow-up, and children treated by a third person or by self medication during follow-up without confirmed parasitaemia.

We examined the efficacy of artesunate combined with chloroquine both given simultaneously and in sequence (study III). As artesunate is rapidly eliminated we would avoid any possible antagonistic effect between the two drugs by administering them in sequence in contrast to the simultaneous administration. As seen in other studies (244), we found a rapid reduction in the parasite count following treatment with artesunate. The initial eradication of the major part of the parasite load would be assumed to have made the subsequent treatment with chloroquine more efficient. The percentages of children with treatment failure during follow-up were still as high in the two groups treated with the combination therapy as in the group treated with chloroquine alone (Figure 5 and Table 4).

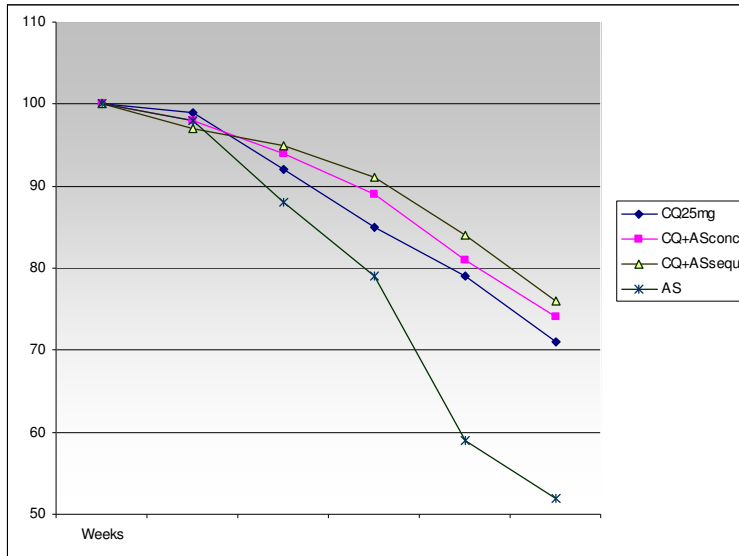


Figure 5:

The ACPR rates following treatment with chloroquine 25 mg/kg (CQ25mg), chloroquine and artesunate concomitantly (CQ+ASconc), or in sequence (CQ+AS sequ), and artesunate monotherapy (AS).

In the Gambia, a reduction in early treatment failure rates was found when testing this combination, but the overall results after 28 days of follow-up were discouraging, prompting the authors to conclude that “one highly effective antimalarial (artesunate) plus one relatively ineffective antimalaria (chloroquine) equals a poor combination” (213). In Burkina Faso, the addition of artesunate to chloroquine increased the efficacy, but probably due to the high background resistance to chloroquine, the treatment failure rate at day 28 was 51 %. In the Ivory Coast and São Tome and Principe similar results have been found (4). Another reason for combining artemisinin with chloroquine could be to reduce post-treatment prevalence and density of gametocytes, and the duration of gametocyte carriage. In the Gambia, it has been shown that treatment with artesunate plus chloroquine for three days gave a transient reduction in parasite transmissibility, but that the combination only delayed, rather than prevented, the emergence of mature gametocytes, as compared to treatment with chloroquine alone (213). So neither our study, nor the results of studies from other African countries support the suggestion of introducing an artemisinin combination therapy including chloroquine, when resistance has already been established.

As artesunate is already available on the market, and the recommended 5 to 7-day treatment courses as monotherapy are unlikely to be complied with, we found it of interest to evaluate the effect of a 3-day course, which is more likely to be used. Failure rates of 41% at day 28 were reported, which compares with results from Thailand and from a study performed in

Gabon (22). However, a study from Burkina Faso including 27 children in a group treated with artesunate for three days did not observe any children with reappearing parasitaemia during 28 days of follow-up (13).

Conclusion of the chloroquine studies

It has been confirmed both from our *in-vivo* studies and from *in-vitro* studies conducted over the same time period that resistance to chloroquine exists in Guinea-Bissau. There is an indication that the level of the resistance *in vivo* diminished following a civil war in the country from 1998 to 1999. However, the level of treatment failures is still too high to recommend chloroquine 25 mg/kg as first line treatment. The treatment with chloroquine 50 mg/kg gave acceptable cure rates.

Giving higher doses of chloroquine will prolong the time that subtherapeutical concentrations of the drug remain in the blood. There is therefore a concern that the level of resistance might increase with higher doses. However, the decrease of *in vivo* failure rates following the war together with the experience from Malawi, Gabon and Mali indicate that mutations associated with drug resistance might to some extent be disadvantageous for the parasites, thus reducing their viability. This can be explained by metabolic resources being diverted to counteract the drug rather than to be used for anabolism and reproduction, hence extending the use of chloroquine might pose no danger for further or total loss of drug response (58).

Another mechanism which might reduce the risk of further development of resistance in spite of increasing the dose of chloroquine is that one of the most important factors determining selection of resistance is the number of parasites exposed to the drug levels which select for resistance, i.e. concentrations between 20% and 80% of the maximal inhibitory concentration (199). Treating with the higher dose will reduce the biomass of the parasite significantly more than treating with a lower dose, thus minimizing the number of parasites exposed to the drug in concentrations within the “selective concentration limits” (199). Increasing the dose of chloroquine from 25 mg/kg to 50 mg/kg would therefore not be expected to accelerate the development of resistance.

As treating with chloroquine in a total dose of 50 mg/kg divided into 2 daily doses for 3 days does not increase the risk of serious adverse effects and as it is very unlikely that such a change in policy will negatively affect the development of resistance to the drug, treatment with chloroquine 50 mg/kg should be seriously considered for first line therapy. Other West-African countries with a moderate level of resistance to chloroquine should also consider adopting this guideline – at least as an interim strategy.

Instead of changing the treatment from quinine to chloroquine when a child is discharged from hospital, or the child for other reasons has been treated with quinine initially, the treatment could be completed with quinine for the full seven days. If adherence is suspected to be a problem this could be overcome by prescribing one daily, oral dose of 15 mg/kg (94). Therefore, quinine should continuously be recommended as the third line treatment for malaria in Guinea-Bissau.

ACT is being strongly advocated (7,9,21,89). A possibility in Guinea-Bissau could therefore be to change to a chloroquine-based combination therapy. However, our study confirmed the results from studies in other African countries that with the present level of resistance to chloroquine this is not an option, as it is not sufficiently efficacious, and as evidence exists which suggests that use of the combination would do little to reduce the transmission of chloroquine resistant parasites (232). Also in Africa, monotherapy for 3 days with artesunate is insufficient to eliminate the parasites and should therefore strongly be discouraged.

The usefulness of sulfadoxine-pyrimethamine

Until 1996, sulfadoxine-pyrimethamine (S/P) was rarely used in Guinea-Bissau. By then the National Malaria Programme introduced the drug as the recommended second line treatment for malaria, i.e. for “cases of non-complicated malaria in whom the first line treatment did not work” (164). The dosage recommended was one tablet for each 20 kg body weight. At the time of introduction no studies existed on the efficacy of S/P either as initial treatment or as therapy for treatment failures following the first line drug.

Sulfadoxine-pyrimethamine for initial treatment

In study I the efficacy of S/P as first line treatment was compared to that of chloroquine 50 mg/kg, which previously had shown good therapeutic results (study II). In East Africa, S/P has become the first line drug in several countries either as monotherapy or as one of the components in a combination strategy. A report from Malawi 10 years after S/P replaced chloroquine as first line treatment claimed that S/P had retained good efficacy (162). This conclusion was, however, challenged, as an adequate clinical response was only seen in 73% at the start of the period and in 60% ten years later, the parasitological cure rates being even lower (172,240).

The fact that S/P has failed in many parts of East Africa is documented by clinical as well as genetic studies. The *East African Network for Monitoring Antimalarial Treatment* has estimated that 6% of the surveyed sites had a 14-day clinical treatment failure rate of more than 25% before 2000, and that this increased to 22% of the sites post-2000 (220). Similar results have been reported from other studies in Uganda (32,209,216), Kenya (228), and Mozambique (2).

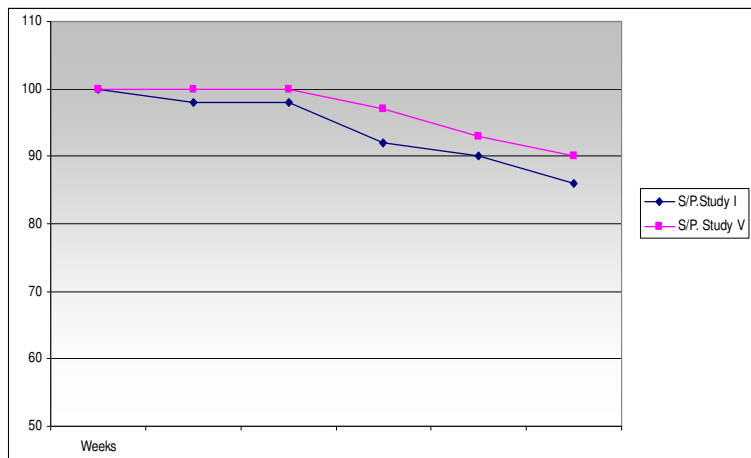


Figure 6:
The ACPR rates following treatment with S/P as initial treatment (S/P.Study I) and as second line therapy (S/P.StudyV).

Resistance to S/P was reported in Senegal in a non-immune tourist as early as 1989 (23). In Liberia and Sierra Leone failure rates over 40% have been found on day 28 in clinical studies (31,33). Thus, resistance exists in West Africa as well. However, very low day 14 clinical and

parasitological treatment failures, comparable to what was found in Bissau, have been detected in Burkina Faso (130,223), and in Ghana (43).

Sulfadoxine-pyrimethamine as second line treatment

Very few studies have evaluated the efficacy of retreatment with S/P even if it is being recommended as second line treatment in several countries. We evaluated the usefulness of S/P for treatment failures following therapy with quinine or chloroquine just before the National Malaria Programme introduced the regimen and again after approximately seven years (study V). We found an excellent efficacy both in the first and in the second part of the study, with ACPR rates of 100% on day 14 and 94% and 91% on day 28 (Figure 6). There is therefore no indication of a decline in the *in vivo* efficacy in spite of the higher utilization of the drug. This corroborates results from Equatorial Guinea where the efficacy of S/P used as second line treatment remained unchanged from 1992 to 1999 (174).

Other studies have recommended S/P as second line treatment. In the Gambia, children who had experienced recrudescence following treatment with chloroquine, amodiaquine or S/P were retreated with S/P. The failure rates were, however, higher for children treated initially with S/P than for children retreated with S/P (131). A recent study from Burkina Faso also “provided further evidence for the justification of continued use of pyrimethamine-sulphadoxine as a second-line treatment for malaria” (130). East African studies, in which the children initially had been treated with antifolates, found higher numbers of clinical treatment failures following retreatment than following initial treatment with S/P, indicating that S/P is unlikely to be very efficacious as second line treatment if the first line treatment is also an antifolate (42,132).

Conclusion of the studies on sulfadoxine-pyrimethamine

S/P is effective for treatment of uncomplicated malaria in children in Guinea-Bissau. No change in efficacy was observed during the first 6 to 9 years after its introduction as second line treatment by the National Malaria Programme.

Table 5: Outcome of treatment with S/P

	Study I (1996– 1999)	Study V (1995/96, 2002/04)
ETF	2% (2/84)	0 % (0/108)
ACPR-d7	98 % (82/84)	100 % (108/108)
ACPR-d14	98 % (81/81)	100 % (107/107)
ACPR-d21	92 % (75/78)	97 % (100/103)
ACPR-d28	90 % (67/70)	93 % (92/96)
ACPR-d35	86 % (62/65)	90 % (85/88)

ETF and cumulative ACPR rates calculated by evaluability analyses. We excluded children admitted to hospital on the day of inclusion, or who did not complete treatment, children who could not be found on the first day of follow-up, and children treated by a third person or by self treatment during follow-up without confirmed parasitaemia.

The prevalence rates of molecular markers for resistance have been found to be higher than the prevalence of *in vivo* drug resistance in most studies (160). In spite of excellent results of the *in vivo* studies on the efficacy of S/P in Bissau (studies I and V) an assessment of the prevalence of mutations in the *dhfr* and the *dhps* genes could therefore be used to predict the risk of an increase in the *in vivo* resistance in the future. As part of study III we determined the mutations in the parasites of 100 children (93). Forty-one percent harboured the triple-mutation in the *dhfr*-gene at codons 51, 59, and 108, and almost 30% had either mutation at codon 436 or 437 in the *dhps*-gene. As in other West African countries no mutations at codon 540 were detected (47,104).

The high prevalence could be explained by the use of sulphonamides and trimethoprim for therapy and as prophylaxis for bacterial or other parasitic infections. Trimethoprim has been shown to partly select for the same mutations as pyrimethamine, indicating a cross-resistance between the two drugs (83,87). In Malawi, high levels of mutations in the *dhps*-gene were associated with the use of sulphonamide containing drugs for treatment of bacterial infections (27). This is supported by the finding in Guinea-Bissau of high levels of resistance to both trimethoprim and sulphadoxine in *E.coli* isolated from children suffering from malaria (93).

The relatively high level of genetic markers for S/P resistance could therefore be explained by the extensive use of antifolates for other infections.

In East-Africa several countries have introduced S/P combined with amodiaquine as first line treatment, but the combination is now increasingly failing. However, at the time of introduction, a high level of resistance to S/P existed (220). In Bissau, 41% of isolated parasites already had triple mutations of the *dhfr*-gene, in spite of the good clinical outcome. According to a theoretical model estimating the useful therapeutic life of antifolate combination therapies, a frequency of more than 5% of the triple mutations associated with resistance to pyrimethamine seriously compromises the usefulness of this antimalarial (236). S/P remains effective as a cheap, safe, and easy to administer second line drug. Nevertheless the use should be restricted in order to prolong the period of usefulness.

Amodiaquine as monotherapy

Amodiaquine is only rarely used in Guinea-Bissau, but with the increasing number of treatment failures to the recommended first line treatment with chloroquine 25 mg/kg, it could be expected to be more popular. The efficacy of treatment with amodiaquine has never been evaluated in Guinea-Bissau.

In study IV we compared the treatment of uncomplicated malaria with amodiaquine 15 mg/kg and 30 mg/kg to establish the efficacy of the different doses, and to compare with the efficacy of chloroquine. The ACPR rates on day 28 and day 35 were 92% and 88% in the group treated with 15 mg/kg vs. 94% and 92% in the group treated with 30 mg/kg, corresponding to 94% and 91% vs. 94% and 92% when PCR-corrected (Figure 7 and Table 6).

The treatment with amodiaquine has been studied in other West-African countries. In Senegal, PCR-uncorrected ACPR rates were 95% on day 14, and 81% on day 28 (3). Studies in Nigeria and Burkina Faso have produced treatment failure rates on day 28 of 5% and 4%, respectively (13,208).

In Sierra Leone, corresponding failure rates varied between 5% and 30% (33). On day 14, treatment failure rates of 14% were found in Ghana (142), and 0% and 10% in Nigeria (63,129). Thus, the efficacy of amodiaquine for treatment of uncomplicated malaria in West-Africa is generally acceptable.

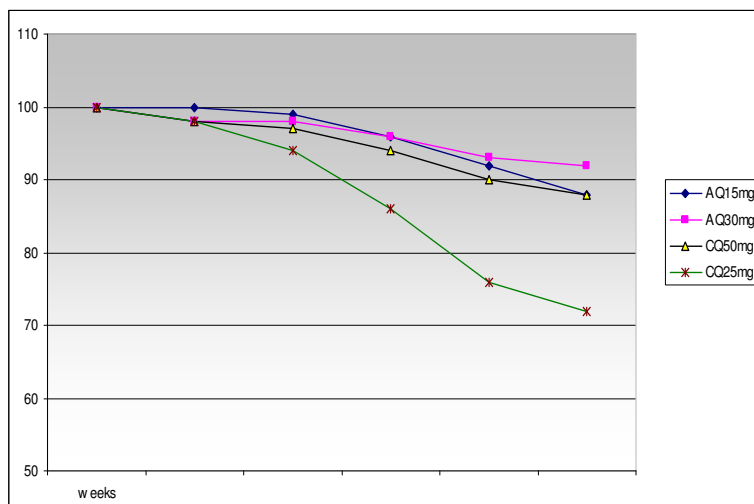


Figure 7:

The ACPR rates following treatment with chloroquine 25 mg/kg and 50 mg/kg compared to treatment with amodiaquine 15 mg/kg and 30 mg/kg.

In East Africa, the average percentages of patients with adequate clinical response at day 28 to treatment with amodiaquine remained constant at about 95% from 1998 to 2003 in spite of increased use of the drug especially in combination therapies (220). However, a PCR-adjusted failure rate of 21% was found in Uganda by day 28 (32); and 42% of Tanzanian children had clinical or parasitological treatment failure by day 14 (133). In Mozambique, Abacassamo found a good clinical effect of amodiaquine (92%), but the parasitological failure rate at day 14 was as high as 26% even if the drug had not been used in the area for 10 to 15 years; however, when co-administered with artesunate or S/P a good response was observed (2).

Many East-African countries have changed their first line treatment to combination therapies since 1998, most of them including S/P and/or amodiaquine. In Uganda, children treated with S/P combined with amodiaquine had adequate clinical response on day 14, but approximately 16% experienced parasitological treatment failure on day 28 (216) whereas Bakyaita reported a PCR-adjusted clinical failure rate of between 13% and 35% from three different districts (11). Yeka and co-workers found the combination artesunate-amodiaquine to be associated with a lower risk of recrudescence but a higher risk of new infections than the S/P-

amodiaquine combination (260). In Zanzibar, amodiaquine was also combined with artesunate, and a PCR-corrected adequate clinical and parasitological response at day 42 of 91% was found, which was just as good as that of a group treated with artemether-lumefantrine (119). A multicentre study (3) showed only modest efficacy of the combination in Senegal where the level of amodiaquine resistance is low. The PCR-unadjusted cure rates at day 28 were 81% and 82% in the group treated with amodiaquine monotherapy and the group treated with amodiaquine combined with artesunate, respectively.

Table 6: Outcome of treatment with chloroquine and amodiaquine in study IV:

	Amodiaquine 15 mg/kg	Amodiaquine 30 mg/kg	Chloroquine 25 mg/kg	Chloroquine 50 mg/kg
ETF	0 % (0/170)	1 % (2/166)	2 % (4/165)	1 % (1/164)
ACPR-d7	100 % (170/170)	98 % (162/166)	98 % (161/165)	98 % (161/164)
ACPR-d14	99 % (161/163)	98 % (162/162)	94 % (150/158)	97 % (157/159)
ACPR-d21	96 % (152/157)	96 % (153/155)	86 % (136/149)	94 % (147/151)
ACPR-d28	92 % (144/149)	93 % (140/145)	76 % (117/132)	90 % (134/141)
ACPR-d35	88 % (131/138)	92 % (132/134)	72 % (105/111)	88 % (127/130)
LTF-d35	9 %	6 %	18 %	8 %
LPF-d35	3 %	1 %	8 %	3 %

ETF and cumulative ACPR, and the cumulative LCF and LPF at day 35 calculated by evaluability analyses. We excluded children admitted to hospital on the day of inclusion, children who did not complete treatment, children who could not be found on the first day of follow-up, and children treated by a third person or by self treatment during follow-up without confirmed parasitaemia.

Conclusion on the amodiaquine study

Amodiaquine has a good efficacy in Guinea-Bissau, as in other West-African countries, in spite of the fairly high level of resistance to chloroquine. Still, the efficacy is no better than treatment with chloroquine 50 mg/kg, and a change of recommended drug from chloroquine to amodiaquine would thus not be relevant at present. Amodiaquine could instead serve as one of the partner drugs in a combination treatment and should therefore not be used as

monotherapy to diminish the risk of development of resistance to the drug. Considering the high level of mutations associated with resistance to S/P, the options would most likely be an artemisinin derivative.

Effectiveness study

Successful antimalarial treatment regimens need to be effective. Apart from drugs given as one single dose, as S/P, chloroquine given as one daily dose for three days is one of the simplest treatment schedules for malaria. Still, in different studies non-adherence to chloroquine has varied between 9% and 79% (39) and has been shown to increase morbidity and mortality (143,163).

Effectiveness studies are therefore essential in order to evaluate the benefit of a treatment for the children. No uniform methodology exists for performing this kind of studies. In study VI, the level of adherence to the treatment recommended by the National Malaria Programme was evaluated. The outcome measures were 1) whether the plastic bags, in which the mothers had been given the tablets, were empty on day seven, 2) the clinical and parasitological treatment failure rates until day 35, and 3) the chloroquine drug levels at day seven in the un-supervised group as compared to children who had been given the treatment under supervision at the health centre.

One third of the mothers did not keep the empty plastic bag. However, the median drug concentration in the children whose mothers had kept the bag and in children whose mothers were unable to show the bag was similar at day seven. Likewise, the drug concentrations in children from the supervised and from the unsupervised groups were the same. The treatment failure rate was not significantly higher in the unsupervised group than in the supervised group. Thus, given proper information the mothers did comply with the treatment recommended.

These results are encouraging, but they were derived from a group of children who had been given good information about a simple treatment schedule and who had received the

medication free of charge at the health centre. Treating with the combination therapy S/P plus artesunate in Zambia showed a “certain adherence” of 40%, and a “possible adherence” of 79% (39). In Uganda the cure rates for children treated with supervised vs. unsupervised artemether-lumefantrine (Coartem™) did not differ, (158). However, the tablets, which are expensive, were provided free of charge for the patients, furthermore Coartem™ is a coformulated combination therapy. Still, the mean lumefantrine concentrations were significantly lower on day 3 and day 7 in the unsupervised vs. the supervised group, indicating an adherence problem.

As most African children with fever are treated at home without professional supervision, and as parents have to pay for the drugs, effectiveness studies ought to be performed before a treatment, which is both more expensive and more difficult to adhere to, is being recommended by the malaria programme in a country.

CONCLUSION

As in other parts of Africa, treatment with chloroquine 25 mg/kg gave too high failure rates to be recommended as first line therapy. As a substitute, ACT is strongly endorsed by WHO and the Roll Back Malaria movement (20,254) and artemether-lumefantrine has now been adopted by several African countries (177). A six-dose regimen is needed and produces PCR-corrected cure rates as high as 93.9% at day 28 (52,151). However, it has been shown that long follow-up periods are necessary to identify recrudescence following treatment with artemether-lumefantrine as between 15% and 58% of the total treatment failures as identified on day 42 would be missed if only assessing until day 28 (119,212). Artemether-lumefantrine has never been tested in Guinea-Bissau.

Other alternatives as first line treatment for uncomplicated malaria are S/P and amodiaquine which both have shown excellent efficacies in our *in vivo* studies (Tables 5 and 6). However, previously we found a high level of mutations associated with S/P resistance in the malaria parasites, indicating that resistance to this antifolate combination might develop rapidly if used to a higher extent than at present. Amodiaquine was shown to be as efficient as chloroquine 50 mg/kg and could serve as a first line drug (Table 6). Combination therapies might be introduced in the future, and an option would then be to combine amodiaquine with artemisinin (4,21,241,254). Reasonably priced, coformulated artesunate-amodiaquine tablets are likely to be produced soon (37). Amodiaquine should not at present be used as monotherapy but saved as the partner drug in a combination therapy.

In study I we found that 3-day courses with quinine were ineffective, which corroborates earlier results demonstrating the length of treatment to be very important, thus at least 7-day courses are needed (96). Quinine would therefore not be very attractive as a first line drug. If chosen for second line therapy, the treatment schedule should be simplified, and treating with one daily dose only should be considered in order to ensure an acceptable adherence (94). Due to the high efficacy, quinine serves perfectly well for third line therapy.

We have repeatedly shown that chloroquine in a total dose of 50 mg/kg administered in 2 daily doses for 3 days results in high cure rates. A PCR-corrected ACPR rate on day 28 of 92% was found in study IV, which is equivalent to the rates found elsewhere following treatment with artemether-lumefantrine (52,151).

Before giving recommendations for a national policy on treatment of malaria the following should be considered:

1. The efficacy: chloroquine in a total dosage of 50 mg/kg is as efficient as other antimalarials including ACT.
2. Dosing complexity: chloroquine should probably be given in two daily doses, as should artemether-lumefantrine. Other ACTs are still not coformulated and the dosing is considerably more complex.
3. Safety: there is limited experience with the new artemisinin combination therapies, and concerns have been raised about the safety of artemisinin derivatives, though the drug is most likely quite safe. Chloroquine is very well known as it has been used as an antimalarial for long, only minor adverse effects are seen.
4. The cost of the treatment: ACT is 10 to 20 times more expensive than chloroquine per treatment course (ACT costs 1.20 – 3.50 US\$ per adult course (261) as compared to 0.12 US\$ for S/P per dose (106) and 0.08 US\$ per dose of chloroquine (14)). The more expensive treatments need to be subsidised, and the funding should be sustainable. However, there is a huge need for further funding (177,196,258) and concerns have been raised about the long-term viability of the *Global Fund to Fight AIDS, Tuberculosis and Malaria*, which is the main funder of ACT (90).
5. Availability of the drug: chloroquine is readily available all over Guinea-Bissau. ACT has to be distributed, not only to the government health system but also to the private sector.
6. Home-based management: Roll Back Malaria advocates that all patients be treated within 24 hours of getting symptoms, and home-based management is an important tool in obtaining this goal (178). Chloroquine is well known by the population and is already used to a high extent for treatment of presumed malaria (97). If a more

expensive treatment is to be implemented a system for covering the costs should be introduced, which might be difficult especially within the private sector.

7. Effectiveness: the efficacy of chloroquine 50 mg/kg is high. Furthermore, the cost is low, the side effects are minor, and the drug is well known by the health personnel and the population. The adherence to the drug and thereby the overall effectiveness would therefore be expected to be higher than if changing to a new, more expensive and to the population unknown therapy.
8. Keeping chloroquine for first line therapy would leave S/P as an efficient, cheap and easy-to-administer second line drug. Finally, quinine could be an efficient third line drug, even considering the longer treatment period of 7 days – reducing the number of daily doses could be considered (94-96).

Chloroquine 50 mg/kg is thus the most attractive and realistic first line treatment at present. It would be an advantage to postpone the decision of shifting to an artemisinin combination therapy until some of the problems have been solved, especially as a good and cheap alternative exists, which can be implemented without delay, thereby giving time for planning of a hypothetical introduction of an artemisinin combination therapy. It will, however be important to monitor the resistance *in vivo* to the therapy given.

ACKNOWLEDGEMENTS

Many years ago I attended a meeting in the Danish Society of Tropical Medicine. A social anthropologist named Peter Aaby spoke about measles and why the medical world had got it all wrong. A few months prior to the meeting, I had participated in a course at the University of London led by Professor David Morley and had learned all about measles in the developing world. It was hard to accept the ideas put forward by this Peter Aaby. However, later I learned to appreciate his innovative, though often controversial and provocative, hypotheses. So I was glad to get the opportunity to work at the Bandim Health Project. It is very inspiring to work with Professor Peter Aaby, and without his support I would never have finished this work.

When living in Bissau I was approached by Dr. Lars Rombo, affiliated with the National Health Laboratory in Bissau as an expert in malaria. He proposed collaboration, initially just to study the efficacy of the commonly used 3-day treatment course with quinine. Discussing with Lars always produced and still produces new interesting and important research questions, always focusing on issues of relevance to the population in the area. By now, we have collaborated for more than 10 years, and I owe my interest in and knowledge of malaria to Professor Lars Rombo.

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Finally I thank my family for having accepted me spending so much time in Bissau and so much effort on the studies during the past ten years.

SAMMANFATTNING

Malaria är ett mycket allvarligt hälsoproblem i Afrika. Behandling av sjukdomen har blivit allt mer svårbemästrad på grund av resistens mot de vanligaste använda malarialäkemedlen. I Guinea-Bissau används i första hand klorokin för behandling av okomplicerad malaria (first line treatment). När denna inte lyckas rekommenderas sulfadoxine-pyrimetamine (S/P) (second line treatment). För behandling av komplicerad och allvarlig malaria används kinin (third line treatment). Under de senaste 10 åren har vi studerat effekten av dessa läkemedel i Bandimdistriktet i utkanten av huvudstaden Bissau.

Klorokin har visat sig vara mindre effektivt när vi använde den totaldos på 25 mg/kg som rekommenderas av WHO eftersom bara 68 % av barnen med malaria inte hade återfått parasiter (adequate clinical and parasitological response (ACPR) rate) 28 dagar efter behandlingsstart i studien från 1996/1997. Motsvarande andel i studier från 2001/2004 var 76 %. En större dos på 50 mg/kg fördelat på 2 dagliga doser under 3 dagar lyckade i 86 % och 90 % under samma perioder. Inga allvarliga biverkningar noterades under behandling med den högre dosen.

Artemisininderivat är en grupp malarialäkemedel som snabbt reducerar antalet parasiter som cirkulerar i blodbanan. Om ett ytterligare malarialäkemedel ges samtidigt når man i teorin både en snabbare klinisk effekt och en minskad risk för resistensutveckling. I Bissau gav klorokin och artesunate emellertid inte bättre resultat än behandling med endast klorokin vare sig läkemedlet gavs samtidigt eller i följd. Tre dagars behandling med enbart artesunate resulterade i att 50 % av barnen åter hade parasiter dag 35. En så kort behandling med enbart artesunate bör inte användas.

S/P konstaterades vara effektivt både för behandling av okomplicerad malaria och för behandling av återfall (med ACPR-rater på 86 % och 90 %) med samma resultat under perioden 1996 till 2004. Vi har i tidigare påvisat en stor andel mutationer associerat med

resistens mot S/P i *P. falciparum* parasiter. Trots att S/P har en god klinisk effekt i Guinea-Bissau bör preparatet därför användas återhållsamt för att i görligaste mån minska risken för resistensutveckling.

Tre dygns behandling med kinin efterföljt av en standarddos klorokin används ofta i Guinea Bissau. Denna regim var inte mer effektiv än behandling med enbart klorokin och bör därför inte användas längre. När initial behandling ges med kinin rekommenderar vi istället att denna behandling fortsätts i 7 dygn vilket visat sig vara effektivt i våra tidigare studier.

När klorokin 25 mg/kg inte längre kan användas på grund av ökande resistens har amodiakin rekommenderats som förstahandspreparat antingen som monoterapi eller som en del av en kombinationsbehandling. Vi fann att amodiakindoser med 15 mg/kg och 30 mg/kg båda var effektiva (dag 35 ACPR-rate på 88% och 92%), men resultaten var inte bättre än efter behandling med klorokin 50 mg/kg (dag-35 ACPR-rate på 88%). Amodiakin kan därför sparas till en eventuell nödvändig senare kombinationsbehandlingsstrategi.

For att värdera om ett malarialäkemedel med god farmakologisk effekt också har god effekt i praktiken måste man utföra effektivitetsstudier. Vi jämförde resultaten av övervakad behandling och behandling som inte övervakades med klorokin 25 mg/kg på en hälsocentral. Vi fann ingen skillnad mellan grupperna, vare sig mellan andelen som återfick parasiter eller läkemedelskoncentrationer i blodet. Om mödrarna i Guinea-Bissau får en bra information ger de en korrekt behandling av sina barn i hemmet.

Konklusion: 1) Klorokin är effektivt och säkert med en dos på 50 mg/kg. Som en tillfällig strategi kan man välja denna behandling för okomplicerad malaria. Denna ändring (från 25 mg/kg) kan snabbt implementeras. 2) S/P är effektivt för behandling om inte klorokin är effektivt men bör endast användas i begränsad omfattning. 3) Amodiakin kan sparas för att istället användas som en av delarna i en senare införd kombinationsterapi.

RESUMO

Em crianças africanas, o paludismo é a principal causa de morbidade e mortalidade. O tratamento da infecção pelo *P.falciparum*, após o aparecimento de cepas multi-resistentes em muitas partes de Africa, ficou mais difícil, sendo necessário o uso de regimes de tratamento alternativos. Uma avaliação dos tratamentos antipalúdicos, tal como recomendados e actualmente usados, parece essencial para fornecer informações ao Programa de Luta Contra o Paludismo e para que este possa dar recomendações para o futuro. Durante 10 anos estudamos a eficácia de cloroquina (antipaludico da primeira linha), de sulfadoxina-pirimetamina (antipaludico da segunda linha) e de quinino (antipaludico da terceira linha) em Bandim, situada em Bissau, capital da República de Guiné-Bissau. Durante a maioria dos estudos utilizamos a mesma metodologia e o mesmo pessoal.

Cloroquina usada numa dosagem total de 25 mg base por kg durante 3 dias demonstrou não ter uma eficácia suficiente para o tratamento de paludismo em crianças, tendo uma taxa de resposta clínica e parasitológica adequada (RCPA) 28 dias depois de iniciar o tratamento de 68% em 1996/1997 e de 76% em 2001/2004, no entanto tratando com cloroquina numa dosagem de 50 mg/kg dividida em duas doses por dia durante 3 dias foi muito eficaz com taxas de RCPA no dia 28 de 86% e 90%, respectivamente. Nenhum efeito colateral significativo foi registado.

Os nossos estudos documentaram que o nível de resistência in-vivo de cloroquina não tem aumentado durante os últimos 10 anos, um resultado verificado por estudos in-vitro e estudos genéticos realizados no mesmo período em Guiné-Bissau, pelo contrário a resistência parece estar a diminuir durante este tempo.

Artemisininas negativam rapidamente a parasitaemia em sangue periférico, em princípio aumentando a eficácia de um antipalúdico administrado em combinação com um derivado da artemisinina. Contudo, tratando com cloroquina em combinação com artesunate não foi melhor do que utilizar cloroquina como mono-terapia no estudo que realizamos. Artesunate

em mono-terapia durante 3 dias, um esquema de tratamento nunca avaliado em Africa antes, produziu taxas de recaídas de 50% no dia 35. Então este esquema não deve ser utilizado.

Sulfadoxina-pirimetamina (S/P) demonstrou ser eficaz tanto para tratamento de primeira linha como tratamento de segunda linha com taxas de RCPA no dia 35 de 86% e 90%, respectivamente. Também, desde 1996 até 2002/2004 a sua eficácia como antipalúdico não diminuiu. No entanto, encontravamos um nível bastante elevado de mutações associadas com resistência de S/P em parasitas de *P.falciparum*, mesmo se esta droga só tenha sido utilizada durante pouco tempo. Então, mesmo que S/P tenha uma eficácia clínica excelente o uso desta combinação deve ser limitado para prolongar o tempo que continua a ser vantajoso.

Na Guiné-Bissau, assim como em outros países africanos existe uma tradição de tratar com quinino durante 3 dias só. Crianças sofrendo de vômito vigoroso ou que são internadas num hospital com paludismo complicado ou grave são tratadas com quinino. Embora, quando as crianças melhorem e podem ser ter alta este tratamento geralmente seja alternado para cloroquina 25 mg/kg. Este esquema de tratamento nunca tinha sido avaliado.

Tratar com quinino durante 3 dias resultou num nível de insucesso terapêutico muito elevado. Tratar com quinino 3 dias, seguido por cloroquina 25 mg/kg não aumentou a taxa de RCPA de cloroquina mono-terapia, e não deve ser utilizado. O tratamento com quinino deve ser continuado até completar 7 dias uma vez iniciado.

Amodiaquina pode ser uma alternativa para tratamento de primeira linha em áreas onde tratamento com cloroquina 25 mg/kg tem de ser abandonado por causa dum nível de resistência elevado. Nós comparamos o tratamento de amodiaquina com o tratamento de cloroquina. O tratamento tanto com amodiaquina 30 mg/kg como com 15 mg/kg resultaram em níveis elevados de RCPA no dia 35 (92% e 88%, respectivamente), mas o resultado tratando com cloroquina 50 mg/kg foi tão satisfatório (RCPA dia 35 de 88%). Então de momento não tem sentido mudar para amodiaquina como tratamento de primeira linha. Em vez disso amodiaquina pode ser reservada como um útil e eficiente candidato para um

tratamento de combinação com um derivado de artemisinina quando ou se fôr necessário no futuro.

Para um tratamento antipalúdico ter um sucesso, não tem somente de ser efectivo, mas também tem de ser aceiteado pela população, tem de ter poucos efeitos secundários, e os esquemas de tratamento têm de ser simples para obter uma adesão suficientemente elevada. Comparamos o resultado de um tratamento com cloroquina 25 mg/kg durante 3 dias supervisionado num centro de saúde com o mesmo tratamento não supervisionado. Não havia nenhuma diferença nem no nível de RCPA, nem na concentração de cloroquina no sangue das crianças no dia 7. Dando uma informação cuidadosa, as mães em Guiné-Bissau podem ser confiadas em dar tratamento correcto para as suas crianças em casa.

Em conclusão: 1) Cloroquina numa dosagem de 50 mg/kg é efectiva e segura, e o custo é muito menor do que o dos novos tratamentos de combinação. Como uma estratégia interina de mudar o tratamento de primeira linha seria fácil a implementar, não traria implicações económicas, não traria problemas de segurança, e a mudança seria fácil a organizar. 2) O uso de S/P deve ser limitado, mas serve como um bom e eficiente tratamento de segunda linha. 3) A amodiaquina pode ser reservada para ser combinada com artesunate no futuro, se for necessário.

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