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Efficacy, Safety, Tolerability, and Acceptability of Two Paediatric Formulations of Artesunate-Mefloquine in African Children with Acute Uncomplicated *Plasmodium falciparum* Malaria

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2 Introduction

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2.1 Treatment of Malaria

The annual mortality from malaria is estimated to range from 0.7 –2.7 million deaths worldwide [1]. This burden is aggravated by malaria morbidity and its disproportionate occurrence in the world's poorest countries [2]. Epidemiological findings indicate that malaria is the principle cause of at least one fifth of all child deaths in Africa and that the number of children dying of malaria rose substantially during the first half of the past decade compared to the 1980s [3]. This trend is most likely attributable to the emergence and spread of _Plasmodium falciparum_ strains resistant to the used antimalarials.

The mainstay of antimalarial therapy for the past 40 years has been chloroquine, but now resistance is widespread [2, 4-9]. Its successor sulfadoxine-pyrimethamine, which is equally cheap, has been a good replacement but is now also becoming increasingly ineffective due to rising resistance [10-12]. Thus newer compounds or combination regimes must be used to treat drug resistant _Plasmodium falciparum_ malaria successfully. However, these drugs are up to 10-fold more expensive [2].

Spreading resistance and the need of new but expensive drugs in the world's poorest countries lead Africa into a disastrous situation. The most important goal for development of new antimalarials is therefore, to have effective and affordable drugs and to use them in a way that will delay the emergence of resistance. [8]

The World Health Organization embraces this goal recommending antimalarial combination therapies instead of monotherapies for the treatment of _Plasmodium falciparum_ [13]. The rational for combining drugs with independent modes of action in order to prevent the emergence of resistance was first developed in antimycobacterial chemotherapy. The observed benefits of combination therapy are, in addition to decreasing risk of emergence of

resistant parasites, the increased efficacy and shortened duration of treatment and thus increased compliance [10].

Present antimalarial combination therapies can be distinguished in two main groups: the artemisinin combination therapies (ACT) and the non-artemisinin based combinations (non-ACT).

Currently available non-artemisinin based combination regimens include sulfadoxine-pyrimethamine, chloroquine-sulfadoxine-pyrimethamine, quinine-sulfadoxine-pyrimethamine, amodiaquine-sulfadoxine-pyrimethamine, mefloquine-sulfadoxine-pyrimethamine, quinine-tetracycline, quinine-clindamycin, atovaquone-proguanil, chlorproguanil-dapsone [10]. In the review on antimalarial combinations by Kremsner and Krishna, current combination regimens were assessed according to safety, tolerability, efficacy, effectiveness, compliance, pharmacokinetic match, use in children, and pregnancy. Sulfadoxine-pyrimethamine and chloroquine-sulfadoxine-pyrimethamine were judged to have unclear efficacy while for quinine-sulfadoxine-pyrimethamine, amodiaquine-sulfadoxine-pyrimethamine, mefloquine-sulfadoxine-pyrimethamine, and chlorproguanil-dapsone efficacy was graded to be acceptable. Desirable efficacy was given to quinine-tetracycline, quinine-clindamycin, and atovaquone-proguanil. Effectiveness was regarded as acceptable for all regimes except quinine-tetracycline, which was given poor effectiveness [10].

Artemisinin or its derivatives are the constant partner in artemisinin-based combination therapies and have been combined with numerous partners such as amodiaquine, atovaquone-proguanil, chloroquine, clindamycin, doxycycline, lumefantrine, mefloquine, piperaquine, pyronaridine, chlorproguanil-dapsone, sulfadoxine-pyrimethamine, and tetracycline.

In a meta-analysis of the International Artemisinin Study Group [14] the effects of adding artesunate to standard treatments of *Plasmodium falciparum* malaria were evaluated and it was summarized that the addition of 3 day artesunate to standard antimalarial treatment substantially decreased treatment failure,

recrudescence, and gametocyte carriage. Up to date the following artemisinin-based combination therapies have been studied most extensively: artemether-lumefantrine, artesunate-amodiaquine, artesunate-mefloquine, and artesunate-sulfadoxine-pyrimethamine. Today artesunate-amodiaquine is recommended by the World Health Organization as first-line medication for most African countries, it is followed by artemether-lumefantrine and artesunate-sulfadoxine-pyrimethamine. The combination artesunate-mefloquine is recommended by the World Health Organization as first-line treatment in Cambodia, Thailand, Venezuela, Peru, and Bolivia [15].

So far one of the best options of antimalarial combinations available is artesunate-mefloquine [10]. Artesunate-mefloquine is the longest used artemisinin-based combination, however, large parts of present data from clinical trials are available only for Asia. The few existing data derived from clinical trials in Africa are mostly referring to use in adults. It is therefore very important to obtain reliable data for artesunate-mefloquine treatment of acute uncomplicated *Plasmodium falciparum* malaria in African children who represent the main target group worldwide.

2.2 Artesunate

Artemisinins represent a group of antimalarials that derive from the Chinese medical herb Artemisia annua (sweet wormwood). In the traditional Chinese medicine this plant was already known and used against fever a 1000 years ago. It was re-discovered some decades ago and in 1972 artemisinin was for the first time purified and chemically characterized as a sesquiterpene lactone with an endoperoxide bridge (C-O-O-C), unique for antimalarials. Dihydroartemisinin is the reduced lactol derivative of artemisinin while artesunate and other semisynthetic derivatives are ethers or esters of the lactol [16].

Current formulations contain artemisinin, dihydroartemisinin, artesunate or artemether; they have been developed in various formulations for oral, rectal, intramuscular, and parenteral administration. After administration the derivatives are converted to the active metabolite dihydroartemisinin.

Recent work found that artemisinins inhibit the malarial parasite's calcium ATPase6 (sarcoplasmic endoplasmatic reticulum calcium ATPase, Plasmodium falciparum (Pf) SERCA) [17]. SERCA-PfATPase6 as the target for artemisinins is also supported by data finding the S769N PfATPase6 mutation associated with raised IC50 (half maximal inhibitory concentration) of artemether in-vitro [18]. Latest findings report that decreasing *pfmdr1* (*plasmodium falciparum multidrug resistance gene 1*) copy numbers in *Plasmodium falciparum* heightens susceptibility to artemisinin and other antimalarials [19].

The reduced in-vitro susceptibility of artemether associated with the PfAPTase6 mutation could be the first alarming sign for possible emergence of resistance. Increased vigilance and a coordinated rapid deployment of drug combinations is needed [18]. However, besides the innate reduced sensitivity in thalassaemia [16], no concerns about reduced efficacy in vivo have been reported in artemisinin treated patients.

The success and increased use of artemisinins is based on different advantageous properties. They are active against all four species of malaria parasites that infect humans and against multidrug resistant *Plasmodium falciparum* strains [13]. Nanomolar plasma concentrations are sufficient to act against all asexual stages of the parasites. Artemisinins reduce gametocyte carriage, but it remains uncertain to what extent this decreases transmissibility and transmission of malaria [20-22]. Artemisinins are very fast acting antimalarial drugs leading to rapid relief from symptoms and fast decrease of parasitaemia reducing the parasite number by a factor of approximately 10000 each asexual cycle [23].

Artesunate and other artemisinin derivatives are known for their very good tolerability and safety. In only very few cases allergic reactions are reported

after artesunate intake. Neurotoxicity of artemisinins was found in in-vitro and animal studies at very high dosages. But as artemisinins have already been used safely in millions of people, neurotoxicity is unlikely to be clinically relevant [16].

In monotherapy artemisinins have high recrudescence rates despite treatment courses of 5 to 7 days [24-28]. The poor efficacy with artemisinin monotherapy is commonly attributed to the intrinsically short half life and increasing drug clearance during reconvalescence [28]. Today monotherapy with artemisinins or derivatives is no longer recommended, efficacy is low and the fear of emerging resistance is high [13, 29].

The average minimal limit of oral artesunate has been found to be at 2 mg/kg to obtain maximal parasite clearance times [30]. However most physicians currently use an oral dose of artesunate of 4mg/kg per day for 3 days for patients with uncomplicated *Plasmodium falciparum* malaria when in combination with a second antimalarial.

2.3 Mefloquine

Mefloquine was the first synthetic quinoline-methanol compound to be developed as an antimalarial drug by the Walter Reed Army Institute of Research (Washington DC) more than 30 years ago. Thailand was the first country to deploy mefloquine. It was introduced for treatment of multidrug resistant *Plasmodium falciparum* after the emergence of drug resistance to sulfadoxine-pyrimethamine. For a long time it was used as efficacious monotherapeutic agent mainly in Asia. Today it is deployed in combination with artesunate in the treatment of uncomplicated and multidrug resistant *Plasmodium falciparum* malaria and in prophylaxis for travelers to endemic regions.

As a distant derivative of quinine it shares the common structure of the bicyclic quinoline ring system. It has activity against the late trophozoite stage of all

species of human malaria parasites. The blood schizonticidal mechanism was found to be comparable to chloroquine and quinine. After uptake to the parasite's food vacuole it binds to free heme polymer and inhibits thus heme polymerization. This leads to toxic accumulation of the highly reactive heme which kills the parasite probably through oxidative damage of membranes or other cell targets [31].

In the late 1980s first resistance occurred on Thai-Cambodian and Thai-Burmese borders. Losses of efficacy of < 71% cure rates in mefloquine monotherapies at a dosage of 15mg/kg were reported [31, 32]. Also in Africa and in South America in-vitro studies found low mefloquine sensitivity [33-35]. However, overall resistance to mefloquine is considered to be rare outside South East Asia. The mechanism of resistance is described as *pfmdr*-gene (*plasmodium falciparum multidrug resistance* gene) polymorphisms and amplifications resulting in reduced drug uptake and thus reduced intracellular concentrations [36]. The first consequence of the emerging resistance in Thailand was to increase the dosage to 25mg/kg [32]. This improved efficacy for some time until cure rates declined again and monotherapy was replaced by combination regimes [37].

The elimination half-life of mefloquine is very long with a median of 20 days [38]. Mefloquine has poor aqueous solubility and can therefore not be given in parenteral form. Splitting the recommended total oral dose of 25mg/kg into 15mg/kg and 10mg/kg with an administration interval of 24h was associated with elevated blood concentration levels and reduced vomiting compared to the single dose treatment regime [39]. In order to preserve mefloquine efficacy in mefloquine sensitive areas and to reduce and prevent further emergence of resistance, today mefloquine should be used only as a combination partner in treatment of *Plasmodium falciparum* malaria [40].

Many dose-related adverse effects are described to mefloquine therapy. Most frequent adverse events are gastrointestinal disorders including nausea, abdominal pain, vomiting, and diarrhea and neuropsychiatric disorders such as

dizziness, headache, and vertigo [13]. However, overall tolerability and safety are good as serious side effects or sequelae are rarely reported.

2.4 The Antimalarial Combination Artesunate-Mefloquine

To find the partners for an ideal combination regime, different characteristics of both partner drugs alone and combined have to be considered. Kremsner and Krishna outline the following characteristics [10]:

- Safety and tolerability need to be respected in order to avoid serious or fatal adverse events and to increase likelihood of compliance.

- Efficacy should exceed a 90% cure rate (evaluated respecting appropriate follow-up periods) and effectiveness in malaria endemic areas should exceed 75%.

- To facilitate therapy, compliance, and treatment at home drugs should be affordable and drug administration should be possible in children, in patients vomiting or in patients suffering from severe malaria. Ideal would be a fixed-dose formulation administered only once or at least in simple and short regimens of maximal 3 days.

- The regimen should be active against all stages of parasites. Additional gametocyticidal activity might reduce transmission. The drugs should have modes of actions that diminish the risk of occurrence of resistance and the elimination half-lives should match in order to provide reciprocal protection from parasite exposure to single-drug low-dose concentrations. There should not be any other significant negative pharmacokinetic drug interactions and the drugs should be stable regarding temperature conditions in the tropics.

Artesunate as a combination partner has several advantages over other antimalarial drugs for use in combination [2]. As mentioned above, advantages are rapid clinical response, good safety, and tolerance, activity on gametocytes and the absence of resistance. In most combinations with artesunate the

partner drug is eliminated more slowly and is not "protected". But as artesunate has a very high parasite reduction rate, only few parasites remain exposed to the longer acting drug. In the case of artesunate-mefloquine combination this residuum of parasites is then exposed to maximal blood mefloquine concentrations, which may be sufficient to eliminate even a partially resistant *Plasmodium falciparum* strain.

Overall studies assessing the interactions of artemisinin-mefloquine combination compared to artemisinin monotherapy concluded that giving mefloquine with an artemisinin drug does not result in earlier parasite clearance compared with the artemisinin drug alone. However, sustained parasite clearance is better when mefloquine is combined with an artemisinin derivative, as long as the dose and duration of combination treatment is adequate [41].

The overall conclusion of studies comparing combination therapy of artemisinin-mefloquine combination to mefloquine monotherapy was that combination regimes achieve earlier and faster parasite and fever clearance times and that in areas of mefloquine resistance the combination improves the rate of sustained parasite clearance compared to mefloquine alone. Severe vomiting was found to be less common with combination regimens [41].

In areas where multidrug resistant *Plasmodium falciparum* strains were widespread and the use of mefloquine monotherapy had high failure rates, combination therapy of artesunate-mefloquine was introduced successfully with high and stable efficacy [42, 43]. Observations in-vivo show that the combination of artesunate-mefloquine has even reversed the previous decline in mefloquine sensitivity [40]. These findings advocate the use of artesunate-mefloquine in combination therapy and are reflected by the World Health Organization's recommendations for artesunate-mefloquine combination therapy as the first-line option for treatment of *Plasmodium falciparum* malaria in Cambodia, Thailand, Bolivia, Peru, and Venezuela [15].

Still the World Health Organization Treatment Guidelines do not support artesunate-mefloquine combination therapy as the first-line treatment of acute uncomplicated *Plasmodium falciparum* malaria in African children because, up to date, there is still insufficient data on its safety and tolerability in this target group [13].

2.5 Artesunate-Mefloquine in a blister

For the current study artesunate and mefloquine are combined in a prepacked single blister for simultaneous co-administration once daily for 3 days. Blister packs have been developed in order to facilitate patient compliance to combination regimens. Provision of blister packs of daily doses is an effective way to improve compliance with short course and drug combinations regimes [44].

Three studies have been performed in order to investigate the efficacy and safety of artesunate-mefloquine in a blister. One comparative study with artesunate-mefloquine in a blister was conducted in Thailand in 204 adults and children (bodyweight > 25kg) with uncomplicated *Plasmodium falciparum* malaria [45]. The second comparative study with artesunate-mefloquine in a blister was performed in 3 African centers (Benin, Cameroon, Côte d'Ivoire) in 104 patients with > 30kg bodyweight suffering from uncomplicated *Plasmodium falciparum* malaria [46]. Both studies compared treatment groups of simultaneous versus sequential administration of artesunate-mefloquine. The collected data did not show any significant difference in efficacy, cure rates for all treatment groups were above 98%. Similarly, tolerability and safety were good in both studies and all treatment regimens. In the African study, the incidence of vomiting was statistically lower in the simultaneous dosing group compared to the reference group [46]. Conclusion was that the 3-day, once daily co-administration of artesunate-mefloquine, with mefloquine starting already on day 1, offers a practical dosing regime, which is highly effective and well tolerated in patients suffering from uncomplicated *Plasmodium falciparum* malaria. The third study evaluated artesunate-mefloquine in a blister in 200

Kenyan patients with a bodyweight > 35kg and confirmed the high efficacy (cure rate on day 28 of 98%) and safety of simultaneous artesunate-mefloquine administration [47].

Today artesunate-mefloquine in a blister is indicated for the oral treatment of uncomplicated *Plasmodium falciparum* malaria as well as for the treatment of multi-drug resistant strains of *Plasmodium falciparum* and malaria caused by mixed Plasmodium pathogens. Prepacked blisters of artesunate-mefloquine for a 3-day treatment course are available in 3 different dosages of 600/1500, 600/750 and 300/750 mg of artesunate and mefloquine, respectively.

The artesunate-mefloquine combination is not indicated for prophylaxis and contraindicated in patients with known hypersensibilities to the contained drugs, in patients with epileptic disorders, and in pregnant women. The most common adverse experiences of artesunate-mefloquine are: gastrointestinal disorders (abdominal pain, nausea, vomiting, and diarrhea), nervous system disorders (headache, dizziness, and insomnia), and general and metabolic disorders (asthenia and anorexia). Concomitant medication to mefloquine may produce electrocardiographic abnormalities and increase the risk of convulsions, therefore halofantrine must be avoided with or after mefloquine for at least 3 weeks.

2.6 Study Objectives

The currently available artesunate-mefloquine dosages could only be tested in children able to swallow tablets and with a body weight of more than 25 kg. However, there is a great need for artesunate-mefloquine combination formulation for smaller children unable to swallow tablets. The new paediatric artesunate-mefloquine formulation, a mango-flavored, taste-masked preparation of granules, containing 50 mg artesunate and 150 mg mefloquine in a stickpack, was developed in order to provide an efficacious regimen with

simple administration, swallowability and good acceptability for young children with a body weight of less than 20 kg.

This clinical trial presented here was conducted in order to evaluate two paediatric formulations of artesunate-mefloquine as treatments for acute uncomplicated *Plasmodium falciparum* malaria in young Gabonese children. For both treatment formulations efficacy, safety, tolerability, acceptability, and pharmacodynamic parameters were studied. In addition a sub-study evaluated pharmacokinetic parameters in 12 patients of each treatment group.

This study was designed to address the urgent need for data on the safe deployment of new effective therapies for *Plasmodium falciparum* malaria in African children. It is as well a promising step towards the development of new formulations especially for combination regimes and facilitating the application to very young patients. Rendering compliance easier could help to improve adequate treatment and thereby decrease mortality and morbidity from malaria and protect potent drugs from losing efficacy due to emerging resistance.

3 Methods

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3.1 Study Site and Time

This open-label, stratified study of two paediatric formulations of artesunate-mefloquine in children with acute uncomplicated *Plasmodium falciparum* malaria was conducted at two different sites in Gabon, Central Africa.

40 children were included at center 1, Centre Hospitalier Libreville. Libreville is the capital of Gabon with about 500.000 inhabitants and is located between the Atlantic Ocean and the African rainforest.

At center 2, the Medical Research Unit of the Albert Schweitzer Hospital in Lambaréné, 31 children were included and followed up in its vicinity. Lambaréné is a town of about 20.000 inhabitants and is located in the province of Moyen Ogooue, a region of dense rainforest.

The predominant Plasmodium species in Gabon is *Plasmodium falciparum* and its mean parasite densities are typical for areas with stable perennial hyperendemic malaria [48, 49]. The entomological inoculation rate is around 50 [50].

In the area of Libreville in-vitro studies, assessing susceptibility of *Plasmodium falciparum* to antimalarial drugs, found parasites resistant at low but not negligible percentages to quinine, mefloquine, and artesunate/artemether and high grade resistance to chloroquine [51-53].

In and around Lambaréné, *Plasmodium falciparum* is highly resistant to chloroquine as shown in several in-vitro studies and clinical trials [6, 7, 54-56]. Quinine is still effective and is mostly used for parenteral treatment of hospitalized patients [54-56]. Mefloquine is available in the area of Lambaréné, but is not used on a large scale mainly because of its high costs. In-vitro studies showed good mefloquine activity to local *Plasmodium falciparum* isolates in the early 1990ies [54, 55]. In 2003 in-vitro activity of quinolines

against *Plasmodium falciparum* was assessed by Ramharter et al., and an increasing number of isolates showing borderline resistance to mefloquine was observed [57]. Besides, in a publication by Uhlemann in 2005 [58], amplification of *Plasmodium falciparum multi drug resistance gene 1* (*pfmdr1*) was reported in > 5% of patient samples from Lambaréné, Gabon, collected in 1995. Non of the samples collected 7 years later showed *pfmdr1* gene amplifications, suggesting that parasites with elevated *pfmdr1* gene copies have not substantially spread through the population [58]. The activity of artemisinins was assessed in clinical trials evaluating monotherapy with artesunate in children with *Plasmodium falciparum* malaria [59, 60]. While the 3-day course of artesunate failed to achieve sufficiently high cure rates, the 5-day course of oral artesunate yielded a cure rate of 90% on day 28. In 1998 one in-vitro study reported good susceptibility of *Plasmodium falciparum* isolates to artesunate [55].

Responding to the need of further evaluations of new antimalarial and combination regimes in patients with *Plasmodium falciparum* malaria, this clinical trial studied the combination therapy artesunate-mefloquine.

The study took place from the 23rd October 2005 to the 1st February 2006.

3.2 Trial Population

The study population comprised a total of 70 Gabonese children able to take oral medication and suffering from uncomplicated *Plasmodium falciparum* malaria.

All patients had to meet the following inclusion criteria:

- Male or female with a body weight ≥ 10 to ≤ 40 kg

- Patients suffering from acute uncomplicated *Plasmodium falciparum* malaria

- Malaria diagnosis confirmed by a positive blood smear with asexual forms of *Plasmodium falciparum*, i.e. identification of asexual parasite count $\geq$ 1000 to 250000/µl according to the Lambaréné method [61]; [62]

- Temperature $\geq$ 37.5° C or a history of fever within the last 48 hours

- Haemoglobin $\geq$ 7 g/dl

- Written informed consent from parents/guardian for the participating child (verbal consent in presence of literate witness was required for illiterate parents/guardians)

Patients who met the following exclusion criteria were not enrolled:

- Patients with signs and symptoms of severe/complicated malaria requiring parenteral treatment defined according to the World Health Organization recommendations [63]

- Patients with known hypersensitivity or allergy to artemisinin derivatives or mefloquine or mefloquine chemically related compounds (for example quinine and quinidine)

- Patients who had received quinine or any artemisinin derivatives within 12 hours prior to study start

- Patients who had received any other adequate antimalarial drug therapy including antibiotics which might be active against malaria infection within 1 week prior to study start

- Patients who had received investigational (unlicensed) drugs as well as mefloquine within 30 days prior to study start

- Patients with known history of psychiatric disorders

- Patients with known history of cardiac diseases and arrhythmia

- Patients with known sickle cell disease

- Patients with clinical signs of or laboratory evidence for any other severe hepatic, renal, pulmonary, cardiac, metabolic, psychiatric, cancer or haematological disease

- Female patients in pregnancy or breast feeding (a pregnancy test was done for every female patient with child bearing potential)

3.3 Allocation and Investigational Therapy

The study medications were Artequin Paediatric® and Artequin® 300/750, two treatment combinations of artesunate and mefloquine developed by Mepha Ltd. (Pharmaceutical Research, Development and Manufacture, Dornacherstrasse 114, P.O. Box 445, CH-4147 Aesch/Basel, Switzerland).

40 children with a body weight of 10 to 20 kg were treated in treatment group A. They received the new paediatric formulation, a taste-masked mango-flavored fixed-dose oral formulation of granules of daily 50mg artesunate and 150mg mefloquine combined in a stickpack. The content of the stickpack was administered directly into the child's mouth. Thereafter the child was encouraged to drink a glass of water in order to swallow remaining granules.

30 children with a body weight of 20 to 40 kg were treated in treatment group B. They received an oral co-blister formulation of tablets of daily 100mg artesunate and 250mg mefloquine. The tablets were taken with a reasonable amount of water and swallowed without chewing.

Both formulations were administered once daily for 3 days. The medication was taken under the supervision of an investigator who documented drug administration time and acceptance. Acceptability was judged according to swallow-ability, compliance, appreciation, and satisfaction of the patient, and was rated as excellent, good, fair or poor.

If a patient vomited any dose of treatment within 1 hour after administration, the full dose was replaced. The dose was not replaced if vomiting occurred more than 1 hour after intake. If a patient vomited twice the daily dose within 1 hour, the study medication was discontinued and a rescue treatment with a

standardized alternative antimalarial was initiated. However, the patient stayed in the study and follow-up visits were done according to the visit schedule.

Any new additional medication taken during the 28-day study period was documented in the case report form.

3.4 Study Design

3.4.1 Visit Schedule

The study was organized in a baseline assessment, a treatment period and a follow up period (Table 1).

An assessment of pharmacokinetic characteristics of dihydroartemisinin and mefloquine was performed in 24 patients (12 in each treatment group) at the Medical Research Unit, Lambaréné.

Baseline assessment (day 0). Baseline examination was done within 24 hours before the first dose of study medication. The informed consent was obtained prior to any study related activity or evaluation. After having performed a blood smear, blood spot on filter paper for polymerase chain reaction analysis (PCR), physical examination, vital signs, laboratory tests, and electrocardiography (ECG) the patient could be included according to the in- and exclusion criteria.

Treatment period (day 1 – day 3). The treatment period started with the first administration of the study medication, defined as "day 1 hour 0". The second drug administration followed 24 hours later and the third and last drug administration at hour 48. Starting from hour 0 on, every 12 hours a thick blood smear was done and vital signs and temperature were measured. In addition physical examination, ECG, and laboratory tests were performed on day 3.

Patients participating in pharmacokinetic analysis had blood samples taken at 30, 60, 90, 120, 240, 360 minutes, and 54 hours after the first drug administration.

Follow up period (day 4 – day 28). Follow-up visits at the hospital or at the patient's home were done on day 7, 14, 21, and 28. On all follow up visits a thick blood smear was performed and vital signs and temperature taken. Follow up visits on day 7 included in addition a physical examination. Day 28 visits required an additional physical examination as well as laboratory tests, ECG, and a blood sample on filter paper for PCR analysis.

Patients participating in the pharmacokinetic analysis had a blood sample taken on day 28.

All patients and respective guardians were asked to present at the study sites in case of any new or worsening disease or injury occurring in-between the scheduled visits.

Table 1: Visit Schedule

	Baseline	Treatment Period			Follow-up Period			
	Day 0	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Day 28
Informed consent	X							
Background information	X							
Inclusion/Exclusion criteria	X							
Blood smear	X	X	X	X	X	X	X	X
Blood sample on filter paper for PCR	X							X
Physical examination	X			X	X			X
Vital signs	X	X	X	X	X	X	X	X
Laboratory tests	X			X				X
Pregnancy test	X							X
ECG	X			X				X
Blood sample for pharmacokinetics	X	X		X				X
Study medication		X	X	X				
Adverse Events		X	X	X	X	X	X	X
Concomitant medication report		X	X	X	X	X	X	X
Study completion								X

For once included patients the following events were considered withdrawal criteria and sufficient reason to discontinue the study treatment:

1. Adverse event(s)

2. Abnormal laboratory value(s)

3. Unsatisfactory therapeutic response

4. Protocol violation

5. Patient withdrawal of consent

6. Lost to follow up

7. Death

If the patient discontinued study treatment before day 3 and/or observation before day 28, every effort was made to get follow up information on the status of the patient. The only events considered as sufficient reasons for a patient to discontinue observation in the clinical study were loss to follow up, death, and withdrawal of consent.

3.4.2 Efficacy Assessments

The primary efficacy endpoint was the 28-day cure rate. It was defined as proportion of patients with clearance of asexual parasitaemia within 7 days and without subsequent recrudescence within 28 days after initiation of study treatment. Recrudescence was defined as a positive blood smear (with or without clinical signs of malaria) after initial clearance of parasites from the peripheral blood.

Secondary efficacy parameters were defined as 14-day cure rate, time to parasite clearance and time to fever clearance. The 14-day cure rate was defined as proportion of patients with clearance of asexual parasitaemia within 7 days and without recrudescence within 14 days after initiation of study treatment.

Time to parasite clearance was defined as time from first study medication until first total and continued disappearance of asexual parasite forms remaining for at least a further 24 hours.

Time to fever clearance was defined as time from first study drug administration until first body temperature measured with values $\leq$ 37.5°C and remaining below $\leq$ 37.5°C for at least a further 24 hours. Temperature was measured sublingually.

For patients in the pharmacokinetic sub-study blood concentrations of dihydroartemisinin and mefloquine were analyzed at predefined time points.

3.4.3 Tolerability and Safety Assessments

Tolerability and safety evaluations were based on measurements of vital signs, physical examination, ECG, recording of all adverse events, and haematology and blood chemistry analysis.

Vital signs (blood pressure and pulse rate) were measured with Philips C3 Patient Monitor (Philips Medical System, 3000 Minuteman Road, Andover, MA 01810 USA). A 12 lead ECG (Cardio Plug, Cardionics S.A., Rue G. Petitstraat 4, 1080 Brussels, Belgium) was performed and evaluated for each patient at day 0, day 3, and day 28.

Adverse events were defined as any symptom, physical sign, syndrome or disease, which either occurred during the study, having been absent at baseline, or, if present at baseline, appeared to worsen. This was regardless of the suspected cause of the event. Adverse events, either reported spontaneously by the patient or discovered as a result of general questioning or by physical examination by the investigator, were recorded in the adverse event form. Each adverse event was described by its duration (start and end dates), its frequency (single episode, intermittent, continuous), and its intensity (mild, moderate, severe, fatal). For each adverse event the cause (the underlying study indication, a coexisting disease, the study medication, or

others), the relationship to the study medication (unrelated, unlikely, possibly, probably or definitely related), and the impact on the course of the study medication were assessed (stop of study medication).

Serious adverse events were adverse events considered fatal or life threatening, requiring hospitalization or lengthening hospitalization, resulting in persistent or significant disability or incapacity, constituting a congenital abnormality or a birth defect, or considered medically significant.

Blood samples for haematological parameters (haemoglobin, haematocrit, red blood cell count, total and differential white blood cell count, and platelets) were taken in S-Monovette® 1.2ml K3E containing 1.6mg EDTA/ml blood (Sarstedt, 51588 Nümbrecht, Germany) and analyzed by CellDyn 3000 (ABBOT).

Blood samples for chemistry parameters (total bilirubin, alkaline phosphatase, SGOT (ASAT), SGPT (ALAT), glucose, creatinine, triglycerides, and cholesterol) were taken in S-Monovette® 2.7ml Z containing clot activator (Sarstedt, 51588 Nümbrecht, Germany), centrifuged 2000 x g for 10 minutes and serum evaluated by COBAS MIRA plus (Roche).

According to the predefined norm ranges and the clinical findings, the measured values were classified as i) normal, ii) abnormal but not clinically significant or iii) abnormal and clinically significant. All values classified as abnormal and clinically significant were reported as adverse events.

At the end of the study the investigator rated the global tolerability, defined as the overall profile of adverse drug reactions of the study treatment, by using a 4-point scale for each participant: very good, good, moderate, and poor. Similarly, conclusions on safety, referring to potentially hazardous adverse drug reactions, were made at the end of study.

3.4.4 Parasitological Examinations

Thick blood films to investigate parasitaemia and gametocytaemia of *Plasmodium falciparum* were done at predefined time points. Parasitaemia and gametocytaemia were evaluated according to the Lambaréné method [62].

Ten microliters of blood were evenly distributed on a 10 x 18 mm area of microscope slide (drawn on a paper underneath the slide) with a micropipette (Karl Roth GmbH, Karlsruhe Germany). The slides were dried, stained with 20% Giemsa (Colorant de Giemsa R en solution, Réactifs Ral, 33650 Martillac, France) for 20 minutes at pH 7.2 and dried again. Afterwards the slides were read with immersion oil on a standard microscope (Olympos CH 30) at x1000 magnification. Thus each microscope field of the thick smear is ~1/600th of a microliter. Parasites were counted in 100 fields if there were $\leq$ 5 parasites per field, in 30 fields if there were 5-50 parasites per field and in 20 fields if there were > 50 parasites per field. If there were no parasites in 100 fields the slide was considered negative. The counted parasites were divided by the number of counted fields and then multiplied by 600 in order to have the parasitaemia per microliter blood. Every slide was read twice by two different microscopists. A third reading was performed if the two counts differed more than 1.5 fold from each other in parasitaemias higher than 1000, or differed by more than 100 parasites in parasitaemias lower than 1000 parasites per microliter. The final parasitaemia was calculated as median of two matching readings. The same procedures were followed for gametocytaemia.

For all patients some blood drops were taken onto filter paper (Whatman®, Whatman International Ltd., Maidstone England) at day 0, day 28 for potential PCR analysis. In case of treatment failure the PCR analysis allows to differentiate recrudescence from re-infection.

3.5 Data Management

The investigators entered all relevant data into the paper case record forms. The field monitor reviewed the case report forms for completeness and accuracy. One copy was sent to the medical data management staff (Swiss Pharma Contract, Lettenweg 118, CH-4123 Allschwil, Switzerland) for further data processing. Data items from the case record form were entered into the study database using double data entry with electronic verification. The database was systematically checked by Data Management staff, using error message printed from validation programs and database listings. Obvious corrections were corrected by the Data Management staff according to the obvious correction document. Other errors or omissions were entered on Data Query Forms, which were returned to the investigational site for resolution.

Concomitant medication entered in the database was coded using the ATC (Anatomical Therapeutic Chemical) dictionary. Coexistent diseases and adverse events were coded using MedDRA dictionary (Medical Dictionary for Regulatory Activities).

The investigator maintains source data, trial documents, and records for at least 15 years or until written permission by the sponsor.

3.6 Statistics

The power calculation was based on the primary efficacy endpoint, the 28-day cure rate: proportion of patients with clearance of asexual parasitaemia within 7 days of initiation of study treatment. A success rate of 95% was expected. If an unexpected high proportion of patients would not be available for follow up, the power calculation was done in addition for a lower cure rate (i.e. 90%). A success rate of 80% or lower would be considered not appropriate.

A success for 63 out of 70 patients, 36 out of 40 patients, and 26 out of 30 patients (or more), respectively, would be consistent with the assumption of a

95% cure rate at a 5% error rate (one-sided). If in addition 5% of the patients would be lost for follow up, a success for 59 out of 70 patients, 33 out of 40 patients, and 24 out of 30 patients (or more), respectively, would be consistent with the assumption of a 90% cure rate at a 5% error rate (one-sided).

Two populations were defined. The per protocol population included all patients having received at least 1 dose of study medication and who in addition did not violate the protocol. Efficacy parameters were evaluated for the per protocol population of both treatment groups.

The intention to treat population included all patients having received at least 1 dose of study medication. All safety parameters and the day 28 and day 14 cure rates were evaluated for this population of both treatment groups.

Summary descriptive statistics for all background variables, secondary efficacy parameters, safety and tolerability evaluations, and acceptability were done separately for each treatment group. Median, arithmetic mean, standard deviation, maximum and minimum values, and confidence intervals were calculated with JMP version 5.0 (SAS Institute, Cary, NC, USA).

3.7 Ethics and Good Clinical Practice (GCP)

Before study implementation the protocol and the informed consent were reviewed and approved on October 10, 2005, by the ethics committee of the International Foundation of the Albert Schweitzer Hospital in Lambaréné. The study was carried out in compliance to the protocol, in accordance with the Declaration of Helsinki and in adherence to the Good Clinical Practice Guidelines [64].

3.8 Amendments

After the occurrence of isolated and transient hypertriglyceridaemias at the beginning of the study, the sponsor and the investigators decided to include the measurements of total cholesterol and triglycerides as part of the standard laboratory safety evaluations. This amendment was reviewed and approved by the ethics committee of the International Foundation of the Albert Schweitzer Hospital in Lambaréné.

4 Results

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## 4.1 Demographic and Baseline Characteristics

A total of 71 Gabonese children suffering from uncomplicated _Plasmodium falciparum_ malaria were enrolled in the study, 41 children with a body weight of 10 to 20 kg in treatment group A and 30 children with a body weight of >20 to 40 kg in treatment group B. The study finally comprised 1 patient more in group A than designated by the protocol due to an initial error of documentation. 40 patients were included in the trial at study center 1, Centre Hospitalier Libreville (24 in group A and 16 in group B) and 31 patients at study center 2, Medical Research Unit in Lambaréné (17 in group A and 14 in group B). All patients fulfilled the respective inclusion and exclusion criteria.

No patient was lost to follow up during the four week observation after study treatment, thus all enrolled patients completed the study. One patient previously enrolled in group A was withdrawn due to the development of severe malaria with convulsions. The study medication was stopped after first drug administration and the patient received rescue medication. Another patient in group A was withdrawn due to vomiting of the study drug twice within 1 hour on day 2. Study medication was stopped and the patient was successfully treated with intravenous quinine as rescue medication. One patient allocated to group B was excluded because he received rescue medication following a misinterpretation of a blood smear on day 7.

Due to the exclusion of these three patients the per protocol population comprised of 68 patients (39 patients in group A, 29 patients in group B) and the intention to treat population comprised of 71 patients (Figure 1).

Figure 1: Study Flow
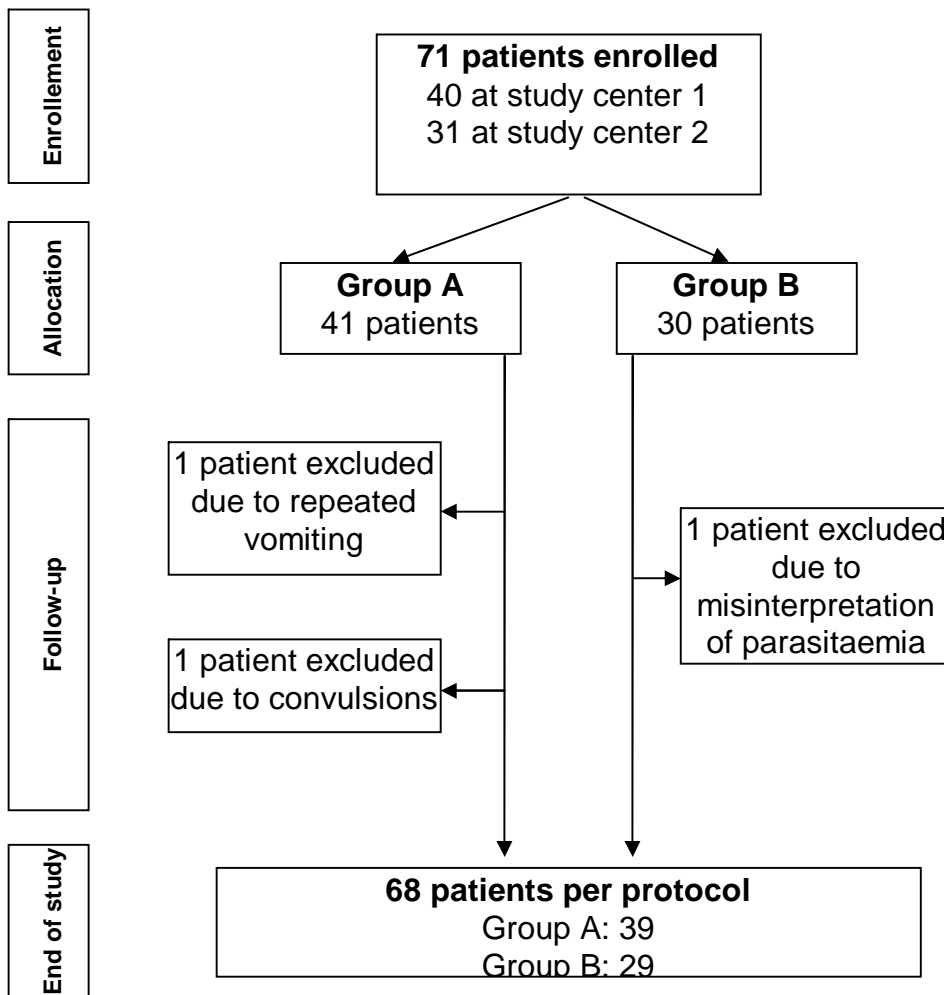
Patients were assigned to the respective treatment group according to body weight. Therefore baseline characteristics for weight, height and age were different in the two treatment groups (Table 2).

Baseline parasitaemia was comparable in the two treatment groups A and B (median 37800 and 36400, respectively, (Table 2)). However, a higher rate of patients with high parasite counts (>150000 asexual forms per microliter of blood) was observed in group A (9 patients in group A and 4 patients in group B).

Fever (oral temperature (T) $\geq$ 37.5°C) was present at baseline examination in 72% and 66% of patients in group A and B, respectively. The median temperature was 39°C in both treatment groups (Table 2).

Table 2: Baseline characteristics (sex, age, weight, height, parasitaemia, fever) in treatment group A and B

		Treatment Group	
		A (n=41)	**B** (n=30)
Sex	Female n (%)	15 (37)	14 (47)
	Male n (%)	26 (63)	16 (53)
Age (years)	Mean (SD)	3.8 (1.6)	8.7 (2.2)
Weight (kg)	Mean (SD)	14.8 (2.4)	26.5 (5.6)
Height (cm)	Mean (SD)	100 (11)	132 (12)
Parasitaemia (asexual forms/µl)	Min - Max	1000 - 249700	2466 - 221550
	Median	37800	36400
Patients with fever at baseline	n (%)	30 (75)*	19 (66)*
Fever (T $\geq$ 37.5°C) (°C)	Min - Max	37.6 – 41.0	37.5 – 40.2
	Median	39.0	39.1

SD = Standard Deviation
*one value missing: 40/41 and 29/30 in group A and B, respectively

Each patient was assessed for haematology and biochemistry, vital signs, physical examination, and ECG in order to evaluate inclusion and exclusion criteria and to have reference values for evaluation of safety during the course of the study.

At baseline haematology and blood chemistry values were representative for children suffering from acute uncomplicated *Plasmodium falciparum* malaria (Table 6). Mean haemoglobin levels and platelets were below the normal ranges (haemoglobin: 9.1g/dl and 10.4g/dl and platelets: 177000/µl and 195000/µl in group A and B, respectively); total bilirubin was within the normal ranges (13.4µmol/l and 11.4µmol/l in group A and B respectively).

However, for 7 patients at least 1 laboratory finding was judged as clinically significant. Four patients (3 group A patients and 1 group B patient) showed low values for haemoglobin, haematocrit, and red blood cell count, interpreted as clinically significant anaemia (haemoglobin: 9.1g/dl, 7.9g/dl, 7.8g/dl and 7.6g/dl; haematocrit: 26.7%, 24.5%, 23.3% and 21.4%; red blood cell count: 3.6Mio/µl, 3.0Mio/µl, 4.1 Mio/µl and 3.1Mio/µl, respectively). One patient in group A presented with elevated liver enzymes judged as clinically significant (SGOT 132UI/l and SGPT 49UI/l) and another patient in group A presented with elevated triglyceride values at baseline judged as clinically significant (triglycerides 3.45mmol/l).

None of the patients had a significant medical history at inclusion although concomitant infections were common. Such infections were acrodermatitis, herpes simplex, schistosomiasis, tinea infection, upper respiratory tract infection, and filiariasis.

4.2 Efficacy

Patients in treatment group A received the new paediatric stickpack formulation with a mean dosage of artesunate-mefloquine of 3.4 (range: 2.6 – 4.9) mg/kg and 10.1 (range: 7.9 – 14.7) mg/kg, respectively. In treatment group B patients were treated with tablets packed as co-blister formulation with a mean dosage of artesunate-mefloquine of 3.8 (range: 2.5 – 4.9) mg/kg and 9.4 (range: 6.3 - 12.3) mg/kg, respectively.

Efficacy measures were evaluated for the per protocol population and the intention to treat population and are summarized in Table 3.

Treatment acted rapidly on *Plasmodium falciparum* parasitaemia. The median parasite clearance time was 36 hours in both groups. Mean parasite clearance time was 3 hours shorter in treatment group B than in treatment group A (mean (SD): 34 (13) hours 31 (9) hours, respectively).

Similarly, fever resolved quickly in both treatment groups. Fever clearance time was evaluated only in patients presenting with fever (temperature $\geq$ 37.5°C) at baseline. The median fever clearance time was approximately twice as long in group A as in group B with 23 hours in group A and 12 hours in group B.

The cure rate at day 28 served as primary outcome measure of efficacy in this clinical trial. In the per protocol population all patients (68/71) were clinically and parasitologically cured on day 28 (clearance of asexual parasitaemia within 7 days and without subsequent recrudescence within 28 days). Similarly, the day 14 cure rate was 100% for the per protocol population in both treatment groups.

Cure rates were also calculated for the intention to treat population employing an extreme case scenario. All patients who received at least 1 dose of the study drug and who were not evaluable on day 28 were considered as treatment failures. Respective cure rates at day 28 and 14 were 95% in group A and 97% in group B.

Table 3: Primary and secondary efficacy measures of artesunate-mefloquine therapy in group A (10-20kg bodyweight) and group B (>20-40kg bodyweight):

		Treatment Group	
		A (n = 39)	**B** (n = 29)
Per protocol population	28-day cure rate, 95 CI (%)	100 (91-100)	100 (88-100)
	14-day cure rate, 95 CI (%)	100 (91-100)	100 (88-100)
Intention to treat population	28-day cure rate, 95 CI (%)	95 (91-100)	97 (88-100)
	14-day cure rate, 95 CI (%)	95 (91-100)	97 (88-100)
PCT (hours)	Min - max	12 – 67	12 – 48
	Mean (SD)	34 (13)	31 (9)
	Median	36	36
FCT (hours)	Min - max	12 – 60	12 – 37
	Mean (SD)	22 (12)	19 (8)
	Median	23	12

CI = Confidence Interval, SD = Standard Deviation, PCT= parasite clearance time, FCT= fever clearance time

4.3 Tolerability and Safety

Adequate tolerability and safety evaluations in the clinical development are fundamental prior to registration of pharmaceutical products. Tolerability defines the overall profile of adverse drug reactions, whereas safety refers to potentially hazardous adverse drug reactions. In this trial tolerability and safety evaluations were based on measurement of vital signs, physical examination, ECG, recording of all adverse events, and haematology and blood chemistry analysis. All tolerability and safety analysis were performed for the intention to treat population.

Values of vital signs (blood pressure and pulse rate) evaluated during the study course were within the expected ranges. Blood pressure and pulse rate decreased in parallel to fever clearance from day 2 onwards, reflecting the recovery from malaria infection (mean systolic/diastolic blood pressure (mmHg) at baseline: 104.8/75.2 and 108.0/74.2, day 2: 101.4/72.0 and 106.4/74.2 and day 28: 99.9/66.7 and 104.1/69.3; pulse rate (beats per minute) at baseline: 108 and 94, day 2: 100 and 88 and day 28: 94 and 84 for group A and B, respectively).

None of the patients had a clinically significant ECG finding at baseline or during follow up.

Adverse events were all newly occurring untoward medical findings during treatment and follow up period. Approximately two third (70%) of the patients experienced at least 1 adverse event during the study. The incidence of adverse events for each treatment group and the most frequently experienced adverse events are listed below in Table 4.

Table 4: Rate of patients experiencing at least 1 adverse event (n (%)) and rate of most frequent adverse events (> 5% in any group) (n)

	Treatment Group	
	A (n=41)	**B** (n=30)
Patients with at least 1 adverse event	28 (68)	22 (73)
Cough	9	6
Pyrexia	7	6
Vomiting	5	7
Headache	4	8
Intestinal helminths	0	10
Diarrhea	6	2
Abdominal pain	1	7
Dizziness	1	5
Hypertriglyceridaemia	5	0
Anaemia	2	2
Eosinophilia	2	2

Other adverse events occurred with a frequency of less than 5 % per group and are summarized here: constipation, nausea, hyperpyrexia, abscess, bronchitis, fungal skin infection, giardiasis, nasopharyngitis, rhinitis, schistosomiasis, acrodermatitis, filiariasis, furuncle, herpes simplex, hookworm infection, strongyloidiasis, urinary tract infection, hand fracture, alkaline phosphatase increased, cardiac murmur, lipids increased, haemoglobin decreased, arthralgia, convulsion, leucocyuria, dysuria, vaginal haemorrhage, tachypnoea, rhinorrhoea, tinnitus, vertigo, depression, erythema, pruritus, and rash.

The majority of adverse events was attributable to the underlying or to concomitant diseases and only a small proportion was judged to be in a causal relation with the study drug. 25 (35%) of the patients experienced at least 1 adverse event judged as related to study medication. No adverse event was of "definite" relation to study drug, 17 of the 25 patients experienced at least 1 adverse event of "possible" relation to study drug, the other 8 patients experienced at least 1 adverse event of "probable" relation to study drug. The most common adverse events judged drug-related were gastrointestinal disorders (vomiting (10), abdominal pain (5), and diarrhea (1)), nervous system

disorders (dizziness (5) and headache (4)), and hypertriglyceridaemia (5). The incidence of study drug related adverse events for each treatment group is listed below in Table 5.

Table 5: Rate of patients with at least 1 drug-related adverse event (n (%)) and rate of drug-related adverse events (n)

	Treatment group	
	A (n = 41)	**B** (n = 30)
Patients with at least 1 adverse event related to study medication	10 (24)	15 (50)
Adverse events related to study medication	12	26
Vomiting	4	6
Abdominal pain	0	5
Dizziness	0	5
Hypertriglyceridaemia	5	0
Headache	0	4
Alkaline phosphatase increased	2	1
Diarrhea	1	0
Anorexia	0	1
Tinnitus	0	1
Pruritus	0	1
Depression	0	1
Vertigo	0	1

One serious adverse event occurred in the course of the study. The patient was allocated to treatment group A and showed good treatment response to the study drugs. However, on day 15 the patient required hospitalization due to a traumatic fracture of digitus II. The patient was operated at the department of surgery at the Albert Schweitzer Hospital in Lambaréné and resolved without difficulties. This serious adverse event was judged as "severe" in intensity and it was not related to the study medication.

The majority of the adverse events were of mild intensity. Less than one fifth (16%) of the reported adverse events were of moderate intensity. Most common adverse events of moderate severity were fever (3 in group A and 1 in

group B), abdominal pain (4 in group B), vomiting (2 in group B), headache (2 in group B), and hypertriglyceridaemia (2 in group A). Other adverse events of moderate intensity (infections, respiratory disorders, anaemia, high SGOT and SGPT and hypereosinophilia) occurred only in 1 patient each.

Nine adverse events of moderate severity were judged drug-related; these were abdominal pain (4 in group B), hypertriglyceridaemia of > 9 mmol/l and 3.8 mmol/l on day 3 (2 in group A), vomiting (1 in group B), headache (1 in group B), and elevated SGOT with 271UI/l and SGPT with 197 UI/l (1 in group A). One patient allocated to group A showed convulsions on day 2. This was judged as an adverse event of severe intensity and treated accordingly. Convulsions were judged to be associated to the underlying *Plasmodium falciparum* infection and not related to the study drugs.

Haematology and biochemistry evaluations showed values within the expected range for children suffering and recovering from malarial infection (Table 6).

Red blood cell count, haemoglobin, and haematocrit values decreased slightly from baseline to day 3 and increased thereafter until day 28, reaching higher values than at baseline (haemoglobin baseline: 9.1g/dl and 10.4g/dl, day 3: 8.6 g/dl and 9.7g/dl, day 28: 10.6g/dl and 11.1g/dl in group A and B, respectively). Mean platelet values were within the normal range already on day 3 measurements (219000/µl and 232000/µl in group A and B, respectively). Compared to baseline values mean reticulocyte counts were lower at day 28 measurements (baseline: 2.5% and 2.0%, day 28: 1.3% and 1.2% in group A and B, respectively). While mean values of white blood cell counts did not show major changes, mean values for eosinophils and lymphocytes rose until day 28 and mean values for neutrophils, monocytes, and basophiles decreased until day 28.

Clinical chemistry was measured at baseline, day 3 and 28. Measured mean values of bilirubin, creatinine, glucose, SGOT, SGPT, and triglycerides were lower at the end of study than at inclusion. Mean cholesterol values increased on day 28 compared to mean baseline values. Abnormal laboratory values

were classified with regards to their clinical significance. Clinically significant findings were coded as adverse events and are described above (eosinophilia, hypertriglyceridaemia, high SGOT and SGPT, and low haemoglobin, haematocrit, and red blood cell count as anaemia). At the end of follow up no abnormal laboratory value was classified as clinically significant.

Table 6: Haematology and biochemistry values (mean (standard deviation) at baseline and day 3 and day 28 after treatment for group A and B (intention to treat population)

	Treatment Group					
	A			B		
	Day 0	Day 3	Day 28	Day 0	Day 3	Day 28
Red blood cells (Mio/µl)	3.93 (0.56)	3.71 (0.52)	4.48 (0.45)	4.21 (0.55)	3.88 (0.52)	4.39 (0.47)
Haemoglobin (g/dl)	9.1 (1.1)	8.6 (1.1)	10.6 (0.8)	10.4 (1.2)	9.7 (1.3)	11.1 (1.0)
Haematocrit (%)	28.1 (3.3)	26.3 (3.4)	31.6 (2.5)	31.6 (3.7)	29.3 (3.8)	32.8 (3.5)
White blood cells (T/µl)	7.2 (2.7)	7.3 (2.4)	8.4 (3.6)	7.3 (2.9)	5.4 (2.3)	7.2 (3.4)
Platelets (T/µl)	177 (119)	219 (100)	323 (96)	195 (115)	232 (136)	258 (71)
Eosinophils (%)	3.4 (5.4)	8.0 (10.4)	12.3 (13.6)	6.3 (12.8)	11.7 (14.9)	11.2 (11.7)
Lymphocytes (%)	34 (15)	54 (14)	50 (16)	28 (10)	48 (13)	50 (13)
Monocytes (%)	6.6 (5.1)	6.4 (4.6)	5.5 (5.5)	6.7 (6.3)	6.6 (4.8)	5.7 (3.9)
Neutrophils (%)	53 (17)	31 (13)	31 (9)	56 (20)	32 (13)	30 (11)
Basophils (%)	1.2 (4.2)	0.6 (1.1)	0.2 (0.4)	1.6 (4.8)	1.1 (2.8)	0.3 (0.6)
Reticulocytes (%)	2.5 (1.8)	2.9 (2.6)	1.3 (1.0)	2.0 (1.8)	1.5 (0.9)	1.2 (1.0)
Alkaline phosphatase (U/l)	330 (155)	303 (155)	424 (193)	402 (217)	338 (171)	243 (222)
Bilirubin total (µmol/l)	13.4 (15.8)	5.1 (3.7)	4.9 (2.5)	11.4 (8.5)	6.6 (4.1)	7.2 (2.6)
Creatinine (µmol/l)	40.8 (13.6)	45.0 (19.1)	36.7 (10.5)	52.2 (14.8)	52.4 (24.3)	48.1 (10.5)
Glucose (mmol/l)	4.8 (1.4)	4.5 (1.1)	4.6 (0.8)	4.7 (1.2)	4.5 (1.1)	4.3 (1.0)
SGOT (ASAT) (U/l)	56 (39)	51 (41)	35 (16)	47 (30)	38 (16)	31 (11)
SGPT (ALAT) (U/l)	32 (34)	28 (33)	17 (10)	22 (9)	22 (10)	17 (8)
Total Cholesterol (mg/dl)	2.6 (0.6)	3.4 (1.0)	4.1 (1.0)	2.8 (0.7)	3.0 (0.9)	3.8 (0.9)
Triglycerides (mg/dl)	1.8 (0.9)	2.6 (1.8)	0.8 (0.3)	1.1 (0.7)	1.5 (1.0)	0.8 (0.4)

Global tolerability was rated for each patient by the investigator at the end of study. Rating was done according to observed adverse events using a standard 4-point scale: very good, good, moderate, poor. All but 3 patients were given very good (52) or good (16) tolerability. One group B patient was given moderate tolerability because he experienced abdominal pain and vomiting judged as study drug related on day 2. One group A patient was given poor tolerability because of repeated vomiting after second study drug administration, this patient was withdrawn from the study and successfully treated with intravenous quinine as rescue medication. For the patient withdrawn due to convulsions on day 2 tolerability was not assessed.

Tolerability of both treatment groups did not show major differences (very good tolerability was 71% and 77% and good tolerability was 27% and 20% in group A and B, respectively). In summary, the global tolerability was therefore judged very good.

According to the character and severity of occurred drug-related adverse events in this trial, both treatments can be judged as safe. No serious adverse events were related to the study drug. No drug-related adverse event was of severe intensity. Most adverse events related to the study drug resolved without further medical intervention.

4.4 Acceptability

Acceptability of the drugs was assessed for the two different drug formulations. Major criteria were ability to swallow study drugs, compliance, appreciation, and satisfaction of the patients in the two different age groups. Intake of study medication was assessed for each patient and each drug administration and was rated by the investigators as excellent, good, fair or poor.

Around 80% of the drug administrations were rated as excellent and around 10% as good. In treatment group A, the group with younger patients, the

acceptability was lower than in group B. Altogether 13 drug intakes were rated "fair", reasons for this judgment were refusal and disapproval by the patients, 1 group B patient had difficulties in swallowing the mefloquine tablet. One drug intake was rated "poor", this rating was given to the patient withdrawn due to repeated vomiting. Acceptability results are presented per treatment group and day of administration in Table 7.

Table 7: Acceptability ratings per treatment group and day of administration (n (%))

		Treatment group	
		A	B
Day 1	Excellent	32 (78)	27 (90)
	Good	4 (10)	2 (7)
	Fair	5 (12)	1 (3)
Day 2	Excellent	27 (66)	28 (93)
	Good	8 (20)	2 (7)
	Fair	3 (7)	0
	Poor	2 (5)	0
	NA*	1 (2)	0
Day 3	Excellent	29 (71)	29 (97)
	Good	7 (17)	0
	Fair	3 (7)	1 (3)
	NA*	2 (5)	0

*NA = not applicable

4.5 Gametocytes

Gametocytaemia at baseline and evolution or newly occurring gametocytaemia was analyzed for all patients throughout the study course.

In the per protocol population 21 (31%) patients (group A 14 (36%) patients, group B 7 (24%) patients) showed gametocytes in at least 1 slide during the course of the study. 13 of these 21 patients showed gametocytes already at pre-dose evaluation, the other 8 patients developed gametocytaemia only after antimalarial treatment was started. Gametocytaemia at baseline as well as gametocyte reduction rates (percentage of patients cleared from gametocytes)

were evaluated for day 7, 14, 21 and 28 for the patients showing gametocytes already at baseline (Table 8).

Table 8: Rate of patients with gametocytes at baseline, baseline gametocytaemia (defined as numbers of *Plasmodium falciparum* sexual forms per microliter of blood), and gametocyte reduction rates (percentage of patients cleared from gametocytes) (per protocol population)

		Treatment Group	
		A	**B**
Patients with gametocytes at baseline n (%)		9 (23)	4 (14)
Patients showing gametocytes only after treatment was started n (%)		5 (17)	3 (10)
Gametocytaemia (sexual forms/µl)*	Min - Max	7 - 200	6 - 335
	Median	28	7
Gametocyte reduction rate (%)*	Day 28	100	100
	Day 21	100	100
	Day 14	93	71
	Day 7	86	71

*evaluated in patients showing gametocytes already at baseline

5 Discussion

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Malaria remains a leading cause of morbidity and mortality worldwide. About 90% of the world's malaria deaths are estimated to occur in tropical Africa south of the Sahara, where the majority of infections is caused by *Plasmodium falciparum*.

Epidemiological evidence shows that malaria causes around 20% of deaths in children younger than 5 years of age in Africa. Young children and pregnant women are at greatest risk of malaria infection and death because of their lower levels of acquired immunity against malaria.

Yet malaria is a curable disease and effective medicines exist. Early diagnosis and prompt and effective treatment of malaria disease shortens its duration and prevents the development of complications and the vast majority of deaths from malaria. Important characteristics of an efficacious treatment are high schizontocidal and gametocyticidal activity, widespread accessibility, good tolerability, affordable price, short regimens and simple administration.

As a response to the worsening situation of antimalarial drug resistance situation, the World Health Organization now recommends that treatment of *falciparum* malaria in all countries experiencing resistance to monotherapies (such as chloroquine, sulfadoxine-pyrimethamine and amodiaquine) should consist of combination therapies, preferably those containing an artemisinin derivative (ACT – artemisinin-based combination therapy).

The advantages of artemisinin derivatives making them ideal combination partners are: rapid reduction of parasite densities, rapid resolution of clinical symptoms, effective action against multi-drug resistant *Plasmodium falciparum*, no documented resistance in vivo, few clinical adverse reactions and reduction of gametocyte carrier rate which may reduce transmission. Yet recrudescence is common when this drug and its derivatives are used in monotherapy.

To the deployment and use of artemisinin-based combination therapy, particularly in Africa, major challenges are limited experience with artemisinin-based combination therapy, operational obstacles to implementation (registration and marketing), reliable supply and drug quality, costs and affordability, and potential problems with adherence to co-administered (non-fixed) drug combinations, particularly at the household level. Therefore, evaluation of safety, efficacy, and effectiveness of artemisinin-based combination therapies in areas of high malaria transmission became one of the high priority issues for operational research by the TDR Scientific Working Group Malaria (WHO Special Programme for Research and Training in Tropical Diseases) [65].

Up to date the antimalarial treatment combination of artesunate-mefloquine has been studied extensively in South East Asia. However, limited data are available for Africa whereas the situation is even worse with regards to African children. The novel paediatric formulation of artesunate-mefloquine allows for the first time the evaluation of oral artesunate-mefloquine treatment in very young African children normally unable to swallow properly treatment in form of tablets.

Baseline

This study comprised 71 patients, all of them Gabonese children between the age of 1 and 13 years. This target age group matches with the World Health Organization's recommendations to emphasize treatment evaluations in young children [65]. The rational behind this requirement is that, even in populations with low acquired immunity, younger children often have a less favorable response to antimalarial drugs than older children and adults.

Follow up rates in our clinical trial were excellent as all included children completed the study and could be analyzed for safety and tolerability over the whole study period. Three patients were, however, withdrawn from the per

protocol population; per protocol efficacy analysis was therefore done for 68 participants.

As the patients were stratified to the treatment groups by body weight (group A: 10-20 kg, group B: 20-40 kg), the distribution of background characteristics was not comparable between the two groups. This resulted in different mean, minimal, and maximal values of age, body weight, and height between the two treatment groups. Hence, parts of the results cannot be compared directly by groups and have always to be considered in relation to the different background characteristics, especially in relation to the age of the patients.

Efficacy

The younger patients included in this study and allocated to treatment group A received the new paediatric stickpack formulation with a mean dosage of artesunate-mefloquine of 3.4 mg/kg and 10.1 mg/kg, respectively. The older patients assigned to treatment group B were treated with tablets packed as co-blister formulation with a mean dosage of artesunate-mefloquine of 3.8 mg/kg and 9.4 mg/kg, respectively. In both groups administration was once daily for 3 days in 24 hour intervals.

Day 28 cure rate served as the primary outcome measure of this study. Results for the 28-day cure rate were highly satisfying with 100% for both treatment groups in the per protocol analysis. Similarly, the 14-day cure rate, as a secondary efficacy objective, was equally high in the per protocol population of both treatment groups (100%). In the intention to treat analysis the cure rates for day 28 and day 14 were lower with 95% and 97% in group A and B, respectively, on day 28 and 14. But even in this extreme case scenario, where all not evaluable patients are defined as treatment failures, the cure rates exceeded the expected cure rate of 95%.

One patient was excluded from the per protocol analysis due to convulsions on day 2. According to the World Health Organization's classification of response to treatment, this patient meets the criteria for early treatment failure as

convulsions are judged "development of danger signs or severe malaria" occurring between day 1 and 3 in the presence of parasitaemia [65]. Hence, the criteria for adequate clinical and parasitological response (absence of parasitaemia on day 14 without meeting any of the criteria of early treatment failure or late clinical/late parasitological failure) are not fulfilled by this patient [65].

The cure rates of this trial are in line with those of previous studies evaluating oral artesunate-mefloquine treatments. Studies assessing the same combination were performed in South America, Asia, and Africa in adults and children and reached cure rates of 89% to 100% [37, 42, 44-47, 66-77]. Efficacy of several artemisinin combination therapies, with other than artesunate-mefloquine compounds, have been assessed in clinical trials. Failure rates of most of these combination therapies were higher compared to failure rates with artesunate-mefloquine therapy.

For artesunate-sulfadoxine-pyrimethamine combination the day 28 cure rates from a trial in children from the Democratic Republic of Congo were 80% [78]. In a study in 2000 in The Gambia, children were treated with artesunate-sulfadoxine-pyrimethamine, the 14-day cure rates were 96% in the one-dose artesunate group and 98% in the 3-dose-artesunate group [79]. Another study in Mozambique achieved a 100% cure rate on day 14 for artesunate-sulfadoxine-pyrimethamine [80]. In both studies cure rates for later timepoints were not analyzed but one may assume that by day 28 cure rates would have decreased as in a former study in Mozambique 21% of the parasite strains were found to be resistant to sulfadoxine-pyrimethamine on day 14 [80].

The combination artesunate and amodiaquine was earlier studied in children from Kenya, Senegal, and Gabon [81]. The cure rates were at acceptable levels on day 14: 91%, 93%, and 98%, respectively, but decreased significantly on day 28: 68%, 82%, and 85%, respectively. In Tanzania and the Democratic Republic of Congo the 28-day cure rate for artesunate-amodiaquine was 89% and 80%, respectively [78, 82].

Another study compared the efficacy of artesunate-trimethoprim to artesunate-mefloquine; both treatments yielded a 28-day cure rate of 97% [83].

In Guinea Bissau co-administration of chloroquine-artesunate reached a 28-day cure rate of 73% [25].

Lumefantrine (benflumetol) was frequently studied in combination with artemether and is the only artemisinin-based combination registered in accordance with internationally recognized guidelines. In a trial by van Vugt in 1998 in Thailand, the 63-day cure rate of artesunate-mefloquine treatment was significantly higher (94%) than the cure rate of the compared treatment artemether-benflumetol (81%) [68]. Artemether-benflumetol was also assessed by Looareesuwan in 1999 but the 28-day cure rates were even lower with 69% [84]. In 2001 Lefevre et al. published data from a study in Thailand comparing artesunate-mefloquine to artemether-lumefantrine with high 28-day cure rates of 100% and 96%, respectively [85]. Artemether-lumefantrine was compared to artesunate-mefloquine in Laos, the 42-day cure rates were 94% and 100%, respectively [71]. This same combination achieved a 28-day cure rate of 97% in a study by Mutabingwa et al. in Tanzania [82], and a 42-day cure rate of 97% in Bangladesh [73].

In 2005 Krudsood et al. assessed artesunate-chlorproguanil-dapsone combination in Thai adults and the 28-day cure rate was 77% [86]. Dihydroartemisinin-piperaquine was evaluated in Thailand, Laos, and Burma and all 28-day cure rates were high with 99%, 100% and 99%, respectively [72, 76, 77].

In conclusion, efficacy is often reduced in the artemisinin combinations with partner drugs such as amodiaquine, chloroquine, and sulfadoxine-pyrimethamine. For these drugs resistance is emerging or already widespread and combination with short course administration of artemisinin derivatives does not provide satisfying cure rates and sustained parasite clearance. Artesunate-lumefantrine generally proves low failure rates and is therefore successfully used. Less common partners for artemisinin combinations are

chlorproguanil-dapsone and piperaquine, both proved good efficacy in combination with artemisinin derivatives. However, chlorproguanil-dapsone might show a rather unfavorable safety profile. Piperaquine, as a new combination partner, appears promising and further evaluations are ongoing.

With regards to efficacy, the observed cure rate results of this trial confirm the rational of combining artesunate with mefloquine, two antimalarial drugs with different modes of action. Thus the high potency of artesunate, which might be compromised by its short half-life, is counterbalanced by mefloquine, which has a long half-life and eliminates the remaining parasites with therapeutic concentrations.

One potential limitation of the efficacy analysis of our trial might be the duration of follow up. Recommended minimum length of follow up in areas of intense transmission is dependent on the treatment drugs. The most suitable duration of follow up for mefloquine is 63 days because of its long half-life [65]. Studies with shorter assessment periods may underestimate the true rates of treatment failure as blood levels of mefloquine might be still in therapeutic ranges before day 63. Evaluation of the pharmacokinetic sub-study in this trial confirmed this with blood mefloquine levels within the therapeutic range over a three week period (data not shown).

For both treatment groups parasite clearance times were short with means of 34 and 31 hours in group A and B, respectively. The median parasite clearance time of 36 hours is similar in both groups. These parasite clearance times are shorter than in other trials with adult patients [45, 46]. The slightly delayed mean parasite clearance time of 3 hours in group A could be explained by the presence of more patients with high parasite counts (> 150000 asexual forms/µl) in group A (21%) than in group B (14%).

Fever clearance times are clinically satisfactory in both treatment groups with means of 22 and 18 hours in group A and B, respectively. Mean fever clearance time is shorter in group B than in group A where a small subgroup of patients with long lasting fever accounts for a prolonged fever clearance time of

3h. The observed difference might be due to the fact that group B patients were older and older children are known to have lower body temperatures. The presence of more patients with high parasite counts (>150000/µ) in group A might have also played a role.

As no patient showed parasitaemia on day 2 higher than on day 0, nor parasitaemia on day 3 with temperature > 37.5°C, nor parasitaemia on day 3 > 25% of count on day 0, none of the patients met the World Health Organization criteria for early treatment failure with regards to parasite and fever clearance. Similarly, no World Health Organization criteria for late clinical failure (presence of parasitaemia on day 3 with development of severe malaria or presence of parasitaemia an temperature > 37.5°C on any day from day 4 to day 14) or late parasitological failure (presence of parasitaemia on day 14 and temperature < 37.5°C) was met by any patient [65]. However, the patient presenting convulsions met a criterion for early treatment failure because convulsions are judged a danger sign of severe malaria.

Gametocyte reduction rate (percentage of patients cleared from gametocytes) was 100% in both treatment groups on day 28 and day 21. At day 14 and day 7 gametocyte reduction rate was somewhat lower in group A, however mean gametocyte clearance time was shorter in group A. The gametocyte reduction rates confirm the good gametocyticidal effect of artemisinins leading to reduced infectivity to mosquitoes by gametocytes in peripheral blood [20, 87]. This gametocyticidal effect of arteminsinins is one of their proposed advantages compared to other drugs such as chloroquine or sulfadoxine-pyrimethamine known to induce gametocytogenesis [88-90]. However, transmission is only reduced but not prevented by artemisinin-based treatments [21] and some authors even claim that the gametocyticidal effect of antimalarials favor selection of resistant strains and therefore spread of resistance [91].

Tolerability and Safety

Antimalarial drug toxicity is one important factor in drug development. Drug-related toxicity and its risks must be balanced against the likely outcome of malaria treatment and the circumstances of clinical practice, causing – at the very least - less harm to the patients than the disease itself.

Clinical research tends to omit two important groups who are particularly vulnerable to malaria: very young children and pregnant women. However, not least due to safety and tolerability concerns, it is of major importance to encourage clinical research for these target groups. Randomized controlled trials are the best way to gather evidence on the effectiveness of a health intervention whereas open-label trials are subject to investigator bias especially regarding adverse events and their relationship to the study drugs. Regarding antimalarial drug tolerability, evaluation is complicated by the common malaria associated symptoms as fever, nausea, vomiting, abdominal pain, anaemia, and diarrhea – all of which could similarly be caused by the study drug. Additionally, the recent development of drug treatment policy changing from use of single agents to combination therapies implicates the question whether two drugs will be more toxic than one. This trial aimed to contribute to the knowledge on the use of artesunate-mefloquine combination therapy in African children.

Adverse events. In this trial tolerability and safety results showed appropriate profiles for both treatment formulations in their respective target population. Approximately two thirds of all patients experienced at least 1 adverse event. Most adverse events were of mild intensity and less than one fifth reached moderate intensity. Most adverse events could be attributed to concomitant diseases or to the underlying *falciparum* malaria infection. Approximately one third of the patients experienced 1 adverse event judged as possibly or probably related to study medication, no adverse event was judged as definitely related to the investigational drug. The most frequent drug-related adverse

events were vomiting, abdominal pain, dizziness, hypertriglyceridaemia, and headache.

As all patients were treated with a combination of the two different drugs, artesunate and mefloquine, the drug-related adverse events could either be induced separately by artesunate or mefloquine or by both. Most of the drug-related adverse events are more likely to be associated with mefloquine then to artesunate, the artemisinins generally show excellent tolerability. Ribeiro et al. reviewed published and unpublished studies of the artemisinin derivatives (n= 8844) in 1998 and reported no serious adverse events nor adverse events dependent of the artemisinins and their route of administration [92]. Further studies found substantially fewer adverse events in artemisinin regimes than in mefloquine containing regimes [93]; oral artesunate was well tolerated and there was no evidence for drug-related toxicity. In 2001 two cases of severe allergic reactions following oral artesunate were reported and the risk of developing an allergic reaction was estimated at 1 in 2833 [94]. Neurotoxicity is often questioned when safety of artemisinins is discussed because neurotoxic effects have been observed in preclinical and animal models after administration of high doses of artemisinin derivatives. Taylor et al. summarized literature of tolerability and safety of artemisinins and concluded that there are no data providing evidence that short course therapies with the artemisinins, either alone or in combination with mefloquine, are associated with neurotoxicity in man [95].

On the contrary to the artemisinins, drug toxicity of mefloquine is evident and drug-related adverse events have been numerously reported. Gastrointestinal and nervous system adverse events, similar to those experienced in this trial, have often been described after intake of mefloquine [32, 45-47, 96, 97]. Reviewing literature, mefloquine is still generally well tolerated by malaria patients [95]. However, in some clinical trials, mefloquine recipients reported a higher rate of certain adverse effects compared to chloroquine, halofantrine, and artemether-lumefantrine recipients [68, 84, 98, 99]. In a review of mefloquine treatment in 3673 patients of all ages on the Thai-Burmese border,

the most important adverse effect was early (<1 hour) drug-induced vomiting, followed by dose-related anorexia, nausea, late vomiting, and dizziness [100].

In our trial 1 patient (1%) experienced early vomiting. As he vomited twice the study drug on day 2 he was withdrawn from the per protocol analysis. This patient meets the following predispositions known for increased early vomiting following mefloquine intake: young age (< 6 years), temperature > 38°C and parasitaemia > 10000/µl at baseline. Several studies showed that the overall risk of vomiting is reduced by splitting up the mefloquine total dose of 25 mg/kg [95]. In our study the total dose of mefloquine was split into three administrations and the incidence of vomiting in treatment group A was 12%. This result is in line with other studies administering low-dose mefloquine (15mg/kg) in children aged 3-4 years of age showing a similar 12% incidence of vomiting [101]. However, in this trial the older patients of treatment group B had an elevated incidence of vomiting with 23%. In general the incidence of vomiting is reduced in older children, with a reported incidence in 5-6 year old children of 5% for low-dose mefloquine (15mg/kg) administration [101]. The high incidence of vomiting in the older patients of this trial might at least in part be attributed to the high rate (33%) of concomitant helminth infections compared to group A where none of the patients had a helminth infection reported.

Neuropsychiatric adverse events are often prematurely associated with mefloquine as its use as prophylactic treatment in travelers and soldiers is widespread and therefore frequently issue to the lay press. However, mefloquine remains acceptable to most patients as neuropsychiatric adverse events are mostly of mild or moderate severity and resolve completely [95]. In this trial dizziness was reported by 1 patient in group A and 5 participants in group B. Headache was observed in 4 and 8 patients in group A and B, respectively. In most studies headache was reported to be more malaria-related than drug-induced [95]. ter Kuile et al. found that high-dose (25mg/kg) mefloquine therapy produced a significant increase in moderate dizziness (feeling of swaying) from 2% to 6% and severe dizziness (inability to

walk unaided) from 0% to 3% in children between day 1 and 3 [101]. Corresponding figures for adults were 15% and 5% indicating that dizziness is more commonly reported in older patients. The difference of incidence of dizziness in the two treatment groups of our trial can therefore most likely be explained by the difference of age as moderate dizziness is a medical finding that needs to be reported by the patient and cannot easily be diagnosed by the physician.

In our study 1 patient in group A experienced 1 episode of convulsions on day 2 and recovered completely; this severe neuropsychiatric adverse event was judged as not drug-related. Dose-related serious neuropsychiatric toxicity of mefloquine has been reported in literature and essential points, summarized by Taylor et al., are: many (40%) neuropsychiatric adverse effects occur soon after the first dose and 75% are manifest by the third dose, most are of mild or moderate severity and resolve completely, some require medical management including hospitalization, < 2% of patients have sequelae and in prospective studies mefloquine-related neuropsychiatric adverse effects were broadly similar to other antimalarials or placebo [95]. As use of mefloquine for prophylaxis is widespread, many reports of tolerability and safety result from assessments of prophylactic treatment in healthy individuals. Considering these trials one has to keep in mind that the risk-benefit ratio is different for patients suffering from malaria.

In this trial drug-related abdominal pain was experienced by 1 and 7 patients in group A and B, respectively. In other trials these adverse events were closely related to malaria and not caused by mefloquine [95]. Again, the difference of incidence between the two treatment groups of this trial is most probably due to the difference of age and its related ability to express complaints.

Hypertriglyceridaemia. Five cases of hypertriglyceridaemia were reported as adverse events. However, considering triglyceride values regardless of classification as clinically significant or not, a noticeable observation occurs.

25 (35%) patients presented at least 1 triglyceride value above the normal range of 2.26mmol/l at baseline or day 3 measurement, while on day 28 triglyceride values of all patients were within the normal range. 13 (18%) patients had elevated triglycerides already at baseline but the values did not exceed 2-fold the upper limit of the normal range. On day 3 19 (27%) patients had elevated triglycerides, 17 were group A and 2 were group B patients. The highest values were measured exclusively in group A patients, with 4 values exceeding 2-fold the upper limit of the normal range, 2 values exceeding 2.5-fold the upper limit of the normal range and 1 value exceeding 3-fold the upper limit of the normal range.

Summarizing these findings it can be concluded that the incidence of elevated triglycerides differed by treatment group: in treatment group A the incidence of elevated triglycerides was 27% at baseline and 41% on day 3, in treatment group B the incidence was 7% on baseline and day 3. Values of triglycerides which were elevated at baseline were risen up higher on day 3 in all but 4 patients, the highest values were measured in group A patients and exceeded up to 3-fold the normal range.

The group of patients showing hypertriglyceridaemia was comparable with patients showing triglycerides within the normal range. However, baseline parasitaemia was significantly different: patients with hypertriglyceridaemia had a mean baseline parasitaemia of 97184/µl compared to patients with normal triglycerides with a mean baseline parasitaemia of 51604/µl. Patients exceeding at least 2-fold the normal triglyceride values had a mean baseline parasitaemia of 141471/µl.

In the past 25 years, dyslipidaemia, including hypertriglyceridaemia and hypocholesterolemia, have been reported as a result of malaria infection [102-110] but only few controlled studies have been published. Triglyceride levels have been compared between healthy individuals and patients suffering from uncomplicated and severe malaria in endemic regions or in returned travelers [103, 107, 110, 111]. In 1983 Onongbu et al. determined triglyceride levels in Nigerian patients infected by *Plasmodium falciparum* and found triglyceride

levels 2-fold increased in moderate and 3-fold increased in severe infection [103]. In India plasma triglycerides were measured significantly higher in severe than in the mild malaria [111, 112]. In 1996 in Sao Tome Island a similar pattern of plasma lipids was found in children; patients with mild or severe malaria had higher triglyceride levels than controls [107]. In 2002 a study by Faucher et al. depicted that low-level *Plasmodium falciparum* infections induce lipid parameter changes in Gabonese schoolchildren [108]. A retrospective study of returned travelers by Parola et al. in 2004 found that triglyceride levels were significantly higher in the malaria group than in the controls, and that triglyceride levels were also significantly higher in severe than in mild malaria [110]. In the latter study hypertriglyceridaemia was observed in all patients with severe malaria, compared to 37% of patients with mild disease. While the former studies did not find significant correlations between lipid plasma levels and parasitaemia or disease severity [102, 103, 107, 111], this last study significantly associated the magnitude of hypertriglyceridaemia with the severity of falciparum malaria for the first time [110].

According to actual data on hypertriglyceridaemia during *Plasmodium falciparum* infection explanations for the difference of triglyceride levels in the two treatment groups remain speculative. The young age, related to lower levels of immunity and the higher incidence of high parasitaemia in treatment group A indicate an increased immune response. Consequently the higher levels of triglycerides in treatment group A could be related to the severity of disease in analogy to results from former studies. Based on current available data it is unlikely that the hypertriglyceridaemias in group A are related to the study drug. Up to date there are no reports about an increase of triglycerides after intake of artesunate or mefloquine. To better differentiate between the biological changes related to *Plasmodium falciparum* infections and drug-induced changes, further controlled age-matched studies with triglyceride measurements under fasting conditions are needed.

Haematology and clinical chemistry. Haematological abnormalities were frequent on admission and consisted mostly of a decrease of haemoglobin, haematocrit, and red blood cell count, and an increase of reticulocyte count. These findings are expected changes induced by *Plasmodium falciparum* malaria. Today malarial anaemia is explained by the cytokine TNF (Tumor Necrosis Factor) induced dyserythropoesis and erythrophagocytosis. The former assumption that anaemia results from haemolysis of parasitized erythrocytes may only play a part in very high parasitaemia [113]. Elevated reticulocyte counts are associated with increased erythropoetin levels [114]. On day 3 red blood cell parameters were similar to baseline or slightly lower and by day 28 the red blood cell parameters were higher than at baseline, but still lower than the normal ranges. The increase of haemoglobin towards the normal range is of high importance as chronic anaemia may influence the development and cognitive abilities of the children.

In this trial mean platelet counts were slightly below the normal range at baseline. Thrombocytopenia in *Plasmodium falciparum* infected patients has been reported several times before and a number of observations have shown its association with the pathophysiology of severe malaria [115-120]. In 2003 data were published from a study assessing thrombocytopenia during acute *Plasmodium falciparum* malaria in children from Libreville (Gabon), Dakar (Senegal), and traveler children from Paris (France) [121]. Initial thrombocytopenia was present in 43-58% and mean platelet counts (/µl blood) were 142000, 121000, and 161000, respectively. These findings are in line with results from our trial showing a baseline thrombocytopenia in 68% and 53% and mean platelet counts of 177000/µl and 195000/µl in group A and B, respectively. On day 28 platelets were within the normal range and therefore consistent with the recovery from malaria.

Regarding clinical chemistry, most abnormalities were not clinically significant with only few exceptions. The adverse events of hypertriglyceridaemia are discussed above. One case of a significant increase in serum transaminases was reported with elevated SGOT and SGPT levels at baseline, levels peaked

on day 3 and were normal again at the end of study. As the elevated values were present already before treatment, they are probably attributable to a preexisting condition, but an aggravation due to the study drug cannot completely be ruled out as mefloquine is associated with the potential to induce liver damage [96].

Overall tolerability and safety. Overall tolerability was judged as very good as most of the occurring drug-related adverse events were of mild intensity. Similarly, overall safety was judged very good as no hazardous adverse events were experienced. This safety profile confirms the good overall tolerability of the artesunate-mefloquine combination in acute uncomplicated *Plasmodium falciparum* malaria, which has already been reported several times from Asia, Africa and South America [37, 42, 45-47, 66, 67, 69, 70, 72, 74, 122, 123]. As there was no major difference of safety and tolerability between the two treatment groups, both formulations appear appropriate from the tolerability and safety point of view.

Tolerability and safety of artesunate-mefloquine combination therapy has been compared to other artemisinin-based combination regimes with drugs such as amodiaquine, sulfadoxine-pyrimethamine, doxycycline, chlorproguanil-dapsone or lumefantrine [14, 68, 71-73, 76, 77, 80-83, 124]. In these trials a tendency of increased incidence of adverse events related to artesunate-mefloquine regimes was observed compared to the other artemisinin-based regimes. However, this less favorable tolerability accounts for mild or moderate and transient adverse events. Conversely the safety evaluation is most often in favor of artesunate-mefloquine combination compared to the other regimes.

A multicentre study evaluated artesunate-amodiaquine and found neutropenia, an adverse event often associated with amodiaquine, in 6% of the patients [81].

Artesunate-sulfadoxine-pyrimethamine combination treatment has also been assessed for tolerability and safety [79, 80, 124, 125]. However, because of drug-resistance, this combination is now used less and as efficacy falters, the

risk-benefit ratio for use of sulfadoxine-pyrimethamine is becoming unfavorable [10]. Rare occurrences of severe and fatal adverse events, mainly Stevens-Johnson and Lyell syndromes, hepatotoxicity, agranulocytosis, and skin-reactions have been reported for sulfadoxine-pyrimethamine treatment or prophylaxis [126, 127].

Artemether-lumefantrine was often studied and is registered in accordance with internationally recognized guidelines [71, 73, 82, 85]. One case-control study found irreversible hearing impairment associated with this treatment combination [128]. However, subsequent studies could not reproduce these findings [129].

Doxycycline has been evaluated in sequential administration with rectal artesunate in Sudanese adults [124], the treatment was well tolerated but doxycycline is contraindicated in children and pregnant women in analogy to tetracycline. The combination of artesunate-chlorproguanil-dapsone was assessed by Krudsood et al in 2005 [86]. However, chlorproguanil-dapsone was previously associated with serious haematological adverse events and can cause methemoglobinaemia and hemolysis in individuals with glucose-6-phosphate dehydrogenase deficiency [130].

In comparison to available combination regimens artesunate-mefloquine therapy remains a well tolerated and safe treatment option as confirmed by this trial.

Acceptability

Drugs can be administered via different routes. Most common are enteral (oral, sublingual, rectal), parenteral (intra-venous, sub-cutanous, intra-muscular), topical (trans-dermal), and inhalative drug administration. The choice of administration depends on the available formulations and the conditions of the patient.

In antimalarial treatment acceptability of drugs is of major importance. Patients with the most urgent need of therapy are mostly those not able to take simple oral formulations because of young age, repeated vomiting or unconsciousness due to severe malaria. One alternative to parenteral administration in these cases, or in case of lack of skilled staff, is rectal administration. In the past 20 years many trials have successfully assessed artemisinin-derivative suppositories in children and adults from Africa and Asia [122-124, 131-145]. Suppositories containing artemisinin-derivatives were administered as monotherapy or followed by oral mefloquine or other antimalarials in patients with acute uncomplicated or severe *Plasmodium falciparum* malaria. All results confirmed the high efficacy and safety of the suppositories and emphasized the valuable practical administration in severe malaria in remote areas where parenteral treatment is impossible. However, in monotherapy with artemisinin-containing drugs recrudescence is high and combination regimes of initial suppository administration with sequential oral administration represent difficult treatment courses. Sustained parasite clearance and adherence to treatment regimes might therefore not be granted in outpatient treatment of uncomplicated malaria with suppository regimes.

Our trial addressed to the need for new antimalarial drug formulations in young children. The novel formulation was developed in order to facilitate oral administration and consequently improve compliance in children. Thus drugs do not have to be swallowed in form of tablets but are masked in well flavored granules which are difficult to be spit out because of transformation into sticky jelly.

Acceptability was assessed for the two different drug formulations. Major criteria were ability to swallow study drugs, compliance, appreciation, and satisfaction of the patients in the two different age groups. Intake of study medication was assessed for each drug administration and rated by the investigators as excellent, good, fair or poor. Overall acceptability was judged better for the tablet formulation than for the stickpack formulation. Acceptability

of drug administration was rated excellent or good in 89% and 98% and rated fair or poor in 11% and 2% in treatment group A and B, respectively.

For interpretation of this difference in acceptability results, the older age of group B patients has to be taken into consideration, which may render it easier to administer any drug compared to more problematic drug intake in the younger children of group A. The still high rating of excellent and good acceptability in group A meets the expectations associated to that new paediatric oral formulation. As a result, this stickpack formulation of artesunate-mefloquine is a promising first step towards new forms of oral application of combination therapies in young children suffering from uncomplicated *Plasmodium falciparum* malaria.

In summary, both, the new paediatric stickpack formulation and the co-blister tablet formulation of artesunate-mefloquine combination are efficacious, well tolerated and safe in the treatment of uncomplicated *Plasmodium falciparum* malaria in Gabonese children. The 28-day cure rate of 100% in both treatment groups is superior to other artemisinin combination therapies. Parasite and fever clearance times are fast and offering rapid clinical relief from the disease. Both treatments were well tolerated and safe, the drug related adverse events were transient and of mild or moderate intensity. Acceptability of both oral formulations was very good, even in the very young patients. Both drug formulations of artesunate-mefloquine offer a practical dosing regimen once daily for 3 days. The convenient stickpack and blister packing will hopefully enhance the adherence of patients to antimalarial regimens and will contribute to reduce morbidity and mortality in paediatric patients and contribute to limit the development of drug resistance.

6 Summary

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This clinical trial was performed in order to evaluate efficacy, tolerability, safety, and acceptability of two paediatric formulations of artesunate-mefloquine in African children with acute uncomplicated _Plasmodium falciparum_ malaria.

Both study treatments, the new stickpack and the blister formulation, showed an excellent efficacy in the treatment of acute uncomplicated _Plasmodium falciparum_ malaria as measured by 28-day and 14-day cure rates of 100% in the per protocol population in their respective target population, Gabonese children with a body weight of 10 to 20 kg for treatment A (n = 39) and 20 to 40 kg for treatment B (n = 29). In the intention to treat analysis the 28-day cure rate was 95% in group A and 97% in group B. Time to parasite clearance and fever clearance time were short in both treatment groups.

Safety results showed appropriate safety profiles in the respective target population. No severe or serious adverse event related to the study medications was reported. Good overall tolerability was demonstrated in both treatment groups. There was a tendency to fewer adverse events in smaller children (group A) concerning gastrointestinal (other than diarrhea, more often reported in group A) and central nervous system disorders. Abdominal pain, headache, and dizziness incidence rates might have increased during the course of the treatment; however, it remains difficult to differentiate such events from the symptoms of the underlying disease. Differences of the incidence of adverse events between the treatment groups may result from the different ages in the two study groups and the associated reduced ability of verbalizing complaints. Most of the observed changes in examination and laboratory tests were likely to reflect the recovery from the malarial disease rather than side effects related to the study treatment. Some reported signs and symptoms may similarly be attributed to concomitant diseases such as helminth infections.

Acceptability of drug intake positively meets the expectations taking into account the low age of the paediatric patients, especially for the fixed-dose paediatric stickpack formulation in group A.

The high cure rates, the clinically important rapid parasite and fever clearances as well as the good safety and tolerability profiles are desirable results for the treatment of *Plasmodium falciparum* malaria. The therapy of uncomplicated malaria in Gabonese children with the combination of artesunate and mefloquine is therefore a satisfying and suitable alternative to current therapies with decreased efficacy. Both treatments, the new fixed-dose stickpack formulation as well as the co-blister tablets, can be advised due to the favorable results of this trial. Also for other African countries looking for new effective and safe medications against *Plasmodium falciparum* malaria, this combination of artesunate-mefloquine could be a good option if the basic prerequisites such as availability, low costs, and efficacy are given in the respective region. However, before being able to recommend this specific combination therapy for the entire continent, larger studies assessing the safety of artesunate-mefloquine in African children would be desirable.

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9 Curriculum Vitae

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