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Pyronaridine-Artesunate combination for the treatment of acute uncomplicated *Plasmodium falciparum* malaria in paediatric patients in Gabon

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Abbreviations

ACPR	adequate clinical and parasitological response
ACT	artemisinin-based combination therapy
AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
bpm	beats per minute
CI	confidence interval
CRF	case report form
DHA	dihydroartemisinin
ECG	electrocardiogram
ETF	early treatment failure
FCT	fever clearance time
GDP	gross domestic product
ICH-GCP	International Conference on Harmonization Good Clinical Practice
IPTp	intermittent preventive treatment in pregnant women
ITT	intention to treat
IU	International Unit
LCF	late clinical failure
LPF	late parasitological failure
LTF	late treatment failure
PCR	polymerase chain reaction
PCT	parasite clearance time
PP	per protocol
PTP	post treatment prophylaxis
QTc	heart rate-corrected QT interval
SAE	serious adverse event
SD	standard deviation
SP	sulfadoxine-pyrimethamine
WBC	white blood cells
WHO	World Health Organization

1 Introduction

1.1 Malaria

Malaria is a widespread vector-borne infectious disease occurring in tropical and subtropical regions of Africa, Asia and South America. A significant proportion of the world's population is affected by this fatal disease: in 2006, about 3.3 billion people worldwide were at risk of malaria and approximately 247 million malaria cases occurred, 86 % of them in African countries. The mortality due to malaria is estimated at nearly one million deaths per year, with more than 90 % occurring in Africa and 85 % being children under five years of age[1].

1.1.1 Life cycle of Plasmodium sp.

Protozoan parasites of the genus *Plasmodium* are the causative agent of malaria. There are several species, but only five affect humans: *Plasmodium falciparum, malariae, vivax, ovale* and *knowlesi.* Until recently, *Plasmodium knowlesi* was only known as monkey malaria since it was often misdiagnosed as *Plasmodium malariae* in humans. Due to new molecular methods the distinction between *Plasmodium knowlesi* and *Plasmodium malariae* became evident[2-4].

Plasmodium falciparum causes the most dangerous form of malaria, the so-called *Malaria tropica*, which is almost exclusively responsible for the high mortality mentioned above. Parasites are transmitted by mosquitoes, female *Anopheles spp.*, which ingest gametocytes during a blood meal from an infected person. Parasites pass through their sexual cycle in the guts of the mosquito and develop to sporozoites. They are transmitted by the anopheline saliva, closing the transmission cycle. After penetration of the skin sporozoites pass to the blood stream to infect liver cells. Subsequently schizonts are formed which rupture and release thousands of merozoites. They break out into the blood and infect red blood cells where they enter the erythrocytic stage of their life cycle and multiply asexually. The newly formed merozoites are released again and infect further erythrocytes. Some merozoites develop into gametocytes which

are taken up by the mosquito during a blood meal and enter the sexual cycle again.

1.1.2 Symptoms of Plasmodium falciparum malaria

Plasmodium falciparum infections show quite unspecific symptoms, which means that definite diagnosis cannot be made clinically, but must be confirmed by direct assessment of parasites, most commonly done by microscopic examination of capillary blood.

One main symptom present in the majority of patients suffering from uncomplicated malaria is fever or history of fever. Additional symptoms are headache, fatigue, myalgia and rigors. Abdominal pain, diarrhoea, vomiting, hepatosplenomegaly, thrombocytopenia and anaemia are also clinical signs that are common in uncomplicated *Plasmodium falciparum* infections[5,6].

If the disease is not rapidly diagnosed and treated, it can turn into severe malaria, which is characterized by acute dysfunction of several body systems.

According to the World Health Organization (WHO) definition severe malaria is present

"…if there are asexual forms of *Plasmodium falciparum* in a blood film and the patient shows any of the following:
Clinical features:

- a change of behaviour, confusion or drowsiness
- impaired consciousness or unrousable coma
- multiple convulsions
- deep breathing, respiratory distress
- difficulty in breathing or pulmonary oedema
- circulatory collapse or shock
- pulmonary oedema (radiological)
- jaundice
- haemoglobinuria
- a bleeding tendency

- prostration, i.e. generalised weakness so that the patient cannot walk or sit up without assistance

Laboratory findings:

- hypoglycaemia
- acidosis, metabolic acidosis
- severe normocytic anaemia (packed cell volume < 20 %, Hb < 7 g/dl)
- haemoglobinuria
- hyperparasitaemia
- hyperlactataemia
- renal impairment…"[7]

To control these complications, intravenous antimalarials and further drugs, blood transfusions, intubation, mechanical ventilation and other intensive care procedures can become necessary. As intensive care units are rare and treatment quite expensive in most of the endemic areas, fatal outcome is most likely in case of severe malaria[7].

Most adults in malaria endemic regions develop semi-immunity against severe malaria through repeated infections during childhood. This fact accounts for the increased malaria related mortality in childhood and a lower affection during adult life in areas of high malaria transmission.

1.1.3 Socio-economic burden

There is a clear correlation between poverty and malaria: the global distribution of malaria corresponds to the distribution of the world's lowest per capita gross domestic product (GDP). Poverty aggravates the malaria problem; conversely, malaria contributes to the lower economic level in the affected countries. Private and public medical costs are enormous; additionally there is a loss of productivity due to the infection. Every 40 seconds a child dies from malaria – this translates into a daily loss of more than 2000 young lives worldwide. Furthermore, the disease has a significant impact on cognitive development and education: in a study in Kenya up to 11 % of missed school days were ascribed to *Plasmodium* infections and there are some indications that even brain

function and cognitive development are affected by malaria[8]. These examples show the high impact of malaria on the daily life of millions of people, especially children, and the need for adequate intervention. It is estimated that the number of malaria cases will double until 2020 in the absence of effective control measures[9].

1.1.4 Control strategies

In 1998 the Roll Back Malaria programme was initiated with the aim to control malaria and to cut back mortality to 50 % by the year 2010[10]. Four major interventions were considered for implementation to achieve this ambitious aim: use of insecticide-treated bed nets, spraying houses with insecticides, intermittent preventive treatment in pregnant women (IPTp) and potent drugs for effective treatment that are affordable and available to all those who need them. About 80 % coverage of each of these health care interventions is needed until 2010 to achieve the target. Eradication of malaria however was not aimed for as this will take considerably longer – perhaps being possible only with a yet still lacking effective vaccine[11].

Impregnated bed nets and indoor spraying are quite promising control strategies; however reduced susceptibility of mosquitoes to commonly used insecticides is a major concern. In order to detect emerging resistance a good monitoring system is needed[12]. Yet another problem is the insufficient coverage of impregnated bed nets due to a lack of information and a lack of sustained availability[13].

Basic requirements for effective treatment are health care facilities that are capable of diagnosing malaria rapidly and accurately. However, febrile episodes are often misdiagnosed and mistreated due to a lack of reliable diagnostic tools. Furthermore, considerable proportions of mainly rural populations do not have easy access to medical services or cannot afford recommended drugs, being then treated with suboptimal drug regimens. Finally, one of the most important reasons for ineffective treatment is the development of drug resistance of *Plasmodium falciparum* against first line antimalarial drugs.

1.1.5 Drug resistance

The first drug used to treat malarial fever in the western hemisphere was quinine, which has been known for more than 350 years[14]. It has been the only antimalarial medication until the beginning of the 20th century. Chloroquine was developed in 1934 and has been used worldwide on a large scale as first-line treatment against malaria infections. In 1957, the first cases of resistance were observed on the Thai-Cambodian border and in the following years also in South America and Africa. In the next decades nearly all countries had to change their recommendations of first line treatment for uncomplicated falciparum malaria to sulfadoxine-pyrimethamine (SP). Unfortunately, resistance against SP emerged rapidly and this drug is now ineffective in most endemic regions[15,16]. Subsequently, resistance to almost all other available drugs (including quinine, amodiaquine, mefloquine etc.) has been observed[6].

Mechanisms of drug resistance are diverse, including point mutations or gene amplifications leading to survival advantage under drug related selection pressure. By this means, binding sites for drug target enzymes are modified or the accumulation or efflux of the drug in the parasite are altered[15]. The development and spread of resistant parasites are influenced by various factors including inadequate treatment with incorrect dosage or ineffective drugs and long half-live drugs resulting in prolonged low drug concentrations unable to kill potential reinfection[17]. Other factors include number of parasites, host immunity factors and transmission intensity[15,18]. Multiple drug-resistance is defined as resistance of parasite strains to chloroquine, SP and another class of antimalarials, most commonly mefloquine or quinine[19,20]. Some parasites are cross-resistant, which means that they show reduced susceptibility for similar drugs of the same class, for example mefloquine and quinine[19].

Several factors intensify the emergence of resistance: as the number of available antimalarials is limited and cross-resistance occurs frequently, patients are often re-treated with the same drug or with another insufficient one when the first therapy fails. If the treatment is not able to kill all parasites at once, the patient becomes chronically ill and resistant parasites survive[19]. Non-

compliance also contributes to increasing failure rates. Often drugs are not taken in the recommended scheme or correct dosage[20] – a practice that may also promote the development of resistance.

Drug resistance has an important impact firstly on the affected individual who does not receive curative treatment and secondly on the whole population exposed to malaria due to a raising number of drug resistant malaria cases[20]. As a result of reduced drug susceptibility, malaria mortality has significantly increased during the 1990s[21,22].

To win the race against evolving drug resistant *Plasmodium falciparum* parasites, different strategies have to be pursued. The development of novel drugs and drug families with innovative modes of action is a long-term approach. The current recommendations for antimalarial therapy are combination therapies, hoping to delay emerging resistance[19].

1.2 Artemisinin-based combination therapies (ACTs)

1.2.1 Combination therapies

Combination therapies have already been used successfully in the treatment of infectious diseases such as tuberculosis[14], leprosy, HIV and in cancer[23]. Combining different drugs may be advantageous in different ways: firstly, improving efficacy and reducing the risk for the development of drug resistance[19] and thereby lengthening the therapeutic lifespan of the drug[24]. Additionally, patients' compliance is improved through shortened treatment courses. Several combination treatments for the use in malaria therapy have been studied in the last years[14]. By now, the combination of an artemisinin derivative with another antimalarial drug became the accepted standard of care[23]. These artemisinin-based combination therapies (ACTs) are now recommended first-line drugs in most malaria-endemic countries[6].

1.2.2 Artemisinins

Artemisinins are extracted from *Artemisia annua*, a medicinal herb that has been used in Traditional Chinese Medicine for more than 2000 years as a

febrifuge[25]. In 1971, Chinese scientists described its high intrinsic activity against malaria parasites. In the following years, the novel derivatives dihydroartemisinin (DHA), artemether, arteether and artesunate proved to be at least as active as artemisinin and are now the most frequently used ones[26]. Artemether, arteether and artesunate are rapidly biotransformed into the active metabolite DHA after ingestion[27]. DHA has a very short elimination half-life of about one hour[28-30].

The mode of action of artemisinins has not yet been completely understood. The integrity of the peroxide bridge, ion-dependant alkylation and free radicals seem to play an important role. The plasmodial enzyme PfATPase6 was shown to be a main target. However, all of these proposed mechanisms do not yet fully explain the antimalarial pharmacodynamics of artemisinins.

Artemisinins are active against all blood stages in the development of the parasite, including the early ring-stage, but exhibit no activity against liver-stages. The ability of artemisinins to reduce gametocyte carrier rates has been discussed extensively, however evidence for epidemiological impact is still lacking[31,32].

Compared to other antimalarials, artemisinins are active against a broader spectrum of parasite stages. The decreased stage specificity translates into a faster parasite reduction in clinical use. It is hypothesized that killing parasites prior to developing into the more pathogenic older stages may prevent more severe courses of malaria[26]. Artemisinin derivatives are available in oral, intramuscular, intravenous[33] and rectal drug formulations. Rectal suppositories have been shown to reduce overall unfavourable outcome when used in the early treatment of severe malaria[34]. Besides the impressive antimalarial properties, artemisinins do exhibit certain activity against trematodes including *Schistosoma spp*., fungi and viruses. Their activity against particular cancer cells has been demonstrated in vitro[35,26].

The overall safety profile of artemisinins is very favourable. Side effects may include a transient suppression of erythropoiesis without causing clinically

significant anaemia. Neurotoxicity, which was described for high doses of artemisinins and intramuscular injections of artemether and arteether in preclinical studies in animal models and in vitro, could not be confirmed in humans[36-38,26]. After the oral ingestion of artesunate, hypersensitivity reactions have been observed[39], but they seem to be quite rare (about 1 in 3000 treatments)[26]. Dizziness, nausea and vomiting, anorexia and diarrhoea have been reported as potential side effects of artemisinins, but it is difficult to discern side effects from symptoms of the underlying disease[38]. Prolonged heart rate-corrected (QTc)-intervals were observed in the treatment of animals with high doses of arteether[36] and in a clinical study with artemether, but not as a significant finding[40].

If artemisinins are used as monotherapies, treatment courses of five to seven days are necessary to ensure sufficient parasite clearance because of very short half-lives. The combination with another, longer-acting partner drug allows shorter regimens of only three days and therefore improves compliance[27]. Due to concerns of resistance against artemisinins when used in monotherapy, WHO recommends not to employ artemisinins without complementary drug[23].

As artemisinins became the cornerstone of all current antimalarial treatments, the potential development of resistance is of great concern. Until now, resistance to artemisinins was only induced at low levels in laboratory studies and in rodent malaria. However, observations from Asia in 2002 and 2003 are disquieting: on the Thai-Cambodian border reduced susceptibility could be detected both in vitro and in vivo and in Western Cambodia treatment failure rates of artesunate-mefloquine already reached nearly 8 % and of artemether-lumefantrine even more. Both rates that cannot entirely be explained by increasing resistance to the partner drugs[41,23,26,42]. More recently, two cases of proved artesunate-resistant malaria with prolonged parasite clearance times were reported from Western Cambodia[43].

1.2.3 Artemisinin-based combinations

Artemisinin-based combination therapies are composed of an artemisinin derivative acting over two asexual cycles of the parasite and reducing the

parasite burden by the factor of about 100 million and a partner drug with a longer half-life eradicating the remaining parasites[26]. The rapid parasite reduction attained by the artemisinin component is responsible for the fast clinical and parasitological response.

Due to the fact that the probability of developing resistance to both drugs at once is very low, the two partner drugs protect each other against resistance during the three days of treatment[23]. However, in areas with high transmission there are concerns that long half-life partner drugs are at an increased risk for selecting resistant mutants due to subtherapeutic drug levels at a time when new infections may already be present[44,27]. An advantage of partner drugs with long half-lives may be a certain post treatment prophylaxis (PTP), which prevents a new infection and ameliorates clinical recovery[23].

The underlying idea of artemisinin-based antimalarial drugs was to add an artemisinin derivative to an already existing standard treatment. The first beneficial effects could be noticed on the Thai-Myanmar border in the 1990s, where the efficacy of widely used mefloquine monotherapy had decreased to about 75 % but could be enhanced to nearly 95 % in combination with artesunate[45,20]. In 1998, a series of clinical trials were conducted[46]. Results from the latter suggested the use and further development of artemisinin-based combination therapies. The first available and recommended combinations were artemether-lumefantrine, artesunate plus amodiaquine and artesunate plus SP for areas with high SP efficacy[47].

Currently one of the major problems for a scale up and worldwide implementation of ACTs are the limited availability of artemisinins and the comparatively high costs. Many of the poorest regions are hardly capable to afford monotherapies with chloroquine or SP, which are nearly tenfold cheaper[24,26]. The costs for artemisinin-based treatments impeded policy changes. Most malaria-endemic countries do not have funds for the implementation of ACTs. However, international donors, foremost the Global Fund, intensified funding for ACTs. Between 2003 and 2007 nearly all African countries changed their treatment policies and recommended ACTs as first-line

treatment[48]. Although costs are higher for ACTs some studies already indicated that these new interventions are cost-effective when compared to conventional SP treatment[49,50].

Drug costs could be reduced by improved horticulture of *Artemisia annua*[26] and by new semi-synthetic artemisinins produced with the help of innovative manufacturing processes[51-54]. New, fully synthetic peroxides with antimalarial activity could also be promising alternatives[55,56].

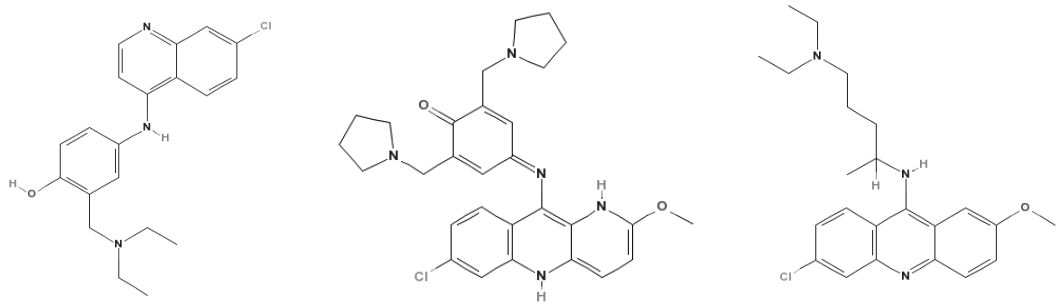
One of the major impediments to improved health care is the underdeveloped and often dysfunctional health care system in many sub-Saharan African countries. Although ACTs are recommended by the government, delivery is not guaranteed[23] and there is a lack of health care workers to care for all malaria episodes[57].

Presently, the following artemisinin-based combination therapies are available and widely used: artesunate-SP and artesunate-amodiaquine, artemether-lumefantrine and artesunate-mefloquine in areas with emerging resistance. A number of novel compounds for the use in combination with artemisinin derivatives are currently in an advanced stage of clinical development, including piperaquine and pyronaridine[23,58].

1.3 Artesunate-pyronaridine combination

1.3.1 Pyronaridine

Pyronaridine is a fully synthetical antimalarial drug that has been created in the 1970s at the Institute of Parasitic Diseases in Shanghai. It is a Mannich base derivative of mepacrine, the first synthetic antimalarial in clinical use, which was based on acridine but was not widely used due to its toxicity. The nucleus of pyronaridine is similar to mepacrine but the side chain was replaced and resembles to that of amodiaquine[59,60].



mepacrine pyronaridine amodiaquine

Figure 1: Chemical structures of mepacrine, pyronaridine and amodiaquine[61]

As for a number of other antimalarial drugs there is evidence that the activity of pyronaridine is attained by its interference with haemoglobin degradation in the parasite's digestive vacuole.

During its intra-erythrocytic life stage, the parasite catabolises haemoglobin, thereby obtaining amino acids for protein synthesis, keeping up the osmotic pressure in the erythrocyte[62] and avoiding the toxicity of free haem[63]. Free haem leads to the inhibition of parasitic enzymes in the digestive vacuole, membrane lysis and finally parasite destruction[64]. It is transformed by the parasite into the non-toxic haemozoin, also called β-haematin or malaria pigment, via biocrystallization. Other antimalarials inhibiting biocrystallization are chloroquine, quinine, amodiaquine, mefloquine, halofantrine and lumefantrine[65].

In vitro studies showed the inhibition of β-haematin formation by pyronaridine, configuration of a haematin-drug complex, increased haematin-induced erythrocyte lysis and inhibition of the glutathione-dependent degradation of haematin. In contrast to chloroquine, much lower concentrations (about 1 %) are necessary to reach these effects[66]. These findings show that pyronaridine is active against haemozoin producing stages, but not against sporozoites or liver schizonts[65]. Contradictory reports have been published on its gametocytocidal effect[67,68].

During the last two decades, pyronaridine has demonstrated to be highly effective against *Plasmodium falciparum*, including chloroquine-resistant strains[69-71], as well as against *Plasmodium ovale*, *malariae*[72] and *vivax*[73]. However, cross-resistance to chloroquine is discussed controversially. Some authors described correlations between chloroquine resistance and pyronaridine[74-76], others did not find relationships[77,78]. Apart from its antimalarial properties, recent studies suggest the potential use of pyronaridine in anti-epileptic treatment[79].

Pyronaridine has been used in clinical practice in Asia since the 1980s as "Malaridine". It can be administered orally, intramuscularly and intravenously and showed very low toxicity. It was effective in acute uncomplicated malaria, and has been used in its parenteral form for the treatment of cerebral malaria[60]. In several Asian studies including more than 1000 patients the following mild side effects were reported: slight diarrhoea, nausea, abdominal pain and vomiting, particularly after oral intake; dizziness, palpitations, allergic skin rash and in some cases slight ECG changes[73,69,60,70]. First studies in Africa showed similar findings. Pyronaridine was very well tolerated in adults as well as in children. However, in addition to the symptoms mentioned above, some patients suffered from pruritus[71,80].

Pyronaridine was mainly used as monotherapy. Quite early, several in vitro and in vivo studies raised fears about the development of resistance. On the China-Lao People's Democratic Republic border, recrudescence rates and fever clearance times rose significantly between 1985 and 1995 in clinical trials. In vitro resistance reached 36.4 % in 1995 whereas all strains were sensitive in 1988[81]. Another Chinese study showed similar results in in vitro analyses[82]. A survey in Thailand revealed a recrudescence rate of 12 % and recommended the use of the drug in combination with other antimalarials[70].

1.3.2 Artesunate plus pyronaridine

The combination of artemisinins and pyronaridine has already been studied in vitro and in vivo. Pyronaridine in combination with artesunate indicated a delay of resistance in rodent malaria[83], and in another study in vitro and in rodent malaria models showed this combination to be more efficacious, even in lower doses, than monotherapies[78]. A clinical study confirmed the superior effect of the artemisinin-combination compared to the use of one drug alone, using dihydroartemisinin instead of artesunate[84]. Based on unpublished data, the World Health Organization cites an efficacy of 100 % for the combination of pyronaridine with artesunate, artemether or dihydroartemisinin[47]. With regard to the interaction of pyronaridine and artemisinins there is conflicting data. Some results indicate a slight trend towards antagonistic effects in vitro, however this does not seem to play a clinically significant role in vivo[78].

Pyramax®, the fixed-dose combination of pyronaridine and artesunate in the ratio 3:1 is currently under development. Medicines for Malaria Venture (Geneva, Switzerland) and Shin Poong Parmaceuticals (Seoul, Republic of Korea) aim at providing a new ACT for adults as well as for children that is highly efficacious and safe, especially in small children. Additionally, it should have a short and simple regimen, low tendency to develop resistance and low costs[85].

After completion of Phase I and Phase II studies in adults showing favourable efficacy and safety data[86], the clinical development for paediatric patients was started. This work presents the descriptive Phase II study in children with pyronaridine-artesunate in Africa. The combination was investigated as an oral tablet formulation and as a novel paediatric granule formulation. The paediatric drug formulation was examined to facilitate the treatment of young children where adequate oral malaria therapy is of major concern.

1.4 Paediatric formulations

Although young children are the group which is most affected by malaria, options for paediatric drug formulations are very limited. Syrups were available

for some antimalarials, for example chloroquine, but they are not longer recommended besides the development of drug resistance also due to non-adherence to dosage instructions[87]. Children often receive adult tablets that have to be broken to adjust dosing, but this also leads to undefined amounts of the active ingredient. Several manufacturers already launched tablets with lower doses, but smaller children may not be able to swallow them. Pulverizing of tablets is not always possible and can worsen compliance due to bitter taste and vomiting.

The masking of the bitter taste of many antimalarials, preferably in new galenic preparations for paediatric patients, and research about pharmacokinetics in children demand more attention. For the combination of artesunate plus mefloquine (Artequin®) a paediatric formulation is already available (Artequin Paediatric®). It is a granule co-formulation with mango taste that has to be administered directly in the mouth. The pharmacokinetic parameters are very satisfying and comparable to the standard tablet formulation, so the granules represent a good alternative for antimalarial treatment especially in small children[30,88].

To broaden paediatric therapy options for malaria, it is important to make similar drug formulations available for other artemisinin-based combination therapies. In addition to the tablets, Shin Poong Parmaceuticals (Seoul, Republic of Korea) therefore developed a fixed dose granule formulation for the new combination of artesunate and pyronaridine. This galenic preparation has an orange flavour to mask the bitter taste of pyronaridine[73] and can be taken either suspended in water or poured directly in the mouth and swallowed with water. It was developed to help ameliorating antimalarial treatment especially for very small children.

1.5 Objectives of this work

Given the increasing resistance against antimalarials, the development of new drugs is – among other control strategies - a very important task to reduce malaria morbidity and mortality and the associated socio-economic problems. In

malaria endemic countries, mainly in subtropical Africa, Asia and South America, children are most affected by this infectious disease, which necessitates special research on the development of paediatric drugs and new galenic preparations for children.

This work presents the first clinical trial in children with the new artemisinin-based antimalarial combination of artesunate and pyronaridine, which was conducted in Lambaréné, Gabon. In three groups with 15 patients each, the tablet formulation was investigated in escalating dosages concerning safety, tolerability and pharmacodynamic endpoints. Pharmacokinetic parameters were also assessed, but will not be described here. In the second part of the study, a new granule formulation was examined in only one group of 15 children, using the same parameters as study endpoints.

This Phase II trial provides first clinical data about the use of pyronaridine-artesunate in children between 2 and 14 years of age. Our promising results are the basis for further Phase III studies that will evaluate efficacy in a larger number of patients and will be the next step towards registration.

2 Materials and methods

2.1 Study site

The study was conducted from June to December 2006 at the Medical Research Unit of the Albert Schweitzer Hospital in Lambaréné, Gabon.

Gabon is located in Central Africa, bordered by the Atlantic Ocean, the Republic of the Congo, Cameroon and Equatorial Guinea. Its climate is tropical, characterised by high humidity, temperatures varying between 18 and 36 °C during the year and two dry and two rainy seasons. The majority of its 270,000 km² surface is covered by a unique form of rain forest with extraordinary biodiversity. Approximately 50 % of Gabon's population of 1.5 million people is situated in the capital Libreville and its close surroundings. The traditional ethnic grouping into tribes is still present and besides French as the official language, many local languages, such as Fang, Yipunu, Eschira, Mitsogo are commonly spoken[89].

The small city of Lambaréné is located in one of the nine provinces, named Moyen-Ogooué, in about 150 km distance from Libreville, and roughly 75 km south of the equator. Its urban area is divided by the large river Ogooué into three parts, one on each riversides and one on the central island, surrounded by dense tropical rain forest and plantations. The patients participating in this study are residents in Lambaréné and various small villages along the main road in up to 40 km distance from the hospital.

The study site is characterised by perennial malaria transmission. The area is hyperendemic for *Plasmodium falciparum* malaria and the major vectors are *Anopheles gambiae* and *Anopheles moucheti*[90,91]. A high degree of drug resistance of *Plasmodium falciparum* to chloroquine, both in vitro and in vivo, was reported[92,93].

2.2 Study design

The trial was designed as an open label, sequential group, dose-escalation study to assess the pharmacokinetics, safety, tolerability and pharmaco-dynamics of the fixed dose combination tablet of pyronaridine-artesunate in the ratio 3:1.

Children considered eligible for the study after verifying the inclusion and exclusion criteria were hospitalized at the Medical Research Unit of the Albert Schweitzer Hospital for the duration of drug administration. The drug was given once daily for three days. Patients were then discharged from the hospital if no clinical signs and symptoms of malaria were present any more. Follow up visits were done at the Medical Research Unit in a weekly interval up to at least 42 days after first drug administration, depending on whether adverse events occurred during this period requiring observation for a longer time span.

A total of 60 male and female patients were included in the study, assigned to one of the four treatment groups A, B, C and D, each consisting of 15 children. After each cohort, safety was monitored by a Safety Oversight group, consisting of representatives of the sponsor and the principal investigator, before proceeding to the next dosing level.

The study protocol was approved by the Ethics Committee of the International Foundation for the Albert Schweitzer Hospital in Lambaréné. Written informed consent, complying with the International Conference on Harmonization Good Clinical Practice (ICH-GCP) guidelines, had to be provided by study participants' parent or legal representative after being informed about trial aims, design and procedures, benefit, risks and alternative treatments. If the respective person was unable to read and write, witnessed consent was permitted. Study participation was voluntary, meaning amongst others that patients could discontinue at any time, without any penalty or loss of benefit.

As this was the first clinical study with the artesunate-pyronaridine combination in children, safety, tolerability and pharmacokinetics were particularly relevant and were defined as primary objectives.

Secondary objectives were pharmacodynamic endpoints, defined mainly in concordance with the WHO Guidelines 2003[94]: proportion of patients with polymerase chain reaction (PCR)-corrected adequate clinical and parasitological response (ACPR) on day 14, 28 and 42 (PCR-corrected ACPR on day 28 being the primary efficacy endpoint), parasite clearance time and fever clearance time, proportion of treatment success or failures, proportion of patients who have cleared parasites or fever cleared at day 1, 2 and 3, number of gametocytes per µl at days 0, 3, 7, 14, 21, 28 and 42, non-PCR corrected ACPR on day 14, 28 and 42.

Study medication – with the active ingredients pyronaridine tetraphosphate and artesunate - was supplied by Shin Poong Pharm Co. Ltd, Republic of Korea. Drug strength per tablet was 48 mg pyronaridine and 16 mg artesunate for group A, 72 mg pyronaridine and 24 mg artesunate for group B and 96 mg pyronaridine and 32 mg artesunate for group C. The sachets with the granules contained 60 mg pyronaridine and 20 mg artesunate. Tablets were administered according to body weight, which corresponds to 6 mg pyronaridine tetraphosphate plus 2 mg artesunate per kilogram body weight for group A, 9 mg pyronaridine tetraphosphate plus 3 mg artesunate per kilogram body weight for group B, 12 mg pyronaridine tetraphosphate plus 4 mg artesunate per kilogram body weight for group C and 9 mg pyronaridine tetraphosphate and 3 mg artesunate per kilogram body weight for group D.

2.3 Inclusion and exclusion criteria

Patients presenting with fever or other symptoms of malaria either at the Medical Research Unit, the paediatric ward of the Albert Schweitzer Hospital or at one of the dispensaries in the vicinity of Lambaréné were examined and the following inclusion and exclusion criteria were checked.

Male and female patients were included in the study if they were two to fourteen years of age and weighing between 10 to 40 kg. Acute uncomplicated monoinfection with *Plasmodium falciparum* had to be confirmed by positive thick and thin blood smears. Additionally, orally measured temperature of ≥ 38.0 °C or history of fever within the past 24 hours was required. The acceptable range of asexual forms of *Plasmodium falciparum* was between 1,000 and 200,000 per µl of blood. Children had to be able to swallow whole oral tablet formulation without chewing and severe malnutrition was excluded by a mid upper arm circumference > 110 mm. Study participants had to be able to comply with the study protocol and the study visit schedule.

Children were not included in the study in case of signs and symptoms of severe or complicated malaria requiring parenteral antimalarial treatment according to the WHO Criteria 2002[7]. Severe vomiting (more than three times in the 24 hours prior to inclusion) or severe diarrhoea (three or more watery stools per day) led to exclusion. If fever was caused by diseases other than malaria or if patients' history and examination showed any clinically significant disorders (such as cardiovascular, respiratory, hepatic, renal, gastrointestinal, immunological, neurological, endocrine, infectious or psychiatric), children were considered not eligible, as well as if hypersensitivity, allergic or adverse reactions to pyronaridine or artesunate or other artemisinins, active Hepatitis A IgM (HAV-IgM), Hepatitis B surface antigen (HBsAg), Hepatitis C antibody (HCV Ab) or seropositive HIV antibody were known. Girls of child-bearing potential being pregnant, confirmed by a urine pregnancy test, or lactating were not included in this study. Further exclusion criteria were restricted liver and renal function, defined as aspartate aminotransferase and alanine aminotransferase (AST, ALT) levels more than 3 times upper limit of normal values or serum creatinine of ≥ 2 mg/dl. The reception of an investigational drug within the past 8 weeks or the use of any other antimalarial drugs within two weeks prior to start of the study (confirmed by the Lignin test and Saker Solomon urine test) did not allow inclusion. Participation in this clinical study was permitted only once.

After drug administration, patients could be excluded due to adverse events or due to protocol violation, such as administration of other antimalarials or antibiotics with antimalarial activity, for example doxycycline, erythromycin, clindamycin, rifampicin, trimethoprim sulfamethoxazole.

2.4 Drug administration

Prior to enrolment, the following baseline characteristics were collected: clinical signs and symptoms of malaria including headache, sweating, loss of appetite, myalgia, nausea or vomiting, medical history and demographic data. A complete physical examination was done, including weight, height, vital signs (blood pressure and heart rate) and oral temperature. Additionally, a 12-lead electrocardiogram (ECG) was performed. Blood samples were taken for clinical chemistry, haematology and pre-dose pharmacokinetic measurement. To determine *Plasmodium* species and the number of asexual forms and gametocytes, thin and thick blood films were prepared and examined. Additionally, a drop of capillary blood for PCR was put on filter paper. Analysis of urine samples was performed and presence of antimalarials ruled out by Lignin and Saker Solomon urine test.

After examination of these baseline parameters and inclusion of the participant, the first dose of the investigational drug was administered with a defined amount of water, tablets of group A, B and C had to be swallowed unchewed. Second and third doses were given on day 1 and day 2 in the morning with at least 12 hours between first and second dose. Drug application was always done under supervision of an investigator.

If a patient vomited the first dose within 0 to 30 minutes after drug administration, a repeat full dose was given. In case of vomiting the re-applied dose or the dose on day 1 or day 2, the child was excluded from the study and given rescue medication. Patients withdrawn for this or any other reason were not replaced.

Drug administration was followed by blood sampling for the pharmacokinetic assessments at the time points 0.25 h, 0.5 h, 1 h, 1.5 h, 2.5 h, 4 h, 8 h and 12 h post-dose. Thick blood films and haemoglobin levels were examined every eight hours until 72 hours or until two consecutive blood film readings were negative, confirmed by a third one 24 hours after the first. Additionally, temperature was taken every eight hours until 72 hours or normalisation (< 37.5 °C) for two readings 8 hours apart and at 24 hours later.

On day 1, 2 and 3, adverse events and clinical signs and symptoms were assessed, vital signs and temperature recorded and haemoglobin levels checked. Between two and four hours after drug administration on day 1 and 2, the 12-lead ECG was performed. Additionally, a sample for PCR was collected on day 1 and a physical examination was done on day 2. On day 3 blood and urine analysis were redone (clinical chemistry, haematology and urinalysis, blood samples for pharmacokinetics).

Patients were then discharged from the Medical Research Unit on day 3 if parasites and fever had cleared and they received an insecticide treated bed net. Weekly follow up visits were scheduled and patients were advised to come back to the study site at any time in case of recurrence of malaria symptoms or appearance of any other illnesses.

2.5 Follow Up

If children did not present at the study site by themselves for scheduled follow up visits, they were invited to come to the Medical Research Unit by staff once a week for follow up visits until at least day 42 after first drug administration.

At each follow up visit, adverse events and clinical signs and symptoms were assessed, temperature and vital signs recorded, thick blood films examined for asexual forms and gametocytes, samples for PCR and pharmacokinetics collected and haemoglobin levels checked. Clinical chemistry, haematology and urinalysis were done on day 7, abnormal values were checked again on day 28 and 42. Physical examination was performed on day 14, 28 and 42 and a 12-

lead ECG was recorded on day 7 and 14 and additionally on day 28 if clinically indicated.

In case of reappearing parasitaemia, patients were withdrawn from the study and given antimalarial rescue medication and followed up until resolution of clinical signs and symptoms.

2.6 Diagnostic methods

2.6.1 Blood tests

At each visit, several blood tests were performed: thick blood films to diagnose malaria and count parasitaemia and thin blood films to determine the *Plasmodium* species *P. falciparum, P. malariae* and *P. ovale*. Haematological and biochemical laboratory assessments were done to detect disorders at baseline or in the course of the study, for example anaemia which is common in *Plasmodium falciparum* infections, blood count changes or any insufficiency of liver or kidney function.

With 1 ml of venous blood a full blood count was done, including haemoglobin, haematocrit, erythrocyte, platelet and leukocyte count, as well as a differential count.

Another sample of venous blood (1 ml) was drawn to perform the following parameters for clinical chemistry: total bilirubin, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase, alkaline phophatase, urea, creatinine, sodium and potassium.

If the scheduled haemoglobin assessments did not coincide with the clinical laboratory assessments, haemoglobin values were measured with a HemoCue haemoglobin photometer with capillary blood, which was usually taken by finger prick.

Thick and thin smears were also prepared with capillary blood taken by finger prick, except when laboratory tests were done at the same time, in which cases the blood films were prepared with venous blood.

For thin films, a small drop of blood was spread on a microscope slide with the edge of another one to get a very thin layer of blood cells. The slide was dried, fixed with methanol to prevent haemolysis and then stained with Giemsa stain (20 %, pH 7.2) for 20 minutes.

Thick smears were prepared with about 10 µl of blood which were distributed on the microscope slide over an area of roughly 1.5 cm², dried and stained in the same way as the thin films.

After staining and drying again, slides were read under the microscope at x1000 magnification and parasites were counted against white blood cells (WBC) in the thick blood film, until 200 WBCs were counted. To calculate the parasite density (parasites per microlitre), the number of counted parasites was divided by the number of counted WBCs and multiplied by the number of WBCs taken from the baseline haematology result. A slide was considered negative when 1,000 WBCs were counted without the presence of any parasites. If less than 10 parasites were counted against 200 WBCs, counting had to be continued until at least 500 WBCs were seen.

Gametocyte density was determined in the same way, except for counting against 1,000 WBCs.

Slides were read independently by two different microscopists and the arithmetic mean was taken as result. If the ratio of densities from the higher to the lower count was greater than 1.5 or if there was a discrepancy in positivity or negativity, the slide was read a third time. The final result was based on the majority verdict for positivity/negativity and the arithmetic mean of the two results which fulfilled the mentioned criteria. Additionally, an external quality control was done by an independent certified institution.

2.6.2 Urine tests

In order to detect the presence of antimalarial drugs, two qualitative tests were performed: the Lignin test for sulfonamides and the Saker Solomon test for chloroquine and its metabolites.

For the Lignin test, two drops of urine were placed on a filter paper and one drop of 10 % hydrochloric acid was added. The appearance of a yellow to orange colour within 20 seconds showed the presence of sulfonamides in urine.

For the Saker Solomon test, 2 ml of urine were mixed with 1 ml of phosphate buffer (pH 8.0) and 0.2 ml of tetrabromophenolphtaleine ethylester (TBPEE), hand-shaken for 15 seconds and left to stand for 15 min to allow phase separation before reading the result. If the organic indicator layer turned red to purple, this indicated a positive result for chloroquin and its metabolites, a yellow-green colour indicated a negative one. Phosphate buffer stock solution was prepared by mixing 162 g of $K_2HPO_4 \cdot 3H_2O$ with 5 g of KH_2PO_4 in 500 ml of distilled water. TBPEE was made by dissolving 50 mg TBPEE in 100 ml chloroform, then shaking this mixture with 10 ml 2 mol/l hydrochloric acid.

Urinalysis was done by common dipstick (Combur-Test; Roche Diagnostics) to identify glucose, protein, pH, blood or haemoglobin, leukocytes and nitrite in urines.

2.6.3 Electrocardiogram

Electrocardiograms were recorded at baseline and follow up in order to find any changes during the study course, especially a prolongation of the QT interval corrected for heart rate (QTc interval).

Children had to stay in a horizontal position, the electrodes of the 12-lead ECG (CT110 CardioConcept; SECA) were placed in the common way and patients were advised not to move while three recordings were done five minutes apart for each scheduled ECG reading.

All ECGs were evaluated by an investigator at the study site as well as by an independent investigator for control.

2.6.4 Polymerase chain reaction

Genotyping was done via polymerase chain reaction (PCR) analysis to distinguish between recrudescence and reinfection in case of reappearing parasitaemia. Blood spots were collected on filter paper (FTA Classic Card;

Whatman International) and shipped to the Department of Parasitology of the Institute for Tropical Medicine, University of Tübingen in Germany where PCR analysis was performed on the basis of *Plasmodium falciparum* merozoite surface antigen (MSA)-2 length polymorphisms and gene-product sequencing.

Samples were anonymized and DNA was extracted from the blood spots using a commercially available kit (QIAmp DNA extraction; Qiagen). According to a family specific nested PCR protocol[95] the MSA-2 genes were amplified on a Biometra Uno thermal cycler and analyzed by gel-electrophoresis in 1.5 % agarose. If the products were of similar size, sequencing was done using forward and backward primers in an Applied Biosystems 3100 genetic analyzer following standard protocols.

2.7 Statistical analysis

No formal sample size calculation was done as this study was intended to be only of exploratory nature without any further testing of hypothesis. The number of 15 patients per treatment group was considered to be sufficient to describe the safety of this antimalarial combination for paediatric use in three different dose levels and to obtain valid measures of pharmacokinetic parameters.

Two populations were defined for data analysis: intention-to-treat (ITT) population which consisted of all patients who received at least one dose of the study drug, and per-protocol (PP) population which included all patients with complete treatment, known PCR-corrected ACPR on day 28 and without major protocol violations. Efficacy analyses were performed on both populations, but the PP analysis was regarded as the primary efficacy analysis. Safety analyses were done only for the ITT population.

Descriptive statistics were used for evaluation of quantitative and categorical variables. Quantitative variables were summarized with mean, standard deviation, median, minimum and maximum and categorical variables with absolute and relative frequencies. Exact 95 % confidence intervals (CI) were calculated according to the Pearson-Clopper method.

Treatment success in PCR-corrected analysis was defined as parasite clearance within seven days post start of treatment, without recrudescence within 28 days and without meeting any criteria of Early Treatment Failure (ETF), Late Clinical Failure (LCF) or Late Parasitological Failure (LPF), in accordance with the WHO guidelines[94]. Parasite clearance time (PCT) and fever clearance time (FCT) in hours were summarized using Kaplan-Meier estimates.

3 Results

3.1 Patient disposition

A total of 173 children were screened for this study. 53 were excluded due to parasitaemia less than 1,000 per µl or due to *Plasmodium malariae* or mixed infection. 11 parents refused consent, 10 children were incapable to swallow whole tablets and 11 were not able to comply with the study schedule because they lived too far away or had planned a journey. In 10 cases clinical or laboratory parameters such as anaemia, thrombocytopenia or the presence of severe malaria made inclusion impossible due to the requirement of parenteral treatment in the paediatric ward. All reasons for exclusion from the study are presented in the following table. Patients considered not eligible received appropriate treatment, especially antimalarials.

Table 1: Reasons for exclusion from the study

Reason for exclusion	***N***
Unability to comply with study schedule	11
Unability to swallow whole tablets	10
Parasitaemia less than 1000/µl or more than 200,000/µl	33
Mixed or *Plasmodium malariae* infection	20
Antimalarial drug intake in the past 2 weeks prior to study	4
No consent	11
Febrile condition caused by diseases other than malaria	4
Severe malaria	1
Anaemia or thrombocytopenia	9
Weight under 10 kg or over 40 kg	5
Others	5
Total	**113**

15 patients were assigned to dose level groups A (pyronaridine+artesunate 6+2 mg/kg, tablets), B (9+3 mg/kg, tablets), C (12+4 mg/kg, tablets), and D (9+3 mg/kg, granule formulation), respectively. In group A, one patient was not

treated with the study drug because of inability to swallow tablets. Two other patients, one of group A and one of group B, vomited the first dose of the administered drug, were given a repeated dose but vomited again. These three children were withdrawn from the study before finishing drug administration and were given appropriate rescue medication. All other 57 children received the full investigational treatment of three doses.

During the follow up period until day 28 three more children were withdrawn: one suffered from a new *Plasmodium falciparum* infection on day 21, a further received quinine in the local hospital, which represents a protocol violation (both group A), and in group B the informed consent was withdrawn once by participant's parents. In group D one patient did not present for the day 28 follow up visit and was therefore counted as "treatment failure" on day 28.

Consequently, in the primary efficacy analysis on day 28, 11 children were included in per-protocol (PP) analysis for group A, 13 for group B, 15 for group C and 14 for group D. Intention-to-treat (ITT) analysis on day 28 was based on 14 patients for group A (the patient not treated was not included) and 15 for group B, C, D, respectively.

Seven more participants discontinued the study prematurely between day 28 and day 42: one patient of group A showed a *Plasmodium ovale* infection on day 39, was treated with antimalarials and therefore withdrawn. In group B one child was lost to follow up after day 35, three others had new *Plasmodium falciparum* infections on day 28 and another case of re-appearing parasitaemia on day 35 was classified as recrudescence by PCR. The second case of recrudescence appeared in group D on day 36.

For day 42 analyses, per-protocol population consisted of 10 patients for group A, 9 for group B, 15 for group C and 14 for group D, intention-to-treat population of 13, 11, 15 and 15 (group A, B, C and D, respectively). The patient of group A with *Plasmodium falciparum* infection on day 21 was considered not cured on day 28, so he was not included in the day 42 analysis. The three patients of group B with *Plasmodium falciparum* infections on day 28 were

counted as cured for day 28 but not taken into account for day 42 analysis as well as the child lost to follow up after day 35. The child of group D which could not be examined on day 28 returned for the day 35 and day 42 visits, so it was considered cured in the day 42 analysis. Patient flow is presented in figure 2.

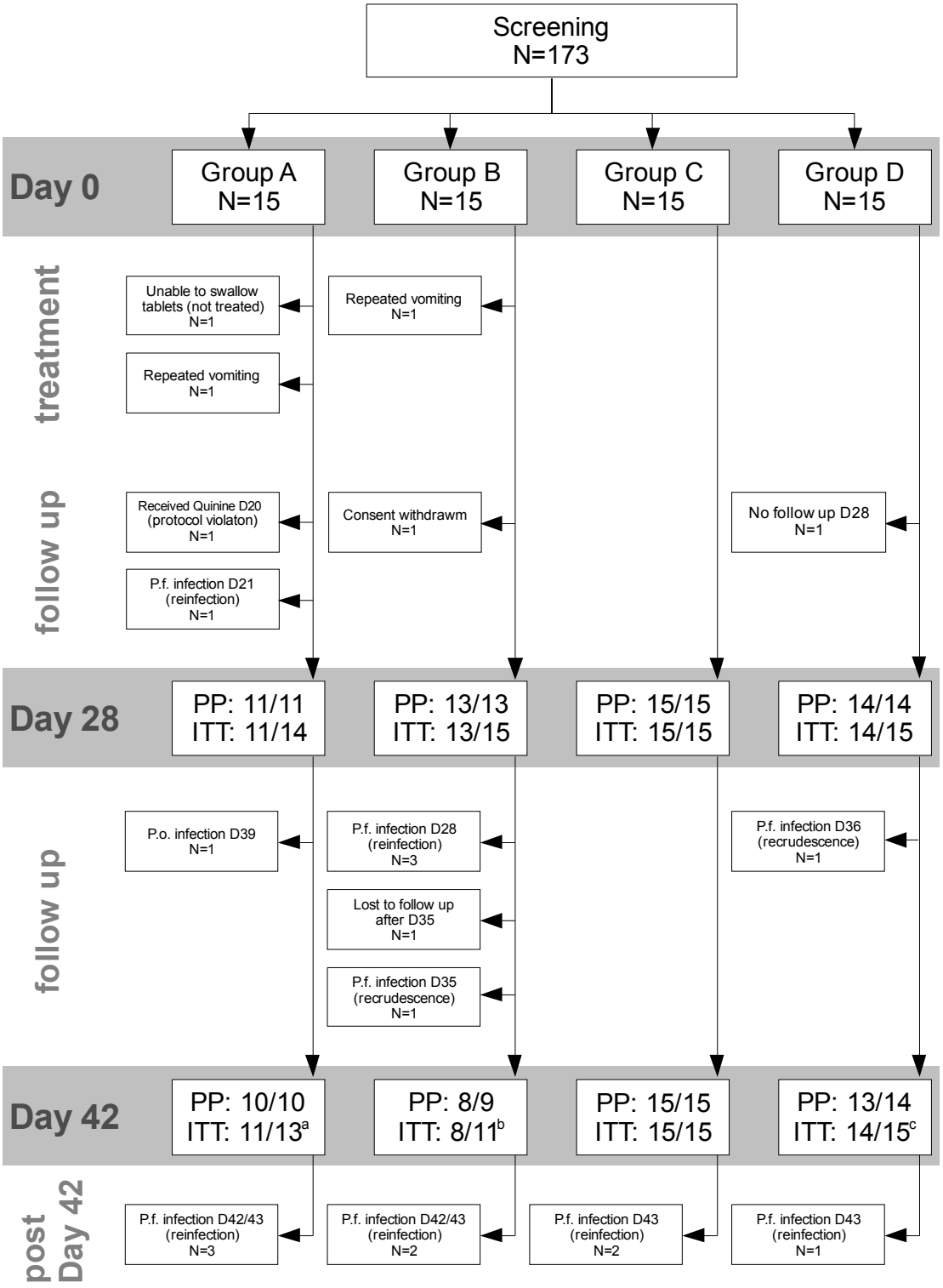


Figure 2: Patient flow (PP: Per protocol population; ITT: Intention-to-treat population)

[a] One patient with a new infection on D21 was considered not cured at D28 and was not taken into account for the D42 analysis

[b] Three patients with a new infection on D28 were considered to be cured at D28 and were not taken into account for the D42 analysis. One patient was not taken into account for the D42 analysis because he was lost to follow up on D35

[c] One patient did not come for the D28 visit but returned for further follow up visits up to D42; this patient was classified as “treatment failure” for the D28 analysis and as “cured” for the D42 analysis

3.2 Baseline characteristics

Comparison of baseline characteristics in all three treatment groups showed no relevant differences, as illustrated in table 2.

All included children were between 2 and 14 years old, with means of 5.8 (group A and C), 5.9 (group B) and 5.5 (group D). Proportions of males and females differed slightly in the four groups, in total there were 30 males and 29 females. Height and weight were as well comparable, with values between 85 to 153 cm and between 10.0 to 36.4 kg, respectively.

We had a wide range of initial parasitaemia – 1,072 being the lowest and 174,241 being the highest number of asexual *Plasmodium falciparum* parasites counted per µl of peripheral blood. More than 50 % of the children had less than 10,000 parasites per µl and 86.4 % between 1,000 per µl and 50,000 per µl. On the basis of these facts, the median is used to describe the parasitaemia of the four populations: 8,144 per µl in group A, 11,090 per µl in group B, 7,020 per µl in group C and 2,971 per µl in group D. Two patients in group A and one in group C and D, respectively presented gametocytes at baseline.

Most of the children did not have fever (defined as an orally measured body temperature of ≥38.0°C) at baseline, but reported fever within the last 24 hours, which is represented by the relatively low means of body temperature in all four groups: 37.6°C, 37.7°, 37.1°C and 37.2°C in groups A, B, C and D. Six patients with fever received paracetamol or acetylsalicylic acid as antipyretic treatment previous to start of the study. No other prior medication was recorded for this study population.

All patients showed at least one sign or symptom of a *Plasmodium falciparum* infection, mainly splenomegaly, headache, loss of appetite, fatigue and sweating. Two thirds of the included children reported at least one episode of malaria in the past 12 months and many presented a medical history, mainly infections and infestations such as Schistosomiasis or fungal and bacterial skin infections, which are common infections in this area.

Table 2: Baseline characteristics (Intention-to-treat population)

	group A *tablets (6+2 mg/kg)* ***N = 14***	***group B*** *tablets (9+3 mg/kg)* ***N = 15***	***group C*** *tablets (12+4 mg/kg)* ***N =15***	***group D*** *granules (9+3 mg/kg)* ***N = 15***
Sex [n (%)]				
Male	8 (57.1)	8 (53.3)	6 (40.0)	8 (53.3)
Female	6 (42.9)	7 (46.7)	9 (60.0)	7 (46.7)
Age [years]				
Mean ± SD	5.8 ± 2.19	5.9 ± 3.48	5.8 ± 2.60	4.6 ± 1.96
Min – max	2 – 11	2 – 14	2 – 10	2 – 10
Height [cm]				
Mean ± SD	110.4 ± 13.2	109.3 ± 18.7	111.8 ± 16.1	103.3 ± 14.3
Min – max	85 – 142	86 – 153	93 – 144	85 – 142
Weight [kg]				
Mean ± SD	18.7 ± 5.54	18.8 ± 7.00	19.0 ± 6.44	16.5 ± 5.15
Min – max	11.4 – 35.0	10.0 – 34.8	11.8 – 36.4	11.2 – 33.0
P. falciparum **asexual forms**				
Median [n/µl]	8,144	11,090	7,020	2,971
Min [n/µl]	1,072	1,152	1,350	1,096
Max [n/µl]	57,890	58,893	118,500	174,241
1,000 – 50,000/µl [n (%)]	12 (85.7)	14 (93.3)	12 (80.0)	13 (86.7)
> 50,000/µl [n (%)]	2 (14.3)	1 (6.7)	3 (20.0)	1 (13.3)
P. falciparum **gametocytes**, [n (%)]	2 (14.3)	0 (0.0)	1 (6.7)	1 (6.7)
Body temperature				
Mean ± SD [°C]	37.6 ± 0.99	37.7 ± 0.86	37.1 ± 0.91	37.2 ± 0.91
Fever [n (%)]	3 (21.4)	4 (26.7)	2 (13.3)	1 (6.7)
Clinical signs and symptoms of malaria [n (%)]				
At least one symptom	14 (100.0)	15 (100.0)	15 (100.0)	15 (100.0)
Splenomegaly	12 (85.7)	10 (66.7)	14 (93.3)	13 (86.7)
History of fever	10 (71.4)	10 (66.7)	13 (86.7)	14 (93.3)
Headache	7 (50.0)	10 (66.7)	6 (40.0)	11 (73.3)
Loss of appetite	7 (50.0)	7 (46.7)	8 (53.3)	10 (66.7)
Fatigue	7 (50.0)	9 (60.0)	6 (40.0)	9 (60.0)
Sweating	2 (14.3)	11 (73.3)	8 (53.3)	8 (53.3)
Cough	9 (64.3)	4 (26.7)	6 (40.0)	9 (60.0)
Nausea	3 (21.4)	5 (33.3)	8 (53.3)	0 (0.0)
Vomiting	5 (35.7)	3 (20.0)	5 (33.3)	1 (6.7)
Hepatomegaly	2 (14.3)	5 (33.3)	4 (26.7)	4 (26.7)
Rigors/chills	4 (28.6)	2 (13.3)	2 (13.3)	1 (6.7)
Jaundice	1 (7.1)	0 (0.0)	0 (0.0)	0 (0.0)
Myalgia (back and limbs)	1 (7.1)	0 (0.0)	0 (0.0)	0 (0.0)
Other	1 (7.1)	1 (6.7)	2 (13.3)	0 (0.0)
Medical history [n (%)]				
At least one malaria episode in the last 12 months	9 (64.3)	8 (53.3)	10 (66.7)	13 (86.7)
At least one medical history	8 (57.1)	6 (40.0)	6 (40.0)	12 (80.0)
Infections and infestations	6 (42.9)	6 (40.0)	6 (40.0)	9 (60.0)
Prior medication [n (%)]				
Antipyretics	3 (21.4)	0 (0.0)	1 (6.7)	2 (13.3)

(SD: Standard deviation)

3.3 Safety and tolerability

3.3.1 Adverse Events and Serious Adverse Events

The majority of children experienced at least one adverse event (AE), 13 in the 6+2 mg/kg tablet group, 11 in the 9+3 mg/kg tablet group, 13 in the 12+4 mg/kg tablet group and 12 in the 9+3 mg/kg granule group, which corresponds to 81,6 % of all included patients.

All adverse events were recorded in Case Report Forms (CRF) with a short description, start and end date and information about seriousness (yes/no), severity (mild, moderate, severe, life-threatening), relationship to study medication (definite, probable, possible, none), action taken (none, study drug discontinued, patient withdrawn from study, concomitant medication, hospitalization required or prolonged, other) and outcome (resolved, resolved with sequelae, continuing, death).

Most adverse events belonged to the categories “infections and infestations”, mainly *Plasmodium falciparum* and other parasitic infections, or to “gastrointestinal disorders”, such as nausea, vomiting, abdominal pain and diarrhoea. An overview of all adverse events is given in table 3.

Table 3: Adverse events (Intention-to-treat population)

	group A *tablets (6+2 mg/kg)* ***N = 14***	***group B*** *tablets (9+3 mg/kg)* ***N = 15***	***group C*** *tablets (12+4 mg/kg)* ***N = 15***	***group D*** *granules (9+3 mg/kg)* ***N = 15***
Any adverse event [n (%)]	13 (92.9)	11 (73.3)	13 (86.7)	12 (80.0)
Mild	8 (57.1)	8 (53.3)	11 (73.3)	10 (66.7)
Moderate	5 (35.7)	3 (20.0)	2 (13.3)	2 (13.3)
Infections and infestations [n (%)]	9 (28.6)	8 (53.3)	10 (66.7)	11 (73.3)
P. falciparum infection	4 (35.7)	6 (40.0)	2 (13.3)	2 (13.3)
Nasopharyngitis	2 (14.3)	0 (0.0)	3 (20.0)	5 (33.3)
Ascariasis	1 (7.1)	1 (6.7)	3 (20.0)	1 (6.7)
Acarodermatitis	0 (0.0)	0 (0.0)	2 (13.3)	1 (6.7)
Abscess	1(7.1)	0 (0.0)	1 (6.7)	0 (0.0)
Schistosomiasis	1 (7.1)	0 (0.0)	1 (6.7)	0 (0.0)
Urinary tract infection	1 (7.1)	0 (0.0)	1 (6.7)	0 (0.0)
P. ovale infection	1 (7.1)	0 (0.0)	0 (0.0)	0 (0.0)
Ear lobe infection	0 (0.0)	0 (0.0)	1 (6.7)	0 (0.0)
Panaritium	1 (7.1)	0 (0.0)	0 (0.0)	0 (0.0)
Pneumonia	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Skin infection	0 (0.0)	0 (0.0)	1 (6.7)	0 (0.0)
Tinea capitis	1 (7.1)	1 (6.7)	0 (0.0)	0 (0.0)
Trichuriasis	1 (7.1)	0 (0.0)	0 (0.0)	0 (0.0)
Gastrointestinal disorders [n (%)]	7 (50.0)	3 (20.0)	3 (20.0)	1 (6.7)
Vomiting	4 (28.6)	3 (20.0)	1 (6.7)	1 (6.7)
Abdominal pain	2 (14.3)	1 (6.7)	1 (6.7)	0 (0.0)
Diarrhoea	1 (7.1)	0 (0.0)	1 (6.7)	0 (0.0)
Nausea	1 (7.1)	1 (6.7)	0 (0.0)	0 (0.0)
Headache [n (%)]	5 (35.7)	1 (6.7)	2 (13.3)	1 (6.7)
General disorders [n (%)]				
Fatigue	1 (7.1)	2 (13.3)	0 (0.0)	1 (6.7)
Pyrexia	1 (7.1)	0 (0.0)	1 (6.7)	2 (13.3)
Chills	0 (0.0)	0 (0.0)	1 (6.7)	0 (0.0)
Anorexia	2 (14.3)	0 (0.0)	0 (0.0)	1 (6.7)
Dehydration	0 (0.0)	0 (0.0)	1 (6.7)	0 (0.0)
Respiratory disorders [n (%)]				
Cough	2 (14.3)	1 (6.7)	2 (13.3)	1 (6.7)
Rhinorrhoea	0 (0.0)	0 (0.0)	0 (0.0)	1 (6.7)
Blood and lymphatic system disorders [n (%)]				
Splenomegaly	0 (0.0)	2 (13.3)	0 (0.0)	1 (6.7)
Anaemia	1 (7.1)	0 (0.0)	0 (0.0)	0 (0.0)
Lymphadenopathy	0 (0.0)	0 (0.0)	1 (6.7)	0 (0.0)
Hepatomegaly [n (%)]	0 (0.0)	0 (0.0)	2 (13.3)	0 (0.0)

No deaths occurred during the study, but two adverse events, both in group A, were classified as serious adverse events (SAE) as they required

hospitalization. A two-year old female patient with known chronic otitis media was hospitalized on day 20 after first drug administration in the local public hospital for acute abdominal pain and fever without knowledge of the study team and received intravenous quinine and antibiotics presumptively (with negative thick blood smear). She recovered completely but was withdrawn from the study because of antimalarial treatment which represents a protocol violation. Another six-year old female patient suffered from a panaritium of moderate intensity. Despite early antibiotic treatment, a surgical intervention became necessary. The patient had full recovery and was able to continue study participation. Both serious adverse events were not considered to be study drug related.

No severe or life-threatening adverse events occurred; most of them were of mild, only 10 of moderate severity. Two moderate adverse events, both in group A, were considered to be drug related – vomiting and anaemia.

All adverse events that are considered to be study drug related, which includes possible, probable or definite relationship to the study drug, are summarized in the following table. The most frequent study drug related adverse events are gastrointestinal disorders, mainly vomiting and abdominal pain, which were both reported for at least one patient in each dose level group.

Table 4: Adverse events considered study drug related (Intention-to-treat population)

	group A *tablets (6+2 mg/kg)* ***N = 14***	***group B*** *tablets (9+3 mg/kg)* ***N = 15***	***group C*** *tablets (12+4 mg/kg)* ***N = 15***	***group D*** *granules (9+3 mg/kg)* ***N = 15***
Any drug-related adverse event [n (%)]	5 (35.7)	4 (26.7)	5 (33.3)	3 (20.0)
Mild	3 (21.4)	4 (26.7)	5 (33.3)	2 (13.3)
Moderate	2 (14.3)	0 (0.0)	0 (0.0)	1 (6.7)
Gastrointestinal disorders [n (%)]	3 (21.4)	2 (13.3)	2 (13.3)	1 (6.7)
Vomiting	1 (7.1)	1 (6.7)	1 (6.7)	1 (6.7)
Abdominal pain	1 (7.1)	1 (6.7)	2 (13.3)	0 (0.0)
Diarrhoea	1 (7.1)	0 (0.0)	0 (0.0)	0 (0.0)
Nausea	1 (7.1)	0 (0.0)	0 (0.0)	0 (0.0)
Headache [n (%)]	1 (7.1)	0 (0.0)	0 (0.0)	1 (6.7)
General disorders [n (%)9				
Fatigue	1 (7.1)	1 (6.7)	0 (0.0)	1 (6.7)
Pyrexia	0 (0.0)	0 (0.0)	0 (0.0)	1 (6.7)
Anorexia	1 (7.1)	0 (0.0)	0 (0.0)	1 (6.7)
Hyperhidrosis	0 (0.0)	0 (0.0)	1 (6.7)	0 (0.0)
Blood and lymphatic system disorders [n (%)]				
Splenomegaly	0 (0.0)	1 (6.7)	0 (0.0)	1 (6.7)
Anaemia	1 (7.1)	0 (0.0)	0 (0.0)	0 (0.0)
Hepatomegaly [n (%)]	0 (0.0)	0 (0.0)	2 (13.3)	0 (0.0)

Two adverse events, both episodes of vomiting, led to study drug discontinuation and withdrawal from the study; six other patients were withdrawn from the study due to re-appearing *Plasmodium falciparum* parasites and one due to a *Plasmodium ovale* infection. All other adverse events could be treated with concomitant medication and hospitalization was only required for the two SAEs mentioned above. All children recovered completely and no sequelae were observed.

3.3.2 Changes in laboratory values

Evaluation of haematologic parameters (including differential blood count) over time did show a clinically significant change in one patient (6+2 mg/kg tablet group). This child experienced a drop in haemoglobin from 7.4 g/dl at baseline to 4.9 g/dl 24 hours after first drug administration, but recovered to higher than baseline values during follow up without specific medical intervention. All other abnormalities and changes in haematologic values were not clinically

significant. Most prominent changes could be observed for platelets, where median values increased from baseline to day 7 in all four treatment groups, for neutrophils, where median values decreased over time, especially in group A and B, and for eosinophils, where medians rose in all groups during the course of the study. Details of median haematology parameters are shown in table 5.

Table 5: Median haematology values over time (Intention-to-treat population)

	group A *tablets (6+2 mg/kg)* ***N = 14***	***group B*** *tablets (9+3 mg/kg)* ***N = 15***	***group C*** *tablets (12+4 mg/kg)* ***N = 15***	***group D*** *granules (9+3 mg/kg)* ***N = 15***
Parameter	*median*	*median*	*median*	*median*
Haemoglobin [g/dl]				
Baseline	10.0	10.5	10.2	9.5
24 hours	9.3	9.3	9.7	8.2
day 3	9.7	10.4	9.9	9.1
day 7	10.1	10.6	9.7	10.0
Haematocrit [%]				
Baseline	29.5	31.3	30.7	27.5
day 3	29.0	31.3	28.2	27.5
day 7	30.4	31.0	28.1	29.5
Red blood cells [10^6/mm³]				
Baseline	4.35	4.39	4.36	3.81
day 3	4.16	4.37	4.12	3.69
day 7	4.35	4.51	3.98	3.97
Platelets [10^3/mm³]				
Baseline	244	210	219	196
day 3	298	560	235	250
day 7	336	370	271	324
White blood cells [10^9/l]				
Baseline	7.6	7.9	9.3	7.5
day 3	7.6	8.1	7.3	8.5
day 7	7.9	9.7	9.4	8.7
Neutrophils [%]				
Baseline	43.3	40.7	29.3	30.7
day 3	28.3	25.1	25.8	32.7
day 7	26.5	29.3	26.1	31.3
Lymphocytes [%]				
Baseline	37.3	35.3	42.0	44.2
day 3	45.6	43.2	44.3	39.5
day 7	49.5	39.7	43.3	45.1
Monocytes [%]				
Baseline	10.7	11.3	9.4	12.8
day 3	8.7	9.6	9.8	10.4
day 7	7.7	9.0	8.4	9.5
Eosinophils [%]				
Baseline	5.7	7.4	5.1	8.7
day 3	9.2	19.4	19.0	14.1
day 7	10.2	13.2	15.4	13.7
Basophils [%]				
Baseline	0.7	0.7	0.8	0.9
day 3	1.0	0.9	0.8	0.8
day 7	0.8	0.9	1.0	1.0

Particular attention was drawn on haemoglobin changes from baseline levels. Overall, a modest fall of median haemoglobin levels could be observed during the treatment period with return to baseline values until day 7. They were classified as decreases of more than -2 g/dl, slight decreases between 0 and -2 g/dl and increases (changes ≥ 0 mg/dl). This categorization shows that most changes were of minor extent, which is illustrated in the following diagrams.

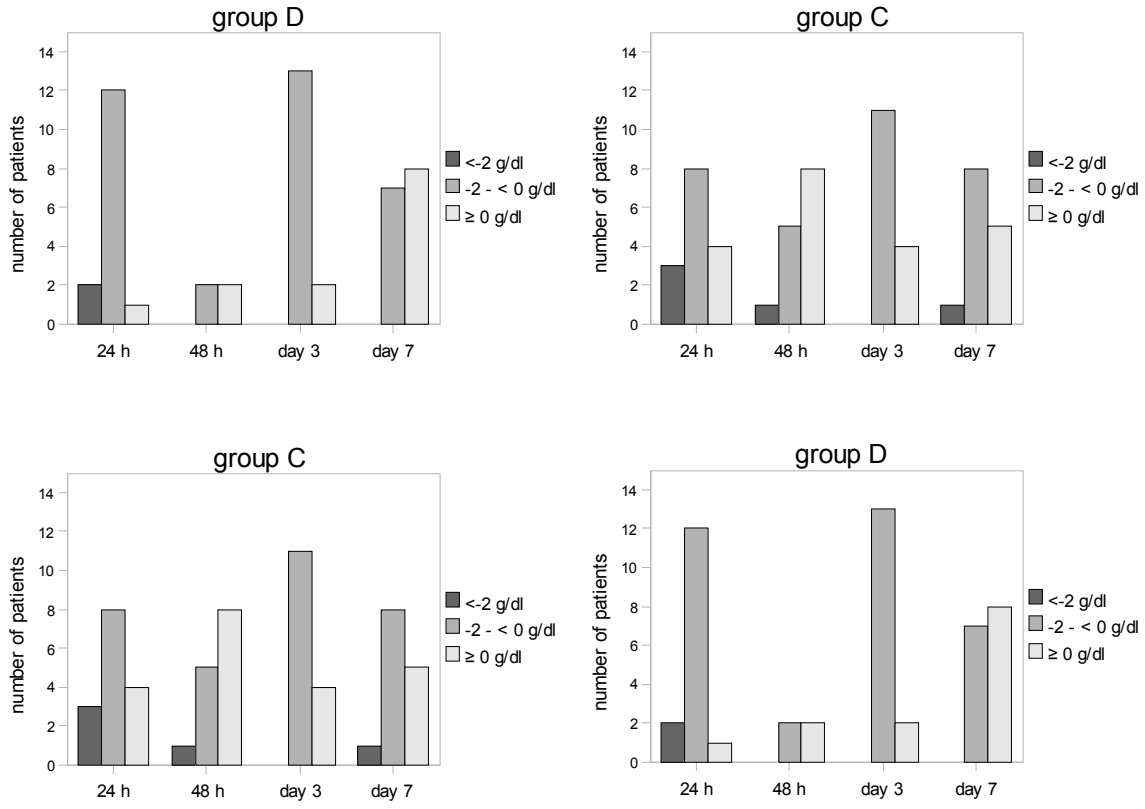


Figure 3: Changes of haemoglobin values from baseline (Intention-to-treat population)

In general, biochemistry parameter showed little variation in the course of the study, except for a decrease from baseline of median total bilirubin values in all treatment groups. For urea, an increase of median values was found in group B and decreases in groups A, C and D. No abnormal biochemistry values or changes were considered to be clinically significant. All median values of biochemistry assessments are summarized in table 6.

Table 6: Median biochemistry values over time (Intention-to-treat population)

	group A *tablets (6+2 mg/kg)* ***N = 14***	***group B*** *tablets (9+3 mg/kg)* ***N = 15***	***group C*** *tablets (12+4 mg/kg)* ***N = 15***	***group D*** *granules (9+3 mg/kg)* ***N = 15***
Parameter	*median*	*median*	*median*	*median*
Total bilirubin [µmol/l]				
Baseline	9.44	10.85	11.60	13.63
day 3	3.54	6.82	6.67	7.54
day 7	4.13	7.44	8.12	6.96
Albumin [g/dl]				
Baseline	3.31	3.99	3.58	3.47
day 3	3.27	4.14	3.49	3.82
day 7	3.41	3.76	3.69	3.49
ALT [IU/l]				
Baseline	19.0	16.6	23.0	21.0
day 3	13.0	18.0	22.0	19.0
day 7	17.0	14.5	23.0	18.0
AST [IU/l]				
Baseline	36.0	28.0	29.0	36.0
day 3	31.0	27.0	31.0	27.0
day 7	10.0	25.0	29.0	33.0
Creatine kinase [IU/l]				
Baseline	72	87	79	61
day 3	69	71	69	48
day 7	72	88	71	67
Alkaline phosphatase [IU/l]				
Baseline	262	274	206	188
day 3	231	237	179	158
day 7	254	298	172	166
Urea [mmol/l]				
Baseline	2.84	2.64	3.38	3.38
day 3	2.52	3.36	3.33	3.59
day 7	2.19	3.96	2.93	2.30
Creatinine [µmol/l]				
Baseline	35.8	38.6	24.8	29.0
day 3	37.1	30.4	28.2	30.2
day 7	27.9	36.0	31.0	29.2
Sodium [mmol/l]				
Baseline	136	137	138	138
day 3	137	139	138	140
day 7	138	138	138	140
Potassium [mmol/l]				
Baseline	4.0	4.1	4.1	3.9
day 3	4.0	4.5	4.2	4.2
day 7	4.3	4.6	4.3	4.4

(IU: International Unit)

Urinalysis yielded some clinically significant findings, mostly blood and *Schistosoma* eggs in the context of *Schistosoma haematobium* infestation and leukocytes and nitrite as signs of urinary tract infection. Comparison of all dose level groups did not show major differences in frequency and severity between the groups.

3.3.3 ECG changes

All electrocardiograms were considered normal or presented clinically non-significant changes as judged by the investigator at the study site and an independent reviewer. Heart rate-corrected QT (QTc)-intervals were specifically interesting, but all values remained within the normal ranges, which means no values over 470 ms for females and over 450 ms for males were detected in the ECG recordings.

3.3.4 Vital signs changes

Mean systolic and diastolic blood pressure were relatively constant at all visits, mean heart rates decreased slightly until day 28 in all four groups as illustrated in the following diagrams.

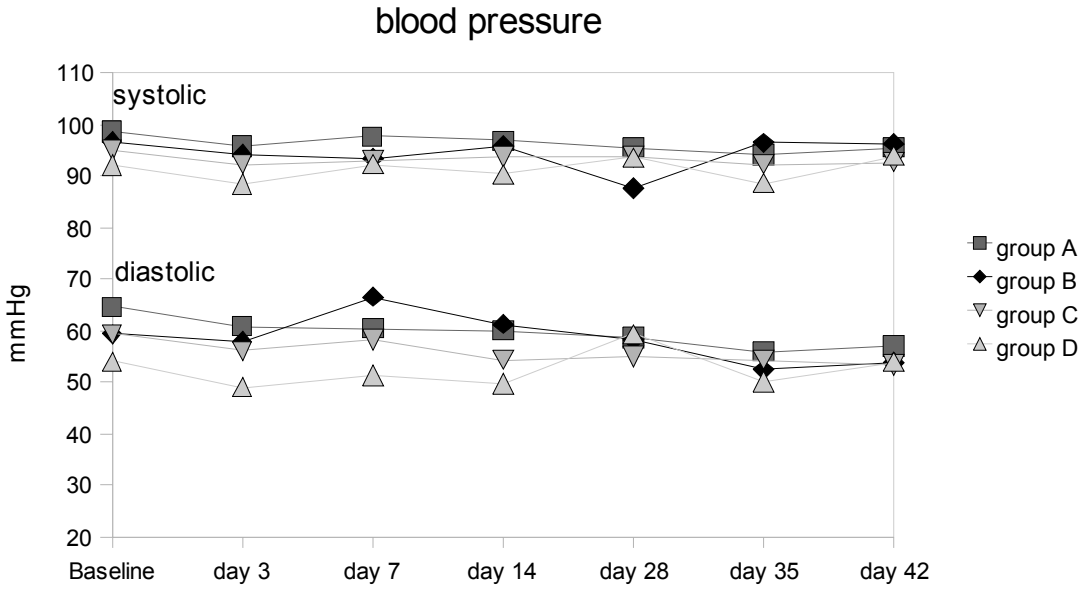


Figure 4: Mean blood pressure over time (Intention-to-treat population)

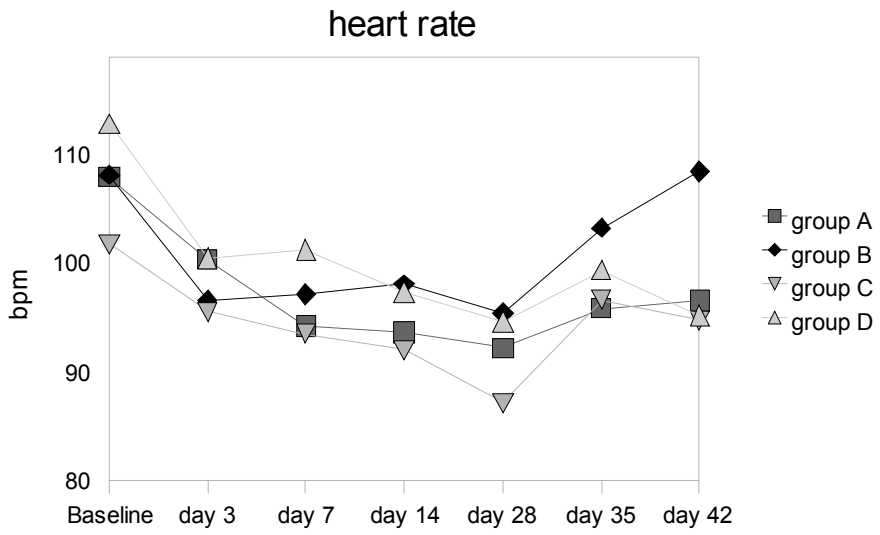


Figure 5: Mean heart rate over time (Intention-to-treat population)

Overall, the pyronaridine-artesunate combination was very well tolerated in all three investigated tablet groups, as well as in the granule group with the same dosage than group B. Particularly, the comparison of the different dose levels did not show any increase in number or severity of adverse events or clinically significant changes in laboratory values, vital signs and ECG readings.

3.3.5 Concomitant medication

Between 26.7 % and 40.0 % of the patients in the different treatment groups were given antipyretic treatment (most commonly paracetamol) at least once during the study. Administration was mainly in the first three days of treatment, only one child of each treatment group A, B and C and three children in group D received antipyretics after day 3.

Most of the patients received at least one concomitant medication during the study; 78.6 % in group A, 53.3 % in group B, 66.7 % in group C and 80.0 % in group D (antipyretics included, antimalarials excluded). Apart from paracetamol the following drugs were the most frequently dispensed ones: praziquantel for the treatment of *Schistosoma haematobium* infestations, albendazole as anthelmintic therapy and antibiotic drugs for simultaneous bacterial infections.

Antimalarials were administered as rescue medication after repeated vomiting (two patients) or in case of re-appearance of parasites (14 patients), mainly a combination of quinine-sulfadoxine/pyrimethamine or artesunate-amodiaquine. One child was treated with quinine in the local hospital for abdominal pain and fever and another patient suffering from *Plasmodium ovale* infection was given chloroquine.

3.4 Efficacy

3.4.1 Cure rates

PCR-corrected adequate clinical and parasitological response (ACPR) in the per-protocol population was defined as the primary efficacy endpoint. All other parameters were secondary efficacy variables.

In per-protocol analysis no treatment failures were observed until day 28, translating into a 100 % efficacy at all four dose levels. PP-analyses on day 14 and 42 showed the same results, except for groups B and D. On day 42, efficacy was 89 % (95 % confidence interval (CI): 52 – 100 %) in group B and 93 % (95 % CI: 66 – 100 %) in group D, due to two children with recrudescent infections on day 35. These two patients experiencing Late Clinical Failure (LCF) were classified as true treatment failure according to results of genotyping.

In intention-to-treat analysis on day 14, three patients (one of group A and two of group B) were counted as treatment failures as the primary endpoint was unknown due to vomiting and withdrawn consent. For day 28 analysis, two additional cases in group A (quinine treatment on day 20 and *Plasmodium falciparum* infection on day 21) were considered to be treatment failure because of the lack of data on the primary endpoint. One child with *Plasmodium falciparum* infection on day 21 was not taken into account for day 42 analysis. These facts led to an efficacy on day 28 of 79 % in group A, 87 % in group B, 100 % in group C and 93 % in group D (95 % CIs : 49 – 95 %, 60 – 98 %, 78 – 100 %, 68 – 100 %, respectively). All other details are summarized in the following table.

Table 7: PCR-corrected ACPR on days 14, 28 and 42 (Per-protocol and Intention-to-treat population)

	group A *tablets (6+2 mg/kg)*		***group B*** *tablets (9+3 mg/kg)*		***group C*** *tablets (12+4 mg/kg)*		***group D*** *granules (9+3 mg/kg)*	
	PP	***ITT***	***PP***	***ITT***	***PP***	***ITT***	***PP***	***ITT***
day 14								
N	11	14	13	15	15	15	14	15
n (%) with ACPR	11 (100.0)	13 (92.9)	13 (100.0)	13 (86.7)	15 (100.0)	15 (100.0)	14 (100.0)	15 (100.0)
Exact 95 % CI [%]	71.5 – 100.0	66.1 – 99.8	75.3 – 100.0	*59.5* – 98.3	78.2 – 100.0	78.2 – 100.0	76.8 – 100.0	78.2 – 100.0
Treatment failures, n (%)	0 (0)	1 (7)	0 (0)	2 (13)	0 (0)	0 (0)	0 (0)	0 (0)
ETF	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
LCF	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
LPF	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Unknown primary endpoint		1 (7)		2 (13)		0 (0)		0 (0)
day 28								
N	11	14	13	15	15	15	14	15
n (%) with ACPR	11 (100.0)	11 (78.6)	13 (100.0)	13 (86.7)	15 (100.0)	15 (100.0)	14 (100.0)	14 (93.3)
Exact 95 % CI [%]	71.5 -100.0	49.2 – 95.3	75.3 – 100.0	59.5 – 98.3	78.2 – 100.0	78.2 – 100.0	76.8 – 100.0	68.1 – 99.8
Treatment failures, n (%)	0 (0)	3 (21)	0 (0)	2 (13)	0 (0)	0 (0)	0 (0)	1 (7)
ETF	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
LCF	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
LPF	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Unknown primary endpoint		3 (21)		2 (13)		0 (0)		1 (7)
day 42								
N	10	13	9	11	15	15	14	15
n (%) with ACPR	10 (100.0)	11 (84.6)	8 (88.9)	8 (72.7)	15 (100.0)	15 (100.0)	13(92.9)	14 (93.9)
Exact 95 % CI [%]	69.2 -100.0	54.6 – 98.1	51.8 – 99.7	39.0 – 94.0	78.2 – 100.0	78.2 – 100.0	66.1 – 99.8	68.1 99.8
Treatment failures, n (%)	0 (0)	2 (15)	1 (11.1)	3 (27)	0 (0)	0 (0)	1 (7)	1 (7)
ETF	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
LCF	0 (0)	0 (0)	1 (11.1)	1 (9.1)	0 (0)	0 (0)	0 (0)	0 (0)
LPF	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (7)	1 (7)
Unknown primary endpoint		2 (15)		2 (18)		0 (0)		0 (0)

(PP: Per-protocol population; ITT: Intention-to-treat population; ACPR: Adequate clinical and parasitological response; CI: Confidence interval; ETF: Early treatment failure; LCF: Late clinical failure; LPF: Late parasitological failure)

Analysis of crude, non-PCR-corrected ACPR on day 28 showed cure rates between 76.9 % and 100 % in PP population and between 66.7 % and 100.0 % in ITT population for the respective cohorts. Day 14 results correspond to those of PCR-corrected analysis and for day 42 lower rates were achieved due to the relatively high amount of new infections on day 42. Details are displayed in table 8.

Table 8: Crude ACPR on days 14, 28, 42 (Per-protocol and Intention-to-treat population)

	group A *tablets (6+2 mg/kg)*	***group B*** *tablets (9+3 mg/kg)*	***group C*** *tablets (12+4 mg/kg)*	***group D*** *granules (9+3 mg/kg)*
Per-protocol				
day 14	11/11 (100.0)	13/13 (100.0)	15/15 (100.0)	14/14 (100.0)
95 % CI [%]	*71.5 – 100.0*	*75.3 – 100.0*	*78.2 – 100.0*	*76.8 – 100.0*
day 28	11/11 (100.0)	10/13 (76.9)	15/15 (100.0)	14/14 (100.0)
95 % CI [%]	*71.5 – 100.0*	*46.2 – 95.0*	*78.2 - 100.0*	*76.8 – 100.0*
day 42	7/10 (70.0)	6/12 (50.0)	13/15 (86.7)	12/14 (85.7)
95 % CI [%]	*34.8 – 89.1*	*21.1 – 78.9*	*59.5 – 98.3*	*57.2 – 98.2*
Intention-to-treat				
day 14	13/14 (92.9)	13/15 (86.7)	15/15 (100.0)	15/15 (100.0)
95 % CI [%]	*66.1 – 99.8*	*59.5 – 98.3*	*78.2 – 100.0*	*78.2 – 100.0*
day 28	11/14 (78.6)	10/15 (66.7)	15/15 (100.0)	14/15 (93.3)
95 % CI [%]	*49.2 – 95.3*	*38.4 – 88.2*	*78.2 – 100.0*	*68.1 – 99.8*
day 42	8/14 (57.1)	6/14 (42.9)	13/15 (86.7)	13/15 (86.7)
95 % CI [%]	*28.9 – 82.3*	*17.7 – 71.1*	*59.5 – 98.3*	*59.5 – 98.3*

(*CI: Confidence interval)*

In both PCR-corrected and crude ACPR analyses confidence intervals showed wide ranges due to the small sample size of the study.

3.4.2 Parasite clearance

Kaplan-Meier estimates of time until parasite clearance showed that patients were free from parasites after comparatively short periods at all dose levels. Median time to parasite clearance was 16.4 hours, 16.1 hours, 8.1 hours and 8.3 hours (95 % CIs: 16.1 – 22.4 h, 8.3 – 16.4 h, 8.0 – 16.0 h, 8.1 – 15.9 h) for groups A, B, C and D, respectively. The shortest parasite clearance time was 7.9 hours, observed in group C and the longest was 32.0 hours, found in group B. Most patients achieved clearance from parasites in the initial 24 hours after first drug administration, which is illustrated in figure 6.

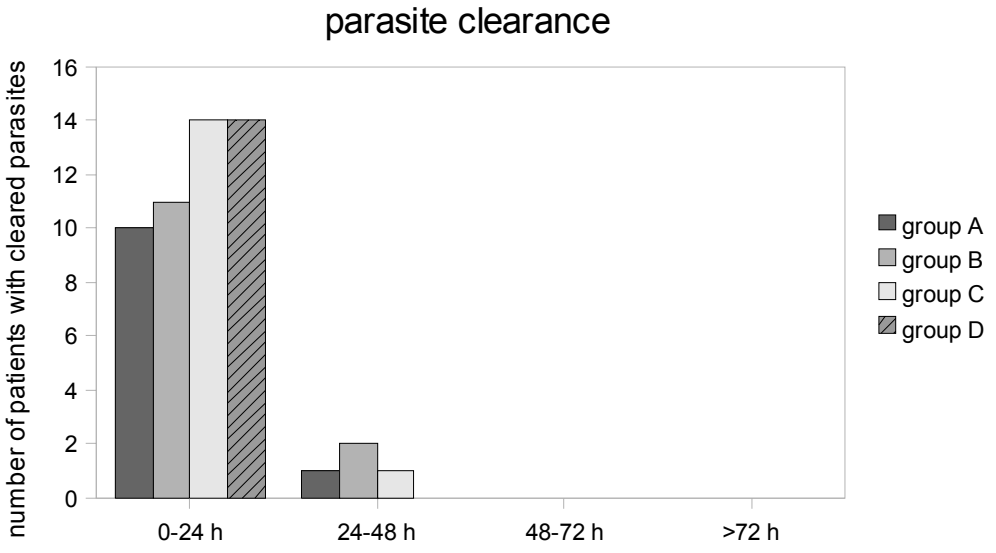


Figure 6: Number of patients with cleared parasites over time (Per-protocol population)

The probability of parasite clearance over time in hours is shown in figure 7 (Kaplan-Meier estimates) and demonstrates that all included patients were free from parasites within 32 hours.

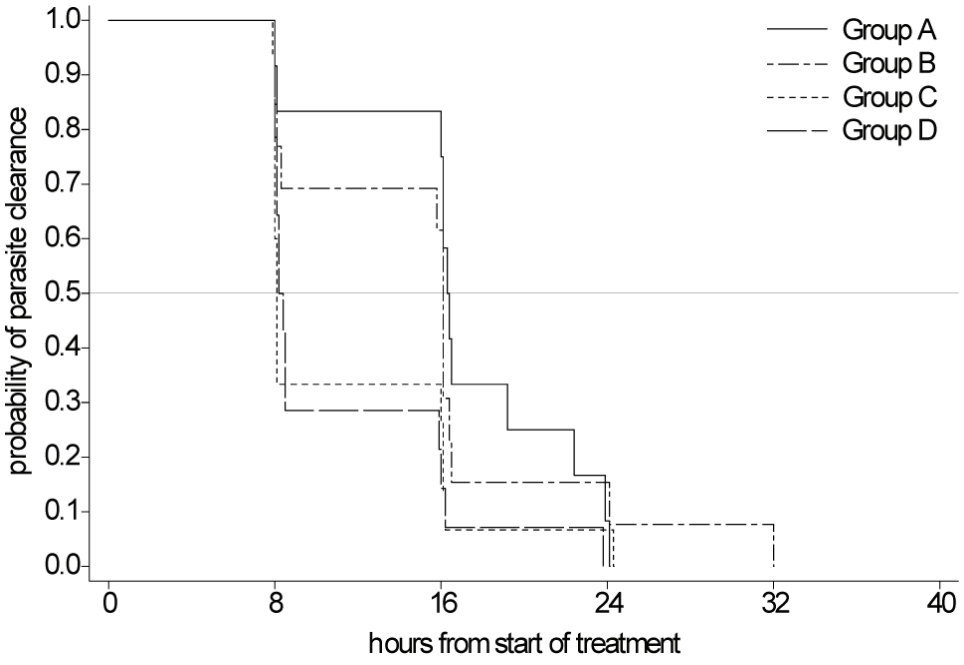


Figure 7: Kaplan-Meier estimates of the time until parasite clearance in hours (Per-protocol population)

3.4.3 Fever clearance

Due to the fact that most children did not present fever at baseline, only 12 patients could be included in this analysis (3, 5, 3, 1 of groups A to D, respectively). Normal temperature was achieved in median times of 8.2 hours in the 6+2 mg/kg tablet group, the 12+4 mg/kg tablet group and the 9+3 mg/kg granule group and in 8.6 hours in the 9+3 mg/kg tablet group. Kaplan-Meier estimates are displayed in figure 8.

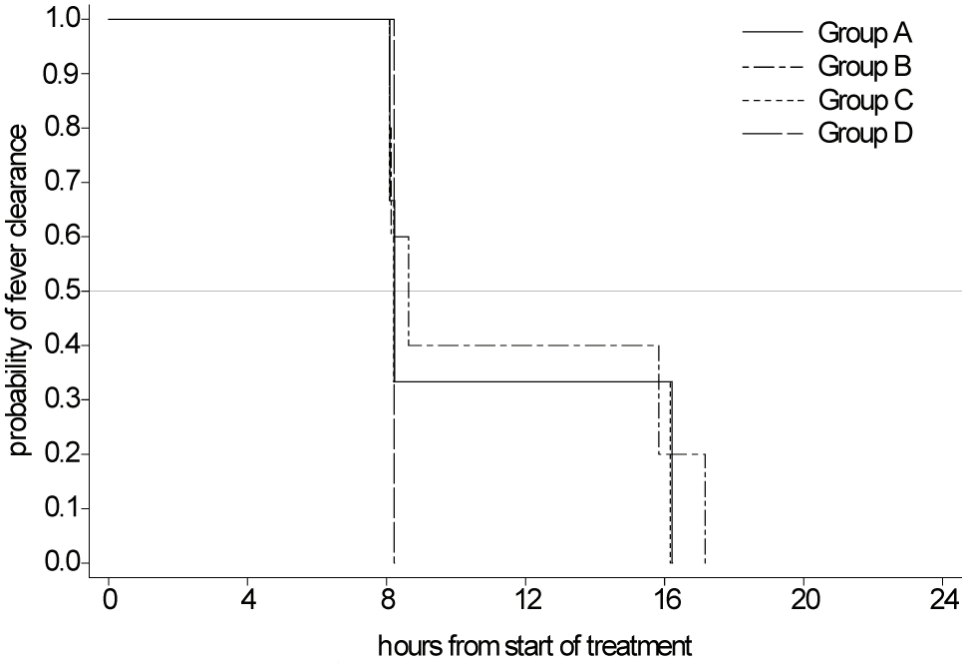


Figure 8: Kaplan-Meier estimates of the time until fever clearance (Per-protocol population)

3.4.4 Gametocyte status assessment

Gametocyte carriers were quite infrequent in this study; only seven patients showed gametocytes at low concentrations in thick blood smears. The two patients of group A presenting with gametocytes at baseline were completely cleared until day 14 and day 22, respectively, the patient of group C was free from gametocytes on day 14 and the one of group D was cleared 32 hours after the first treatment dose. In the course of the study three more patients showed solitary gametocyte-positive counts, whereas all other counts were negative: two of group A, in the first case in association with *Plasmodium falciparum* re-

infection on day 21 and the second case was detected 7.9 hours after first treatment; the third patient was included in group B and had gametocyte-positive thick smears at the time points 16 hours and 60 hours post start of drug administration.

4 Discussion

Malaria is a serious health problem, mainly throughout the world's tropical areas, and jointly responsible for severe medical, social, and economic problems in the affected countries.

The Roll Back Malaria programme aims at halving malaria mortality by the year 2010. A major step to reach this goal is the development of new antimalarial drugs, especially drug combinations in order to reduce the risk of emerging resistance. Currently, the most important and intensively investigated class are the artemisinin-based combination therapies, consisting of a short acting artemisinin derivative and a partner drug with longer half life. Ideally, these new drugs should meet several criteria: safety and good tolerability, high efficacy and effectiveness, and a low risk for the development of drug resistant parasite strains. To safeguard adequate patients' compliance it is of high importance that antimalarial combinations are available in fixed dose formulations and have simple dosing regimens of not more than one drug intake per day for a maximum of three days. Costs and better delivery systems are further essential factors for the implementation of new drugs to guarantee access for those most in need[14,96].

Children are arguably the most important target population of any new antimalarial treatment. However, only few clinical development programs are under way for the registration of paediatric drug formulations of antimalarial drug combinations. Due to a different pharmacological response to drugs in paediatric patients[97], results from adult studies cannot be simply extrapolated to the use in children. Paediatric studies are urgently needed to obtain data about safety, efficacy, tolerability and pharmacokinetics. Special attention should be focussed on the development of paediatric formulations for small children who are not yet able to swallow whole tablets. Such formulations will help to increase compliance and hence lead to better treatment success.

During a long history of clinical use, both artesunate and pyronaridine have proved a very good safety profile, little side effects and high efficacy. They are

ideal partner drugs, combining the fast parasite reduction of short acting artesunate with the prolonged action of pyronaridine and protecting each other against resistance. After having already shown its assumed properties in Phase I and Phase II adult studies, this work provides the first data about the use of pyronaridine-artesunate in children. This new combination has the potential to become an alternative to commonly used malaria treatment, especially for paediatric use in endemic areas.

4.1 Study design and baseline

This clinical Phase II trial was designed as an open-label, dose-escalating study evaluating a pyronaridine-artesunate combination for the treatment of children. The tablets and the new granule formulation contained pyronaridine and artesunate in the ratio 3:1. This study was designed to evaluate the safety and tolerability of pyronaridine artesunate. In addition, first indications of the potential efficacy profile could be obtained, which permits an inclusion of this age group in further Phase III studies. However, the study was not designed for a comparative evaluation of efficacy. Efficacy parameters were investigated in line with the standard guidelines provided by the WHO[94] and are therefore comparable with other clinical trials employing this trial methodology.

The study population ranged from two to fourteen years, whereas half of all included patients actually were between two and five years old. This age range was chosen to account for WHO recommendations emphasizing the importance of including this age group in clinical trials, as they often have a worse therapeutical response compared to older children[94].

The qualitative and exploratory nature of this study also explains the absence of sample size calculations for the treatment groups, as no statistical hypothesis was to be tested. The predefined objective to obtain at least 12 evaluable patients per dose level was obtained. 60 patients were included; one patient in group A turned out to be unable to swallow the investigational tablets and was therefore excluded from all analyses. During the treatment phase two more

patients discontinued the study due to repeated vomiting. All other patients received the complete three doses of the investigational drug.

During the follow up period until day 42, ten patients were withdrawn from the study prematurely in the four treatment groups. Nearly all patients presented for each follow up visit, except for one child of group A that missed day 14, one of group B that did not come for day 42 and one patient of group D who was travelling on day 28 and day 35, but returned for day 42 visit.

Patients presenting at the Medical Research Unit of the Albert Schweitzer Hospital and considered eligible for the study were assigned to the four subsequent treatment groups. This approach led to incidentally mixed groups that were yet comparable with regard to sex, age, height and weight, *Plasmodium falciparum* parasitaemia and gametocyte count, body temperature, clinical signs and symptoms of malaria, medical history and prior antipyretic medication. Median parasite counts were as well comparable (8.144 per µl, 11.090 per µl, 7.020 per µl, 2,971 per µl in groups A, B, C, D respectively), but the number of asexual forms of *Plasmodium falciparum* showed a wide dispersion (from 1.072 per µl to 174.241 per µl in all four groups). Most of the patients had quite low parasite counts, which might have been the cause for the low mean body temperature and have had an influence on the relatively fast parasite clearance.

4.2 Safety and tolerability

Safety and tolerability results were very favourable. Main safety aspects in this study were the occurrence of adverse events and serious adverse events as well as significant changes in laboratory values and ECG recordings.

Adverse events were analyzed regarding their seriousness, severity, required treatment and outcome. Particular attention was laid on the relationship of any untoward finding to the study drug. The majority of adverse events in this study were classified as mild or moderate. Concomitant infections, infestations and

gastrointestinal disorders were most prominent and they were all transient in nature.

The 17 adverse events considered at least possibly drug related were mostly gastrointestinal or general disorders and none of them was severe or life-threatening. As gastrointestinal irritations and general disorders like headache, fatigue or pyrexia are also common symptoms of malaria, it is very difficult to distinguish between side effects caused by the administered drugs and signs of the *Plasmodium falciparum* infection. These problems of interpretation have already been described in previous reports[98]. No signs of neurotoxicity or hypersensitivity reactions could be observed.

Two serious adverse events requiring hospitalization occurred during the study. Their relationship to therapy was investigated carefully but any causal relation to the study drug could be excluded.

Safety and tolerability was similarly judged based on biochemistry analysis during the study period. Significant changes were observed for haemoglobin, platelet and eosinophil counts. All other values, including biochemical assessments, did not show any relevant changes during the study period. In previous studies there were indications for an elevation of liver enzymes in the course of antimalarial treatment with artemisinins[99]. In this study no such findings occurred.

Slight falls in haemoglobin levels, mostly less than -2 g/dl, could be observed in many patients. They were all considered not clinically significant except for one: a two-year old girl had a haemoglobin drop from 7.4 g/dl at baseline to 4.9 g/dl 24 hours after drug administration. An intervention was not necessary, she recovered clinically and her haemoglobin value returned to 7.7 g/dl after one week and reached 10.9 g/dl on day 14. Anaemia is a common symptom of malaria and similar trends of haemoglobin drops during therapy have already been reported by several authors[100,101]. This effect is considered to be rather related to the disease and the activity of the treatment than to toxic effects of the drug itself.

Increasing platelet counts during the first week after start of treatment occurred in all four groups. *Plasmodium* infections are known to be associated with thrombocytopenia[102,103]. As an indication for successful therapy platelet counts were expected to rise in the course of the study.

Another interesting finding is the rising eosinophil count over time. This has already been described in literature and a positive effect on the recovery of anaemia caused by malaria is assumed[104]. The eosinophil rise may also have accounted for the decline in percent neutrophils as white blood cell count did not change significantly. Cases of severe neutropenia after therapy with artemisinin derivatives can be found in literature review[99], but we did not observe any serious neutrophil drops.

ECG readings showed no significant findings. In particular, all QTc measurements were within normal ranges. Prolonged QTc intervals have been reported as rare side effects of artemisinins in preclinical studies for pyronaridine.[25] In this clinical trial, mean heart rates decreased until day 28 – most likely due to a drop in body temperature after treatment of malaria.

One major finding in this study was the absence of an increase in severity or incidence of adverse events during the dose-escalation procedure. Safety data of all dose level groups were discussed by a Safety Review group before proceeding to the next cohort.

Based on these data from Phase II, treatment of paediatric patients with pyronaridine-artesunate combination can be regarded as safe. However, this finding must be considered as a preliminary result, as patient numbers in our study were quite small, but these data were the basis for entering to paediatric Phase III studies. It is in these controlled studies with larger sample size that a more complete picture on the safety and tolerability profile may be understood.

These data correspond well to other studies on pyronaridine and artesunate treatment. Reports on these two drugs when given alone show a similarly favourable tolerability and safety profile[105,73,70,71,106].

4.3 Efficacy

Efficacy was a secondary outcome parameter in this study. Pharmacodynamic parameters such as PCR-corrected and crude ACPR on day 14, day 28 and day 42, PCT, FCT and incidence rates of gametocytes were examined. These results can be interpreted as indicators for the efficacy of the pyronaridine-artesunate combination in the treatment of acute uncomplicated malaria in children.

PCR-corrected ACPR on day 28, defined as the primary efficacy endpoint, was 100 % in all four treatment groups in per-protocol population. Similarly high cure rates are reported in many studies on ACT efficacy from Africa and Asia including artesunate-amodiaquine[107], artesunate-clindamycin[108] or artesunate-mefloquine and dihydroartemisinin-piperaquine[30,109].

Reappearing parasitaemia on day 35 in one patient in group B and on day 36 in one child in group D were identified as true recrudescence by PCR genotyping. According to WHO recommendations treatment outcome is categorized as Early Treatment Failures (ETF) in the first three days after start of treatment and Late Treatment Failures (LTF). Late Treatment Failures can be separated into Late Clinical Failures (LCF), defined as “presence of parasitaemia and axillary temperature ≥ 37.5 °C (or history of fever) on any day from day 4 to day 28, without previously meeting any of the criteria of early treatment failure” and Late Parasitological Failures (LPF), defined as “presence of parasitaemia on any day from day 7 to day 28 and axillary temperature < 37.5 °C, without previously meeting any of the criteria of early treatment failure of late clinical failure”[94]. According to this classification, the patient of group B is regarded as Late Clinical Failure and the patient of group D as Late Parasitological Failure. These cases led to 88.9 % efficacy in group B and 92.9 % in group D on day 42 per-protocol analysis while the other groups achieved 100 % efficacy in all analyses. Except for these two patients, no further cases of true recrudescence were observed in this study.

Cure rates for groups A, B and D did not reach 100 % in the intention-to-treat population (78.6 %, 86.7 %, 100 %, 93.3 % in groups A, B, C, D on day 28) due to vomiting, withdrawn consent, protocol violation, new infections and loss to follow up. Although intention-to-treat analysis is underestimating the efficacy of drugs, it is more likely to reflect the “real life” situation, as non-compliance and protocol violations are daily problems in clinical practice[110].

Parasite clearance as well as fever clearance was surprisingly fast. All children reached complete absence of parasites within 7.9 to 32.0 hours (median times 16.4 h, 16.1 h, 8.1 h and 8.3 h in group A, B , C and D), most of them in the first 24 hours. Normal temperatures were observed for all patients until 8.2 to 8.6 hours.

Impressively fast PCT and FCT are known for artemisinins. In a study with artesunate-clindamycin and quinine-clindamycin a superiority of artesunate-clindamycin to quinine-clindamycin concerning PCT and FCT was reported[108]. In this study a PCT of 29.3 h and a FCT of 21.2 h were achieved, in another trial evaluating artesunate-amodiaquine and artesunate-sulfadoxine/pyrimethamine all children were free from parasites by day 3 and had fever cleared within 2-3 days[107].

Our results show an even faster activity, both for PCT and FCT. This finding may at least in part be due to the relatively low parasitaemia at baseline. In addition it must be noticed that a high percentage of patients were not febrile at baseline, reporting only a history of fever.

These results suggest a very promising efficacy profile of pyronaridine-artesunate combination therapy at all dose levels. However, when focussing on intention-to-treat analysis, a trend towards higher cure rates at the higher dose levels was observed.

Only six patients in total had *Plasmodium falciparum* gametocytes, three already at baseline and three additional patients developed gametocytaemia in the course of the study. All baseline gametocyte carriers were completely

cleared until day 22, the other cases were detected only in one or two thick smear examinations. Artemisinins are supposed to reduce gametocytes[31,32], for pyronaridine contradictory findings have been reported on its gametocytocidal effect[67,68]. The incidence rates of gametocytes and the number of patients in our study were too low to evaluate the gametocytocidal potential of the pyronaridine-artesunate combination therapy.

4.4 Granule formulation

Adequate paediatric antimalarial combinations are needed to guarantee effective malaria treatment in children. Treatment success highly depends on patient's and parent's compliance, which can be improved through fixed dose packs, simple regimens, good swallowability and a taste that is well accepted by children.

Drugs showing these characteristics are also the precondition for home-based management of malaria – a programme encouraged by WHO – enabling caregivers to administer antimalarial treatment within the first 24 hours after onset of symptoms. Particularly in areas where professional health care services are not accessible or affordable, this policy is regarded as an important measure to reduce malaria associated morbidity and mortality[88].

Shin Poong Pharmaceuticals developed tablets of pyronaridine and artesunate and simultaneously a paediatric granule formulation. This special paediatric drug formulation meets all key requirements mentioned above and could be a promising treatment alternative for small children. It would be one of the first artemisinin-based combinations in a particular formulation for children, in addition to artesunate-mefloquine (Artequin Paediatric®) and artemether-lumefantrine (Coartem dispersable®), which are already available.

In this study, only 15 patients received the granule formulation with 9 mg/kg pyronaridine and 3 mg/kg artesunate (group D), the same dosing strength as in group B. This is a very small number of patients, which has to be taken into consideration for the discussion of these results. Compared to the tablet

formulation, there are no significant differences concerning safety and efficacy parameters. Pharmacokinetic analysis only showed one significant difference: the maximal blood concentration (C_{max}) of pyronaridine was 168 ng/ml in granule group D versus 119 ng/ml in tablet group B, all other bioavailability parameters were similar[86].

In conclusion, all findings concerning safety, tolerability and efficacy are very promising, both for the tablet and the granule formulation. However, they must be regarded only as first evidence that has to be confirmed in further trials as the number of included patients was too small to detect rare clinical adverse events or significant laboratory changes. Efficacy also has to be confirmed in larger study populations and be compared to another established artemisinin-based combination in randomized, double-blind studies.

Additionally, if the drug turns out to be a safe and efficacious alternative in antimalarial treatment for the investigated age group between two and fourteen years, further trials could show if this combination could also be a treatment option for even smaller children and infants.

5 Summary

On a global scale, African children less than five years of age are the age group most affected by malaria, especially *Malaria tropica* caused by *Plasmodium falciparum*. The development of novel efficacious treatment options is one major effort to reduce malaria morbidity and mortality and associated socio-economic problems. Today artemisinin-based combination therapies (ACTs) are recommended by the WHO and became first-line treatment in most of the endemic regions. This Phase II study intended to provide first clinical data about the new combination of pyronaridine and artesunate for the treatment of acute uncomplicated *Plasmodium falciparum* malaria in children.

In addition to fixed-dose tablets in three different dose levels (pyronaridine+artesunate 6+2 mg/kg in group A, 9+3 mg/kg in group B and 12+4 mg/kg in group C), a granule formulation in sachets (group D, drug strength 9+3 mg/kg) was examined. Such novel paediatric preparations could be treatment alternatives particularly to improve compliance in small children not yet being able to adequately swallow tablets.

Safety results, defined as primary endpoints in this study, are very satisfying. In the majority of cases, adverse events were only of mild extent and were most often infections and infestations, including reappearance of parasites. Adverse events considered to be at least possibly study drug related were most frequently gastrointestinal disorders such as nausea, vomiting, abdominal pain and diarrhoea. However, these symptoms are also common clinical signs of *Plasmodium* infections, so that it is difficult to state a clear association with the investigational drug. Changes of haematology and biochemistry parameters were not clinically significant, except for one low haemoglobin value that occurred in a child of group A. ECG recordings, mean blood pressure and heart rate did not show major changes.

Excellent cure rates were achieved in all treatment cohorts. In per-protocol analysis on day 28, PCR-corrected ACPR was 100 % in all four groups. A total of 13 patients had reappearance of parasites during the course of the study, but

only two cases (one in group B and one in group D, both on day 35) were classified as recrudescence, confirmed by PCR.

Parasite clearance time was between 8.1 h and 16.4 h in the different treatment groups and fever clearance time between 8.2 h and 8.6 h indicating rapid activity - possibly influenced by the relatively low parasitaemia in this study population.

In summary, the combination of pyronaridine and artesunate showed very satisfying results, concerning both safety and efficacy parameters. Particularly the dose escalation procedure did not reveal any dose-related toxicities and the novel paediatric drug formulation was well accepted. Given the relatively small sample size of 60 patients, our findings must be interpreted with caution. However, these first data indicate that the pyronaridine-artesunate combination therapy might be a novel efficacious and safe treatment option for *Plasmodium falciparum* malaria in children. Larger Phase III studies are needed to confirm the promising efficacy results and to detect possible rare adverse effects.

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