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Safety of the combination of chloroquine and methylene blue in healthy adult men with G6PD deficiency from rural Burkina Faso

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3 Summary

New drug combinations against falciparum malaria which are both effective and affordable for sub-Saharan African populations are urgently needed. The combination of the well-known drugs chloroquine and methylene blue is such a promising new regimen. There is however some concern, that the application of methylene blue could be followed by the development of haemolysis in glucose-6-phosphate dehydrogenase deficiency, a condition which is prevalent in the malaria-endemic regions. Against this background we have exposed 81 glucose-6-phosphate dehydrogenase deficient but otherwise healthy adult men to a three day oral regimen of total 1.500 mg chloroquine and 780 mg methylene blue in the District Hospital of Nouna in north-western Burkina Faso. This drug regimen was well tolerated and in particular no haemolysis was observed. Methylene blue appears to be safe glucose-6-phosphate dehydrogenase deficient African populations with predominantly class III glucose-6-phosphate dehydrogenase deficiency.

4 Introduction

Malaria remains the most important parasitic disease and is globally responsible for 300-500 million fever episodes and 1.5-2.7 million deaths per year (WHO 1997). Most malaria deaths occur in young children of rural sub-Saharan African (SSA) areas with little access to health services (WHO 1997; Greenwood et al. 1987; Snow et al. 1999; Müller et al. 2003a).

Early diagnosis and prompt treatment with effective antimalarial drugs has remained the basis for malaria control (Greenwood and Mutabingwa 2002). This strategy is now complicated through the increasing development of resistance by *Plasmodium falciparum* to existing and affordable first-line drugs such as chloroquine and sulphadoxine/pyrimethamine in most countries of SSA (Trapé 2001). Moreover, treating malaria with a combination of effective drugs has become a new paradigm in malaria control, with the particular aim to delay and possibly reverse the development of drug resistance (White et al. 1999; Nosten and Brasseur 2002). Artemisinin drugs in combination with a variety of partner drugs have been shown in numerous studies to be effective if compared to existing drug regimens and have been considered the current first choice (International Artemisinin Study Group 2004). However, non-Artemisinin combinations have been shown to be equally or even more effective compared to Artemisinin-based combinations in some areas of SSA (Dorsey et al. 2002; Rwagacondo et al. 2003; Gasasira et al. 2003). Moreover, there are a number of operational problems in implementing malaria combination therapies particularly in SSA (Bloland et al. 2000). As the costs of respective malaria drugs are a major determinant for programme

effectiveness in the poor rural communities of SSA, alternative drug regimens which are both effective and affordable still need to be defined (Bloland et al. 2000; Winstanley 2001; Müller et al. 2003a).

Methylene blue, which has already been successfully used against malaria about 100 years ago, is a particular promising malaria drug candidate (Guttmann and Ehrlich 1891; Ehrlich 1913; Vennerstrom et al. 1995; Schirmer et al. 2003). *In vitro* experiments have confirmed the antimalarial potency of methylene blue (Amaral et al. 2001). Methylene blue has been shown to specifically inhibit the glutathione reductase of *Plasmodium falciparum* and has furthermore the potential to reverse grade I/II chloroquine resistance (Färber et al. 1998; Meierjohan et al. 2002). Methylene blue is registered in most countries for the treatment of methemoglobinemia and for the treatment and prevention of ifosfamide-induced encephalopathy in cancer treatment at single dosages of 1-2 mg/kg (Schirmer et al. 2003). The combination of chloroquine and methylene blue cannot be patented and would be an affordable drug combination even for the poor SSA populations.

Glucose-6-phosphate dehydrogenase (G6PD) deficiency occurs in many allelic variants which are geographically very heterogeneously distributed (Fleming and Silva 2003). Variants of public health importance – associated with intermittent haemolytic crises triggered by oxidant stresses - are mainly from the G6PD deficiency class II (severe) and class III (moderate) (Fleming and Silva 2003). As the G6PD gene is on the X chromosome, clinically significant enzyme deficiencies are most often found in males. In most of SSA, the class III G6PD deficiency dominates (Fleming and Silva 2003). Severe haemolysis episodes occur mainly in class II deficiency and after exposure to fava beans or certain drugs (Fleming and Silva 2003). Drugs reported to cause severe haemolysis are sulfones like dapsone, and nitrofuranes like benzocaine, while phenacetin, methylene blue, pamaquine and primaquine were also associated with haemolysis in G6PD deficient subjects (Fleming and Silva 2003).

The aim of this study was to investigate the safety profile and pharmacokinetics of chloroquine (CQ) in combination with methylene blue (MB) in G6PD deficient healthy adult men of Burkina Faso, West-Africa. In accordance with the “Note for Guidance on Clinical Investigation of Medicinal Products in Children” the current safety study was conducted in adults before proceeding to a subsequent study in children.

The results are already published (Mandi et al. 2004) but this report contains more specific information.

5 Material and Methods

5.1 Study area and study subjects

The study was conducted in September/October 2003 in Nouna Health District in northwestern Burkina Faso. The Nouna area is a dry orchard savannah, populated almost exclusively by subsistance farmers of different ethnic groups. Formal health services are restricted to the district hospital in Nouna town and to a few village-based health centers. Access to formal health services is rather limited, particularly during the rainy season (Müller et al. 2003a). The rainy season usually lasts from June to October. The area is holoendemic for malaria, with *P. falciparum* being the dominant species (Müller et al. 2001). Chloroquine treatment failure rates were recently shown to be around 10% (Müller et al. 2003b). The prevalence of G6PD deficiency in randomly chosen persons from 5 representative villages of the Nouna study area was 138/1018 (13.6%) (Schirmer et al. 2003).

Healthy adult volunteers were recruited from three villages close to Nouna, the capital of Nouna Health District (Bourasso, Bagala and Ouette). All volunteers were initially screened for G6PD deficiency through a modified Beutler-Mitchell test (Beutler and Mitchell 1968; Scheiwein 2001). G6PD deficient volunteers were brought by car to Nouna. Inclusion criteria were: Age 18-60 years, male, G6PD deficiency, Burkinabe nationality, written informed consent. The G6PD status was reconfirmed through the same test from a venous blood sample in the Nouna Hospital. Exclusion criteria were: Any apparent significant disease, anaemia (haemoglobin < 8 g/dl or haematocrit < 24%). Study subjects were hospitalised for four days.

5.2 Study objectives

The primary objective in this one-group, single-centre trial is to study the risk for life-threatening haemolysis after administration of CQ-MB in G6PD deficient healthy adult men. The secondary objectives are to study the risk of other serious adverse events and the risk for adverse events after administration of CQ-MB in G6PD deficient healthy adult men as well as to study the pharmacokinetics of the combination of CQ-MB in healthy adults of western Africa.

5.3 Endpoints

The primary endpoint is the incidence of acute life-threatening haemolysis (definition: haemoglobin ≤ 5 g/dl and haematocrit ≤ 15%, or received blood transfusion according to clinical judgement of a study physician) during treatment until 24 hours of last drug

application. Secondary endpoints are the incidence of other serious adverse events, the incidence of other adverse events as well as changes in haemoglobin and haematocrit during follow-up. Additionally whole blood chloroquine and methylene blue population kinetics (average AUC, C_{max}, T_{max}, elimination half life) in study subjects as assessed by at least one blood sample of each participant once a day for the four consecutive days of hospitalisation, and whole blood chloroquine and methylene blue individual kinetics (AUC, C_{max}, T_{max}, elimination half life) in a subgroup of 12 volunteers measured on study day 3 following intake of the last medication were analyzed. All data regarding pharmacological analyses will be presented in a separate report. Further Endpoints are:

- Frequency and proportion of subjects with a least one serious adverse event:

  acute haemolysis

- Other serious adverse events
- Frequency and proportion of subjects with at least one (other) adverse event, observed or self-reported adverse event
- post hoc endpoints (specified in the analysis plan but not in the protocol): change in haemoglobin, change in haematocrit, correlation between haemoglobin and haematocrit, regression of haemoglobin on haematocrit

5.4 Study design and statistics

As the aim was to be able to stop the trial early in case of a high risk of haemolysis, the sample size calculation was based on *Simon's two-stage design* (Simon 1989). It was planned to reject the Null - Hypothesis (risk of haemolysis > 10%) in case of a low risk (2%) with a power of 90% on a level of 5%. A maximum of 20 and 53 subjects were to be recruited during the first and second stage of the trial, respectively. The trial was to be stopped in favour of the null-hypothesis in case of two or more events in the first stage, or in case of 4 incidences of haemolysis in total during the remaining trial. Otherwise (less than 4 events for the total of 73 subjects) the null hypothesis was to be rejected showing that the incidence rate for haemolysis is below 10%. To account for losses to follow-up, a maximum of 80 study subjects was targeted. The 10% as margin is justified by having a prevalence of G6PD deficiency of about 10% in the study area. Therefore 1% of the whole population would be failure (untreated haemolysis is usually leading to death) which is the current death rate in young children due to malaria.

The primary analysis is based on the full analysis set containing all 74 subjects who received trial medication and were G6PD deficient. The 70 subjects out of 74 who fulfilled the

eligibility criteria were used for a sensitivity analysis (per protocol). The safety analysis is based on all 81 subjects who received the study medication (=SAF). Descriptive statistics are presented with mean and standard deviation or median. The minimum and maximum of the values are given as well. All used statistical tests apart from the primary analysis are nonparametric and have only explorative character and are not adjusted for multiplicity.

5.5 Interventions

All participants received a total dose of 1500 mg of CQ orally (first and second day: 600 mg/day, third day: 300 mg). Additionally a total dose of 780 mg orally MB over three days was administered, divided into morning and evening doses (130 mg per dose). The MB dose (2mg/kg) is thus identical to the dose used in many countries for standard indications (Schirmer et al. 2003). All treatments were directly observed by the study physician. CQ tablets were taken from the essential drug stock of the Nouna Health District pharmacy, MB capsules were produced by the pharmacy of the Medical School in Heidelberg. All indicated drugs were allowed as concomitant treatments, except dapsone and other sulfones, acetanilide and phenacetin, nalidixic acid, niridazole, nitrofurantoins and sulphonamides (themselves known to cause haemolysis in G6PD deficiency) (Fleming and Silva 2003). One urine sample was taken in the morning on day 2 to assess MB compliance through visual observation by the study physician.

5.6 Procedures

Study subjects were questioned for side effects and examined for signs of haemolysis by the study physician in the morning and in the evening of every study day.

On day 1, an experienced laboratory technician took a venous blood sample for malaria diagnosis (thin and thick blood film), haemoglobin and creatinine (photometer), haematocrit (centrifuge), and G6PD deficiency confirmation (modified Beutler-Mitchell test and PCR confirmation). In the mornings of day 2-4 and always before treatment, venous blood samples were taken for haemoglobin and haematocrit determination, and for pharmacological data (whole blood chloroquine and methylene blue determined by HPLC or mass spectrometry for calculation of population kinetics). In the evening of day 3 and after administration of the study medication, venous blood samples were taken hourly for 9 hours in a subgroup of 12 volunteers for determination of the individual pharmacokinetic of study drugs (whole blood chloroquine and methylene blue determined by HPLC or mass spectrometry for calculation of individual kinetics).

The modified Beutler-Mitchell test: The G6PD status was determined at the laboratory of the Centre de Recherche en Santé de Nouna (CRSN), using the NADPH fluorescence test of Beutler and Mitchell (1968) in miniaturized form (Coulibaly et al. 2005). 20-50µl blood was collected in an Eppendorf cap containing 1-2µl 70mM EDTA. The sample was stored at 4°C (it is stable for 3 weeks), and diluted before testing with phosphate-buffered saline to 4g Hb/dl. This standardization is necessary to account for the high prevalence of anaemia. A 10µl sample was mixed with 100µl screening solution, and a 20µl spot dropped on filter paper. Two additional spots were prepared from the mixture after 5 to 10 minutes. In G6PD normal subjects, the first spot was slightly and the additional spots were brightly UV-fluorescent. G6PD deficient samples showed little or no fluorescence in either spot. Control samples were run in parallel.

For confirmation of the G6PD status: Blood was collected on filter paper and examined by PCR genotyping at the Institute of Tropical Medicine in Berlin (Germany). The allele Gd^{A-} was distinguished from Gd^{A} and Gd^{B} by PCR restriction fragment length polymorphism. Sequences flanking the potential mutations were amplified in two separate nested PCR assays (Kotea et al. 1999). Mutations at nucleotides 202 (G A) and 376 (A G) were detected by digestion with *Nla*III and *Fok*I (New England Biolabs, Schwalbach, Germany), respectively.

All data was double entered from the case report forms in Nouna. Quality checks were created and solved. For the analysis the statistical software SAS 8 was used in Heidelberg.

5.7 Ethical aspects

The trial was conducted in accordance with local laws and the internationally established principles for Good Clinical Practice which had their origin in the Declaration of Helsinki of the World Medical Association. The protocol was approved by the ethics committees in Heidelberg and Burkina Faso. Study subjects received a financial compensation for participating on the study. A standard blood transfusion service was available at the hospital and study physicians and emergency medications were available 24 hours per day.

6 Results

6.1 Recruitment of volunteers

In total 81 volunteers from the rural area were included in the trial. The recruitment of volunteers was done in groups by 12 to 15 healthy adult men (Table 10.1).

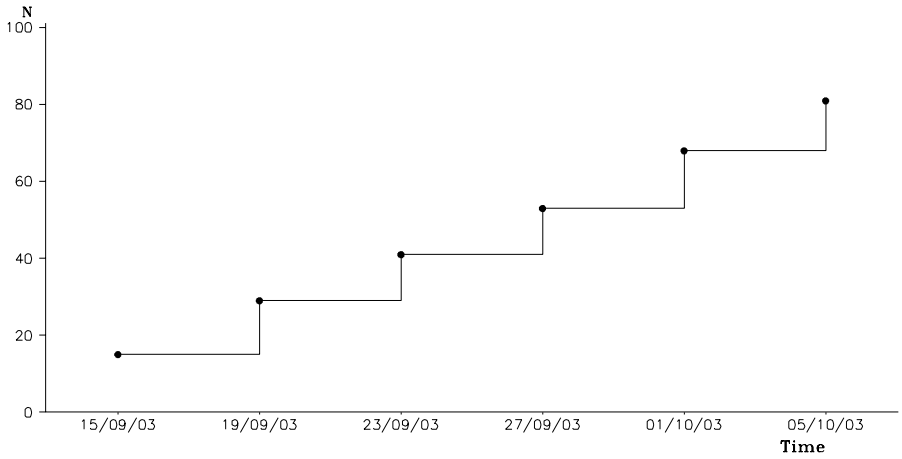

Figure 6.1: Recruitment of volunteers in groups.

6.2 Quality of Data

After the double data entry in Nouna, Burkina Faso only 18 queries had to be solved. Four volunteers did not match the eligibility criteria regarding age but were included. All included men received the study medication. No lost to follow up were observed (Table 10.2). After first data base closure (22.4.2004) one additional adverse event was found (subject 19, nausea, Listing 10.2) but added (reopening and final closure: 6.8.2004) and included in this report.

6.3 Demographic characteristics of study subjects

They were considered for safety analysis only. In 7/81 volunteers the G6PD deficiency status was not confirmed (Table 10.2). G6PD deficiency status was confirmed by PCR in 70 of the remaining 74 men. For the main analysis the 74 volunteers were used regarding our protocol. The mean age of the remaining 74 study subjects in the full analysis set was 31 +/– 12 years (range: 17 to 66), Table 10.4. One man was under 18, three men were over 60 years old. Although they did not fulfil the eligibility criteria formally, it was decided to include them into the analysis. The mean body weight was 62 +/– 7.7 kg (range: 43 to 82).

6.4 Laboratory baseline data

On the first day 60 (81%) volunteers were malaria parasitaemic. The median parasite density (P. faliciparum) was 455 (range: 0 to 55.200), with 54 volunteers having less then 2000 and six men having had more then 2000 parasites. Mean haemoglobin [g/dl] was 14.8 +/– 1.4 (range: 11.8 to 17.7), Mean haematocrit [%] 40.9 +/– 2.4 (range: 36 to 46) and mean creatinine [mmol/l] 72.2 +/– 13.7 (range: 31.5 to 104.5). (Table 10.6, Table 10.8).

6.5 Primary endpoint

There were no cases of acute life-threatening haemolysis leading to an estimated incidence of 0% with the exact one sided 95% confidence interval [0%, 4.9%] (Table 10.11). As a sensitivity analysis the confidence interval based on all volunteers fulfilling the eligibility criteria (N=70) was calculated to be [0%, 5.1%] (Table 10.12). Both upper margins are lower than 10%.

6.6 Laboratory follow-up

An increase in haemoglobin [g/dl] of 0.4 +/– 1.1 with a 95% confidence interval of [0.15, 0.68] was observed over the four days (Table 10.10, Figure 10.1, Figure 10.2). Haematocrit values did not change over time 0.1 [–0.36, 0.58] (Table 6.1). The means kept stable between 40.4% and 41.0% over the five measurements (Table 10.10, Figure 10.3, Figure 10.4).

Table 6.1: Hb and Hct at baseline, after four days, and change with pairwise U-tests.

	Day 1 (N=74)	Day 5 (N=74)	Change (N=74)
Haemoglobin [g/dl]			
Mean +/– SD	14.8 +/– 1.4	15.2 +/– 1.2	0.4 +/– 1.1
Median	15.0	15.3	0.5
Min, Max	11.8, 17.7	12.0, 18.3	–2.4, 3.6
95% CI	[14.4; 15.1]	[14.9; 15.5]	[0.15; 0.68]
p-Value	-	-	0.002
Haematocrit [%]			
Mean +/– SD	40.9 +/– 2.4	41.0 +/– 2.1	0.1 +/– 2.0
Median	40.0	40.0	0.0
Min, Max	36.0, 46.0	38.0, 46.0	–4.0, 6.0
95% CI	[40.3; 41.1]	[40.5; 41.5]	[–0.36; 0.58]
p-Value	-	-	0.681

The correlation between haemoglobin and haematocrit is very low (r=0.41). We also calculated a linear regression of haemoglobin on haematocrit in order to investigate whether one can simply measure Hct instead of Hb (Hb = 4.85 + 0.24 Hct). Only r^2=17% of the variability of Hb can be explained using the linear model with Hct as a independent variable.

Figure 6.2 shows the scatter-plot with the 95% prediction interval. Without an intercept the fitted linear model is Hb = 0.36 Hct.

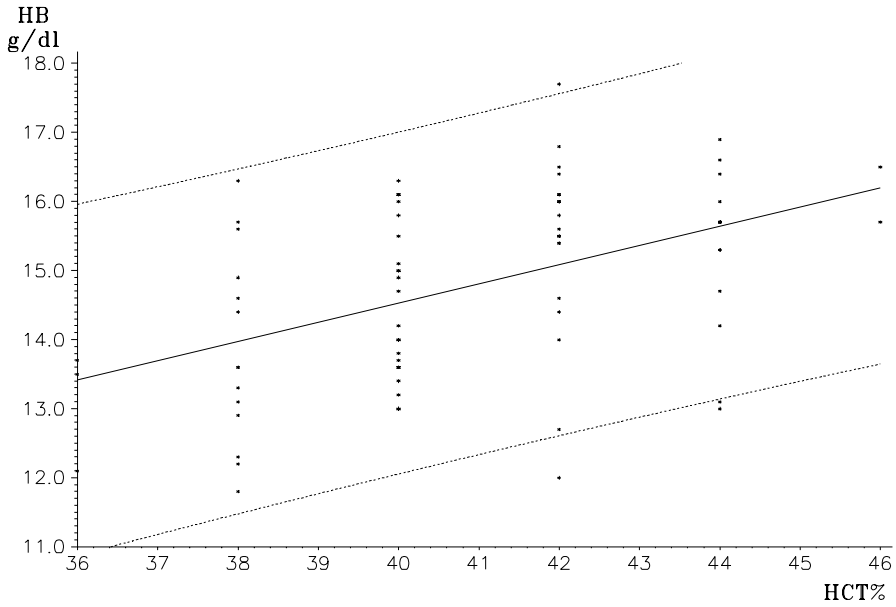

Figure 6.2: Regression of haemoglobin on haematocrit

6.7 Safety analysis

For the safety analysis all 81 subjects who received CQ+MB were considered. No serious adverse events were reported but 12 (14.8%) subjects showed at least one adverse event (Table 10.13). Nine (69.2%) of the adverse events were mild, 4 (30.8%) were moderate. No adverse event was classified as "definitely related" but 4 (30.8%) were assessed as "probably related". Categorizing the adverse events by the MedDRA system organ classes gave: 3 (23.1%) gastrointestinal disorders, 1 (7.7%) injury, poisoning and procedural complications (put his hand in the running fan, Listing 10.2), and 9 (69.2%) nervous system disorders (Table 10.14).

7 Discussion

We have not observed any serious adverse events and in particularly no life-threatening haemolysis during exposure of healthy adult West-African men with G6PD deficiency to the combination of chloroquine and methylene blue. This does not mean that we can exclude any risk for serious adverse events but the likelihood is rather small and certainly smaller than the risk of young SSA children dying from malaria (Snow et al. 1999). Although this is good news regarding the safety profile of this new drug combination in SSA populations with

dominating class III G6PD deficiency, such an outcome is not guaranteed in populations where G6PD deficiency class II occurs (Fleming and Silva 2003).

The combination of dapsone and chlorproguanil (Lapdap) has recently been registered for malaria therapy in SSA (Lang and Greenwood 2003). With regard to the potential of haemolysis development in G6PD deficient populations, methylene blue belongs to the same risk category as dapsone (Fleming and Silva 2003). Thus our findings may also be reassuring regarding the safety of Lapdap in SSA populations.

We did not see many adverse events associated with the study medication and most of them were mild. The MB dose used (2mg/kg) is known to be safe for the treatment of methemoglobinemia and for the treatment and prevention of ifosfamide-induced encephalopathy in cancer treatment (Schirmer et al. 2003). Moreover, we studied MB in combination with a standard CQ dose before in a phase I trial in volunteers at the Heidelberg University Hospital where we observed no increase of chloroquine blood levels compared to chloroquine alone (Rengelshausen et al. 2004). However, this does not necessarily mean that higher doses of MB are without risk in comparable populations as doses exceeding 7 mg/kg have been associated with distressing symptoms such as dyspnoea, chest pain, tremor, cyanosis and haemolytic anaemia in one study and as under real life conditions repeated dosing of malaria drugs is rather common (Goluboff and Wheaton 1961). Thus, in case that MB will be used on a large scale against malaria, further safety studies with higher dosages are needed.

We did not see any major change in the mean haemoglobin or haematocrit levels over the study period of four days. This is further encouraging as it does make it unlikely that the study medication will be associated with sub-clinical haemolysis. We have also not seen any significant relation between malaria parasitaemia and haemoglobin or haematocrit values which is likely to be explained by the semi-immunity of adults in such a malaria endemic area. As there was no control group planned for this study, a follow-up of malaria parasitaemia was considered unnecessary.

The correlation between haemoglobin and haematocrit in our data was weak. It can be concluded that for further studies the haematocrit value alone is not sufficient for the measurement of haemolysis.

In conclusion, we have shown that the combination of chloroquine and methylene blue is safe in the chosen dosage regimen in a population of G6PD deficient healthy men in West-Africa. The next step will be to study the safety of this new regimen in the target population of young children with uncomplicated falciparum malaria.

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10 Appendix (Tables, Listings, Figures)

Table 10.1: Recruiting of subjects

	N	N(%)cumulative
Date of informed consent		
15/09/2003	15	15(18.5%)
19/09/2003	14	29(35.8%)
23/09/2003	12	41(50.6%)
27/09/2003	12	53(65.4%)
01/10/2003	15	68(84.0%)
05/10/2003	13	81(100.0%)

Table 10.2: Protocol compliance & populations

	N(%)
Included subjects (informed consent)	81(100.00%)
Subjects without trial medication	0(0.00%)
Subjects in Safety analysis Set	81(100.00%)
Subjects G6PD sufficient	7(8.64%)
Subjects in Full Analysis Set	74(91.36%)
Violating other inclusion/exclusion criteria	
- younger than 18 years	1(1.23%)
- older than 60 years	3(3.70%)
Subjects in Per-Protocol Set	70(86.42%)
Other violation of Protocol	0(0.00%)
Premature termination	0(0.00%)

Table 10.3.: Demographics (SAF)

Characteristic	N=81
Age [years]	
- N	81
- Mean +/- SD	31.7+/-12.1
- Median	30.0
- p5, p25, p75, p95	18.0, 21.0, 40.0, 57.0
- Min, Max	17.0, 66.0
Weight [kg]	
- N	81
- Mean +/- SD	62.0+/- 7.7
- Median	62.0
- p5, p25, p75, p95	50.0, 58.0, 65.0, 75.0
- Min, Max	43.0, 82.0

Table 10.4: Demographics (FAS)

Characteristic	N=74
Age [years]	
- N	74
- Mean +/- SD	31.2+/-12.5
- Median	29.0
- p5, p25, p75, p95	18.0, 21.0, 40.0, 57.0
- Min, Max	17.0, 66.0
Weight [kg]	
- N	74
- Mean +/- SD	61.7+/- 7.7
- Median	62.0
- p5, p25, p75, p95	50.0, 57.0, 65.0, 75.0
- Min, Max	43.0, 82.0

Table 10.5: Baseline Diagnoses and Laboratory (SAF)

Characteristic	N=81
Prior Illness	
- Yes	0(0.0%)
- No	81(100.0%)
Parasitaemia Density	
- No	17(21.0%)
- <2000	58(71.6%)
- >=2000	6(7.4%)
Parasitaemia Density	
- N	74
- Mean +/- SD	1477.1+/- 6430.8
- Median	455.0
- p5, p25, p75, p95	0.0, 120.0, 1000.0, 3440.0
- Min, Max	0.0, 55200.0

Characteristic	N=81
Haemoglobin[g/dl]	
- N	81
- Mean +/- SD	14.8+/- 1.4
- Median	15.0
- p5, p25, p75, p95	12.2,13.6,15.8,16.6
- Min, Max	11.8, 17.7
Haematocrit[%]	
- N	81
- Mean +/- SD	40.9+/- 2.4
- Median	40.0
- p5, p25, p75, p95	38.0,40.0,42.0,44.0
- Min, Max	36.0, 46.0
Creatinine[µmol/l]	
- N	81
- Mean +/- SD	72.9+/- 13.7
- Median	75.7
- p5, p25, p75, p95	56.1, 63.1, 80.4, 96.4
- Min, Max	31.5, 104.5

Table 10.6: Baseline Diagnoses and Laboratory (FAS)

Characteristic	N=74
Prior Illness	
- Yes	0(0.0%)
- No	74(100.0%)
Parasitaemia Density	
- No	14(18.9%)
- <2000	54(73.0%)
- >=2000	6(8.1%)
Parasitaemia Density	
- N	74
- Mean +/- SD	1477.1+/- 6430.8
- Median	455.0
- p5, p25, p75, p95	0.0, 120.0, 1000.0, 3440.0
- Min, Max	0.0, 55200.0

Characteristic	N=74
Haemoglobin[g/dl]	
- N	74
- Mean +/- SD	14.8+/- 1.4
- Median	15.0
- p5, p25, p75, p95	12.2,13.6,16.0,16.6
- Min, Max	11.8, 17.7
Haematocrit[%]	
- N	74
- Mean +/- SD	40.9+/- 2.4
- Median	40.0
- p5, p25, p75, p95	38.0,40.0,42.0,44.0
- Min, Max	36.0, 46.0
Creatinine[µmol/l]	
- N	74
- Mean +/- SD	72.2+/- 13.7
- Median	72.4
- p5, p25, p75, p95	52.2, 60.3, 80.4, 96.4
- Min, Max	31.5, 104.5

Table 10.7: Baseline Parasitaemia density (SAF)

	Baseline Parasitaemia group			
Characteristic	0	<2000	>=2000	total
N	17	58	6	81
Mean +/- SD	0.0+/-0.0	618.0+/-420.6	12523.3+/-21014.1	1370.2+/-6153.4
Median	0.0	510.0	3520.0	440.0
Min, Max	0.0, 0.0	50.0, 1800.0	2400.0, 55200.0	0.0, 55200.0
p5, p25, p75, p95	0.0, 0.0, 0.0, 0.0	100.0, 320.0, 880.0, 1500.0	2400.0, 2400.0, 8100.0, 55200.0	0.0, 120.0, 880.0, 2400.0

Table 10.8: Baseline Parasitaemia density (FAS)

	Baseline Parasitaemia group			
Characteristic	0	<2000	>=2000	total
N	14	54	6	74
Mean +/- SD	0.0+/-0.0	632.7+/-429.0	12523.3+/-21014.1	1477.1+/-6430.8
Median	0.0	510.0	3520.0	455.0
Min, Max	0.0, 0.0	50.0, 1800.0	2400.0, 55200.0	0.0, 55200.0
p5, p25, p75, p95	0.0, 0.0, 0.0, 0.0	120.0, 320.0, 903.0, 1500.0	2400.0, 2400.0, 8100.0, 55200.0	0.0, 120.0, 1000.0, 3440.0

Table 10.9: Laboratory data by visit (SAF)

Characteristics	DAY1	DAY2	DAY3	DAY4	DAY5
HB[g/dl]					
- N	81	81	81	81	81
- Mean +/- SD	14.8+/- 1.4	15.2+/- 1.3	15.0+/- 1.3	15.1+/- 1.3	15.2+/- 1.2
- Median	15.0	15.2	15.0	15.3	15.3
- p5, p25, p75, p95	12.2,13.6,15.8,16.6	13.1,14.2,16.0,16.9	13.1,14.0,16.0,16.9	13.0,14.2,16.0,16.7	13.1,14.3,16.0,16.7
- Min, Max	11.8, 17.7	11.5, 18.3	11.2, 18.2	12.0, 18.0	12.0, 18.3
HCT[%]					
- N	81	81	81	81	81
- Mean +/- SD	40.9+/- 2.4	40.6+/- 3.0	41.0+/- 2.8	40.4+/- 2.6	41.1+/- 2.2
- Median	40.0	40.0	40.0	40.0	42.0
- p5, p25, p75, p95	38.0,40.0,42.0,44.0	36.0,38.0,42.0,46.0	36.0,40.0,42.0,46.0	36.0,38.0,42.0,44.0	38.0,40.0,42.0,46.0
- Min, Max	36.0, 46.0	32.0, 48.0	32.0, 46.0	32.0, 46.0	38.0, 46.0

Table 10.10: Laboratory data by visit (FAS)

Characteristics	DAY1	DAY2	DAY3	DAY4	DAY5
HB[g/dl]					
- N	74	74	74	74	74
- Mean +/- SD	14.8+/- 1.4	15.0+/- 1.2	14.9+/- 1.3	15.0+/- 1.3	15.2+/- 1.2
- Median	15.0	15.2	15.0	15.3	15.3
- p5, p25, p75, p95	12.2,13.6,16.0,16.6	13.0,14.1,16.0,16.8	13.0,14.0,16.0,16.8	12.5,14.1,15.9,16.7	13.0,14.3,16.0,16.8
- Min, Max	11.8, 17.7	11.5, 17.1	11.2, 17.3	12.0, 18.0	12.0, 18.3
HCT[%]					
- N	74	74	74	74	74
- Mean +/- SD	40.9+/- 2.4	40.5+/- 2.9	40.7+/- 2.8	40.4+/- 2.6	41.0+/- 2.1
- Median	40.0	40.0	40.0	40.0	40.0
- p5, p25, p75, p95	38.0,40.0,42.0,44.0	36.0,38.0,42.0,46.0	36.0,38.0,42.0,46.0	36.0,38.0,42.0,44.0	38.0,40.0,42.0,46.0
- Min, Max	36.0, 46.0	32.0, 48.0	32.0, 46.0	32.0, 46.0	38.0, 46.0

Table 10.11: Primary analysis (FAS)

	Baseline Parasitaemia group			Total
Characteristic	0	<2000	>=2000	(N=74)
Haemolysis				
- Yes	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
- No	14(18.9%)	54(73.0%)	6(8.1%)	74(100.0%)
- 95% CI	[0.00; 0.23]	[0.00; 0.07]	[0.00; 0.46]	[0.00; 0.05]
Change in HB[g/dl]				
- N	14	54	6	74
- Mean +/- SD	0.2+/-1.2	0.5+/-1.2	0.3+/-0.9	0.4+/-1.1
- Median	0.0	0.5	0.3	0.5
- Min, Max	-1.7, 2.3	-2.4, 3.6	-1.0, 1.3	-2.4, 3.6
- p5, p25, p75, p95	-1.7, -0.6, 1.1, 2.3	-1.7, -0.1, 1.3, 2.5	-1.0, -0.3, 1.3, 1.3	-1.7, -0.3, 1.3, 2.3
- 95% CI	[-0.50; 0.87]	[0.17; 0.81]	[-0.66; 1.30]	[0.15; 0.68]
- p-Value	0.575	0.002	0.531	0.002
Change in HCT[%]				
- N	14	54	6	74
- Mean +/- SD	0.1+/-1.8	0.0+/-2.1	0.7+/-2.1	0.1+/-2.0
- Median	0.0	0.0	0.0	0.0
- Min, Max	-2.0, 4.0	-4.0, 6.0	-2.0, 4.0	-4.0, 6.0
- p5, p25, p75, p95	-2.0, -2.0, 2.0, 4.0	-4.0, -2.0, 2.0, 4.0	-2.0, 0.0, 2.0, 4.0	-4.0, -2.0, 2.0, 4.0
- 95% CI	[-0.92; 1.20]	[-0.54; 0.61]	[-1.50; 2.83]	[-0.36; 0.58]
- p-Value	1.000	0.966	0.750	0.681

p-Values are created using Signed-Rank Test

Table 10.12: Primary analysis (PP)

	Baseline Parasitaemia group			Total
Characteristic	0	<2000	>=2000	(N=70)
Haemolysis				
- Yes	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
- No	14(20.0%)	50(71.4%)	6(8.6%)	70(100.0%)
- 95% CI	[0.00; 0.23]	[0.00; 0.07]	[0.00; 0.46]	[0.00; 0.05]
Change in HB[g/dl]				
- N	14	50	6	70
- Mean +/- SD	0.2+/-1.2	0.5+/-1.2	0.3+/-0.9	0.4+/-1.2
- Median	0.0	0.5	0.3	0.5
- Min, Max	-1.7, 2.3	-2.4, 3.6	-1.0, 1.3	-2.4, 3.6
- p5, p25, p75, p95	-1.7, -0.6, 1.1, 2.3	-1.7, -0.3, 1.2, 2.5	-1.0, -0.3, 1.3, 1.3	-1.7, -0.3, 1.2, 2.3
- 95% CI	[-0.50; 0.87]	[0.13; 0.81]	[-0.66; 1.30]	[0.12; 0.68]
- p-Value	0.575	0.004	0.531	0.004
Change in HCT[%]				
- N	14	50	6	70
- Mean +/- SD	0.1+/-1.8	0.1+/-2.1	0.7+/-2.1	0.2+/-2.0
- Median	0.0	0.0	0.0	0.0
- Min, Max	-2.0, 4.0	-4.0, 6.0	-2.0, 4.0	-4.0, 6.0
- p5, p25, p75, p95	-2.0, -2.0, 2.0, 4.0	-4.0, -2.0, 2.0, 4.0	-2.0, 0.0, 2.0, 4.0	-2.0, -2.0, 2.0, 4.0
- 95% CI	[-0.92; 1.20]	[-0.47; 0.71]	[-1.50; 2.83]	[-0.31; 0.65]
- p-Value	1.000	0.740	0.750	0.497

p-Values are created using Signed-Rank Test

Table 10.13: Adverse events (SAF)

	Baseline Parasitaemia group			Total
	0 (N=17)	<2000 (N=58)	>=2000 (N=6)	(N=81)
Subjects with at least one AE(1)	3(17.6%)	8(13.8%)	1(16.7%)	12(14.8%)
Number of AE's (=100%)	3(100.0%)	10(100.0%)	1(100.0%)	14(100.0%)
SAE				
- No	3(100.0%)	10(100.0%)	1(100.0%)	14(100.0%)
- Yes	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
Severity				
- mild	2(66.7%)	7(70.0%)	1(100.0%)	10(71.4%)
- moderate	1(33.3%)	3(30.0%)	0(0.0%)	4(28.6%)
- severe	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
Relationship				
- unrelated	0(0.0%)	3(30.0%)	0(0.0%)	3(21.4%)
- possibly related	2(66.7%)	3(30.0%)	0(0.0%)	5(35.7%)
- probably related	0(0.0%)	4(40.0%)	1(100.0%)	5(35.7%)
- definitely related	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
- cannot be assessed	1(33.3%)	0(0.0%)	0(0.0%)	1(7.1%)
AE's per Study day				
- Day1	0(0.0%)	1(10.0%)	0(0.0%)	1(7.1%)
- Day2	3(100.0%)	1(10.0%)	1(100.0%)	5(35.7%)
- Day3	0(0.0%)	5(50.0%)	0(0.0%)	5(35.7%)
- Day4	0(0.0%)	3(30.0%)	0(0.0%)	3(21.4%)
- Day5	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
MedDRA SOC				
- Gastrointestinal disorders	1(33.3%)	3(30.0%)	0(0.0%)	4(28.6%)
- Injury, poisoning and procedural complications	0(0.0%)	1(10.0%)	0(0.0%)	1(7.1%)
- Nervous system disorders	2(66.7%)	6(60.0%)	1(100.0%)	9(64.3%)

(1) percent based on number of subjects by group; otherwise the percentages are based on the number of AE's by group.

Table 10.14: Number of subjects with adverse events by day (SAF)

	N=81
Combination of study	
- 11111	69(85.2%)
- 11121	2(2.5%)
- 11211	3(3.7%)
- 11221	1(1.2%)
- 12111	5(6.2%)
- 21111	1(1.2%)

The combination 12111 means that 5 volunteers had their adverse event(s) on the second day.

Listing 10.1: Concomitant Medication

Subject	Age(years)	Baseline parasitaemia group	Medication	Onset date	Stop date	Ongoing
8	26	<2000	paracetamol	17/09/03	17/09/03	No
15	22	<2000	Paracetamol	17/09/03	17/09/03	No
16	49	No	Paracetamol	20/09/03	20/09/03	No
19	27	<2000	Quinine sulfat	22/09/03	.	Yes
21	26	No	Paracetamol	20/09/03	20/09/03	No
22	32	>=2000	Paracetamol	20/09/03	20/09/03	No
27	43	>=2000	Erythromycin	22/09/03	.	Yes
40	19	<2000	Paracetamol	25/09/03	25/09/03	No
60	21	<2000	Paracetamol	28/09/03	28/09/03	No

Listing 10.2: Adverse Events

Subject	Age(years)	Baseline parasitaemia group	Description	SAE	Severity	Onset date	Stop date	Ongoing	Causality	Therapy
2	62	<2000	Traumatisme main droite (VENTILATEUR)	No	mild	16/09/03	.	Yes	unrelated	surgery
8	26	<2000	headache	No	mild	17/09/03	17/09/03	No	unrelated	medication
13	20	<2000	Vertiges	No	moderate	17/09/03	17/09/03	No	prob. related	no action
13	20	<2000	Vertiges	No	mild	18/09/03	18/09/03	No	prob. related	no action
15	22	<2000	headache (CEPHALEES)	No	moderate	17/09/03	17/09/03	No	poss. related	medication
16	49	No	Headache	No	mild	20/09/03	20/09/03	No	not assessed	medication
19	27	<2000	Vomissement	No	mild	21/09/03	21/09/03	No	prob. related	no action
19	27	<2000	NAUSEES	No	mild	22/09/03	22/09/03	No	prob. related	.
20	29	<2000	Douleurs abdominales	No	mild	22/09/03	22/09/03	No	poss. related	no action
21	26	No	Headache (CEPHALE)	No	mild	20/09/03	20/09/03	No	poss. related	medication
22	32	>=2000	Headache (CEPHALE)	No	mild	20/09/03	20/09/03	No	prob. related	medication
40	19	<2000	Headache (CEPHALEES)	No	mild	25/09/03	25/09/03	No	poss. related	medication
60	21	<2000	Headache (CEPHALEES)	No	moderate	27/09/03	28/09/03	No	unrelated	medication
83	35	No	Douleurs abdominales	No	moderate	06/10/03	06/10/03	No	poss. related	no action

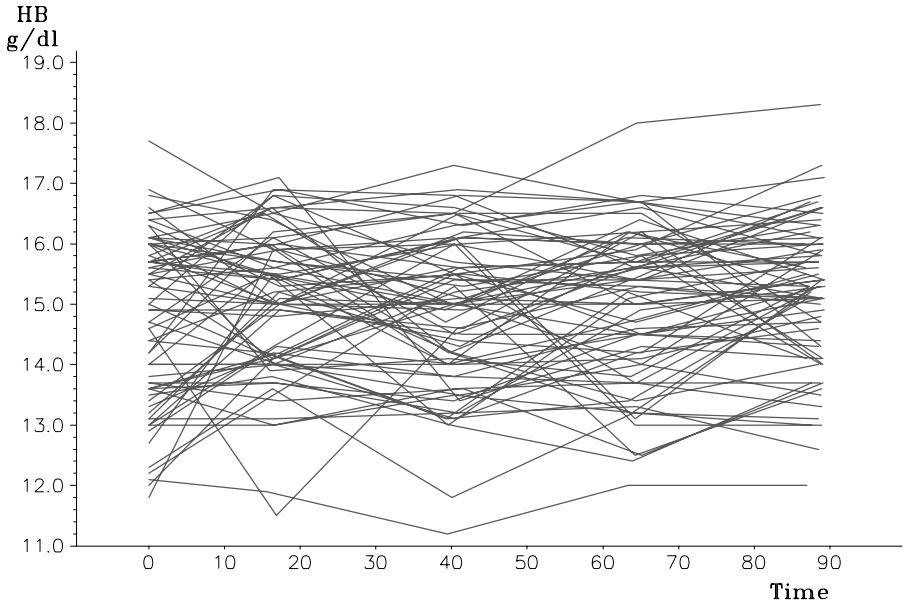

Figure 10.1: Haemoglobin by volunteer over time

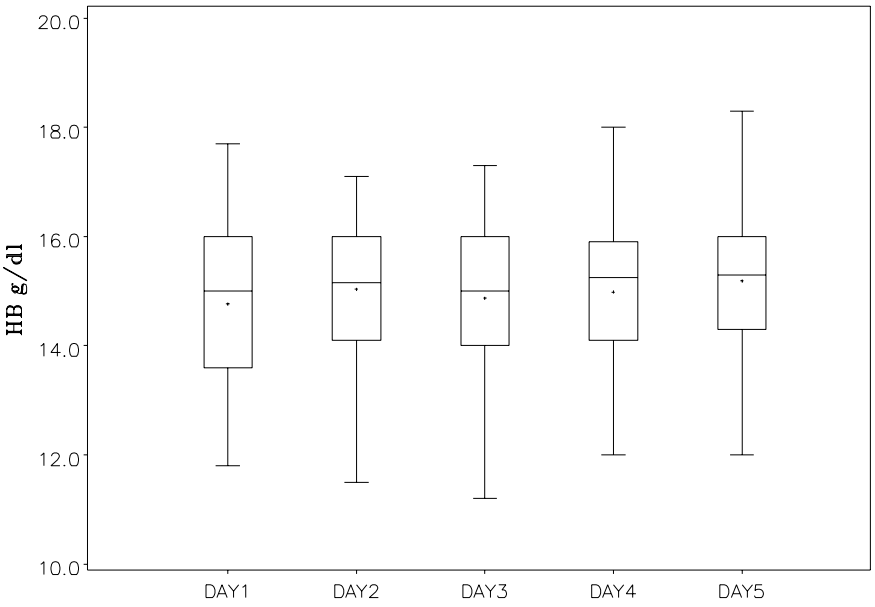

Figure 10.2: Boxplots of haemoglobin over time

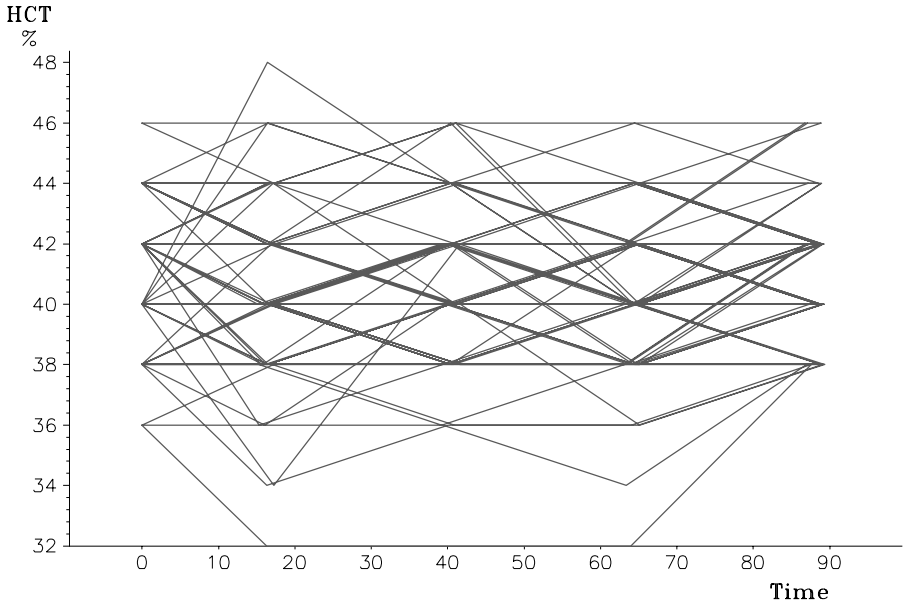

Figure 10.3: Haematocrit by volunteer over time

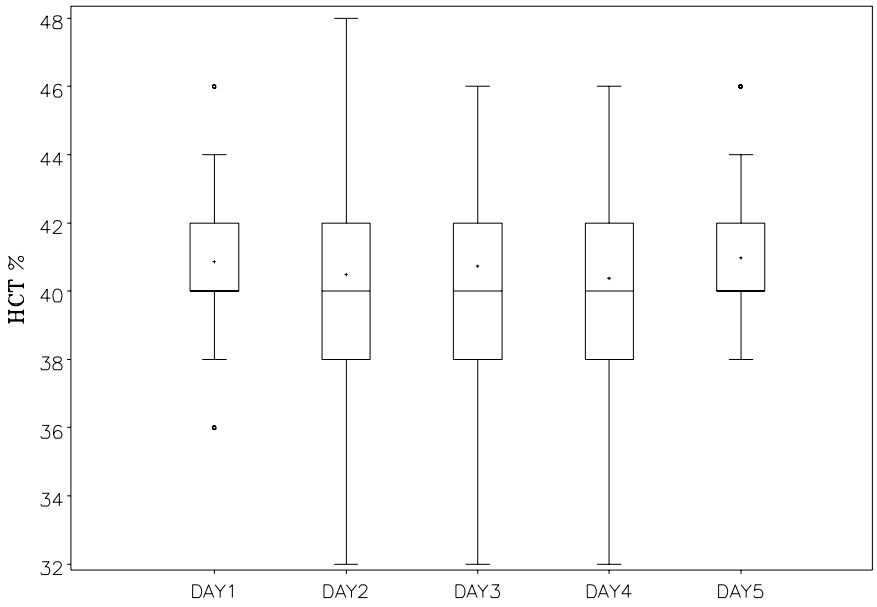

Figure 10.4: Boxplots of haematocrit over time