HEMATOLOGICAL CHANGES IN PATIENTS WITH SEVERE MALARIA FROM BANGKOK HOSPITAL FOR TROPICAL DISEASES

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HEMATOLOGICAL CHANGES IN PATIENTS WITH SEVERE MALARIA FROM BANGKOK HOSPITAL FOR TROPICAL DISEASES

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ABSTRACT

The purpose of this retrospective study was to elucidate the dynamic hematological changes in severe *P. falciparum* malaria and to determine the role of abnormal hemoglobin. The samples were 204 patients from Thailand and Myanmar aged 13 to 53 years. Data were collected by reviewing hospital charts of patients admitted to the Hospital for Tropical Diseases, Bangkok, in 2003-2004. Statistical analysis was performed using the Mann-Whitney "U" test, Chi-square analysis, independent t-test and Pearson's Chi-square.

The results revealed no association between total white blood cell count on day of presentation and severity of malaria. Median white blood counts remained within the normal range throughout the 28-day follow-up period. A statistically significant difference was found for bandforms and segment forms of PMN, eosinophils, lymphocytes, and monocytes. Bandforms and segment forms of PMN were significantly higher and eosinophils, lymphocytes, and monocytes were lower in severe patients. Low platelet count was associated with complications, although platelet transfusions were not required (94.1% of all patients had reached normal concentrations by day 7). Anemia was also not a predictor of severity; it only became significant one week after initiation of treatment. Biochemistry data showed an association between high BUN, creatinine, direct, indirect and total bilirubin, protein, AST and ALT.

No association was found between abnormal hemoglobin types or G6PD deficiency and complicated malaria.

It was therefore concluded that total WBC count cannot be used as a predictor for severity. Thrombocytopenia can implicate complications, but platelet transfusions are generally not required because they recover quickly. Anemia on day 0 is also not suitable for assessing the severity of malaria. There was no difference between patients with abnormal hemoglobin or G6PD deficiency. To evaluate the role of these factors a much larger sample size is needed.

KEY WORDS: SEVERE MALARIA/ HEMATOLOGICAL CHANGES/ WHITE BLOOD COUNT/ THROMBOCYTOPENIA/ ANEMIA

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LIST OF ABBREVIATIONS

%	percent
/	per
<	less than
>	more than
ALT	alanine aminotransferase
AP	alkaline phosphatase
ARF	acute renal failure
AST	aspartate transaminase
Baso	basophils
BUN	blood urea nitrogen
°C	degree celsius
CM	cerebral malaria
d	day(s)
DIC	disseminated intravascular coagulation
dl	deciliter
ed.	editor
ELISA	enzyme linked immunosorbent assay
Eos	eosinophils
et al.	et alii
FB	fingerbreadth
fl	femtoliter
g	gram
G6PD	glucose-6-phosphate dehydrogenase
Hb	haemoglobin
Hct	Hematocrit
hrs.	hours
i.e.	id est
l	liter

LIST OF ABBREVIATIONS (cont.)

Lym	lymphocytes
MCH	mean concentration of hemoglobin
MCV	mean corpuscular volume
mEq	milliequivalent
mg	milligram
min	minute
Mo	monocytes
µl	microliter
P.	plasmodium
PCR	polymerase chain reaction
pg	picogram
Plt	platelets
PMN	polymorph nuclear neutrophils
PTA	prior to admission
RBC	red blood cell
RDT	rapid diagnostic test
RDW	red blood cell distribution width
SD	standard deviation
SGOT	serum glutamic oxaloacetic transaminase
SGPT	serum glutamic pyruvic transaminase
spp.	species
SPSS	Statistical Package for the Social Sciences
TNF-α	tumor necrosis factor–α
U	unit
vs.	versus
WBC	white blood cell

CHAPTER I
INTRODUCTION

Malaria, a disease caused in humans by four different species of a protozoan called Plasmodium (*P. falciparum, P.vivax, P.ovale and P.malariae)* is known to be one of the world's major causes of death. Each year an estimated number of 1.1-2.7 million deaths occur as a result of severe malaria (caused by *P. falciparum)*, the main burden of morbidity and mortality being borne by pregnant women and young children in sub-Saharan Africa, where the disease is increasingly implicated in social, economic and even intellectual impoverishment (Breman et al., 2001). Severe malaria is the result of extensive multi-system involvement. The manifestation of malaria is not uniform but extremely variable. These differences include many factors, such as plasmodium species, geographical region, mosquito biting and breeding behavior, drug treatment and immunity, but also genetic host factors like hemoglobin typing and G6PD status.

Hematological changes in malaria have been described, yet, not all of them have been studied clearly and compared to each other, according to severe and uncomplicated malaria. This applies to all four human Plasmodium spp. Nor has the influence of abnormal types of hemoglobin or enzyme defects like G6PD deficiency on those changes been studied thoroughly.

Usually, the patient presents with clinical features suggestive of the diagnosis, which is confirmed by evidence of parasites in the peripheral blood. The correct and prompt diagnosis of malaria is very important because the infection may rapidly develop into a life-threatening form of the disease, requiring urgent medical attention.

Microscopy remains the crucial methodology for malaria diagnosis since it can detect parasitemia as low as 0,0001% when performed under optimal conditions. It is

not only remarkably sensitive but also has a high specificity of the parasite identification at the species level. It is also quantitative, reasonably easy to perform and cheap.

Other diagnostic means are rapid diagnostic tests (RDTs) based on antigen detection and used as dipstick or test strip, nuclear methods (PCR), serology (hemagglutination assays, indirect fluorescent antibody test, ELISA…). Although helpful, they are not widely used since they require various laboratory facilities, are often very expensive or cannot distinguish between current and past infection. None of them has yet out-performed microscopy in all its features, the main disadvantage being a low sensitivity at low levels of parasitemia.

Malaria is transmitted by female anopheline mosquitoes. The mosquito transmits sporozoits to the human via injection into the bloodstream. The sporozoits immediately invade the liver (hepatozoits), remaining in the peripheral blood for only about 30 min. In the liver, they undergo an initial pre-erythrocytic or exo-erythrocytic cycle. Sporozoits of the species *P. vivax* and *P. ovale* can remain dormant in hepatocytes (thus called hypnozoits) causing a latent infection which may result in relapses months or even years after the primary infection. After 7-10 days of development in hepatozoits, schizonts emerge, which eventually rupture and release merozoits. Merozoits invade new erythrocytes, where they develop to trophozoits and finally to multi-segmented schizonts, which rupture again and continue the life-cycle of the parasite. Parasites of the *vivax* and *ovale spp.* only invade reticulocytes, whereas *P. falciparum* causes various changes in all stages of red blood cells: altered membrane transport mechanisms, decreased deformability, development of knobs beneath the surface membrane, rheological changes, expression of strain-specific variant surface antigens and cytoadherent and rosetting properties. The latter two cause sequestration of parasitized erythrocytes in deep vascular beds. Furthermore, these changes result in stimulation of the reticuloendothelial system, changes in regional blood flow, anemia, hypoxia in tissues and organs and a systemic inflammatory response, mainly triggered by TNF-α and interleukins (Day et al., 1999).

Hemoglobinopathies and G6PD deficiency

The normal hemoglobin of adults (HbA) comprises two α-chains and two β-chains. Many genetic variations of red blood cells have been described (Weatherall et al., 1987). Most interesting are those variants that have achieved polymorphic status, i.e. alternative versions of the same gene co-exist in a population at frequencies well above those that could be explained simply by the repeated occurrence of the mutation which produces the variant. The polymorphisms mentioned above include the rates of synthesis of globin chains (α-thalassemia, β-thalssemia), conditions that affect the structure of the β-globin chain of hemoglobin (HbS, HbC and HbE) and the level of a red cell enzyme called glucose-6-phosphate dehydrogenase (G6PD).

1) Thalassemias

These globin chain production disorders are found in Mediterranean, Indian, Southeast Asian, Middle Eastern, Melanesian and some parts of African populations (English et al., 1996). Carrier frequencies for β thalassemia in these areas range from 1-20%, though rarely greater. Those for the milder forms of α thalassemia are much higher, ranging from 10-20% in parts of sub-Saharan Africa, through 40% or more in some Middle Eastern and Indian populations. In northern Papua New Guinea and isolated groups in Northeast India the carrier frequency reaches as much as 80%.

The production of one of the two main types of globin chains(α or β) is reduced as a result of defects or small deletions in the genes. Humans have four α-globin genes in which these changes may occur. This can result in a wide spectrum of effects, ranging from asymptomatic individuals with one α-gene affected to a condition which is incompatible with life (stillbirths). These diseases are thus called α-thalassemias.

In β-thalassemias, the underproduction of β-globin chains of hemoglobin leads to microcytic, hypochromic red blood cells. Furthermore, anemia is worsened by ineffective erythropoiesis due to globin chain imbalance in β-thalassemia.

These disorders of hemoglobin are inherited defects, but the mechanisms of protection are not well understood. However, reduced parasite growth has been found in β-thalassemia cells, particularly under oxidant stress. Furthermore, infected red blood cells from patients with either α- or β-thalassemia have shown enhanced antigen expression at the surface of the cells, which possibly leads to enhanced immune clearance.

2) Hemoglobin E

A single mutation in the β-chain causes this hemoglobin variant, which is common throughout South-east Asia. The aminoacid Glutaminacid is replaced by Lysin in the β-chain. The hemoglobin variant id moderately unstable when exposed to oxidants. Clinically, it results in mild anemia. Liver and spleen size are normal. MCV and MCH are lower, the blood film shows target cells, hypochromic and mycrocytic cells. Treatment is usually not required. The mechanism of protection is not clear.
People with homozygous (HbH-trait) are asymptomatic.

3) Hemoglobin H

In HbH disease, three of the α-globin genes are non-functional. As a result, tetramers of β-chain are built. Since HbH is unstable, anemia can be aggravated by hemolysis. There may be moderate anemia with Hb levels between 8 – 9 g/dl. MCV and MCH are usually decreased. Other clinical features are hepatosplenomegaly, chronic led ulceration and jaundice (reflectingunderlying hemolysis). The blood film shows hypochromia, target cells and increased reticulocytes.

HbH disease is an example of thalassemia intermedia, where patients usually suffer from a moderate hypochromic, microcytic anemia and may be dependent on blood transfusion when suffering from malaria infection. Splenomegaly commonly occurs and hypersplenism may lead to complications.

4) Glucose-6-phosphate dehydrogenase deficiency

Glucose-6-phosphate dehydrogenase plays a critical role in the production of NADPH. Numerous variants of the enzyme have been described, some of which lead to G6PD-deficiency. This X-linked disorder that affects millions of individuals in tropical countries is evidently protective against *P. falciparum* malaria. Both, hemizygous males and heterozygous females have been found to be protected against severe malaria in East and West Africa (Dolan et al., 1990). Under most circumstances, the malaria parasite is thought to use host pathways for NADPH production. It should be susceptible to changes in the level of the enzymes, but the precise mechanisms of protection are still unclear. Since variant genes only reach polymorphic frequencies in malaria-endemic regions, Ruwende and colleagues state the hypothesis that these genes have been selected for by malaria (Ruwende et al., 1995).

CHAPTER II
SPECIFIC OBJECTIVES

This retrospective study was aimed to evaluate the following blood parameters and their changes with time in patients from Bangkok, Hospital for Tropical Diseases, suffering from severe *P. falciparum* malaria:

-Complete blood count:
- RBC
- Hemoglobin (Hb)
- Hematocrit (Hct)
- White blood cells (WBC):
  - Polymorph nuclear cells (PMN)
  - Lymphocytes (Lym)
  - Eosinophils (Eo)
  - Monocytes (Mo)
  - Basophils (Ba)
- Platelets (Plt)

-Glucose-6-phosphate-dehydrogenase deficiency (G6PDD)
-Hemoglobin-typing

The primary objective was to detect dynamic changes in those hematological parameters mentioned above in patients suffering from severe malaria.

The secondary objective was

a) to detect the change in those parameters in uncomplicated malaria and
b) compare them with patients with *P. vivax, P. ovale* and *P. malariae.*

Furthermore, we aimed to investigate whether there was any significant change in these parameters in the presence of Glucose-6-phosphatase dehydrogenase deficiency or abnormal Hb-Types.

CHAPTER III
REVIEW OF LITERATURE

Malaria is a mosquito-borne febrile illness caused by infection with one (or more) of four species of *Plasmodium* (a protozoan), transmitted by the *Anopheles* mosquito. The parasite evades extinction by the human immune system by presenting different specific proteins at different stages and antigenic variation. However, semi-immunity exists in areas with high transmission rates, such as sub-Saharan Africa.

In 2002, the total number of malaria patients in Thailand was 47 948, according to the Ministry of Public Health. 21 785 of them were diagnosed with *P. falciparum*, 25 916 with *P. vivax*, 49 with *P. malariae* and 198 patients had contracted a mixed infection. Between 2000 and 2001, the death rate rose from 201 to 429 cases.

Not many studies have been conducted concerning hematological changes in malaria according to different species, severity, hemoglobinopathies and G6PD deficiency.

Anemia occurs as a consequence of parasitized RBCs rupturing. However, enhanced splenic clearance, dyserythropoiesis and also loss of blood contribute to anemia. Even the life-span of non-parasitized erythrocytes is reduced. Splenomegaly was shown to accelerate erythrocyte clearance (WHO 2000). In uncomplicated, acute *Plasmodium falciparum* malaria, the hemoglobin and packed cell volume are usually normal within the first 24 hours of the onset of fever. Treatment results in the clearance of parasitemia after about 3 days.

Thrombocytopenia is also commonly found in patients suffering from falciparum and vivax malaria (WHO 2000). In severe falciparum malaria platelet survival was found to be reduced to 2 – 4 days. A mild or moderate thrombocytopenia without

hemorrhagic manifestations frequently occurs in acute malaria and is usually not associated with DIC (Beal et al., 1972; Kueh et al., 1982). Platelet counts between 60 - 109/l are found in 5% of cases, and the extent of thrombocytopenia correlates with the degree of parasitemia.

Thrombocytopenia is often found in patients without a palpable spleen but is more marked in those with palpable splenomegaly (Kueh et al, 1982). The reduction in the platelet count seems to be mainly due to a reduced platelet life-span and splenic pooling (Skudowitz et al., 1973). The reduced platelet life-span may be caused by the binding of malarial antigens onto platelets, followed by interaction with anti-parasite antibodies and platelet phagocytosis (Kelton et al., 1983) or to platelet activation in vivo (Essien et al., 1981). Macrophage activation and hyperplasia (especially in the spleen) may also play a role. Whether dysmegakaryocytopoiesis contributes substantially to the thrombocytopenia remains unknown. There is a rapid increase in the platelet count following treatment (Essien et al., 1979).

Neutrophil leucocytosis is an important abnormality in patients with severe *falciparum* malaria and is associated with a bad prognosis. However, mild leucocytosis may also occur in patients with uncomplicated forms of malaria. Earlier studies have shown that during the first 2 days of fever the neutrophil count may be increased. Subsequently, it may be reduced. Some cases of acute and chronic *P. falciparum* infection may also have monocytosis (Charmot et al., 1979; Abdalla et al., 1988; Sen et al., 1994).

Eosinophilia has been shown to occur in *P.falciparum* malaria within one week after initiation of therapy, whereas patients with acute infection (on presentation) did not show eosinophilia in general, even when infested with intestinal parasites (Camacho et al., 1999). A strong positive correlation between eosinophil counts on day 7 and hemoglobin concentrations on day 28 was found and suggests an important relationship between the immune response and erythropoiesis. Eosinophilia is assumed to be part of a health immune response and may exert an antiplasmodial action

The assumption that WBC counts are identical during infections with different plasmodium species has been examined only minimally and tangentially. A recent study conducted by McKenzie (McKenzie et al., 2005) conducted in Thailand and Peru between 1998 and 1999 showed that the WBC counts in *P. falciparum* infected patients were lower than those in *P. vivax* infected patients, and those were lower than in uninfected patients. The sample size for that study was 4697. It also showed a positive relationship between the WBC count and the parasite density.

As a response to this publication, Rodriguez-Morales et al. (Rodriguez-Morales et al., 2005) strongly agree that WBC quantitative abnormalities may impact the estimation of parasite densities and add their experience in north eastern Venezuela, where they have observed that severe thrombozytopenia is associated with *P. vivax*. They also found that leucopenia (defined as WBC count < 4000cells/mm^3) was more common among patients with *P. vivax* infection than in patients with imported *P. falciparum* infection (4.5% vs. 0.0%). According to their observations the mean WBC count was lower in patients with *P. vivax* infection than that in patients with *P. falciparum* infection (6537 vs. 8663 cells/mm^3).

An earlier study by Camacho et al. with a sample size of 72 patients with *P. falciparum* malaria, found that the mean parasite clearance time was 1.8 days in patients with uncomplicated malaria and 2.6 in patients with severe malaria. The mean fever clearance time was 1.7 and 3.7 days. The mean hemoglobin and hematocrit concentration decreased within the first week and then increased again. 55% of the patients were still anemic on day 28 and 97% were found to be anemic at some point of the study. All patients with initially low platelet counts showed normal counts by day 7. They also screened patients for G6PD deficiency, low mean corpuscular volumes and hemoglobinopathies(HbE trait, β-thalassemia trait and Hb Constant Spring) and found, that they were evenly distributed between the uncomplicated and severe malaria groups (Camacho et al., 2002).

In 2002, Bashawri conducted a study with 727 patients with malaria in Saudi Arabia, where 59.2% were anemic at presentation. 55.6% showed thrombocytopenia.

The vast majority showed WBC findings within the normal range. 5.5% of all patients were suffering from severe anemia, and they found anemia to be more common in patients with *P. falciparum* than in other plasmodium species. They, too, found that platelet counts were lower in *P. vivax* patients than in *P. falciparum* patients (74.7% compared to 59.9%) (Bashawri et al., 2002).

Another study in 2003 could not find a statistically significant difference in the WBC counts and neutrophil count of 264 patients with *P.falciparum* and *P.vivax* malaria (Jadhav et al., 2003). Mean WBC counts of 6120 cells/mm^3 in *P. vivax* and 6010 cells/mm^3 in *P. falciparum* were recorded.

In 2004, a study conducted in Mae Sot/ Thailand was published (Erhart et al., 2004). It showed low WBC, RBC and platelets in all types of malaria. The mean platelet count was found to be significantly lower in high parasitemia.

CHAPTER IV
MATERIALS AND METHODS

1) Study site and study design

This retrospective study was conducted at Bangkok, Hospital of Tropical Diseases between November 14, 2005 and January 6, 2006.

2) Study population

Hospital charts from patients included in larger clinical trials at the Hospital for Tropical Diseases between 2003 and 2004 were used. All the clinical trials had been approved by the Ethical Committee and the signature of an informed consent form was required for inclusion. For each case included in the study, demographic and morphological data, as well as biochemical and hematological data, had been recorded on admission. The blood pressure, fever and pulse rate had been recorded according to the hospital guidelines, which is every 4 hrs. Patients had been followed up until day twenty-eight. We reviewed these forms for inclusion into our retrospective study.

Inclusion criteria were:

- patients with parasitological diagnosis of severe malaria, uncomplicated *P. falciparum* malaria, *P. vivax* malaria and *P. malariae* malaria
- having been admitted for at least seven days
- al least 12 years of age
- a follow-up period of at least 28 days

Diagnosis of malaria infection had been made by reading thin and thick blood film. Diagnosis of uncomplicated and severe malaria had been made according to the WHO criteria (WHO 2000).

Exclusion criteria were:

- Pregnancy or lactation
- mixed infection
- known hypersensitivity to any of the drugs used for the study
- known concurrent significant illness (including superimposed or concomitant non-malarial infection)

The study was submitted to the Ethical Committee of the Faculty of Tropical Medicine, Mahidol University, Bangkok

3) Investigations

Baseline investigations on admission included measurement of temperature (axillary), respiratory rate, blood pressure, assessment of dehydration, jaundice, anemia, neurological manifestations, liver- and spleen enlargement. Vital signs (temperature, respiratory rate and pulse rate) were taken four-hourly, blood pressure was measured once a day. The following clinical findings were recorded on day 0, day 7. day 14, day 21 and day 28: weakness, chills/ rigor, dizziness, abdominal pain, diarrhea, nausea, vomiting and palpitations. Blood samples were drawn on day 0, day 7, day 14, day 21 and day 28 to determine the hematological parameters mentioned above as well as blood chemistry. Parasites were counted approximately 6-hourly during parasitemia until the first negative result, then once a day.

For blood chemistry our laboratory currently uses the Roche Cobas Integra 400 machine, for the CBC and other blood parameters it uses the sysmex XT-2000i

4) Diagnosis of malaria

Diagnosis of malaria infection was done parasitologically by preparing thick and thin blood films. Both were stained in Field’s staining method. For the thin film, parasites were counted against 1000 red blood cells, for the thick film, parasites were

counted against 200 white blood cells. Calculation of parasitemia (no./µl) was done according to the formula:

For the thin smear: The number of asexual parasites per microlitre =

Number of asexual parasites per 1000RBC X Total RBC ($X10^6$/µl)

For the thick blood smear: The number of asexual parasites per microlitre =

Number of asexual parasites per 200 WBC X Total WBC ($X10^3$/µl)

5) Diagnosis of G6PD deficiency

G6PD deficiency was diagnosed using the R&D Diagnostics Kit which uses fluorescence as a method of detecting the enzyme. The assay procedure is according to reaction described by Beutler (Beutler, 1966), where the enzyme determined is glucose-6-phosphate dehydrogenase, which converts glucose-6-phosphate + $NADP^+$ into gluconate-6-phosphate + NADPH + H^+. NADPH fluoresces under long-wave UV-light. No fluorescence is observed when there is a marked deficiency of this enzyme. False negative results may occur in case of bubble formation in the test well, if the patient has received a blood transfusion within the last 30 days or if the patient has experienced a hemolytic crisis. A hemolytic crisis must be treated first because in some forms of G6PD deficiency, young erythrocytes may manifest normal enzyme activity. Blood samples containing a prevailing population of young erythrocytes may occur falsely negative.

6) Hemoglobin electrophoresis

Hemoglobin-typing was done using electrophoresis by cellulose acetate at an alkaline pH of 8.6. The various hemoglobins move at different rates depending on their net negative charge. The specimen used is anticoagulated whole blood, which must be hemolysed. After electrophoresis of 45 min the cellulose acetate strip is cut into various hemoglobin zones which are then eluted in water. The absorbance of each

eluate at 415 nm using a spectrophotometer is measured. The HbA_2 concentration is calculated as follows:

$$\% HbA_2 = O.D.\ HbA_2/[(O.D.\ HbA_2 \times Volume\ A_2) + (O.D.\ Hb\ A \times Volume\ A)]$$

(O.D. = slopes)

With this method concentrations of HbA and HbA_2 can be determined (Lea & Febiger, 1993; Dacie JV &Lewis SM, 1995)

7) Test for HbF

HbF is determined using the Alkali Denaturation Test (Singer et al., 1951), since it is resistant to denaturation by strong alkali solutions while other hemoglobin types are not. A red blood cell hemolysate solution is filtered, KOH and 50% saturated acid Ammonium sulfate are added. This “Filtrate” is examined in a spectrophotometer at wavelength fo 540 nm. A “Total” is prepared adding destilled water. Cyanide solution as mixed with both. The result is calculated as follows:

% HbF = O.D. “Filtrate” x 20/ O.D. “Total”

8) Test for HbE

HbE is determined using the “KKU-DCIP-Clear Reagent Kit”. HbE is associated with an increased liberation of reactive (-SH) groups. Upon oxidation with a dichlorophonolinophenol (DCIP) dye HbE precipitates. Precipitation results in visible changes of the solution: a turbid solution means precipitation and is interpreted a positive result, a clear solution means no precipitation and is considered a negative result.

9) Treatment

All patients with falciparum malaria were treated with a combination therapy of Artesunate 600 mg as a total daily dose for 3 – 5 days, given orally or intravenously according to the severity of illness, plus a single dose of Mefloquine 1250 mg. Patients with vivax and ovale malaria received 1g of Chloroquine (600 mg base), then 500mg (300mg base) 6 hrs later and 500mg at 24 hrs and 48 hrs plus Primaquine 15mg base for 14 days. Patients with *P. malariae* infection were treated with the same dose of Chloroquine as patients with vivax and ovale malaria. All patients received the same dosage. Supportive treatment was given as indicated.

10) Sample size

The Hospital for Tropical Diseases was able to provide about 200 cases for the period mentioned above.

11) Data analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS Version 12). All the p-values were expected to be from two-tailed tests and the statistical significance level was set at 0.05. The distribution of data was assessed for normality using the Kolmogorov-Smirnov test. It showed no normal distribution for most of our data so we used non-parametric tests: 1) the Mann-Whitney“U”test to compare the rank of two groups, 2) chi-square analyses to test differences between two groups of qualitative variables, 3) the Pearson’s correlation to find a correlation between, at least, two continuous variables.

12) Significance of the research

By evaluating and correlating blood parameters (hematocrit, hemoglobin, MCV, white blood count, differential count, platelet count) we aimed to find out the factors

associated with severe malaria. We also aimed to identify the role of abnormal types of hemoglobin and G6PD deficiency in severe and uncomplicated *falciparum* malaria

13) Operational Definitions

Hyperparasitemia

Parasite density of more than 5 % in non-immune patients (WHO, 2003).
(It is the same meaning of more than 50 asexual forms per 1000 RBC)

Fever clearance time

Fever clearance time was taken as the period from the start of treatment until the oral temperature decreased to 37.5 C and remained below this temperature for the next 48 hours.

Parasite clearance time

The parasite clearance time was defined as the time from the start of treatment until the first negative blood film and remained negative for the next 24 hours.

14) Research fund

The research fund was provided by the Faculty of Tropical Medicine.

CHAPTER V
RESULTS

204 patients that matched our inclusion criteria were included in this retrospective study. All patients with significant concurrent illness that might affect the white blood count or patients that had a parasitologically proven mixed malaria infection were excluded. The only concurrent illness that does affect the differential white blood count but was still accepted was helminthic infestation, since 70.6% of all cases had a positive stool examination (mostly for Ascaris lumbricoides, Trichuris trichiura, Hookworm or Strongyloides stercoralis). 62.3% of patients were male and 37.7% were female. Out of these 204 patients 98 (48%) suffered from severe malaria and 106 (52%) had uncomplicated malaria, meaning they had a mono-infection with either *P. falciparum*, *P. vivax*, *P. ovale* or *P. malariae*. Overall, only one patient was diagnosed with *P. ovale* and two with *P. malariae* during the time studied. 57 (58.2%) of the patients suffering from severe malaria were male and 41 (41.8%) were female, whereas in the group of uncomplicated malaria 70 patients (66%) were male and 36 (34%) were female. The median age in the group of severe malaria was 21 (the youngest patient was 13 and the oldest patient in this group was 53 years old), compared to 24 years in uncomplicated malaria (13-49 years) (Table 1).

37 (18%) of all patients were from Thailand (26 of them had uncomplicated malaria and 11 had severe malaria), 40 (19.6%) were from Myanmar 21 uncomplicated and 19 severe cases), 59 (28.9%) were Karen (25 uncomplicated and 34 severe cases) and 68 (33.5%) were Mon (34 uncomplicated and 34 severe cases). The recorded residence province of most of the patients was Tak (56.4), followed by Kanchanaburi (35.3%). 8.5% of patients were from Ratchaburi. When looked at it from the view of uncomplicated and severe malaria there was an association between the residence province and severity: 58 out of 115 patients from Tak, 39 out of 72 from Kanchanaburi and 1 out of 17 from Ratchaburi had severe malaria. The overall p-value was 0.001 (Table 2). When recoded and further analyzed we found out that

only the p-value for Ratchaburi showed statistical significance ($p<0.001$). We then calculated the residence province against previous malaria infections and found that there was a highly significant overall p-value of < 0.001. From Ratchaburi 3 out of 17 patients (17.6%) had a positive and the remaining 14 patients (82.4%) had a negative malaria history.

Most of the patients worked as laborers (83.3%). The remaining patients were farmers (14.7%) and monks (2%) (Table 2).

There was no difference between the uncomplicated and severe malaria group in sex, ethnic group and occupation (Table2).

Furthermore, there was no significant difference in age, height, weight and days of fever prior to admission, but there was a significant difference in previous malaria infections ($p=0.002$). 142 patients (70.1%) claimed they had no previous malaria infection. 61(29.9%) mentioned at least one previous malaria infection: 18 patients in the severe group (29.5% of patients with previous malaria infections) and 43 (70.5%) in the uncomplicated group (Table 1).

Table 1
Demographic data and baseline investigations

	Uncomplicated			Severe			p-value
	Median	**Range**	**n**	**Median**	**Range**	**n**	
Demographic data							
Age years	24	13-49	106	21	13-53	98	0.671
Weight (kg)	51	35-83	106	50	25-84	98	0.302
Height (cm)	161	131-181	106	160	146-186	98	0.287
Day of fever prior to admission	4	1-30	106	5	2-14	98	0.114
Previous malaria infections	0	0-10	101	0	0-5	98	0.002
Baseline investigations before treatment							
Temperature (°C)	38.5	36-40	106	38.3	36-41.9	98	0.273
Respiratory rate(/ min)	20	20-34	106	24	18-40	98	<0.001
Pulse rate(/ min)	90	72-120	106	100	72-208	98	0.050
Systolic blood pressure (mmHg)	110	90-130	106	100	67-143	98	0.005
Diastolic blood pressure (mmHg)	70	50-90	106	60	26-90	98	<0.001
Parasitological data							
Initial parasite count (no./µl)	8520	1-245960	106	170640	60-1277500	98	<0.001
Parasite clearance time (hrs)	42	18-331	106	48	9-96	98	<0.001
Fever clearance time (hrs)	24	4-160	105	68	4-232	89	<0.001

Table 2
Demographic data and percentage

	Uncomplicated	Severe	p-value
	n (%)	n (%)	
Sex			0.246
male	70 (66%)	57 (58.2%)	
female	36 (34%)	41 (41.8%)	
Ethnic group			0.064
Thai	26 (24.5%)	11 (11.2%)	
Myanmar	21 (19.8%)	19 (19.4%)	
Karen	25 (23.6%)	34 (34.7%)	
Mon	34 (32.1%)	34 (34.7%)	
Residence province			0.001
Tak	57 (53.8%)	58 (59.2%)	0.437
Kanchanaburi	33 (31.1%)	39 (39.8%)	0.197
Ratchaburi	16 (15.1%)	1 (1%)	<0.001
Occupation			0.634
Laborer	86 (81.1%)	84 (85.7%)	
Farmer	18 (17%)	12 (12.2%)	
Monk	2 (1.9%)	2 (1%)	

When baseline investigations were analyzed, significance was found in respiratory rate, pulse rate and blood pressure (Table 1). Significant hematological p-values were found in median hematocrit, platelets, band forms and segment forms of PMN, eosinophils, lymphocytes and monocytes. The median initial parasite count was 8520 in uncomplicated and 170640 in severe malaria. The parasite clearance time for uncomplicated and severe malaria was 42 and 48 h, the fever clearance time was 24 and 68 h. P-values for all those parasitological data were <0.001, showing significance.

When median white blood cell counts on day 0, 7, 14, 21 and 28 were compared, they only showed significant p-values for day 14 and 28. Initial WBC counts were slightly lower in severe malaria but tended to rise to a higher lever the 3 following weeks (*Figure 1*).

P-values for band forms of PMN only showd significance for day 0, where the median percentage was 1% in uncomplicated and 3% in severe malaria (*Figure 2*).

As for segment forms of PMN, p-values were significant for day 0, 7, 14 and 28. Initially, they were higher in severe malaria but decreased to a lower level for the following three weeks until they increased again on day 28 (*Figure 3*).

Eosinophils were significantly lower in severe malaria, especially during the first two weeks of illness. The median percentage on day 0 was 1% (with a minimum of 0% and a maximum of 17%), compared to 2% (minimum 0%, maximum 10%) in uncomplicated malaria. There then seemed to be an approximation of percentage of eosinophils but levels were still lower on day 28 (*Figure 4*).The highest percentage was 49%, measured on day 28 in a patient with severe malaria. We took a look at the absolute eosinophil count and it showed a very similar pattern (*Figure 17*). At no point was there any positive association between eosinophil count and severity. 70.6% of all cases had a proven parasitic (helminthic) co-infection. They were only treated with anthelminthics after completion of the study period so there would not be any unexplained hematological or biochemical changes. When eosinophil counts were

compared according to intestinal parasite infection it showed that in both groups median eosinophil counts were slightly above the normal range on day 0 but then constantly rose to significantly higher levels in patients with helminthic co-infection (*Figure 18*). Still, on day 28 even patients without helminths showed markedly increased eosinophil counts. P-values were significant from day 7 to day 28.

Regarding basophils, the median percentage was 1% throughout the whole study period in uncomplicated and severe malaria. Statistically significant p-values were calculated for days 14 and 21 (*Figure 6*).

Lymphocytes showed a different pattern: they were significantly lower in severe malaria on the day of admission, but rose to a significantly higher level on day 7, 14 and 21. On day 28 they were below the lymphocyte count in uncomplicated malaria again (*Figure 5*).

Monocytes were also significantly lower in severe malaria than in uncomplicated malaria, but, however, a significantly higher level was shown one week later. On day 14, 21 and 28 the numbers were identical (*Figure 7*).

The median platelet count in severe malaria on day 0 was 30 x 10^9/l, compared to 98 x 10^9/l. The lowest number was as low as 5 x 10^9/l in severe malaria. However, they seemed to recover quite quickly, since on day 7 the median platelet count in severe malaria was 322 x 10^9/l, compared to 336 x 10^9/l in uncomplicated malaria, though the difference was still significant. On day 14 and 28 platelets were even higher than in uncomplicated malaria (*Figure 8*).

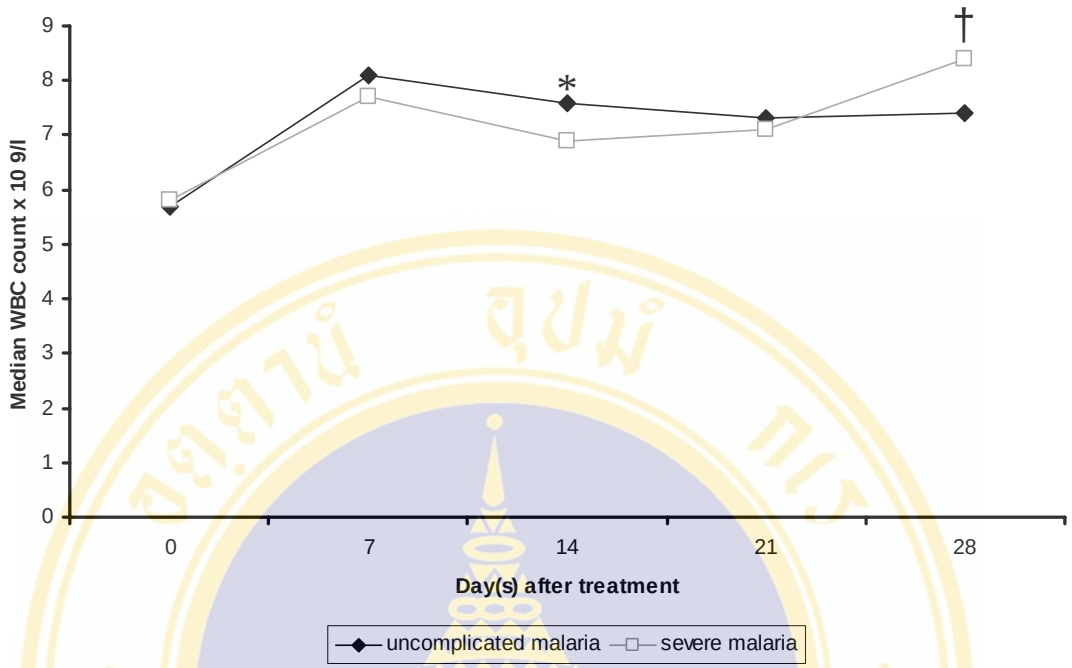

Figure 1. **Median white blood count in uncomplicated and severe malaria**
*= p-value <0.05
†= p-value 0.01

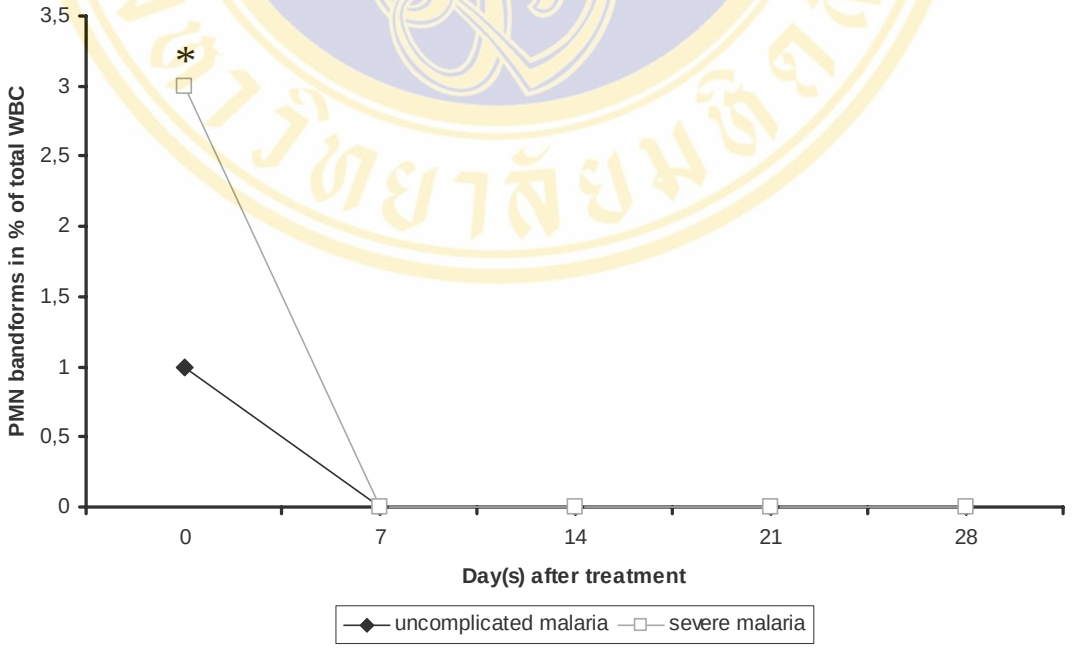

Figure 2. **Median percentage of band forms of PMN in uncomplicated and severe malaria**
*= p-value <0.001

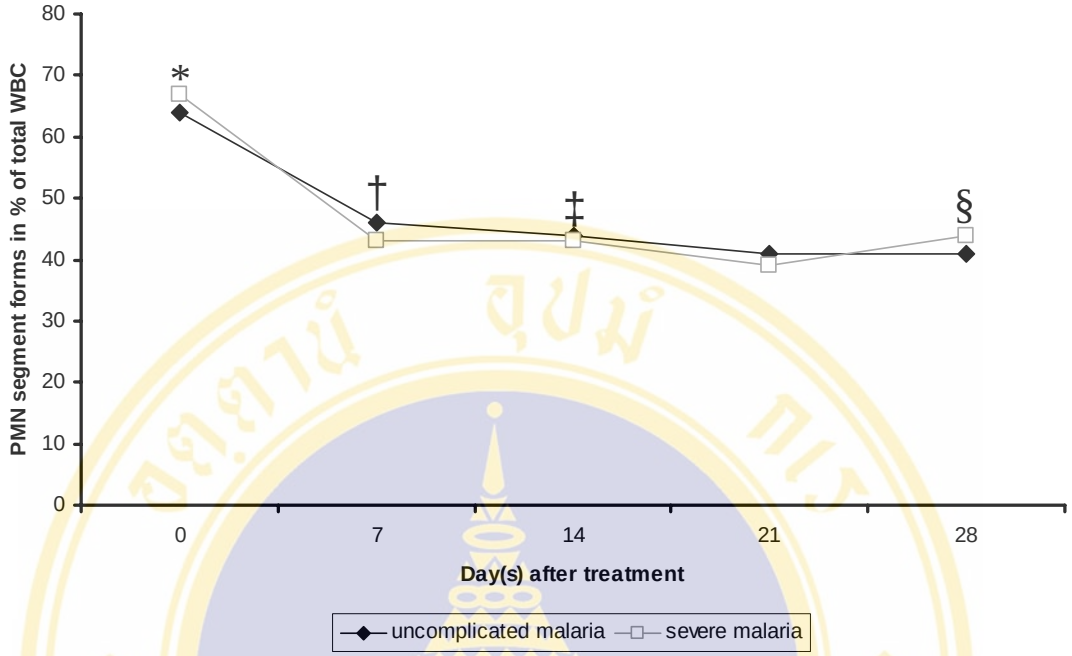

***Figure 3*. Median percentage of segment forms of PMN in uncomplicated and severe malaria**
*= p-value <0.05
†= p-value <0.05
‡ = p-value <0.05
§ = p-value <0.05

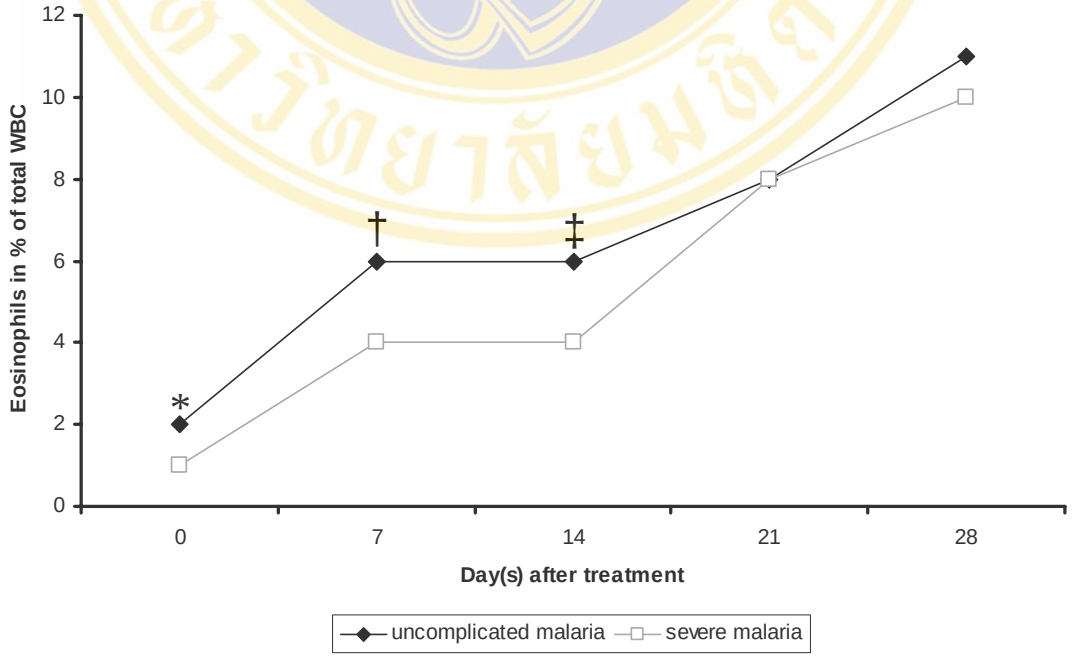

***Figure 4*. Median percentage of eosinophils in uncomplicated and severe malaria**
*= p-value 0.005
†= p-value <0.001
‡= p-value <0.001

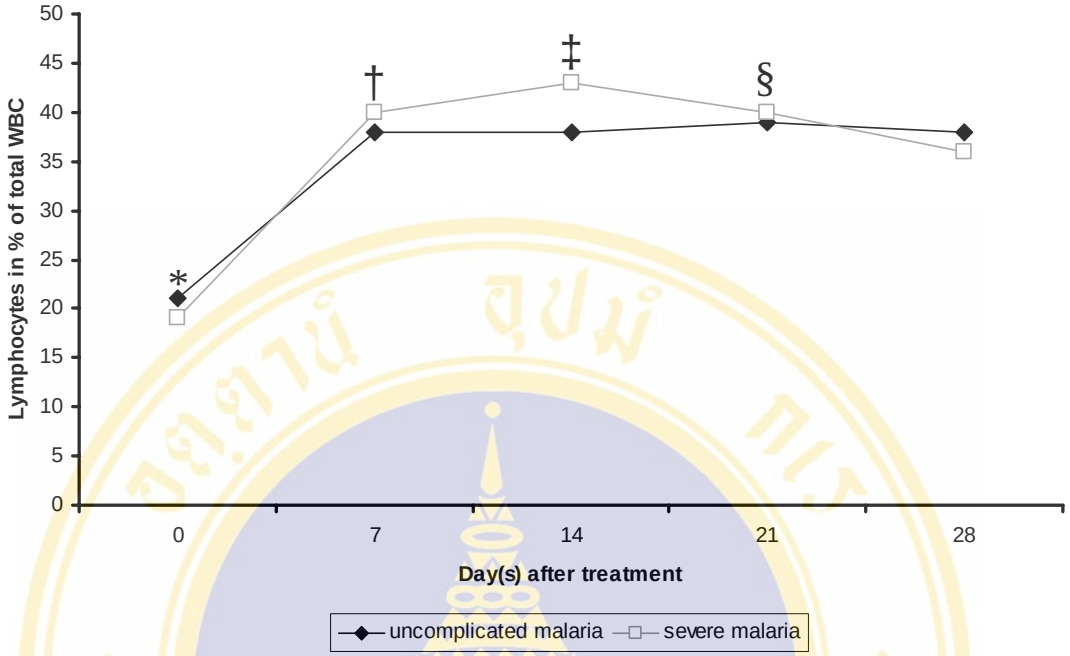

***Figure 5*. Median percentage of lymphocytes in uncomplicated and severe malaria**
*= p-value <0.01
†= p-value 0.005
‡= p-value <0.001

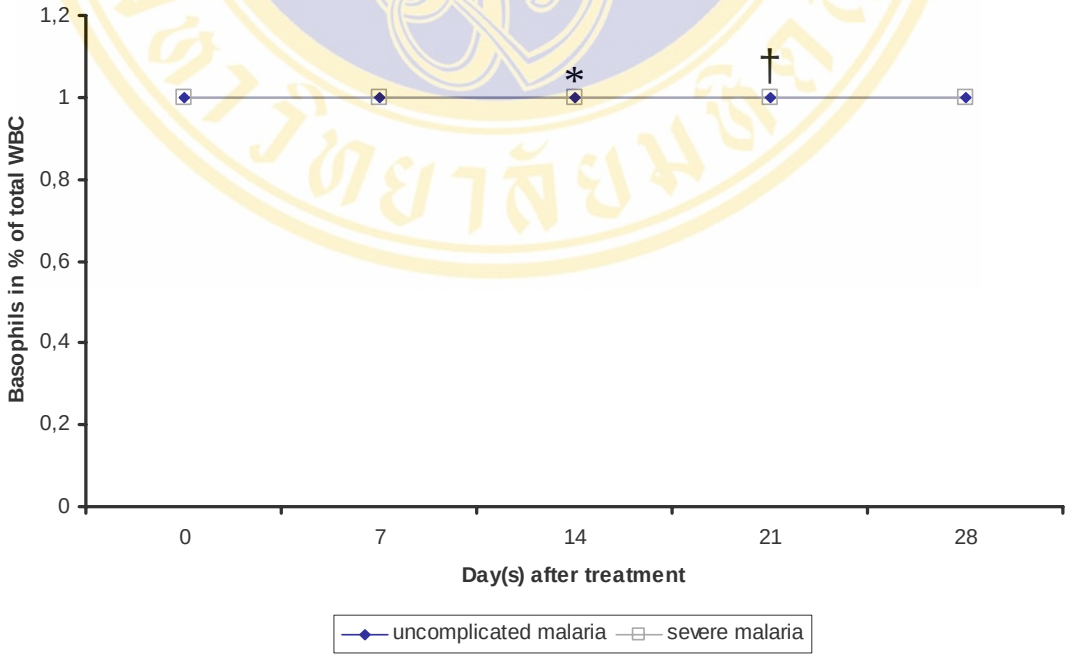

***Figure 6*. Median percentage of basophils in uncomplicated and severe malaria**
*= p-value <0.01
†= p-value <0.05

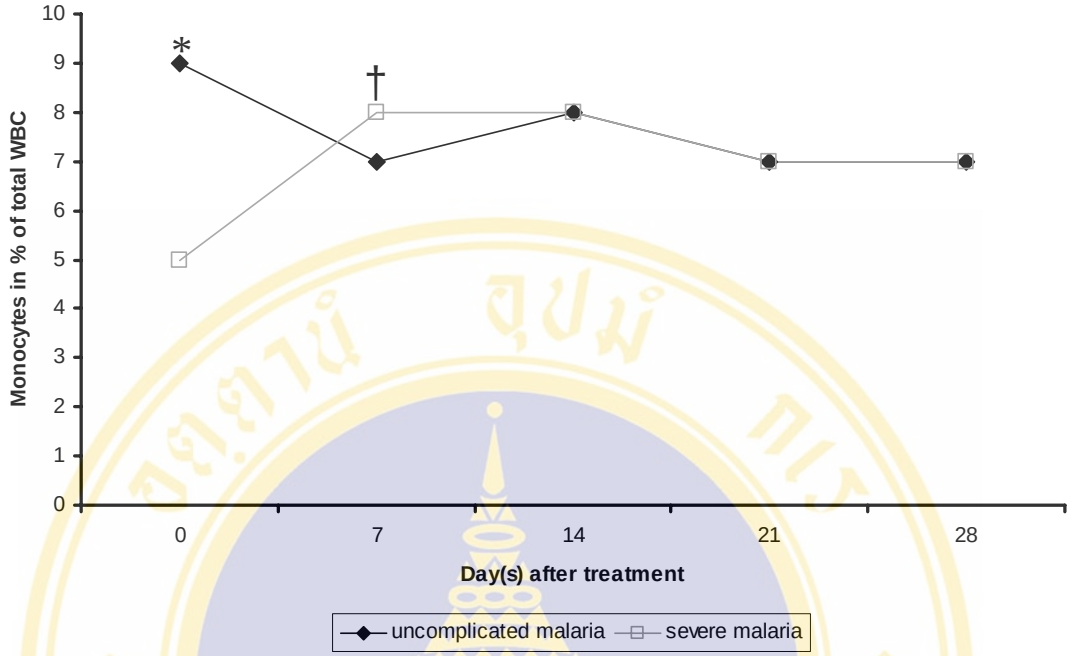

***Figure 7*. Median percentage of monocytes in uncomplicated and severe malaria**
*= p-value <0.001
†= p-value <0.05

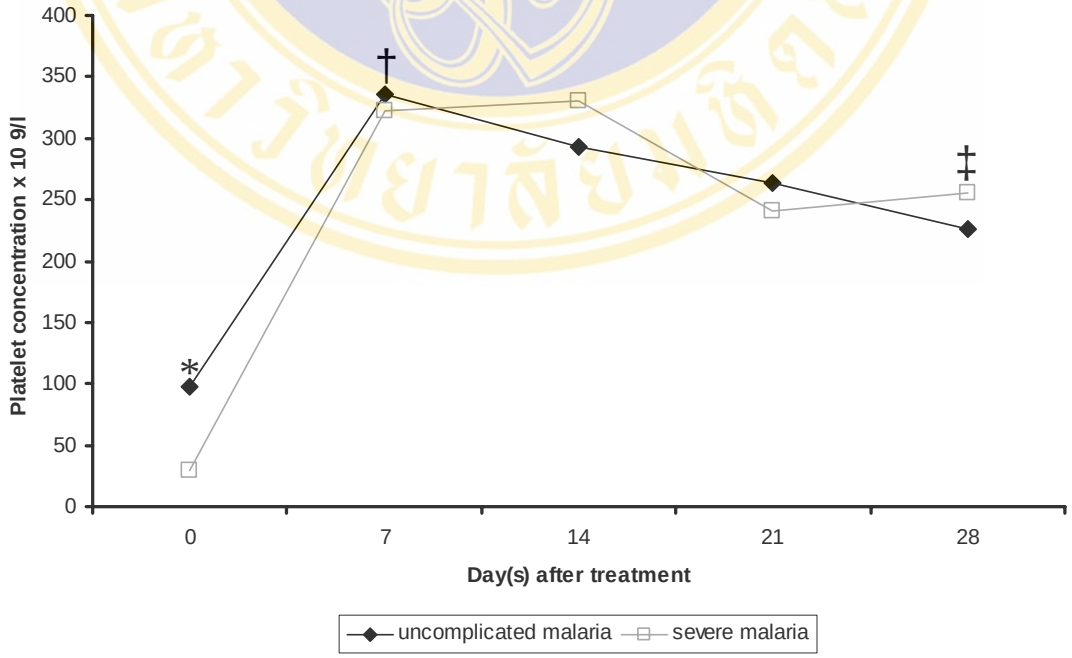

***Figure 8*. Median platelet count in uncomplicated and severe malaria**
*= p-value <0.001
†= p-value <0.05
‡= p-value <0.01

Median haemoglobin concentrations where identical on day 7, but the range was different (5.3-17.1 mg/dl in severe and 6.7-16.5 mg/dl in uncomplicated malaria). However, no significance was shown for day 0. On day 7, 14, 21 and 28 hemoglobin levels were lower in severe malaria, with significant p-values for day 14, 21 and 28 (*Figure 9*). Median hematocrit levels showed a very similar pattern, only this time significantly lower p-values were shown for each day (*Figure 10*) 60 patients (61.2%) in the severe group appeared clinically pale, indicating anemia, 38 (38.8%) didn't. In the uncomplicated group on the other hand only 12 patients (11.3%) appeared clinically anemic. When looked at the hemoglobin concentration (Hb<10 mg/dl) 31 out of 98 patients (31.6%) in the severe group showed values below the normal range and the remaining 67 had normal values, compared to uncomplicated malaria, where 23 out of 106 patients (21.7%) were anemic. P-values were highly insignificant. 77 patients (78.6%) in the severe group had a decreased hematocrit, whereas 21 (21.4%) had normal values. In the uncomplicated group 71 (67%) patients had a low and 35 (33%) a normal hematocrit. Again, p-values were not significant. Pallor and clinical jaundice on day 0 were not associated at all (p-value 1), nor were they when the cut-off point of 3mg/dl total bilirubin was used. Jaundice was associated with dehydration, cerebral malaria, acute renal failure, hyperparasitemia, but not with shock and acidosis. P-values for blackwater fever, pulmonary edema, respiratory distress, hypoglycemia, disseminated intravascular coagulation/ bleeding and generalized convulsion could not be calculated because there were 0 patients in one or both groups (i.e. none of our patients suffered from disseminated intravascular coagulation/ bleeding or generalized convulsion).

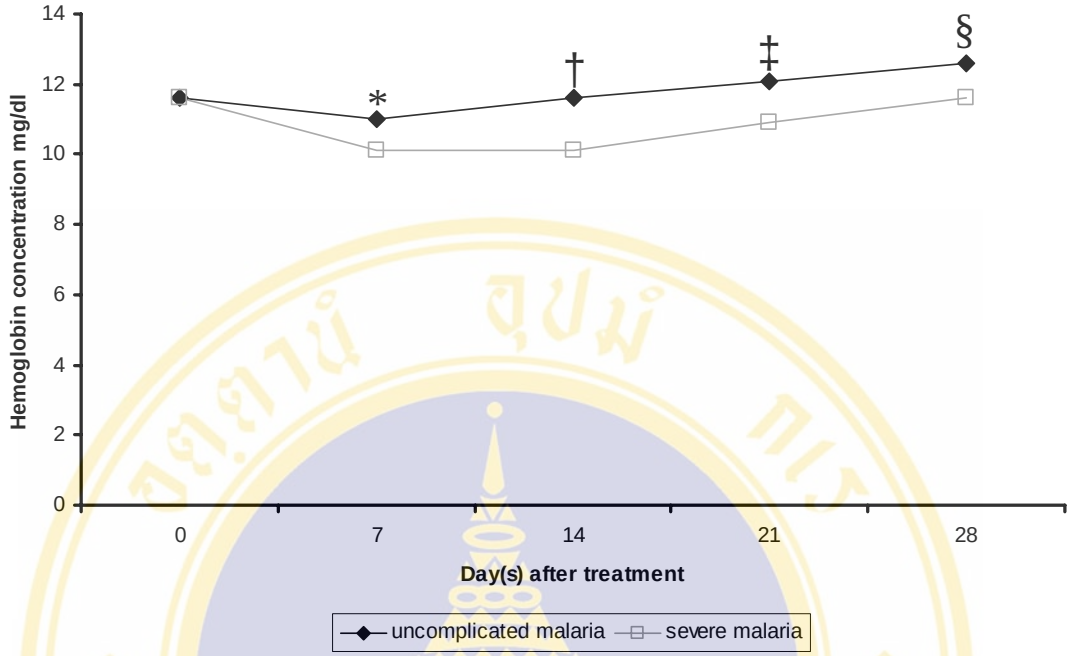

***Figure 9*. Median hemoglobin concentration in uncomplicated and severe malaria**
*= p-value <0.001
†= p-value <0.001
‡ = p-value <0.001
§ = p-value <0.001

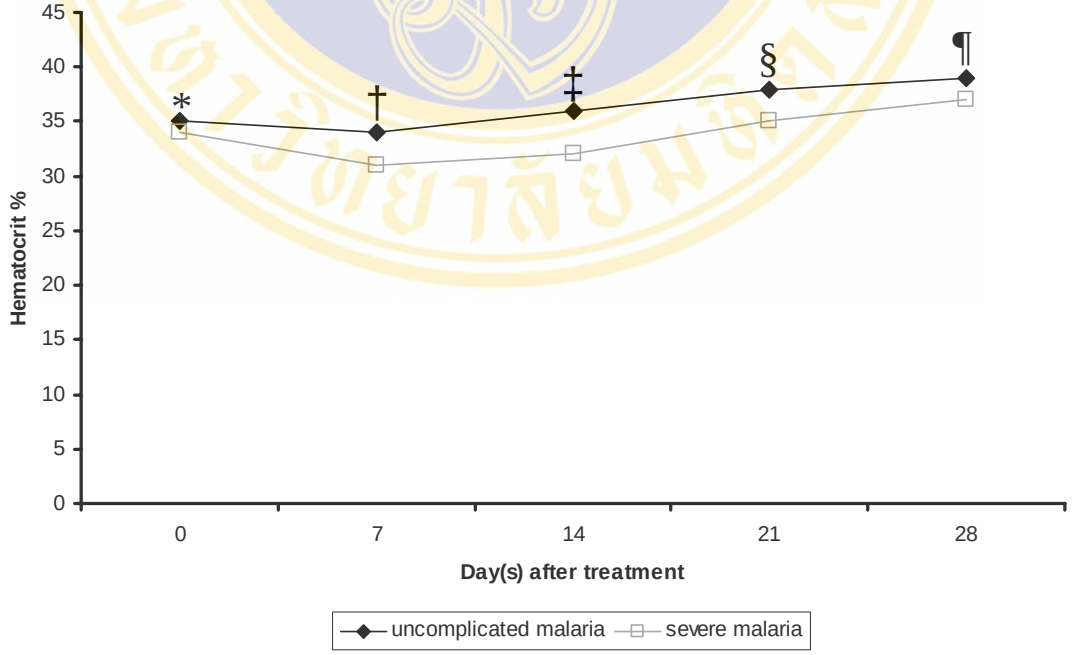

***Figure 10*. Median hematocrit level in uncomplicated and severe malaria**
*= p-value <0.05
†= p-value <0.001
‡ = p-value <0.001
§ = p-value <0.001
¶= p-value <0.001

All patients underwent hemoglobin electrophoresis, but this methodology cannot identify α-thalassemia. 108 patients (53%) were found to have some kind of abnormal hemoglobin: 42 had β-thalassemia trait 27 in the uncomplicated and 15 in the severe group), 18 had homozygous β-thalassemia (6 versus12 in the severe group), 18 had HbE trait (8 versus 10), 5 had homozygous HbE (all uncomplicated), 1 had HbH (severe) and 1 patient (uncomplicated) had α-thalassemia trait. (We strongly suppose that this patient has α-thalassemia trait due to specific changes in his blood picture, but there are limitations concerning out laboratory facilities.) 23 patients could not be classified with our laboratory means but iron deficiency anemia is the most probable reason for the changes in MCV, hematocrit and/ or hemoblobin. All p-values were insignificant. P-values for HbE disease, HbH and α-thalassemia trait could not be calculated because there were no cases in either severe or uncomplicated malaria. The overall p-value for abnormal hemoglobin in uncomplicated and severe malaria was also insignificant (p=0.077). 23cases (11.3%) had to be labeled “unidentified” because they showed an MCV-, hemoglobin- and/ or hematocrit pattern not consistent with any of the known hemoglobinopathies and hemoglobin electrophoresis could not detect abnormal hemoglobin either. All but one patient were screened for G6PD deficiency. 4.9% were found to be positive, 7 patients in uncomplicated and 3 in severe malaria. The p-value showed no significance (0.248) (Table 3).

All patients in each group received the same total antimalarial drug dose. Concurrent symptoms were treated symptomatically. However, some patients needed special treatment: 18 patients (8.8%) had to receive one unit of (PRC), 21 patients (10.3%) received more than one units. 80.9% of all patients did not need any packed red cells (PRC) transfusion. All except one female patient, who suffered from *P.v.* malaria but had a very low Hb level of 5.7 mg/dl and hct of 19%. and, in addition, had G6PD-deficieny, were severe malaria patients. This patient needed two units of PRC. Her hemoglobin typing was normal. The maximum of packed red cells units given was 10. 4 patients (4.08% of all severe patients) received whole blood transfusions. The maximum number of units was 2. 46 patients (46.94% of all severe patients) received fresh frozen plasma (FFP) transfusions, 17 needed only one transfusion, 29 of them had to have more than 1 unit, the maximum of units being 11. None of the patients

received platelet transfusions since none of our patients showed any signs of disseminated intravascular coagulation (DIC) or abnormal bleeding. Some patients, though, received FFP and PRC transfusions together.

Baseline biochemistry data were also obtained from all patients on day 0, 7, 14, 21 and 28. There were significant p-values for BUN, creatinine, direct and total bilirubin, total protein, albumin, AST, ALT, sodium and chloride on day 0 (Table 4).

Of all patients with severe malaria 71 (72.4%) had a raised BUN (>19 mg/dl) compared to only 17 patients (16%) in uncomplicated malaria (*Figure11*). The p-value was highly significant. The highest BUN value recorded in this study was 119.8 mg/dl. On follow-up there was a clear assimilation of BUN values, still, they remained minimally above the ones in patients with uncomplicated malaria until day 28. The median creatinine value was also significantly higher in severe malaria on day 0 (0.44-4.78 mg/dl), but had decreased even below values in uncomplicated malaria by day 7. On day 21 it was significantly lower in severe malaria than in uncomplicated (*Figure12*). 5 patients (2.5%) had to have hemodialysis due to renal failure. The median number hemodialysis sessions was 5, the longest duration was 12 sessions of dialysis. When clinicians examined patients on admission, 61 patients had at least mild jaundice, 53 (86.9%) of them were severe patients and 8 (13.1%) were uncomplicated. Median total bilirubin concentrations were 1.37 mg/dl (0.22-4.39) and 3.53 mg/dl (0.51-32.58). They remained significantly higher than concentrations in uncomplicated malaria cases (*Figure 13*). A very similar pattern was found in direct bilirubin concentrations. P-values were <0.001 on day 0, 7, 14 and 21, on day 28 it was 0.043 (*Figure 14*). The median indirect bilirubin concentrations on the day of presentation were 0.4 and 0.65 mg/dl for uncomplicated and severe patients. Levels remained stable for one week, then they showed a rise in both groups on day 21 until they continuously decreased again until day 28. P-values showed significance for each day of measurement (*Figure15*). At no time was the median indirect bilirubin concentration higher than the median direct bilirubin concentration.

The median albumin levels on day 0 were 3.8 g/dl (2.2-4.7) and 2.9 g/dl (1.4-4.3). They remained significantly lower in severe than in uncomplicated cases until day 21 (all p-values <0.001). Only on day 28 was the level insignificantly lower (*Figure 16*).

There were also significant p-values on the day of admission for sodium and chloride (both were lower in severe patients). The difference in potassium was insignificant.

Table 3
Number of patients with abnormal hemoglobin or G6PD-deficiency

	Uncomplicated		Severe		p-value
	n	(%)	n	(%)	
Hb-typing					
Normal	49	(51)	47	(49)	0.804
β-thalassemia homozygous	6	(33.3)	12	(66.7)	0,098
β-thalassemia trait	27	(64.3)	15	(35.7)	0.073
HbE	5	(100)	0	(0)	NA
HbE trait	8	(44.4)	10	(55.6)	0.504
α-thalassemia trait	1	(100)	0	(0)	NA
HbH	0	(0)	1	(100)	NA
unidentified	10	(43.5)	13	(56.5)	0.387
G6PD-deficiency	7	(70)	3	(30)	0.248

NA = not assessed

Table 4

Baseline laboratory data before treatment

	Uncomplicated			Severe			p-value
	Median	**Range**	**n**	**Median**	**Range**	**n**	
Hematology							
Total WBC (x 10^9/l)	5.7	2.6-11.6	106	5.8	1.6-25	98	0.478
Bandforms (%)	1	0-17	106	3	0-30	98	<0.001
Segments (%)	64	23-85	106	67	29-88	98	0.014
Eosinophils (%)	2	0-10	106	1	0-17	98	0.005
Basophils (%)	1	0-2	106	1	0-3	98	0.198
Lymphocytes (%)	21	5-51	106	19	5-49	98	0.002
Monocytes (%)	9	2-13	106	5	1-12	98	<0.001
Hemoglobin (mg/dl)	11.6	6.7-16.5	106	11.6	5.3-171	97	0.375
Hematocrit (%)	35	21-51	106	34	17-49	98	0.029
Platelets (x 10^9/l)	98	26-309	106	30	5-457	98	<0.001
Biochemistry							
BUN (mg/dl)	13.1	5.2-24.7	106	24.2	9.7-119.8	98	<0.001
Creatinine (mg/dl)	0.83	0.56-1.46	106	0.95	0.44-4.78	98	<0.001
Direct bilirubin (mg/dl)	0.45	0.12-1.5	105	1.29	0.22-11.79	98	<0.001
Indirect bilirubin (mg/dl)	0.4	0.13-2.9	106	0.65	0.13-3.42	98	<0.001
Total bilirubin (mg/dl)	1.37	0.22-4.39	106	3.53	0.51-32.58	98	<0.001
Albumin (g/dl)	3.8	2.2-4.7	105	2.9	1.4-4.3	98	<0.001
Globulin (g/dl)	3.1	2-4.9	106	3.3	2.4-4.7	98	0.140
Alkaline phosphatase (U/l)	119	37-281	106	129	41-513	98	0.151
AST (U/l)	36	13-112	106	65	11-458	98	<0.001
ALT (U/l)	31	2-186	106	52	4-252	98	<0.001
Na^+ (mmol/l)	137	119-143	104	134	118-145	98	<0.001
K^+ (mmol/l)	3.6	2.5-4.9	104	3.6	2.6-4.9	98	0.654
Cl^- (mmol/l)	102	95-110	103	99	85-109	98	<0.001

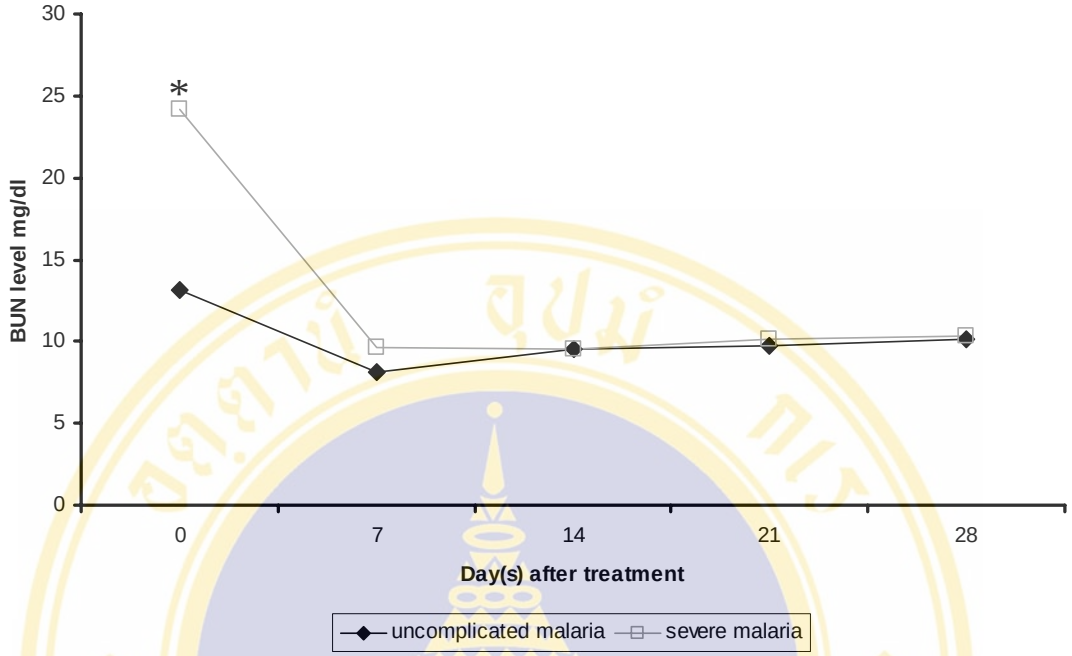

***Figure 11*. Median BUN values in uncomplicated and severe malaria**
*= p-value <0.001

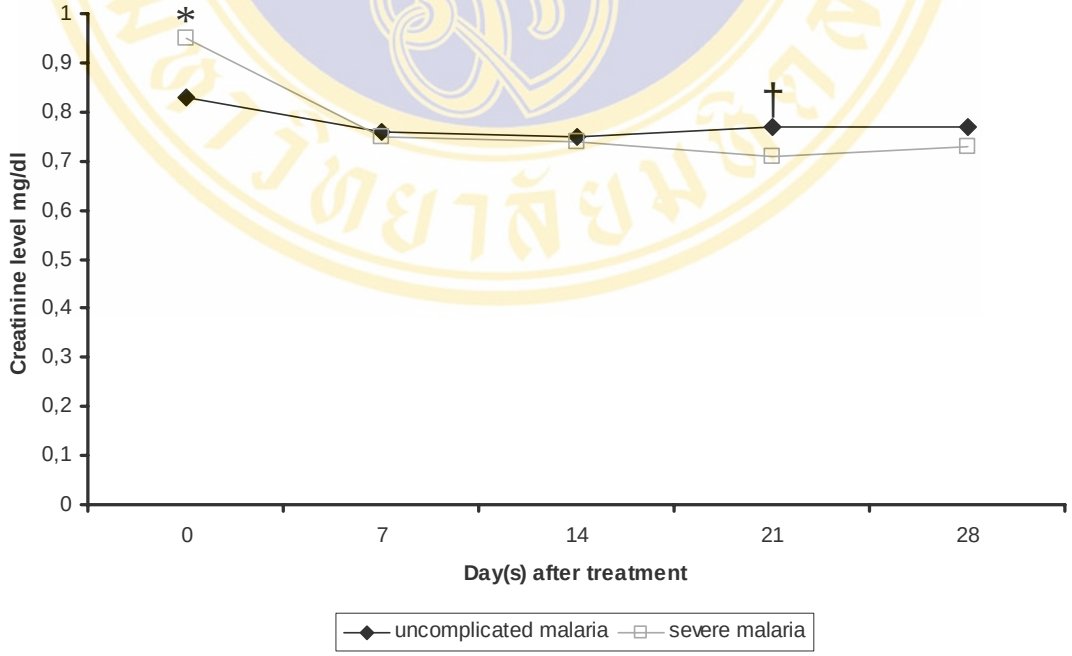

***Figure 12*. Median creatinine values in uncomplicated and severe malaria**
*= p-value <0.001
†= p-value <0.05

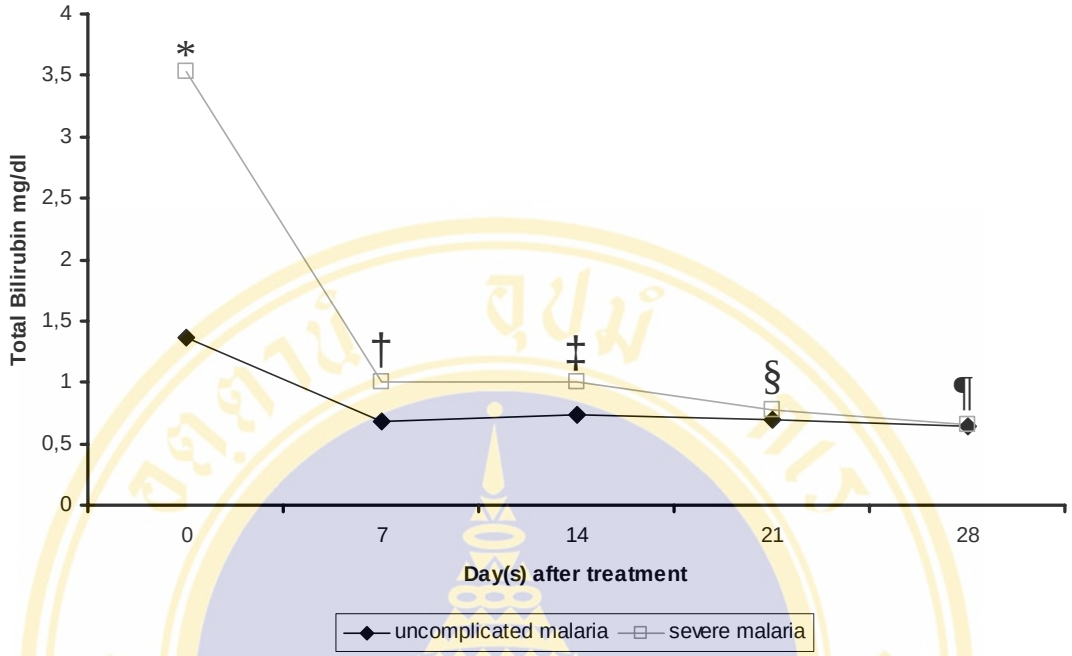

Figure 13. **Median total bilirubin concentration in uncomplicated and severe malaria**
*= p-value <0.001
†= p-value <0.001
‡ = p-value <0.001
§ = p-value <0.01
¶= p-value <0.05

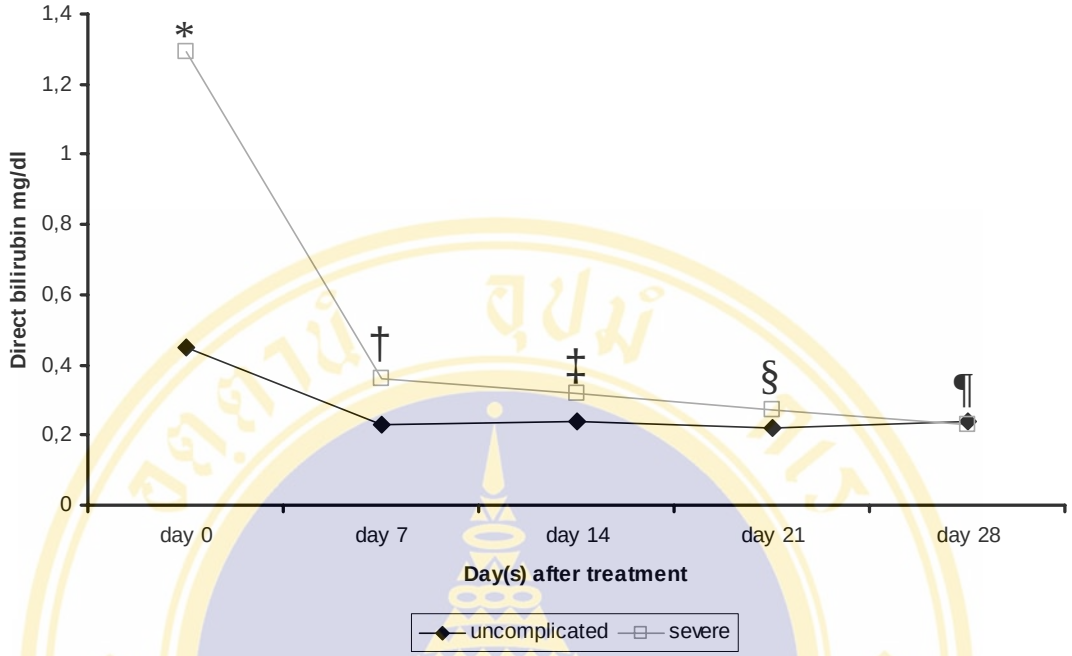

***Figure 14*. Median direct bilirubin concentration in uncomplicated and severe malaria**
*= p-value <0.001
†= p-value <0.001
‡ = p-value <0.001
§ = p-value <0.001
¶= p-value <0.05

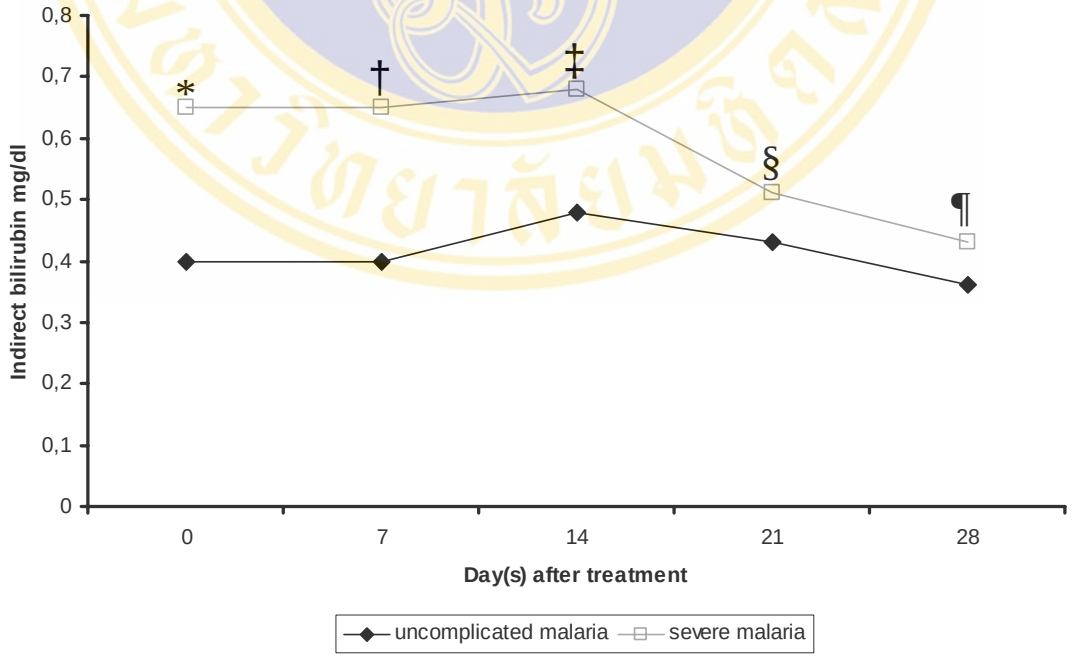

***Figure 15*. Median indirect bilirubin concentrations in uncomplicated and severe malaria**
*= p-value <0.001
†= p-value <0.001
‡ = p-value <0.001
§ = p-value <0.05
¶= p-value <0.05

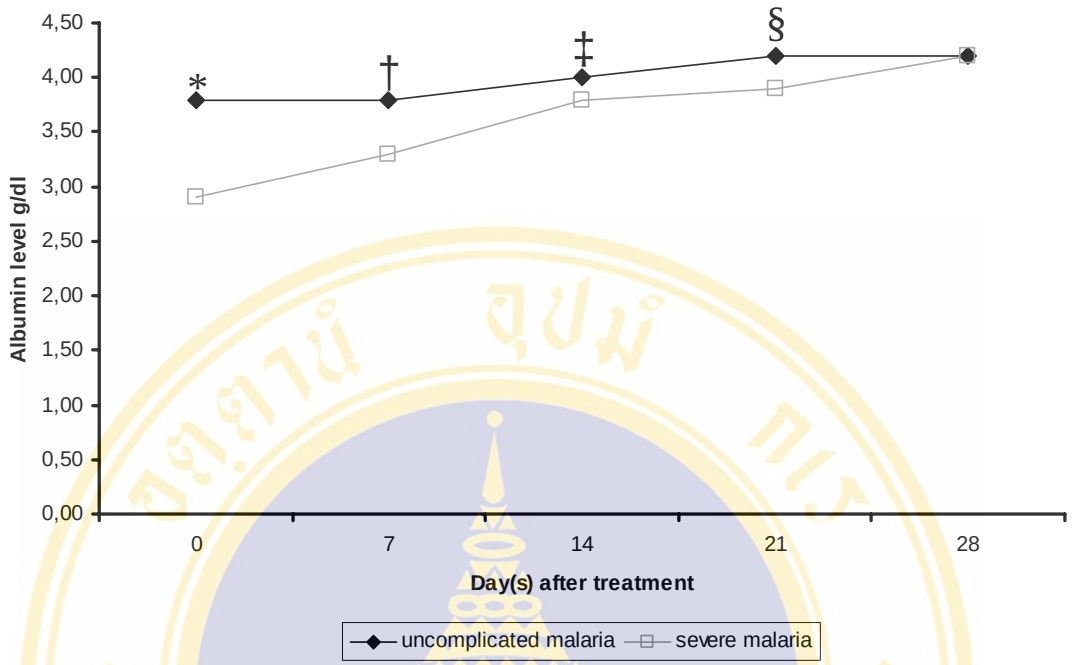

Figure 16. **Median albumin concentration in uncomplicated and severe malaria**
*= p-value <0.001
†= p-value <0.001
‡ = p-value <0.001
§ = p-value <0.001

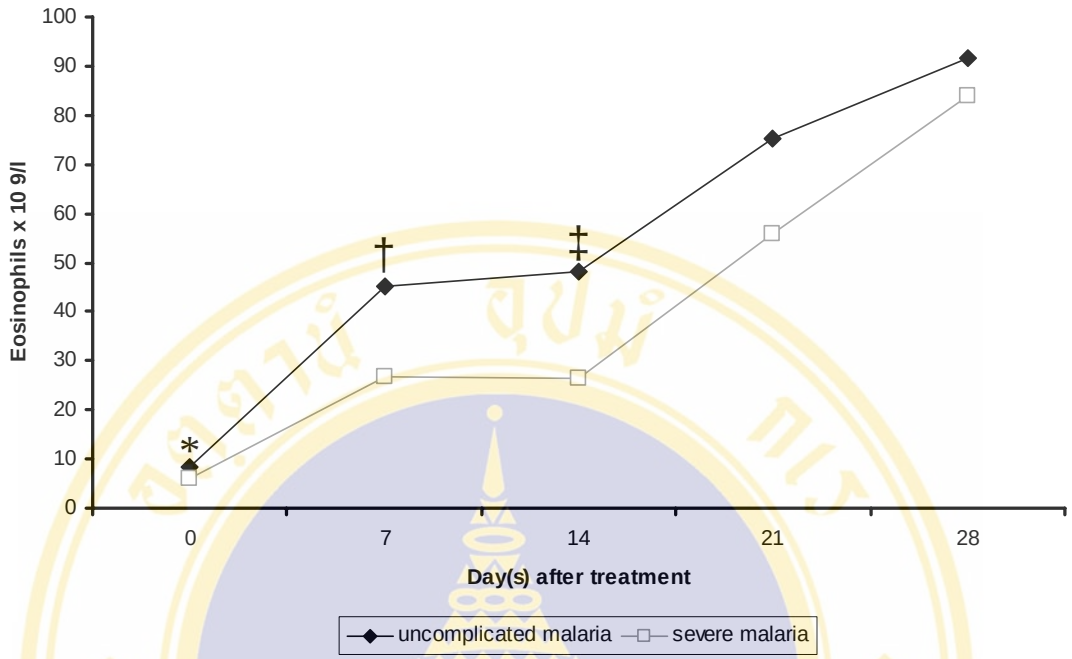

Figure 17. **Absolute eosinophil count in uncomplicated and severe malaria**
*= p-value <0.05
†= p-value <0.001
‡ = p-value <0.001

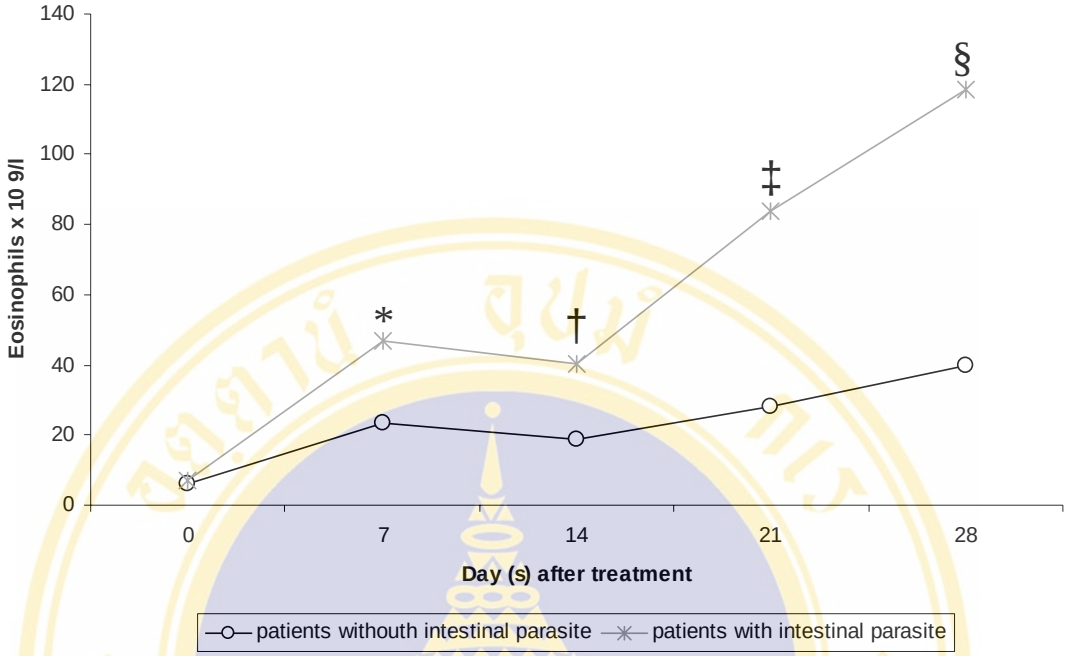

Figure 18. **Absolute eosinophil count in patients with and without intestinal parasite infection**
*= p-value <0.001
†= p-value <0.001
‡ = p-value <0.001
§ = p-value <0.001

CHAPTER VI
DISCUSSION

Our main objective was to detect hematological dynamic changes in malaria – especially in severe cases. The findings in this retrospective study show that there is no association between the severity of malaria and the total white blood count on the day of admission until one week after treatment is started. The median total white blood counts were initially fairly low but still within the normal range. After one week the numbers had increased to higher levels, but also not exceeding the upper normal limit. Levels in severe patients stayed below the ones in uncomplicated patients until day 21. When taken a look at the differential blood count (bandforms of PMN, segment forms of PMN, basophils, lymphocytes and monocytes) we found that none of the mentioned showed a rise after day 7. Instead, all of them would decrease either after day 0 or day 7 and would decrease further or stay on that level. Only eosinophils showed a constant rise. We then calculated the absolute number of eosinophils and the pattern remained quite the same (see *Figure 17*). Concentrations were significantly lower in severe malaria until day 14, but on day 28 the p-value was only 0.752 (insignificant), which would explain the increased median percentage of eosinophils and thus the even higher level in the total WBC count on day 28. That suggests that eosinophils, indicating a parasitic (helminthic) co-infection, remain low in acute malaria infection in favor of other white blood cells, but rise after the more acute illness has been treated. Elevated eosinophil concentrations have been observed earlier (Anantrakulsin et al., 2005; Nacher 2004). Kurtzhals et al. observed a significant increase in eosinophil frequency in children with asymptomatic parasitemia, whereas a significant drop of eosinophils was found in acute illness, returning to initial values after cure. Low eosinophil counts were associated with cerebral malaria, severe anemia or uncomplicated malaria. No change was observed in uninfected children. They conclude that *P.falciparum* infections induce eosinophilia due to immunological stimuli (Kurtzhals et al., 1998). In contrast to our study their's was conducted in a

hyperendemic area. It is only mentioned that approximately 18% of healthy children excrete eggs of intestinal helminthes, whereas our study population showed intestinal helminth infestation in more than 70%. We do agree though that during acute illness there is no rise of eosinophils. In our study population eosinophil cationic protein or eosinophil protein X were not measured so we can not make any further comments concerning eosinophil activity. But when we compared eosinophil counts in patients with and without helminthic co-infection it was clear that the helminthes caused significantly higher eosinophil levels. Still, even patients without intestinal parasites showed constantly increasing eosinophil counts which indicates the important role eosinophils play in malaria infections. Nacher suggests a very interesting link between helminths and malaria: He concludes that by protecting the host, helminths protect themselves and their reproductive potential, but also favor the dissemination and reproduction of Plasmodium falciparum. Protection, according to him, is mediated by IgE, the CD23/NO pathway, Il-10 and possibly the hematological changes in worm-infected patients. The chronic activation of the CG23/NO pathway might be instrumental in down regulating the expression of cytoadherence receptors thus reducing sequestration of parasitized red blood cells in the deep organs. Mild anemia on the other hand might favor gamatocytogenesis (Nacher, 2004).

The p-values that indicate a statistically significant difference between uncomplicated and severe malaria cases on presentation were found for bandforms of PMN, segment forms of PMN, eosinophils, lymphocytes and monocytes.

Elevated monocyte counts are often found in malaria infection, in our study about 20% of all patients had monocyte concentrations above the normal range, most of them (83%) were uncomplicated cases. By day 7 the pattern had changed: now only 34% were uncomplicated patients and the remaining 66% were severe cases. Also, the number of patients that showed monocyte counts above the normal range had increased though the median concentrations in uncomplicated and severe malaria were much lower on day 7. From day 14 on monocyte counts were identical in uncomplicated and severe patients. As monocytes represent the bone marrow function it is no surprise that the concentration was significantly lower in severe patients.

The cut-off point for leucopenia is set at 4.5 x 10^9cells/l. 54 patients (26.5%) were found to have leucopenia on the day of admission, 27 in each group. The p-value was highly insignificant, meaning there was no association between leucopenia and severity of the disease.

Thrombocytopenia is a common feature in *P.falciparum* malaria. It was as low as 30 x 10^9 cells/l (5-457 x 10^9/l). It is a well known phenomenon and assumed to be the result of bone marrow suppression, splenic pooling, reduced platelet life-span and dysmegakaryocytosis (Wickramasinghe et al., 2000). As observed in previous studies (Moulin et al., 2003) platelets did recover within one week after treatment was started, the median platelet count in severe and uncomplicated malaria being above 300 x 10^9 cells/l. Still, it was statistically significantly lower in severe patients. They reached the peak on day 14, when it was even higher than in uncomplicated patients. On day 28 again, it was significantly higher than in uncomplicated patients (as shown in *Figure 8.*). Why this is the case remains unexplained, but the results mainly show that levels are within the normal range. Platelet transfusions were not required in any of the cases since none of our patients showed any signs of DIC or abnormal bleeding. Some patients, though, received FFP or whole blood transfusions. The p-value being 0.001 on day 0 indicates that there is a strong association between thrombocytopenia and severe malaria.

We also found that on the day of admission there was no association between anemia and the severity of the disease, though severe patients had a slightly lower median hemoglobin concentration. The difference became statistically significant only after one week after treatment was started and it remained significant until day 28. On day 14 more patients – especially in the severe group – were found to be anemic, so the median hemoglobin concentrations decreased slightly during the first week and then gradually increased until day 28 (patients that received any kind of blood transfusion were included). These findings partly confirm the findings of an earlier study (Camacho et al., 1998), where a similar pattern was shown. Since on admission 140 patients (68.6%) were dehydrated it is possible that some of the anemic cases

were masked by that fact. Other factors that might well have contributed to anemia are malnutrition and helminthic infestation (>70% of our patients had parasitic co-infection, i.e. hookworm).

Hb-typing and G6PD-deficiency were not associated with severity of the disease. P-values for HbE, heterozygous α-thalassemia and HbH could not be calculated since there were no cases in either severe or uncomplicated malaria (all p-values are shown in table 3). Despite very low number of cases with HbH disease (1 *P.f.*-patient) the result is not surprising, since HbH is very unstable, which would also contribute to severiy of the disease. In addition, this particular patient also had G6PD-deficiency. On admission his hemoglobin was 8.3 mg/dl, which is below the normal range of 14-18 mg/dl for men and his hematocrit was 28% (normal 40-54%). Interestingly, no pallor was noted, but what classified him as a severe patient was his hyperparasitemia of 170640 parasites/µl. 23 patients (11.3%) had to be categorized "unidentified" regarding hemoglobinopathies because they showed abnormalities in MCV, hemoglobin concentration and/or hematocrit but electrophoresis could still not detect any abnormal hemoglobin types. We therefore suggest iron deficiency anemia, but confirming tests were not conducted. Our findings do not confirm what has been published earlier (Weatherall et al., 1987; Weatherall et al., 2002), but the number of patients with abnormal hemoglobin in our study is very small, though.

While there was no association between the severity of malaria in Tak and Kanchanburi, in Ratchburi severe malaria seemed to be highly related to the residence province. Out of 17 patients from Ratchaburi, 16 had uncomplicated malaria and only one suffered from severe malaria. The numbers might be coincidental since 17 is a rather small sample size but the p-value for the residence province and the number of previous malaria infections is highly significant (p= <0.001). So, in Ratchaburi it would implicate that malaria is highly endemic there and people are less prone to severe malaria due to immunization during previous infections.

A high initial parasite count, low parasite clearance time and low fever clearance were highly associated with severe illness. That does not surprise since hyperparasitemia is one of the criteria of severe malaria.

Clinical jaundice occurred in about one third of all patients, most of them were severe cases. That confirms the findings of earlier studies (Wilairatana et al., 1994), where the complications observed in jaundiced patients included cerebral malaria, acute renal failure, pulmonary edema, shock and other severe malarial complications . Jaundice was associated with cerebral malaria ($p < 0.05$), acute renal failure ($p < 0.01$), and hyperparasitemia ($p < 0.01$) similar to the results of our study. High direct, indirect and total bilirubin-, as well as AST- and ALT concentrations as signs of hepatic dysfunction were highly associated with severe malaria, but hemolysis also contributes to jaundice. Low serum albumin in our cases may be the result of liver disease, renal dysfunction and/ or stress response. It is also low in dialysis patients. Renal failure due to acute tubular necrosis is one of the major causes of death (WHO 1990; Trang et al., 1992). Although 43.1% of our patients had BUN concentrations above the normal range, only 4.9% actually suffered acute renal failure. That was less than expected, according to earlier studies, where a much higher percentage of patients with renal impairment was observed (WHO 1990; Trang et al., 1992). Our results rather confirm the findings of another study, where definite renal failure was observed only in 6.1%. The median number of hemodialysis sessions was 5 (-12), which is identical with results of an earlier study.

CHAPTER VII
CONCLUSION AND RECOMMENDATIONS

We conclude that on presentation the total white blood cell count in malaria is no reliable parameter to determine the severity of the disease. Our results even showed a minimally higher WBC count in severe malaria that decreased during the follow-up period. Elevated monocyte concentrations should not be used as an indicator of uncomplicated malaria for it can not distinguish malaria from other infectious diseases (typhoid, tuberculosis, trypanosomiasis…) or myelodysplasia.

Our study showed again that thrombocytopenia frequently occurs in acute malaria infection and is even more profound in severe cases. It can not only be used as a predictor but also as an important clue to the diagnosis of malaria. Still, a normal platelet count does not exclude the disease! Platelet transfusions are usually not required (unless there are bleeding complications) because platelets do recover within one week after treatment is started.

Anemia is also not a good predictor for severe malaria, although hemoglobin concentrations were slightly lower in severe patients. Furthermore, malnutrition and helminthic infestation, factors that contribute to anemia, were not among the exclusion criteria. Packed red cell-, whole blood- or fresh frozen plasma transfusions may be required in some cases.

Against our expectations we could not find any association between abnormal hemoglobin types/ G6PD deficiency and the severity of malaria, the major drawback being the relatively small number of patients with hemoglobinopathies or G6PD deficiency. We therefore suggest, in order to evaluate the role of these two factors in severe malaria, a much bigger sample size is needed.

Eosinophilia seems to play a more important role in malaria than expected, especially when associated with helminthic co-infestation. It is not entirely clear whether the rise after treatment of acute malaria is more associated with intestinal parasitic infestation or malaria itself and certainly deserves more attention in the future.

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APPENDIX

CASE RECORD FORM

Patient Data:

Patient Code : …….

Date of admission: …../…../…..

Age: …….

Gender: …….

Weight: …….kg

Height: …….cm

Residence Province: ……..

Nationality: ……..

Occupation: ……..

History:

No. of previous malaria infections: …….

Origin of current infection: ……..

Days of fever PTA: …….days

Alcohol: …….

Smoker: …….

(0 = no, 1 yes, 99 = data missing)

Physical examination on admission:

Level of consciousness: …….(0 = conscious, 1 = semiconscious, 2 = coma)

Temperature: …….°C

Respiratory rate: ……./min

Pulse rate: ……./min

Blood pressure: ……./…….

Anemia: …….yes □ no □

Jaundice: …….yes □ no □

Dehydration: …….yes □ no □

Neurological manifestation: ……. (if abnormal, specify……)

Liverenlargement yes □……..cm
no □

Spleenenlargement: yes □……..cm
no □

Clinical findings:

	Day 0	Day 7	Day 14	Day 21	Day 28
weakness					
Chills/rigor					
Dizziness					
Abdominal pain					
Diarrhea					
Nausea					
Vomiting					
Anorexia					
Palpitations					
Other					
Other					

(0 = no, 1 = mild; 2 = moderate, 3 = severe, 99 = data missing)

Hematology:

Malaria:

P.falciparum ☐

P.vivax ☐

P.ovale ☐

P.malariae ☐

Complications: …….CM

…….ARF dialysis no ☐

yes ☐

…….pulmonary edema

…….days

…….respiratory distress

…….severe normocytic/ hypochromic anemia

…….hyperparasitemia

…….hypoglycemia

…….circulatory collapse (shock)

…….spontaneous bleeding/ DIC

…….repeated generalized convulsions

…….acidosis

Parasite count on day 0: …….MP/µl

Parasite clearance time: …….hrs

Fever clearance time: …….hrs

	Day 0	Day 7	Day 14	Day 21	Day 29
WBC					
RBC					

Hb g/dl					
Hct.					
MCV fl					
MCH pg					
Plt					
G6PD					
Band %					
Segm %					
Eos %					
Baso %					
Lym %					
Mo %					
RDW %					

WBC x 10^9/l; RBC x 10^{12}/l; Plt x 10^9/l;

(99 = data missing)

Hb-Typing:

A: …….%

A2: …….%

HbH: …….%

HbE: …….%

(99 = data missing)

Interpretation:……

Blood transfusion:

transfusion:…….

(0 = no, 1 = whole blood, 2 = packed red cells, 3 = plasma)

Units:…….

day after treatment:…….

Plt transfusion:…….

(0 = no, 1 = yes)

Units:…….

day after treatment:…….

(99 = data missing)

Chemistry:

	Day 0	Day 7	Day 14	Day 21	Day 28
Glu mg/dl					

BUN mg/dl					
Cr mg/dl					
D Bili mg/dl					
T Bili mg/dl					
Prot g/dl					
Alb g/dl					
Glob g/dl					
Alk P U/l					
AST U/l					
ALT U/l					
Na mmol/l					
K mmol/l					
Cl mmol/l					

Summary:

Type of malaria:……

Parasite clearance time:…….

Fever clearance time:…….

Complications:……..

……

…….....

Hb-Typing (interpretation)……..

G6PD status:…….

(0 = normal, 1 = deficiency)

Patient outcome: survived □

died: □

…….days after treatment

BIOGRAPHY

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