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CLINICAL AND PATHOPHYSIOLOGICAL ASPECTS

OF SEVERE FALCIPARUM MALARIA

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A Thesis Submitted in Partial Fulfilment of the Requirement

of the Open University, UK

for the Degree of Doctor of Philosophy

Wellcome Trust Major Overseas Programme

Oxford University Clinical Research Unit

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Viet Nam

August 2009

ProQuest Number: 13889933

ProQuest 13889933

ABSTRACT

Malaria is a major global public health problem. One-fifth of the world's population is at risk of malaria and drug resistance is spreading. Nearly five times as many cases of malaria were reported in 2000 as tuberculosis, AIDS, measles and leprosy cases combined. In the time it takes to say the word "malaria," ten children will contract the disease and begin fighting for their lives. Since the cinchona alkaloids were introduced as a specific treatment for agues 350 years ago, the treatment of severe malaria has changed little and therapy remains largely empirical. Quinine and quinidine remain the drugs of choice for severe chloroquine-resistant malaria due to *Plasmodium falciparum*, and with the spread of these resistant parasites, the usage of these drugs is increasing. In 1972 scientists in China discovered the antimalarial properties of a group of sesquiterpene lactone peroxides derived from the qinghao plant *(Artemisia annua)*. The principal component, qinghaosu (artemisinin), and two derivatives - the water-soluble hemisuccinate artesunate and the oil-soluble artemether - are the most rapidly acting and potent of all antimalarial drugs. Although much research effort has been invested in optimizing antimalarial drug regimes, severe malaria remains a major cause of adult mortality in the Asiatic tropics. Despite many clinical trials reducing the mortality from severe malaria has proved difficult. Artemether has been shown to be as good as quinine but not better. This thesis set out to determine whether Artemether or Artesunate was the the better drug, how to manage acute renal failure in severe malaria, to design a severity score for malaria and assess whether there had been any change in the parasite clearance

times in Viet Nam over a 18 year period. The results from a series of clinical trials and research from one hospital in Viet Nam are presented.

This thesis undertook the following studies:

1. A randomised clinical trial of artesunate vs artemether in the treatment of severe malaria in adults in Viet Nam.

2. A randomized comparison of pumped venovenous haemofiltration and peritoneal dialysis in acute renal failure associated with severe infection.

3. Fluid management in severe malaria.

4. The stage of the development of the falciparum parasites at the time of clinical presentation with severe malaria is important in predicting outcome.

5. Development of a predictive score of outcome in adults with severe falciparum malaria.

6. Assessment of the efficacy of the artemisinin derivatives in the treatment of severe falciparum malaria in Viet Nam 1991-2008.

ACKNOWLEDGEMENTS

DECLARATION

Other than the assistance outlined in the acknowledgements, the work described in this thesis is my own work. I participated in designing, implementing, treating and caring for all patients involved in the studies, taking and preparing blood samples and blood smears, performing the peritoneal dialysis and haemofiltration, doing all parasite staging in peripheral blood smears, collecting specimens with autopsy from death patients, and conducting the follow-up examination of the patients. I have also been involved in all the data analysis.

My work described in this thesis has not been submitted for a degree or other qualification to this or any other university.

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ABBREVIATION

ACT: Artemisinin based combination therapy

AD: Anno Domini

AIDS: Acquired immune deficiency syndrome

An: Anopheles

ARTM: Artemether

ARTS: Artesunate

ALT: Alanin aminotransferase

AST: Aspartate aminotransferase

BBB: Blood brain barrier

BC: Before Christ

BUN: Blood urea nitrogen

CD: Cluster of differentiation

DDT: Dichloro-diphenyl-trichlorethane

DHA: Dihydroartemisinin

DNA: Deoxyribonucleic acid

ECG: Electrocardiography

FCT: Fever clearance time

GCS: Glasgow coma scale

GDP: Gross domestic product

Hb: Haemoglobine

HF: Heamofiltration

HIV: Human immunodeficiency virus

HLA: Human leucocyte antigen

HRP2:   Histidine – Rich Protein 2

HTD: Hospital for Tropical Diseases of Ho Chi Minh City, Viet Nam

ICAM: Intercellular adhesion molecule

IC: Inhibition concentration

IMPE: Institute of Malariology – Parasitology – Entomology (Viet Nam)

IL: Interleukin

IFN: Interferon

NMCP: National Malaria Control Programme, Viet Nam

P.:   Plasmodium

PCR: Polymerase chain reaction

PCT: Parasite clearance time

PD: Peritoneal dialysis

Pf EMP1: *Plasmodium falciparum* Erythrocyte membrane protein 1

PMNS: Post malaria neurological syndrome

QHS: Qinghaosu

RBC: Red Blood Cell

RDT: Rapid diagnosis test

RNA: Ribonucleic acid

SPR: Smear positive rate

SD: Standard deviation

TNF: Tumor necrosis factor

WBC: White Blood Cell

WHO: World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Malaria

Malaria is a disease of human beings caused by parasites, more specifically: protozoan parasites (Plasmodium parasite). There are four types of malaria parasite, which commonly cause malaria in human: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium ovale*. *Plasmodium knowlesi* is the fifth major human malaria parasite that was firstly reported in 1965. Malaria is transmitted by Anopheles mosquitoes, and remains a serious infectious disease in tropical areas in this 21st century. Malaria transmission is reported from over a 100 countries and to cause nearly 300 million cases (5% of global population) each year, with 1.2 – 2 million deaths, mostly from severe and complicated falciparum malaria. Malaria is found in all ages, with a particular burden in pregnant women and young children. In the hyperendemic areas, 25% of the deaths of children less than 4 years of age are associated with severe falciparum malaria, and women particularly in their first pregnancy are at particular risk.

In the 1950s, the World Health Organization (WHO) attempted to develop a strategy for the global eradication programme for malaria. It was however not easily implemented, only developed countries had much success and the programme failed in the poor and developing world. Later with the development and spread of drug resistant strains of *Plasmodium falciparum* the problem grew and the eradication

strategy was quietly dropped. During the 1960-1990s the slogans were changed and people discussed "Malaria Control". Malaria Control Programmes were established around the world with the aim of controlling or containing the disease by reducing morbidity and mortality. However despite new slogans and many new international initiatives malaria has continued to affect adversely public health, economic and social development in malaria endemic countries. Malaria and economic development are inter-related. High rates of malaria are almost exclusively reported from countries with poorly developed economies and social conditions and the relationship between malaria and social and economic development has been the subject of much debate in recent years. Malaria rarely occurs in developed countries. Climate change, insecticide-resistant mosquitoes and the continued emergence of parasites resistant to anti-malarial drugs will continue to make the malaria control programmes relevant in the future. However with the widespread use of impregnated bed-nets, artemisinin combination therapies and economic development in malaria endemic countries offer us the best hope in generations to truely roll back (some would argue even eradicate) malaria. The development of an effective vaccine would complement these efforts but so much could be achieved by implementation of what we already know works.

1.1.1 History of malaria

Malaria is an old disease (Knell, 1991; Poser and Bruyn, 1999). References to malaria can be found in the ancient Chinese, Egyptian, Roman and Indian writings. One of the oldest cuneiform script described malaria as Nergal, the Babylonian god of destruction and pestilence. The Chinese Nei Ching (The Canon of Medicine) edited 4,700 years ago referred to enlarged spleens with repeated fevers. The Egyptian and

Sumerian texts, dated 3,500 years ago, referred to fevers and splenomegaly, possibly due to malaria. The Indian Vedic (dating from 2,800 to 3,500 years ago) and Brahmanic (1,900 to 2,800 years ago) mentioned the autumnal fevers as the "King of disease". The ancient Hindus were aware of the danger of mosquitos. The Charaka Samhita, one of the ancient Indian texts on Aynvedic medicine (written about 300 BC) and the Susruta Samhita (written about 100 BC) referred to diseases with different types of fevers: continuous, remittent, quotidian, tertian and quartan. The Susruta Samhita indicated the link between fevers and the bites of the insects. The Greeks associated malaria with swamps about 500 BC. Hippocrates, the Father of Medicine, also described the malaria fevers by 400 BC. He distinguished the intermittent fever of malaria from the continuous fever of other diseases and he noticed the fever was related to the season and the areas. He also wrote: "Those who drink stagnant water have always large, stiff spleens and hard, thin, hot stomachs, while their shoulders, collarbones, and faces are emaciated; the fact is that their flesh dissolves to feed the spleen...," he believed in the connection between fevers and marshes. Although he misunderstood the cause of malaria, he was named as the first malariologist. Plato (427-347 BC) and Aristotle (384-322 BC) reported malaria when it occurred in Attica. Plato differentiated continuous from recurrent fevers. The ancient Romans, Varro (116-27 BC) attributed malaria to the swamps.

Galen (130-200 AD), a Greek physician recognized the relation of fevers to season and to jaundice. He believed that malaria was the result of disorder of yellow bile of the body. Unfortunately, his belief that malaria had an internal cause was accepted for the next 1,500 years.

In 1666, Thomas Sydenham still believed the imbalance of the humors as the cause of malaria but he insisted the Cinchona stopped the fevers in his book Methodus curandi

febres. The "Peruvian fever tree", Cinchona, was found by the Jesuit missionaries in the South America when they learned how to treat fever with Cinchona from Inca herbalists in the early 17th century. Spanish Cardinal Juan de Lugo brought Cichona, Jesuit's powder, to the Hospital Santo Spirito (Rome) in 1637, and it became widely used throughout Europe. The British and Dutch introduced it to India later. By 1677, Cortex Penuvianus (an official name of Peruvian bark) appeared in the London Pharmacopeia. The discovery of the Cinchona is one of the most important events in the history of malaria. Richard Morton, an English physician, firstly described the clinical picture of malaria and the treatment of malaria with Cinchona in 1692. Francesco Torti, professor of medicine at Modena, accurately described the course of the disease that was curable by the Cinchona in 1709.

In 1716, Giovanni Maria Lancisi, personal physician of Popes Innocent XI, Innocent XII and Clement XI, first described the black pigment of brain and the spleen of the malaria patients. He also thought that mosquitoes might have caused malaria.

In 1796, John Crawford, an American physician, wrote essays on the cause of malaria. He thought that "when the mosquitoes bite, they lay some eggs into the human body......and those eggs produce malaria..." His theory was echoed by two other Americans, Josiah Clark Nott and Lewis Daniel Beauperthy in 1850 and 1854.

While doing an autopsy of a patient in 1847, a German physician named Heirich Meckel noticed some round, ovoid structures containing black pigments in the blood and the spleen.

In 1879-1880, Edwin Klebs (a German pathologist) and Corrado Tommasi Crudeli (an Italian bacteriologist) announced that malaria was caused by a microbe isolated from the soil, which they named *Bacillus malariae*. But George Sternberg (an American bacteriologist) confirmed that their findings were wrong in 1881.

Finally, the first scientist who noticed the malaria parasite in the blood of a patient suffering from malaria in 1880 was Charles Louis Alphonse Laveran, a French army surgeon. Laveran arrived at Constantine, Algeria in 1878, and worked in a military hospital full of malaria patients. He also found the pigmented structures in the brain and spleen and black bodies in the blood of the malaria patients. On November 6th, 1880, Laveran saw a moving object on the slide of fresh blood specimen from malaria patient and named the parasite *Oscillaria malariae*. In Italy, on December 24th, 1880 Laveran announced the findings of pigmented erythrocytic cells, trophozoites and gametocytes in malaria patients. Later, he concluded that quinine could cure malaria. Laveran wrote a letter of communicating his findings to the Academy of Medicine in Paris. Laveran received the Nobel Prize in 1907 for his discovery. In 1882, Albert King Freeman Africanus at the George Washington University, USA, again noticed the role of mosquito in malaria transmission. Laveran's discovery was confirmed by Louis Pasteur in 1884 and William Osler in 1886. In 1886, Camillo Golgi, an Italian neurophysiologist, was the first to describe the tertian and quartan fevers and asserted that those two types of fevers were caused by two different parasites. He also observed that fever coincided with the rupture and release of merozoites into the blood stream. Giovanni Batista Grassi and Raimondo Filetti, both were Italian, first named two human malaria parasites as *Plasmodium vivax* and *Plasmodium malariae* in 1890. Romanowsky described the methods for staining of malaria parasites in 1891.

Sir Patrick Manson, a British scientist, thought that the malaria disease was transmitted by mosquito. But on the 20th August of 1897, his colleague, Surgeon Major Ronald Ross, was the first to demonstrate that malaria patients could transmit the malaria parasite to mosquitoes. He described the oocysts existed in the gut of

Anopheles mosquito and the sporozoites in salivary glands of Anopheles mosquito and proved that mosquito was the vector of malaria while he was working in Secunderabad, India. His report was published on the British Medical Journal on the December 18th, 1897. Thus, the mechanism for malaria transmission was found. Ross was awarded the Nobel Prize in 1902. And in 1900, Patrick Manson transmitted malaria to volunteers in London from infected mosquitoes brought from Italy. In 1897 William Welch, an American, named the malignant tertian malaria parasite as *Plasmodium falciparum*. Giovanni Battista Grassi, an Italian physician, reported that *Anopheles claviger* mosquito was one malaria vector. Grassi, Amico Bignami and Giuseppe Bastianelli accomplished the observation of the life cycle of *P. falciparum, P. vivax* and *P. malariae* in 1899. They collected mosquitoes and fed them on malaria patients in Rome, Italy, then sent them to London where they infected on two volunteers, both of whom developed malaria. In 1922, John William Watson Stephens introduced the fourth human malaria parasite, *Plasmodium ovale*. An Austrian neurologist, Julius Wagner-Jauregg was offered the Nobel Prize in 1927 "for his discovery of the therapeutic value of malaria inoculation in the treatment of dementia paralytica."

A German investigator, Paul Hermann Muller, discovered the insecticide effect of Dichloro-diphenyl-trichloroethane (DDT) in 1948, and DDT was widely used for malaria control since 1945. Muller received the Nobel Prize for Medicine in 1948. Despite all the achievements of the last 60 years Muller was the last Nobel Prize to be awarded for work directly on malaria.

The liver stage of vivax parasite was demonstrated by Henry Edward Shortt and Percy Cyril Claude Garnham in 1948 at the Ross Institute of the London School of Hygiene and Tropical Medicine, UK. In 2002, the genome of *Anopheles gambiae* and *P.*

falciparum were sequenced. Over the last 40 years a number of candidate malaria vaccines have been developed and tested. Perhaps for the first time at the start of the 21st Century, there is some degree of realistic optimism that a vaccine might be feasible, although probably still many years away.

From the 18th through to the 21st century, we have learnt a great deal; the cause of malaria, the transmission of disease, the role of female Anopheles mosquito, the discoveries of quinine, choloroquine and qinghaosu, massive investments in genomics and development of vaccines, but malaria still kills 1-2 million poor people in the world each year. There remains much to be done on this old disease.

1.1.2 Current malaria situation in the world

During 2006 there were an estimated 247 million cases of malaria (range: 189-327 million) in the world with 881,000 deaths. Nearly 91% of these malaria deaths were in Africa, 85% of deaths were children under 5 years of age, and 4% of deaths were in Southeast Asia region. Malaria was present in 109 countries (Figure 1.1) (World Malaria Report, 2008). Those alarming and increasing figures might be due to the increase of the anti-malarial drugs resistant parasites, the development of resistance to insecticides of mosquitoes, the weakening of public health systems, the population movements into malaria endemic areas, migration, continued economic problems in the world's poorest countries, armed conflict and climate change.

With the implementation of artemisinin-based combination therapy (ACT), the long-lasting insecticidal nets, the indoor residual spraying of insecticide and the intermittent preventive treatment in pregnancy, malaria cases have declined in some endemic countries. The number of deaths has fallen in Thailand, Laos, Cambodia,

Viet Nam, Philippines and Suriname over the last decade (World Malaria Report, 2008).

However, in 2006, half of the global population remains at risk of malaria. With 40% of the global population at risk of malaria residing in the Southeast Asia region, malaria is still a major public health concern across Asia. With global climate change and continued migration of people an additional 260-320 million people worldwide could be living in malaria endemic areas by 2080, thus malaria continues to remain a major public health problem worldwide.

Figure 1.1 Malaria – free countries and malaria – endemic countries in phases of control, pre-elimination, elimination and prevention of reintroduction, end 2007.
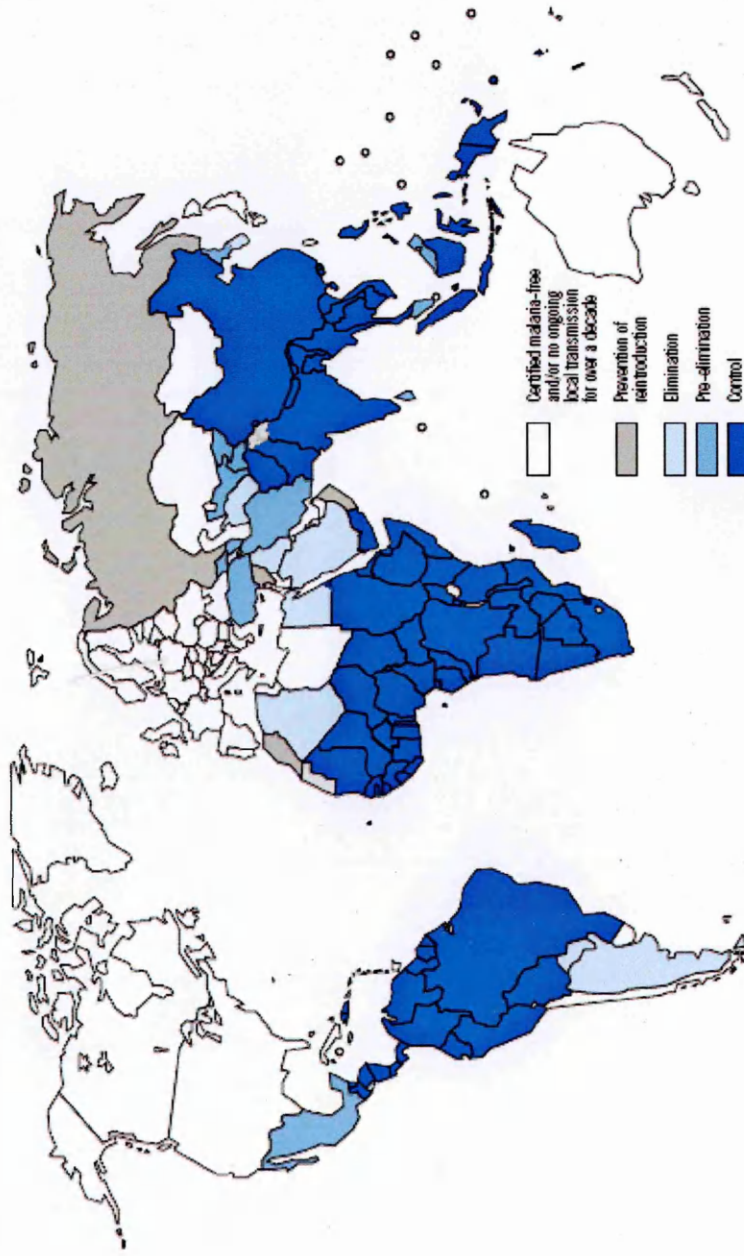
- China, Indonesia, Philippines, Solomon Islands, Sudan, Vanuatu and Yemen have subnational elimination programmes.

(Source: World Malaria Report 2008)

1.1.3 History of malaria in Viet Nam

Long ago the Vietnamese people assumed that malaria originated from the forest as they recognized most patients had fever, rigors, chill as a result of their exposure to the jungle. The disease was since called "SỐT RÉT RỪNG" (Jungle Chilly Fever). At that time and maybe even today, some Vietnamese people remain convinced that malaria is caused by drinking poisonous water from the forest.

The French colonists invaded Viet Nam in 1858 and occupied the Red River Delta of North Viet Nam in 1875. During that time the French physicians noticed that more soldiers in the northern highlands got "Jungle Chilly Fever" than elsewhere. In 1940, Pierre Gourou, a French scientist, finally explained to the the authorities: "The only propagating agent of malaria is mosquito, neither the forest spirits nor the poisoned water feared by Vietnamese, has the least responsibility. The sunlit terraced rice fields with running water of hill regions are veritable breeding sites for the larvae of dangerous Anopheles, in contrast with the stagnant paddies of the plains." At that time the anti-malaria activities of the French focused mainly on their soldiers, emigrants and for parts of Vietnamese society working in large economic projects designed to develop the plentiful natural resources. They were not dedicated to providing support for the health and safety needs of the Vietnamese people. There were two typical projects: one building the rail road route linking Hai Phong in Viet Nam with Yuanam, China and the other to establish rubber plantations in Viet Nam. When the French built the rail road, they realized that up until 1904 the progress of the project was very slow because of the high sickness and fatality rate of the

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Vietnamese workers caused by the "jungle malaria." After 1904 the French changed their policies from violent beatings of sick workers to supplying antimalarial medications. However, these steps could only limit the morbidity and the number of fatalities. In the first decade of 1900, the French colonists discovered that some land areas in Indochina, especially in the Central Highlands, Viet Nam, were very suitable for the planting of the rubber plant and they developed a great number of the rubber plantations. A lot of the Vietnamese plantation workers also contacted and died of the dreaded "jungle malaria." The phrase "jungle malaria" originated from these plantations. The number of sick and dying Vietnamese workers in the rubber plantation development projects steadily greyed which angered the Vietnamese people. This was one of the contributing factors that were believed to have lead to the Vietnamese people finally rebelling against the French.

Since 1954, both North and South Viet Nam implemented the malaria eradication programme. The South Viet Nam got funds from the USA and the WHO but their programme was less successful because the main battlefields in the South were also the malaria endemic areas and thus not easily accessible to the malaria control programme. Thousands of Vietnamese soldiers from both sides died of malaria. Even with the best protective measures available, nearly 40,000 malaria cases were reported in US Army troops between 1965 and 1970. With the number of cases increasing the effectiveness of the US was compromised and from the early 1960s onwards US troops also faced the rise of antimalarial drug resistance. The US Army set up a malaria drug research programme and Mefloquine, a new antimalarial drug, was developed as a direct result of the Viet Nam war. The North Vietnamese forces received the majority of their supplies from the Soviet Union and China. In the

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Northern provinces, the eradication programme was based mainly on house spraying with residual insecticides which lead to a remarkable reduction in the number of malaria cases. Because malaria was the biggest cause of casualties among the North Vietnamese troops, the North Vietnamese asked for help from Chinese scientists to defeat malaria, and a secret military project, named "Project 523," was set up following a meeting between Cho En Lai and Ho Chi Minh. Eventually this lead to the discovery of a completely new antimalarial compound, Qinghaosu, later called Artemisinin. The North Vietnamese troops received artemisinin compounds in the final stages of the war (Li, personal communication). The drugs were also used very early as combination therapy through a series of mixtures labelled CV1, CV2, with the final version CV8 (standing for China-Viet Nam) an early version of what is now called Artekin and increasingly used globally.

The Viet Nam War ended in 1975. It was estimated that one half of a total of 65 million Vietnamese people were at risk of malaria. Malaria became an obstacle to the economic development of the highland areas by people sent by the government from the lowland areas.

1.1.4 Current malaria situation in Viet Nam

In Viet Nam, malaria remains a public health problem particularly in areas such as forested areas in the highlands along the borders with Cambodia and Laos and also on the northern border with China. Malaria cases also come from coastal areas. Woodcutters and poor farmers suffer the most in these epidemic areas. In remote areas with few nearby hospitals, relatives still have to travel far with malaria patients.

It is estimated that one fifth of Vietnamese people are at risk of malaria, and approximately 15 million people live in low transmission endemic areas.

Parasites

In 2003, the annual incidence rate of confirmed malaria was 0.46/1000 countrywide. *P. falciparum* accounted for 79% of malaria cases and *P. vivax* for 21%. In different provinces, the ratio of *P. falciparum* to *P. vivax* cases varied from 1:1 to 1:2 (source: NMCP, 2004). *P. falciparum* causes the majority of malaria infections and is responsible for most severe cases and mortality. *P. vivax* is dominant in the Red River delta, and recently, in the Mekong River delta. *P. falciparum* is highly resistant to chloroquine, sulfadoxine − pyrimethamine, and less sensitive to quinine in Central and Southern Viet Nam. The level of chloroquine resistance of *P. falciparum* is variable in Viet Nam. As in China, chloroquine *in vitro* sensitivity has improved, presumably due to the reduction in drug pressure. In 1986, 83% of 496 isolates tested *in vitro* were resistant to chloroquine, with an IC99 of 900nmol/L, as opposed to 17% resistance with an IC99 of 359nmol/L in 1998. (WHO, 2005) but the clinical use of chloroquine for treatment of falciparum malaria is not recommended. The development of resistance to Mefloquine has been documented since 1997 (Le et al., 1997; Trung et al., 2001). *P. vivax* is still sensitive to chloroquine. *P. malariae* and *P. ovale* cases are rarely reported. *P. knowlesi* has not been noticed.

Vectors

There are three main malaria vectors in Viet Nam. *Anopheles (An.) dirus*: a species complex, breeds in shaded water container, found in the forest and the forest-fringe. Its density is related to rainfall, and it is highly anthropophilic, highly exophilic and

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exophagic. Mainly found in the Central and South of Viet Nam. *An. minimus*: only breeds in running water, found only in the mountains, hilly areas and forest-fringe. It is partly zoophilic, partly exophilic and exophagic. *An. sundaicus*: found in brackish water regions, such as the Mekong river delta and the southern coastal areas. It is mainly endophilic and resistant to DDT.

The other secondary vectors are *An. varruna, An. jeyporiensis* (Central of Viet Nam), *An. nimpe* (South), *An. lesteri and An. subpictus* (North), *An. vagus* (plain areas), *An. macculatus, An. aconitus, An. hyrcanus var. sinensis, An. indefinitus* (unpublished data from IMPE of Ho Chi Minh City, Viet Nam).

Epidemiological patterns

Malaria in Viet Nam is associated with specific social and ecological factors. The urban areas and the plain areas around Hanoi and Ho Chi Minh City are non-endemic areas. Stable malaria affects the indigenous populations living in the forested, mountainous regions, and all age groups can be infected. Malaria epidemics occur in case of largely uncontrolled migration from plains to endemic areas. Severe malaria cases have also been seen in non-immune person who travelled to endemic areas, and in some occupational groups (such as plantation workers, mining, road-building, hunting, forest workers).

The division of Viet Nam into malaria zones has some validity and facilitates planning for the Malaria Programme. On the basis of the entomological, ecological and social information, the malaria patterns can be characterized region by region.

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Hills and mountains of the North Viet Nam: The transmission is of low intensity. Malaria outbreaks occur occasionally. The main vectors are *An. minimus* and *An. dirus*. Malaria control programme is inadequate because of the migration of the population due to many hydro-electric projects and economic migrants. Malaria risk is associated particularly with sleeping in plot huts even though bed nets are widely used by different ethnic group. The sleeping patterns of these people (late to bed) and the biting times of the mosquito (early evening and morning) may explain why bed nets have had a limited impact.

The Red river delta and other plains: Here transmission is very rare. Most of malaria cases are imported.

The Central and Southern highlands: The two main vectors are *An. minimus* and *An. dirus*. The transmission is more intensive with peaks occurring during the rainy season. Many ethnic minority groups inhabit these areas of dense, primary forests and suffer expose to intense malaria transmission so that the immunity develops in early childhood. Infants and pregnant women are more at risk more akin to the situation in Sub-Saharan Africa. Because of the new economic activities, malaria risk is high in the immigrant groups moving into these regions from lowland areas.

The Mekong delta: Malaria occurs in foci along the riverbanks and estuaries. *P. vivax* persist in the coastal areas with the main vector is *An. vagus*.

Morbidity and mortality

During the Viet Nam War, in the North of Viet Nam a programme of malaria eradication was started in 1958. For the first 5 years, the programme appeared to be successful, the smear positive rate (SPR) dropped from 5.6% (in samples of 10% the population) to 0.28%. But, because of many difficulties the programme lacked

investment but the SPR was always kept less than 1% until 1975. In South Viet Nam, the malaria eradication campaigns were less successful. Malaria was found most in the areas which were also areas of intense and repeated battles and many soldiers from both sides and civilians died from malaria.

In 1975, after reunification, the National Malaria Programme was implemented across the whole country. From the end of 1975 to 1996, many surveys were done by the National Programme for Malaria Eradication, and the results showed the positive slide rate was 11.7% in 1976 and 2.9% in 1980. The malaria situation worsened after 1981. There were many factors associated with the resurgence but major issues included the terrible economic situation, the establishment of many newly economic zones in the central and southern highlands with migration of people, the diminishing resources for peripheral health services and the programme for prevention, the emergence of drug-resistant strains of *P. falciparum* and the return of large number of Vietnamese soldiers from the Cambodian war. Malaria had re-emerged in the whole country and became the most important public health crisis affecting the nation. Malaria peaked in 1991, when the number of reported malaria deaths reached 4,646. Many cases remain unreported and this figure is undoubtedly a huge underestimate. Malaria was the leading cause of death caused by infectious diseases in the country. In 1992, the number of malaria cases increased to 225,928, but by the mid-1990s the morbidity and mortality began to decline. Again the reasons for this change are complex and multifactorial and it is a shame that we still do not know the relative contribution of these various interventions to the rolling back of malaria in Viet Nam. The factors that are widely thought to have made a major contribution include the implementation of a vertical and vigorous malaria control programme, economic development, deforestation, and widespread early deployment (before global

organisations gave their blessing) of artemisinin drugs. The new malaria control programme provided free antimalarial drugs as well as insecticides for treating bed nets and spraying. Access to treatment was improved and the more effective artemisinin-based drugs were deployed. By 1994, the Government budget for malaria control was US$ 5.2 millions, making it the biggest categorical programme. The World Bank provided US$ 4.6 million per year from 1996 to 2000. External support reached US$ 1.8 million per year. The national economy began to improve in 1998. The contribution of these factors dramatically reduced malaria morbidity by 60% and mortality by 97%. Only 148 deaths from malaria were reported in 2000 (NMCP report, 2000). Malaria in Viet Nam is shown in Table 1.1, and malaria report in 2006 (World Malaria Report, 2008) is shown in Table 1.2.

Table 1.1 Malaria indicators for Viet Nam 1976-2006

Year	Clinical malaria cases	Confirmed malaria cases	Deaths
1976	615,147		1,513
1977	909,501		3,120
1978	727,984		2,291
1979	807,600		1,125
1980	511,557		1,138
1981	657,064		1,152
1982	747,228		1,368
1983	835,606		1,659
1984	880,713		1,256
1985	964,849		1,413
1986	1,424,919		1,838
1987	1,274,034		2,133
1988	1,310,387		2,465
1989	965,999		3,434
1990	902,789	123,796	3,340
1991	1,091,251	187,994	4,646
1992	1,294,426	225,928	2,658
1993	1,111,500	156,069	1,054
1994	858,000	140,120	604
1995	666,200	100,116	348
1996	532,900	76,356	198
1997	445,200	65,859	152
1998	383,300	72,091	183
1999	341,500	75,534	190
2000	293,000	74,316	148
2001	188,122	53,601	91
2002	184,901	46,902	50
2003	164,282	37,416	50
2004	128,382	24,909	34
2005	99,061	19,497	18
2006	91,350	22,637	41

Table 1.2 Estimated and reported cases and deaths, 2006

Estimated	Population	84.155.800
	Fever cases	8,753,023
	Malaria cases	70,324
	Malaria death	167
Reported probable and confirmed	Outpatient malaria cases	91,350
	Inpatient malaria cases	285
	Malaria attributed deaths	41
Reported laboratory confirmed	Mic. Slides/RDTs taken	2,972,429
	Mic. slides/RDTs positive	22,637
	P. falciparum	17,911
	P. vivax	4,497
	Mixed	229

1.2 Malaria parasite

Plasmodia arise from the Coccidian stem (Table 1.3) and present a sexual phase in the vector, a first asexual phase in the tissue of the host, followed by the main asexual phase in the blood of the host. There are four species of the genus *Plasmodium* that infect humans: *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale and Plasmodium malariae*. The characteristics of the four human malaria parasites are shown in Table 1.4. Recently the fifth species that can infect human is *Plasmodium knowlesi*, commonly found in Southeast Asia, and usually is misdiagnosed by microscopy mainly as *Plasmodium malariae*. This is an emerging infection that was reported for the first time in humans in 1965 (Chin et al., 1965). The morphological resemblance of early trophozoites of *P. knowlesi* to *P. falciparum* and later erythrocytic stages to *P. malariae* makes it extremely difficult to identify *P. knowlesi* infections by microscopy alone (Lee et al, 2009).

Transmission

The main vector of human malaria is female anopheles mosquito. More than 60 species of Anopheles (*An.*) can transmit malaria, but their distributions vary between countries. Mosquito bites are the main way transmission occurs. The other ways are rare but include blood transfusions, needle sharing (among drug abusers) and congenital malaria, especially during the first pregnancy and in non-immune patients. The three main vectors in Viet Nam are: *Anopheles dirus* (forestry), *An. minimus* (near forest) and *An. sundaicus* (coastal region and Mekong river areas). The others also can be found: *An. varruna, An. jeuporiensis* (Central Viet Nam), *An. nimpe* (South Viet Nam), *An. lestein* and *An. subpictus* (North Viet Nam). Malaria transmission does not occur at altitudes higher than

2000m and at temperature below 16°C or above 33°C because the development in the mosquito is not efficient enough.

Host Factors

All humans can get malaria, only a few are partially protected from malaria such as those with the Duffy blood group, and those with certain forms of haemoglobinopathy (HbS, HbF). There is enormous interest in host factors that may reduce or increase the susceptibility to malaria however a detailed review of this topic is beyond the scope of this thesis. Non-immune person (children, people who live in non-endemic area but travel to endemic areas), pregnant women and people who leave endemic areas and then return are more likely to develop severe malaria.

Life cycle of malaria parasite

The life cycle of malaria parasite is complex, but it is similar between the five species of human malaria. There is a sexual phase in the mosquito (sporogony) and an asexual phase in the human body. In the human, schizogony phase starts in the liver cells (exo or pre-erythrocytic schizogony or tissue schizogony) and later develops in the red blood cells (erythrocytic schizogony).

The **asexual phase** includes a pre-erythrocytic schizogony and an erythrocytic schizogony. When the female anopheles mosquito bites the human host it inoculates the parasite sporozoites into the human blood stream. Most of the sporozoites are destroyed. Some of them avoid the immune system and migrate to the liver cells within 30 minutes. Why the sporozoites target the liver cells and how they invade the hepatocytes so quickly remains unknown. Sporozoites pass through several liver cells before invasion and only 8-10 sporozoites invade successfully (Mota et al., 2001). There are 2 co-receptors on

sporozoites that mediate the invasion, the circumsporozoite protein (CSP) and the thrombospondin-related protein (TRP). Once inside the liver, each sporozoite can develop into thousands of merozoites (pre-erythrocytic schizogony or tissue schizogony). These merozoites will be released from liver cells at the end of this initial phase. The complete time of this phase is between 8-25 days for *P. falciparum*, 8-27 days for *P. vivax*, 9-17 days for *P. ovale,* and 15-30 days for *P. malariae.* Each merozoite released from hepatocytes will invade red blood cells. Some of the sporozoites of *P. vivax* and *P. ovale* transform to hypnozoites, a dormant form, which remain in the liver cells for months or years, then can be reactivated and cause relapsing malaria. The mechanism of hibernation is also not known yet.

The erythrocytic schizogony: Thousands of merozoites released from hepatocytes invade the RBC and go through a sequence of changes which results in the clinical manifestations of malaria. In the RBC, the parasite develops into ring forms (young trophozoites), trophozoites, schizonts, and erythrocytic merozoites. This erythrocytic cycle takes 24 hours for *P. knowlesi*, 48 hours for *P. falciparum*, *P. vivax* and *P. ovale*; 72 hours for *P. malariae.* Then, these merozoites are released by the rupture of RBC and invade the uninfected RBCs. The erythrocytic cycle continues synchronously. Some merozoites transform into male and female gametocytes in the RBC. The gametocytes will infect the mosquitoes when they bite malaria infected patients.

Sexual phase (Sporogozy): The sporogonic cycle begins when the female and male gametocytes enter the female Anopheles mosquitoes during a blood meal. In the mid gut of the mosquito, the female gametocyte shed the RBC and becomes a macrogamete. The male gametocyte divides into flagellated microgametes, each of which may fertilize a macrogamete and form a zygote. A zygote develops slowly into motile ookinete, which penetrates the wall of the mid gut and transforms into a non-motile oocyst. The nucleus of

this oocyst divides into thousands of sporozoites. The sporozoites migrate to the salivary gland of the mosquito. When the mosquito bites an individual, those sporozoites will inoculate into the blood stream and start another asexual phase.

Table 1.3 Classification of the genus *Plasmodium*

Sub-kingdom	Protozoa
Phylum	Apicomplexa
Class	Sporozoasida
Sub-class	Coccidiasina
Order	Eucoccidiorida
Sub-order	Haemospororina
Family	Plasmodidae

Table 1.4 Characteristics of human malaria parasites

	P. falciparum	*P. vivax*	*P. malariae*	*P. ovale*
Tissue schizogony (days)	5.5	8	15	9
Erythrocytic phase (days)	2	2	3	2
RBC affected	All	Reticulocytes	Old RBC	Reticulocytes
Hypnozoites	No	Yes	No	Yes
Merozoites per schizont	8-32	12-24	6-12	4-16

1.3 The pathophysiology of malaria

Early hypotheses to explain the pathophysiology of falciparum malaria were based on pathological observations in fatal cases of falciparum malaria; the cerebral capillaries were observed to contain a high proportion of parasitized red blood cells, even when the peripheral parasitaemia was low (Marchiafava and Bignami, 1894). Furthermore, the predominant forms in the cerebral capillaries and venules were large "late" trophozoites and schizonts forms that were seldom seen in peripheral blood smears. It was suggested that these parasitized erythrocytes stuck together and had difficulty passing through the capillary bed. Flow was consequently reduced, and finally stopped (Dudgeon and Clarke, 1918; Gaskell and Millar, 1920). The pathological event was considered to be the results of obstructed microcirculatory flow or the local release of unidentified toxic materials from the malaria parasites. Normal erythrocytes must undergo considerable deformation in order to traverse the capillary, and when erythrocytes are rigid (as a result of being infected with the malaria parasites), obstruction may occur. It has been argued that erythrocytes containing mature parasites might be retained in capillaries by similar mechanisms. But if obstruction were due to cell rigidity only, the site of the obstruction would be expected to be the site of minimum cross-sectional area, the mid-capillary. The tail of erythrocytes stacked behind the obstructing cell should have a similar proportion of parasitized red cells as peripheral blood (White, 1986). Furthermore, such obstruction should occur uniformly as capillaries have similar internal diameter whereas pathological observations show preferential sequestration in different vascular beds, that post-capillaries are often packed solely with infected erythrocytes (Pongponratn et al., 1991; Sein et al., 1993).

The pathophysiological processes in malaria result from the destruction of erythrocytes, the liberation of parasite and erythrocyte material into the blood circulation, and the host reaction. During the intraerythrocytic phase of parasite, *P. falciparum*- infected red blood cells (Pf-IRBCs) disappear from the peripheral blood and localize in deep vascular beds of the brain and other organs in a process of receptor-mediated cytoadherence to endothelial cells (ECs). In falciparum malaria, the parasitized erythrocytes sequester in the capillaries and venules of vital organs, interfering with host tissue metabolism and blood flow (White and Ho, 1992).

1.3.1 Parasite multiplication

When schizonts in hepatocytes rupture, approximately 10^5 to 10^6 merozoites are liberated into the circulation. The liberated merozoites invade passing red blood cells immediately. In nonimmune individuals, the multiplication rate of asexual stages of *P. falciparum* usually reaches 20-fold per cycle (Kitchen, 1941 and 1949). On average, microscopy can detect parasites in the blood (20-50 parasites/µl of blood) on the 11th day after sporozoite inoculation. At this stage the host may still feel well or may complain of vague symptoms such as malaise, headache, myalgia or anorexia. The fever usually begins two days later. At the onset of fever, the adult patient has approximately 10^8 parasites in the body, which corresponds to 20-20,000 parasites/µl of blood. In endemic areas the host with immunity may tolerate parasitaemias up to 10,000/µl of blood without feeling ill (Smith et al., 1994; Amstrong-Schellenberg et al., 1994). The rise in parasite count is logarithmic initially, with a rising sine wave pattern of parasitaemia (Fairley, 1947), with considerable amplitude in synchronous *P. falciparum* infections. In most cases, the parasite multiplication terminates abruptly to limit the infection at a parasitaemia of 104-105/µl. Only *P. falciparum* has the capacity for such multiplication, and parasite counts may

exceed 50% in some cases. Several factors limit the parasite multiplication. The host mobilizes non-specific then specific immune defences. High fever can damage the parasite schizonts and meronts, and brake parasite expansion (Kwiatkowski, 1990). Another limiting factor is the availability of suitable red cells; *P. falciparum* and *P. vivax* prefer younger red cells and *P. malariae* prefer older cells. Although natural infections often contain two or more genetically different parasite strains, the development of the parasite population tends to be synchronous from the onset (White et al., 1992). Further synchronization takes place within 1-2 hours in untreated infections in nonimmune patients. This is associated with malaria paroxysm (fever and rigors). Although one brood predominates, in *P. falciparum* infection there is usually at least one minor brood or subpopulation cycling 24 hours out of phase with the major brood (Li et al., 1982).

1.3.2 Parasite biomass

In the benign malarias, the number of parasites in the body may be estimated simply by multiplying the parasitaemia by the estimated blood volume (White, 1997). An unresolved question about severe malaria is the relationship between parasitaemia and stage of development (biomass), and disease severity. In falciparum malaria there are large discrepancies between the number of parasites in the peripheral blood and the number of parasites in the body (the total parasite biomass). In synchronous *P. falciparum* infection the parasite numbers in the peripheral blood fall at the time of sequestration, and rise at the time of schizogony or merogony (White et al., 1992). The total parasite biomass comprises the circulating parasites and the more mature sequestered parasites that are responsible for pathology. Some patients who die may have had large numbers of sequestered parasites, with very low peripheral parasite count. To estimate the total body

parasite biomass in acute falciparum malaria, plasma histidine-rich protein 2 (PfHPR2) concentrations may be useful (Dondorp et al., 2005).

1.3.3 Toxicity and Cytokines

For many years malariologists considered that malaria parasites contain malaria toxins that cause the periodical "paroxysm", but no toxin has ever been identified. Malaria parasites induce release of proinflammatory cytokines (Kwiatkowski et al., 1989). Cytokines, and in particular tumour necrosis factor (TNF), may play an important role in causing some of the pathological changes that characterise malaria. Tumor necrosis factor (TNF-α), interleukin (IL)-1 and gamma interferon (γ-IFN) are produced and these induce the release of a cascade of "proinflammatory" cytokines including IL-6, IL-8, IL-12, IL-18. These are balanced by production of the "anti-inflammatory" cytokines IL-10 (Kern et al., 1989; Ho et al., 1998). The concentration of the cytokines in plasma is elevated in both vivax and falciparum malaria (Grau et al., 1989, Karunaweera et al., 1992). One of the main toxic serum components appear to be lipid peroxides which are formed by the interaction of lipoproteins with reactive oxygen intermediates. Malaria parasites are extremely susceptible to free radical damage (Malhotra et al, 1988) and lipid peroxidation has the effect of stabilizing the reactive oxygen groups, thus creating a more stable cytotoxic molecule. Soluble antigens of *P. falciparum* released into in vitro culture supernatants, and also found in the plasma of patients with acute malaria, are potent inducers of TNF release from monocytes or macrophages (Bate et al, 1988; Kwiatkowski et al., 1989). Some studies have confirmed a positive association between plasma concentrations of TNF and other pro-inflammatory cytokines and mortality in severe falciparum malaria (Grau et al., 1989; Kern et al., 1989; Kwiatkowski et al., 1990). In most of these studies plasma concentrations of TNF have been correlated with several

indicators of severity, namely hypoglycaemia, hyperparasitaemia, and anaemia. Plasma concentrations of IL-1, IL-6, and IL-8 have also been shown being correlated with disease severity. IL-10 inhibits the ability of malaria antigens to induce release TNF (Ho et al., 1995). A study in the Gambia a genetic polymorphism in the TNF promoter region has been shown to confer a seven fold increased risk of either death or neurological deficit from severe malaria (McGuire et al., 1994). This strongly suggests a link between TNF production and its neutralization by tissue bound and circulating receptors is an important determinant of the biological effects. If pro-inflammatory cytokines do play a central role in severe malaria, then these pathological events are likely to take place locally and may not be reflected in systemic cytokine measurements. Preliminary studies indicate that these are antipyretic confirming a central role for TNF in fever. Results of studies of anticytokine and anti-inflammatory agents (anti-TNF antibodies, pentoxifylline, and dexamethasone) in the treatment of severe malaria have been disappointing (Warrell, 1999). Acute malaria is associated with high levels of most cytokines, but the balance differs in relation to severity. IL-12 and TGF-P 1 (transforming growth factor), which may regulate the balance between pro- and anti- inflammatory cytokines, are higher in uncomplicated than in severe malaria (Perkins et al., 2000). Cytokines are responsible for many of the symptoms and signs of malaria such as fever and malaise. There is no direct evidence that systemic release of TNF or other cytokines causes coma in human. In a recent study on severe malaria in adults, elevated plasma TNF concentrations were associated specifically with acute renal failure and TNF levels were actually lower in patients with pure cerebral malaria than those of other complications (Day et al., 1999). Cytokines may be involved in placental function, suppression of erythropoiesis and inhibition of gluconeogenesis. Cytokines also up-regulate the endothelial expression of vascular ligands for *P. falciparum* infected red blood cells, notably ICAM-1 and may

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promote cytoadherence. But studies have also called into question the asscociation of pro-inflammatory cytokines with a poor prognosis (Lyke et al., 2004). The role of inducible nitric oxide synthase (iNOS) and nitric oxide (NO) in the pathophysiology of severe falciparum was first described in 1991 (Clark et al., 1991), and there was a relation between reactive nitrogen intermediates (RNI) levels with disease severity (Yaman et al., 1996). But other trials found a negative association (Anstey et al., 1996; Taylor et al., 1998; Boutlis et al., 2004).

1.3.4 Sequestration

A characteristic feature of falciparum malaria is the lack of mature forms of the parasite in the peripheral circulation of the patients. The essential pathological feature of severe falciparum malaria is the abundance of erythrocytes containing mature forms of the parasite in the deep vascular beds of vital organs (Berendt et al., 1994). This phenomenon is known as sequestration. Sequestration is not distributed uniformly at a microvascular level or amongst the vital organs; it is usually least in the skin and greatest in the brain. Quantitives studies of sequestration in different organs from fatal cases confirmed that sequestration of parasitized RBC in cerebral malaria (MacPherson et al., 1985; Pongponratn et al., 1991 and 2003), which may explain why cerebral malaria is a prominent feature of severe falciparum malaria in man. Some vessels are completely packed with parasitized red blood cells while adjacent vessels are clear (Silamut et al., 1999). This may cause more gradually reduction in blood flow perhaps with less hypoxia because of preserved flow in adjacent capillaries. The severity of falciparum malaria appears to be proportional to the density of the parasite in the internal vessels, and not necessarily related to the parasitaemia of the peripheral circulation (Pongponratn et al., 1991 and 2003). In the human body, the microvessels of the heart, lungs, kidneys, small

intestine, and liver are the other principal sites of sequestration (Sherman et al., 1992).

Unfortunately, despite considerable interest in immunopathological mechanisms in rodent

models of severe malaria (Grau et al., 1990) and also the sequestering parasite *P. coatneyi*

in the rhesus monkey (Sein et al., 1993) there is no animal model which satisfactorily

reproduces the clinical features of cerebral malaria in man (White and Ho, 1992).

1.3.5 Cytoadherence

The binding of mature red blood cells to endothelial cells in post- capillary venules,

called as cytoadherence, is considered as a virulent factor. Parasitized erythrocytes stick

to the surface of vascular endothelial cells and this explains the process of sequestration.

The post-capillary venule is the first site in a red cell's route from the heart where the

shear forces at the vessel wall drop markedly in a way that should permit adherence and

this is the site of preferential sequestration. Uninfected erythrocytes will also bind to the

surface of erythrocytes containing mature forms of the parasite causing "rosetting"

(David et al., 1988). Numerous stage-specific changes occur in the infected red cell

membrane at the transition from non-sequestering ring form to the sequestering

trophozoite. Electron-dense sub-membranous structures appear and enlarge, resulting in

called "knobs," which are the sites of red cell to endothelial cell adhesion (Pongponratn et

al., 1991; Ho et al., 1992). These knobs are considered essential for cytoadherence by

facilitating the initial attachment of the infected erythrocytes to the vascular endothelial

cell, and by concentrating the parasite ligands at a particular site. Cytoadherence is an

important mechanism for parasite escape from the macrophagic phagocytosis of the first

mechanism, and the spleen plays a major role in mulating the erythrocyte surface

alterations responsible for parasite sequestration (David et al, 1983). The families of

molecules, which mediate cytoadherence, are a series of antigenically variant high

molecular weight proteins exported by the parasite to the red cell exterior. These are called *Plasmodium falciparum* erythrocyte membrane protein 1(PfEMP-1) (Howard and Gilladoga, 1989). The molecule is trypsin sensitive and trypsin will inhibit cytoadherence. Cytoadherence can be inhibited by strain specific anti-sera. PfEMP-1 is also thought to be the major surface antigen determining the population structure of *P. falciparum*. It has been shown to undergo antigenic variation at a rate of approximately 2.4% of per parasite life cycle (Roberts et al., 1992).

The interaction between parasitized red blood cells and endothelial cells involves specific parasite ligands and host receptors. At least six potential receptors have been identified to date. The first to be discovered was CD36. The expression of CD36 on the surface of cells correlates with their ability to act as targets for cytoadherence of infected cells. Recent studies have shown that nearly all freshly isolated parasites from patients with acute falciparum malaria bind strongly to CD36 (Ockenhouse et al., 1991). Immunohistochemical studies indicate that parasite sequestration co-localises with CD36 with vascular expression of CD36 outside the brain, whereas within the brain there is very little CD36 expression and other ligands are involved presumably. This suggests that CD36 is probably the major cytoadherence ligand outside the brain.

The second candidate molecule to be discovered was thrombospondin, a multi-functional glycoprotein which is synthesized by many adherent cell types including endothelium and which is released on platelet activation. Thrombospondin may contribute to cytoadherence, possibly by stabilizing the PfEMP-1 – CD36 interaction but it does not mediate it alone. Intercellular adhesion molecule 1 (ICAM-1) is also a cytoadherence receptor for *P. falciparum* (Berendt et al., 1989). This is the natural receptor for rhinovirus invasion, although most natural isolates bind less avidly to ICAM-1 than to CD36 (Ockenhouse et al., 1991), recent immunohistochemical studies indicate highly

significant co-localisation of parasite sequestration within the brain and vascular expression of ICAM-1. This suggests that ICAM-1 is probably the major vascular ligand for cytoadherence within the brain (Turner et al., 1994). The interaction between ICAM-1 and infected red cells is of a "rolling" type in which the cell rolls along the vascular endothelium somewhat analogous to the movement of a polymorphonuclear leukocyte before it penetrates at sites of inflammation (Cooke et al., 1994). This contrasts the static adhesion resulting from binding to CD36. Recently other potential ligands have been identified: E selectin, vascular adhesive molecule 1 (V-CAM 1), chondroitin sulphate A (CSA), and hyaluronic acid (HA). The role of these adhesions is less clear. *P. falciparum* derived molecules on the RBC surface which mediate adherence include PfEMP-1, sequestrin, modified RBC band 3, rosettins, Pf 332 (White, 2003).

Parasites sequester themselves in various organs such as brain, heart, lung, liver, kidney, subcutaneous tissue and placenta. Cytoadherence results in sequestration of parasitized red blood cells in the deep vasculature of organs, and therefore it localizes parasites in sites of low oxygen tension that is ideal for their growth. It also prevents infected parasitized red blood cells being destroyed in the spleen and it is thought to be the cause of organ dysfunction through microcirculation obstruction, endothelial cell activation and damage, cytokine release. Extensive binding of parasitized RBC to placental chondroitin sulfate A (CSA) is associated with physiopathology during pregnancy (Pouvelle et al., 2000). Febrile temterature may induce cytoadherence of parasitized RBC (Udomsangpetch et al., 2002).

1.3.6 Red cell deformability

P. falciparum infected erythrocytes have reduced deformability, which is directly proportional to the maturity of the intracellular parasite (Cranston et al., 1984). Several factors are likely to account for reduced deformability of parasitized erythrocytes, notably changes in the cytoskeleton (Maguire et al., 1991), increased membrane stiffness, increased cytoplasmic viscosity resulting from changes in membrane permeability (Kutner et al., 1983), reduced surface area to volume ratio (increased sphericity), principally the rigidity of the parasite itself (Nash et al., 1989), and the enlarging and relatively viscous intra-erythrocytic parasite (Paulitschke and Nash, 1993). The parasitized red cells become more spherical and rigid, and the surface is irregular and covered in small protrusions. This explains why infected cells are less able to pass through micropore filters than uninfected cells (Lee et al., 1982). The uninfected red cells also have a reduction in deformability (Dondorp et al., 1997). Reduced deformability alone cannot explain sequestration in venules (White and Ho, 1992).

1.3.7 Rosetting

Red blood cells containing mature malaria parasites adhere to unparasitized red cells. This event leads to the formation of "rosettes" (David et al., 1988; Handunnetti et al., 1989). Rosetting starts in venules and reduce flow, which may enhance anaerobic glycolysis, reduce pH and facilitate adherence of parasitized red cells to venular endothelium. Rosetting is mediated by PfEMP-1 and rosettins, and involves the complement receptors, heparin sulphate, blood group A antigen and other red cell surface molecules (Rowe et al., 1994 and 1995). Rosetting is inhibited by heparin subfractions and calcium chelators. All four Plasmodium species infecting human can induce

rosetting, but only *P. falciparum* causes severe malaria. Rosetting in *P. falciparum* is associated with cerebral malaria (Carlson et al., 1990; Ho et al., 1991).

1.3.8 Genetic factors and Immunity

The development of clinical disease is dependent on the interplay of the infecting parasite with the immune status and genetic background of the host. Following repeated exposure to malaria parasites, individuals residing in endemic areas develop immunity. Naturally acquired immunity provides protection against clinical disease, especially severe malaria and death from malaria, although sterilizing immunity is never achieved. In non-immune individuals, *P. falciparum* may cause severe and life-threatening disease. The other risk groups are children and pregnant women, particularly during their first pregnancies.

The thalassaemias are inherited blood disorders that result from mutations in either the α-globin or β-globin genes. Thalassaemia and sickle cell disease are not protective against malaria. It is the heterozygote carriers of these genes that are protected. The alpha- and beta-thalassaemias and the structural variant haemoglobins S, C and E are believed to provide protection against malaria (Clegg and Weatherall, 1999). Both HbC and HbS affect the early development of naturally acquired immunity against malaria (Verra et al., 2007). The protective effect conferred by glucose-6-phosphate dehydrogenase (G6PD) deficiency remains controversial, but early phagocytosis of G6PD deficient erythrocytes parasitized by *P. falciparum* may explain this protection (Cappadoro et al., 1998). Human leucocyte antigens (HLA), such as class I antigen HLA-BW53 and class II antigen HLA-DR B1*1302, may confer protection against severe malaria (Hill et al., 1991, 1992).

It seems that severe malaria does not result from specific immune-mediated damage. Proliferative glomerulonephritis, the classical pathological marker of an immunological disease, was reported in falciparum malaria (Hartenbower et al., 1972) but it was very

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rarely seen. Intravascular accumulations of white blood cells are seen occasionally in severe malaria patients who died after many days of treatment (Patnaik et al., 1994) and malaria antigens may be found on the endothelial basement membranes (Igarashi et al., 1987). However, histological evidence of vasculitis is absent in fatal cases of facliparum malaria. Although immune complexes and free malarial antigen may be detected, the glomerulonephritis is rare (Stone et al., 1972). There are a reduction of T cells and an increase of gamma-delta T-cells (Ho et al., 1990) in malaria. Hypergammaglobulinaemia was seen in people who live in hyperendemic malaria areas but this antibody is not directed against malaria antigens. There is an immune suppression in severe malaria, with defects in monocytes and neutrophil chemotaxis, reduced monocytic phagocytic function and a tendency to bacterial superinfection. The classical and alternative pathways of the complement system are profoundly activated in severe malaria but there is no relation between clinical disease severity and complement fragments existed (Wenisch et al., 1997). Recently, molecular biology study found that matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) may be relevant in the pathogenesis of severe falciparum malaria, either as proteolytic enzymes that degrade the extracellular matrix or as effectors and regulators of the immune response (Dietmann et al., 2008). HIV-infected nonimmune adults are at increased risk of severe malaria. The risk is associated with a low CD4+ T cell count (Khasnis and Karnad, 2003; Cohen et al., 2005).

1.3.9 Permeability

Systemic vascular permeability increases slightly in severe malaria (Davis et al., 1992). Cerebral oedema is a common finding at autopsy in cerebral malaria (Spitz, 1946; Walker et al., 1992; Sengupta and Narqi, 1992). Pathological studies in the 1940s (Rigdon, 1944) and experimental studies in the 1960s led to a new hypothesis (Maegraith and Fletcher,

1972). There was an increase in blood-barrier permeability of 125l-albumin in severely ill

P. knowlesi infected rhesus monkeys and increased penetration of the brain by water-

soluble dyes which were reversed rapidly by hydrocortisone, mepacrine, and chloroquine

(Migasena and Maegraith, 1967). It was suggested that there was an increase in cerebral

capillary permeability with outward leakage of plasma in cerebral malaria. This was

considered that the extravasation of plasma into the cerebral interstitium, local

haemoconcentration and reduced microcirculatory blood flow result in cerebral oedema.

The factors responsible for this were considered to be kinins (Maegraith and Fletcher,

1972). This theory became widely adopted and was the basis for the widespread use of

corticosteroids in cerebral malaria. Clinical and experimental observations in man argue

the permeability hypothesis. In the absence of spinal block, cerebral oedema leading to

raise intracranial pressure must raise lumbar cerebrospinal fluid pressure, but opening

pressures at lumbar puncture are within the normal range. Papilloedema is relatively

unusual (Looareesuwan et al., 1983). Computerized tomography of the brain rarely shows

evidence of cerebral oedema (Looareesuwan et al., 1983; Newton et al., 1994). Finally, in

the studies of blood-CSF barrier permeability in cerebral malaria, no increase in

permeability was observed with a variety of marker substances that included 125l-

albumin (Badibanga et al., 1986; Warrell et al., 1986). These observations demonstrate

that cerebral oedema resulting from "leaky" cerebral capillaries is not a consistent, or

even a common feature of cerebral malaria, and cannot be the cause of coma in cerebral

malaria.

1.3.10 Cerebral malaria

The major lethal complication of malaria infection particularly in children is cerebral

malaria, a diffuse usually reversible encephalopathy. The essential underlying

pathological process is the sequestration of red blood cells containing mature forms of *P. falciparum* in the cerebral microvasculature. Coma is a characteristic feature of falciparum malaria. Death rate in cerebral malaria is above 20% (Warrell, 1992; Hien et al., 1996), but over 98% of adult survivors recover without sequelae (WHO, 1990). Many hypotheses have been proposed to explain the loss of consciousness in cerebral malaria. None are completely satisfactory. Ischaemia alone would not account for the excellent neurological recovery. Similar cerebral metabolic findings to those in cerebral malaria have been recorded in volunteers breathing low concentrations of oxygen (White and Ho, 1992). Inflammatory processes are not compatible with the time course or pathology. Increased systemic levels of pro-inflammatory cytokines may obtund but do not lead to profound coma. Cerebral malaria most closely resembles a metabolic or anaesthetic encephalopathy. Ultimately abnormalities of neuro-transmitter synthesis, release or binding may be implicated. It was suggested that the ubiquitous messenger nitric oxide might be important (Clark et al., 1994). Studies on Kenyan children with cerebral malaria have proposed that the imbalances of excitatory mediators may be implicated in the initiation and maintenance of seizures and neuronal degeneration and might contribute to neurological symptoms in cerebral malaria (Dobbie et al., 2000). Studies of cerebral blood flow and metabolism and acid-base status (White et al., 1985; Warrell et al., 1988; Taylor et al., 1993) suggested that there is an anaerobic cerebral glycolysis in cerebral malaria; and this presumably results from cerebral hypoxia. There is also a significant contribution from the parasites' glycolytic metabolism (White and Ho, 1992). Cerebral hypoxia may well result from either "patchy" microcirculatory obstruction with adjacent areas of increased perfusion, or alternatively a more diffuse and homogenous process in which the adherent erythrocytes interfere with gas and substrate exchange throughout the brain but do not increase vascular resistance. The most likely scenario is of vasodilation

to accomodate the increased intracerebral blood volume (Looareesuwan et al., 1995).

Magnetic resonance imaging is a more sensitive measure of brain water, but there was no

evidence of cerebral oedema in cerebral malaria (Looareesuwan et al., 1995). Double-

blind placebo-controlled trials of medium and high dose dexamethasone in cerebral

malaria showed no effect on mortality (Warrell et al., 1982, Hoffman et al., 1988). In one

of the trials, coma was prolonged in the steroid-treated patients (Warrell et al., 1982).

Studies in adults with severe falciparum malaria in which transcapillary escape rates for

radio-labeled albumin, fluoroscein angiography to demonstrate retinal capillary leakage,

and urinary micro-albumin excretion were measured did indicate a small but significant

increase in generalized capillary permeability, but this was small and would be unlikely to

account for any of the major pathological features of severe malaria. This is consistent

with the observations in some studies on the structural integrity of the blood-brain barrier

(BBB) and ultrastructure of the brain in fatal cases of falciparum malaria (Brown et al.,

1999; Medana et al., 2001 and 2002). Thus there is little evidence for a severe and

generalized increase in capillary permeability in severe falciparum malaria. It is likely

that the post-mortem observation of cerebral oedema in fatal cases of cerebral malaria

usually results from agonal events (hypotension, severe acidosis, etc.) which occurred in

the hours preceding death, but there is no clinical evidence that oedema precipitates

death. However it is also worth considering that coma may be neuroprotective. Neurones

stressed by an inadequate supply of oxygen and nutrients, and an unfavourable metabolic

milieu may preserve themselve by reducing energy demands. Premature reversal of coma

might increase the risk of neuronal damage.

Immune mechanisms have not been proved to have a role in the pathogenesis of cerebral

malaria. The neuropathological findings in cerebral malaria had been interpreted as

resulting from a reaction of the central nervous system to the antigenic challenge of *P.*

falciparum infection (Toro and Roman, 1978) but the predicted increase in brain water associated with this was not observed on magnetic resonance imaging in vivo (Looareesuwan et al., 1995). The proposed mechanism for this was an immune complex vasculitis of the cerebral vessels. However, the perivenous demyelination described by these authors could have been explained by terminal hypoxia. Furthermore histopathological studies, immunohistochemical studies, and ultrastructural studies usually show no evidence of an inflammatory cell infiltrate despite abundant evidence of endothelial cell activation (MacPherson et al., 1985; Turner et al., 1994). However another study reported that mononuclear cell margination along the cerebral microvascular walls was prominent in 5 of 23 fatal cases of cerebral malaria and speculated that this may have contributed to pathology (Patnaik et al., 1994).

Sequestration results from the adherence of parasitized red cells to vascular endothelium. Histopathological observations in fatal falciparum malaria suggest that the red blood cells containing more mature parasites are sequestered in the brain and other organs. Post-mortem studies have given conflicting results on the predominant stage of parasite in the brain. A study reported almost exclusively schizonts (Lemercier et al., 1966) whereas another study found that both trophozoites and schizonts predominate (MacPherson et al., 1985). Microvascular sequestration was assessed in the brains of 50 Vietnamese and Thai patients who died from severe malaria; the results have shown that within the same brain different vessels had discrete but different populations of parasites indicating that the adhesion characteristics of cerebrovascular endothelium change asynchronously during malaria. Furthermore there were significantly more ring form parasites in the cerebral microcirculation than expected. Sequestration probably has a major role in the pathophysiology of cerebral malaria, particularly in adults (Silamut et *al.*, 1999), but it is less consistently implicated in children (Crawley et al., 2001; Taylor et al., 2004). The

clinical syndromes and the variation in pathological features in severe malaria have caused extensive controversy. The increased cerebral lactate production reflected in increased jugular venous and cerebrospinal fluid concentrations of lactate indicates significant anoxic metabolism, but it is not clear whether these changes provide sufficient explanation for coma (White and Ho, 1992). In a retrospective study of 261 Vietnamese adult patients with severe malaria three metabolites, the excitotoxin quinolinic acid (QA); the protective receptor antagonist kynurenic acid (KA) and the proinflammatory mediator picolinic acid (PA) were measured and related to the incidence of neurological complications, CSF lactate level and the disease outcome. The results have indicated that there was no difference in the levels of KA between groups; no association between QA concentrations in CSF and convulsion or depth of coma, and that the significant elevation of QA, PA levels and QA: KA ratio in patients with poor outcome was only a consequence of impaired renal function in this group (Medana et al., 2002). A post-mortem study on 54 Vietnamese patients who died from severe falciparum malaria the authors found P-amyloid precursor protein (P-APP), a marker of potentially reversible axonal damage, normally transported along the axon. P-APP accumulates at the site of axonal injury, that is significantly associated with cerebral malaria in adults and that this distinguishes cerebral malaria from non-cerebral malaria. This marker highlights the internal capsule and pons as areas of primary involvement in axonal injuries. Unlike other pathological correlates such as neuronal stress markers, axonal injury does not seem to purely reflect the systemic contribution of severe malaria to the specific neurological syndrome of cerebral malaria (Medana et al., 2002). Study of cerebrospinal fluid markers of brain parenchymal damage confirmed that axons are the cellular component most severely affected (Medana et al., 2005) and axonal damage may play an important role in the mechanism of cerebral malaria. Recently, a pathological study reported the evidence

of perturbation of human BBB integrity by the adherence of Pf-IRBCs to brain endothelium through a multifactorial and multistep process (Tripathi et al., 2007).

1.3.11 Renal failure

Renal impairment is a common complication and a sensitive prognosis indicator in severe falciparum malaria in adults; half of these patients have evidence of renal dysfunction (Trang et al., 1992). Renal failure is rarely seen in children (Sowunmi, 1996). The basic pathology is acute tubular necrosis (Sitprija et al., 1967; Stone et al., 1972; Day et al., 1997) and reports of glomerulonephritis are very rare (Hartenbower et al., 1972). Renal impairment may be caused by renal hypoperfusion due to dehydration and hypovolaemia. Renal failure may be compounded by severe anaemia, haemoglobinuria, intravascular haemolysis and clogging of the tubules by the products of haemolysis. The pathological processes of acute tubular necrosis in severe malaria have not been clearly known. Cytoadherence of parasitized red cells in the glomerular capillaries is occasionally seen (MacPherson et al., 1985). Studies of renal cortical blood flow (Sitprija et al., 1977) and radiological studies (angiography and contrast urography) (Arthachinta et al., 1974) had shown renal cortical vasoconstriction. The oxygen consumption of the kidneys is reduced in acute renal failure (Day et al., 1996) and it is not improved by administration of dopamine that induces arteriolar vasodilatation, consequently increases the renal blood flow and glomerular filtration rate (Day et al., 2000). The role of local cytokine release and altered regulation of renal microvascular flow is unclear.

1.3.12 Hepatic dysfunction

Enlargement of liver occurs in malaria. Hepatic dysfunction and jaundice is more common in adults with severe malaria than in children. Jaundice is caused by haemolysis. There are elevations of unconjugated bilirubin and aspartate aminotransferase levels; and reductions in clotting factor synthesis, albuminaemia, metabolic clearance of drugs, biliary excretion. Liver blood flow, measured by indocyanine green clearance, is reduced during acute malaria, and is significantly lower in severe malaria than in uncomplicated malaria. This event is associated with highly venous lactate (Pukrittayakamee et al., 1994). There may be sequestration and consequent microcirculatory obstruction in the portal and hepatic circulations, or portal venoconstriction. The failure of gluconeogenesis contributes to hypoglycaemia and lactic acidosis. Liver biopsy usually reveals the dilation of hepatic sinusoids containing hypertrophied Kupffer cells and parasitized red cells, mononuclear cell infiltration. Other studies have shown either no structural changes or slight hepatocyte swelling (Sherlock, 1975). Small areas of centrilobular necrosis have been reported (Joshi et al., 1986). The overall prevalence of Hepatitis B surface antigen (HbsAg) among Vietnamese patients with severe falciparum malaria was higher than that in general catchment population for the study hospital and patients admitted with cerebral malaria had a greater risk of registering positive HbsAg to other manifestations of severe malaria (Barcus et al., 2002).

1.3.13 Pulmonary oedema

Acute pulmonary oedema in severe malaria results from a sudden increase in pulmonary capillary permeability (James, 1985). It usually develops in severe falciparum malaria, particularly in pregnant malaria patients. The left ventricular function is usually normal so the cause of pulmonary capillary permeability is not known (Charoenpan et al., 1990).

Interstitial oedema and hyaline-membrane formation are seen. Pulmonary capillaries and venules are packed with inflammatory cells and parasitized red cells. Acute respiratory distress syndrome is a major cause of death in adults with severe malaria, and it usually develops after the start of antimalarial treatment. Pulmonary vascular occlusion occurs from sequestration of both RBC and WBC. There is impaired alveolar-capillary membrane in severe malaria patients (Maguire et al., 2005).

1.3.14 Hypoglycaemia

Hypoglycaemia is an important manifestation of falciparum malaria and is associated with hyperlactataemia. There are many explanations of hypoglycaemia such as increased metabolic demands of febrile illness, increased peripheral requirement for glucose consequent on anaerobic glycolysis, the glucose consumption of malaria parasites, the failure of hepatic gluconeogenesis and glycogenolysis (White et al., 1983; Davis et al., 1993)

Quinine/quinidine-induced hyperinsulinaemia: quinine stimulates the pancreatic β-cells to release insulin in healthy individuals and in patients with falciparum malaria, both in pregnant women with severe or relatively mild disease, and in adults and children with severe disease (Davis et al., 1993; Krishna et al., 1994; Looareesuwan et al., 1985; Okitolonda et al., 1987; White et al., 1983). Children with severe malaria have considerably increased glucose requirements compared with adults. Hypoglycaemia in African children with severe malaria, and in adults who have not received previous quinine treatment, is associated with appropriately low plasma concentrations of insulin (Taylor et al., 1988).

1.3.15 Metabolic acidosis.

Acidosis, mainly lactic acidosis, results from a compromised microcirculation caused by sequestration of parasitized red blood cells. Lactic acidosis is an important cause of death in severe falciparum malaria (White et al., 1983 and 1987; Taylor et al., 1988; Krishna et al., 1994; Day et al., 2000) and results from several processes: the tissue anaerobic glycolysis due to microvascular obstruction, the failure of lactate clearance due to liver dysfunction (Day et al., 2000), acute renal failure, the reduction of oxygen carriage due to anaemia and the production of lactate by parasites (Pfaller et al., 1982; Van der Jagt et al., 1990; White and Ho, 1992) and the adhesive forces between red cells and the decreased erythrocyte deformability (Dondorp et al., 2000). The acidosis is caused mainly by lactic acid, impaired hydrogen ion excretion because of renal failure (English et al., 1996). The high level of lactate concentration in venous or arterial blood, or cerebrospinal fluid is a poor prognosis in severe malaria (White et al., 1985; Waller et al., 1995) and prolonged hyperlactataemia may be the best overall prognostic indicator of outcome. A recent study indicated that unidentified anions are also the most important contributors to metabolic acidosis in severe malaria in adults (Dondorp et al., 2004).

1.4 The clinical aspect of malaria infection

The clinical manifestations of malaria depend on many factors: parasites, host immunity, and intensity of malaria transmission. Malaria incubation periods in naturally acquired infections vary between 13 – 28 days. All clinical features of malaria are associated with the multiplication of blood-stage parasites and especially with the often synchronous, bursting of large number of parasitised RBC (Kwiatkowski et al., 1989).

1.4.1 Asymptomatic infection

In the constantly intense *P. falciparum* malaria transmission areas many infected semi-immune adults are asymptomatic, even infected pregnant individuals have no symptoms but the birth weight of babies born to primagravidae is low. Clinical aspects of malaria are different in non-immune and semi-immune patients. The parasitaemia at which fever occurs is termed the "pyrogenic density", and it is widely different between parasites and host immunity.

1.4.2 Uncomplicated malaria:

The first symptoms of malaria are non-specific and resemble influenza, and are similar for all five species of *Plasmodium*. Feeling unwell, malaise, loss of appetite, headache, fatigue, lethargy, muscle and joint aches, abdominal discomfort often precedes fever. In some cases, the patients complain of chest pain, cough, abdominal pain, arthralgia, myalgia, diarrhoea, vomiting or nausea. The characteristics of typical features of uncomplicated malaria illness included three stages: cold stage, hot stage and sweating stage. The classical malaria fever chart, the "paroxysm", is unusually seen now. The fever is irregular at the onset of illness. The non-immune patients and children may have high fever. Childhood febrile convulsions may be seen. Anaemia with palpable spleen is common in children living in high transmission areas. Mild jaundice and slight enlargement of the liver is also commonly seen in adults. Overall the clinical symptoms of non-complicated malaria are similar to other infectious diseases. In tropical areas any fever should be considered to be malaria until the proof of malaria is excluded.

1.4.3 Severe and complicated malaria

Falciparum malaria can lead to multiple organ dysfunction syndromes (MODS). The outcome of severe malaria depends on many factors such as the number of vital organ affected, the degree of vital organ failure, the host immunity and the appropriate management. However, outcomes are better than for similar degrees of organ failure in bacterial sepsis.

1.4.3.1 Cerebral malaria

Cerebral malaria is one of the most common non-traumatic encephalopathies. The term "cerebral malaria" is used to describe any impairment of consciousness or other disturbance of central nervous system in falciparum malaria, after hypoglycaemia is corrected and other meningo-encephalopathies are excluded. Cerebral malaria is the most prominent feature of severe falciparum malaria, manifests as a diffuse symmetric encephalopathy but focally neurological signs are unusual. Headache may be severe in malaria but there is no neck stiffness. Nausea and vomiting are common. The onset of coma is sudden or gradual, or may follow a generalized convulsion. The patient develops drowsiness, confusion, delirium or agitation, disorientation, followed by unconsciousness with divergent gaze and variable tone (Figure 1.2). Despite treatment, coma is associated with death rates above 20% (Hien et al., 1996; Warrell, 1992). Pout reflex and bruxism are common, but other primitive reflexes are usually absent. The tendon reflexes are variable, and the plantar reflexes may be flexor or extensor. Decortication (flexor) or decerebration (extensor) posturing is also documented. The corneal reflexes are preserved in adult patients but lost in children with deep coma (White, 1996). Approximately 15% to 20% of patients have retinal haemorrhages (Looareesuwan et al., 1983; Schemann et al., 2002). Pupilloedema and cranial nerve abnormalities are rare. Convulsions, usually

generalized and often repeated, occur in up to 50% of children with cerebral malaria (Asindi et al., 1993) and prolonged seizures are associated with poor outcome. Seizures, coma, severe anaemia and metabolic acidosis presenting as respiratory failure are common in African children. More covert seizure activity is common, particularly in children, and may manifest as repetitive tonic-clonic eye movements (Crawley et al., 1996). While neurological sequelae is rarely seen in adults (3%), about 10% of children surviving after cerebral malaria particularly those with prolonged hypoglycaemia, severe anaemia, repeated seizures, and deep coma have some neurological sequelae (Molyneux et al., 1989; Brewster et al., 1990 and Bondi, 1992) such as hemiparesis, cerebral palsy, cortical blindness, deafness, tremor and impaired cognition and learning. There was no clear clinical evidence of raised intracranial pressure, and no evidence of deterioration immediately following lumbar puncture. Nevertheless brain swelling, and consequent brain-stem compression, may contribute to a fatal outcome in cerebral malaria particularly in those children who die from sudden respiratory arrest (Waller et al., 1991). Post-malaria neurological syndrome (PMNS) was seen in approximately 3% of adults and 10% of children following cerebral malaria (Molyneux et al., 1989; Brewster et al., 1990). A prospective study in Viet Nam showed that the overall incidence of PMNS was 1-2 per 1000. The syndrome was self-limiting and was associated with use of mefloquine after parenteral treatment (Mai et al., 1996). The overall mortality of cerebral malaria is 20% (up to 50% in pregnancy) and depends on the associated vital organ failure and the availability of medical intensive care facilities (White, 1996). However, all falciparum malaria patients with neurological manifestations of any degree should be treated as cerebral malaria. Lumbar puncture may be done to rule out associated meningitis. CSF opening pressure is normal to elevated; fluid is clear; WBCs are fewer than 10/μL. CSF

lactate remains an independent and significant predictor of poor outcome in severe malaria (Medana et al., 2002).

Figure 1.2 A cerebral malaria patient (N.H.Phu)

1.4.3.2 Acute Renal Failure

Renal impairment is a sensitive prognostic indicator, and is common among adults with severe falciparum malaria but rarely seen in children (Molyneux, 1990; Sowunmi, 1996; Phuong et al., 1997). Renal dysfunction was also recently reported in knowlesi malaria (Daneshvar et al., 2009). Recent study on malaria in India reported that renal failure was seen in 14% of children (Tripathy et al., 2007). Renal failure in severe falciparum malaria is caused by hypoperfusion, sequestration or massive intravascular haemolysis in black water fever. Pathologically, this syndrome manifests as acute tubular necrosis (Sitprija et al., 1967; Stone et al., 1972; Day et al., 1997). Acute renal failure usually manifests as oliguria with urine output of less than 400 ml in 24 hours and serum creatinine of more than 264μmol/L, failing to improve after rehydration; however, it may be non-oliguric or polyuric in some cases. Renal failure is associated with hepatic dysfunction, metabolic

acidosis and pulmonary oedema in many fatal cases. Without renal replacement therapy the mortality rate may rise up to 70%, this was halved to 35% after the introduction of peritoneal dialysis (Trang et al., 1992). Peritoneal dialysis (Jackson et al., 1962; Canfield et al., 1968; Trang et al., 1992) or other renal replacement therapy should be considered if the patients get hyperkalaemia, fluid overload, metabolic acidosis and clinical signs of uraemia syndromes. Dialysis considerably improves patient's survival.

1.4.3.3 Jaundice

Mild jaundice in malaria is caused by haemolysis. There is elevation of unconjugated bilirubin levels and AST. Severe jaundice is associated with *P. falciparum* infections and is more common in adults than in children (Figure 1.3). However, in severe cases both serum unconjugated and conjugated bilirubin levels are high with marked elevation of both AST and ALT, and there is prolongation of prothrombin time. Jaundice was associated with cerebral malaria, acute renal failure, and hyperparasitemia, after effective malaria treatment, liver profile returned to normal within a few weeks. Jaundiced malaria patients had transient liver profile impairment that indicated predominantly haemolysis rather than liver damage (Wilairatana et al., 1994). Jaundice and hepatomegaly were significantly associated with renal failure, and jaundice may have potentiated the effects of hypovolemia (Nacher et al., 2001; Prommano et al., 2005). Hepatic dysfunction contributes to hypoglycaemia, lactic acidosis, and impaired drug metabolism. When accompanied by acute renal failure and metabolic acidosis, liver dysfunction carries a poor prognosis. Jaundice was also recently reported in knowlesi malaria (Daneshvar et al., 2009).

Figure 1.3 A severe malaria patient with jaundice (N.H.Phu)

1.4.3.4 Hypotension

The heart is remarkable resilient and cardiac function appears well preserved even in the face of a heavy parasite burden and other vital organ dysfunction (Bethell et al., 1996). Hypotension in severe malaria may be due to dehydration, bacterial coinfection, pulmonary oedema and metabolic acidosis. This is called "algid malaria" (Gage, 1926) but the pathophysiology of algid malaria is not well understood. Orthostatic hypotension is associated with impaired reflex cardioacceleration and is worsened by the quinoline antimalarial drugs (Supanaranond et al., 1993). Shock in severe falciparum malaria in adults is associated with peripheral vasodilation and carries a poor prognosis (Bruneel et al., 1997).

1.4.3.5 Metabolic Acidosis

Metabolic acidosis, predominantly lactic acidosis, is identified as an important cause of death in severe falciparum malaria (White et al., 1983 and 1985; English et al., 1997). In adults, it may result from renal impairment (White, 1996). Acidosis also commonly coexists with severe hypoglycaemia in patients with malaria (Krishna et al., 1994). Lactic acidosis can lead to respiratory distress syndrome and shock. Sustained hyperlactataemia is the best prognostic indicator of outcome in severe malaria. The prognosis of lactic acidosis is poor. The strong anion gap is also a powerful prognostic indicator in severe malaria (Dondorp et al., 2004).

1.4.3.6 Acute pulmonary oedema

Pulmonary oedema in falciparum malaria was first reported in the early years (Watson, 1905). Adults, particularly pregnant women, with severe falciparum malaria may develop non-cardiogenic pulmonary oedema (Von Mach et al., 2003). The mortality rate is >80%. It is rarely due to fluid overload as a result of rehydration, and may develop even in severe malaria patients at relative low or normal filling pressures, with normal central venous pressure and normal pulmonary artery occlusion pressure. The mechanism of pulmonary oedema in severe malaria is not clearly understood. It is one form of the adult respiratory distress syndrome (Brooks et al., 1968; Gurman et al., 1988; Fein et al., 1978; James, 1985; Charoenpan et al., 1990; Asiedu et al., 2000) and may be difficult to differentiate with aspiration pneumonia. Chest x-ray shows increased interstitial shadowing. Tachypnoea is the first sign. Then patient develops hyperventilation or Kussmaul's breathing, hypoxia and cyanosis. Overall, pulmonary oedema carries a poor prognosis (Aursudkij et al., 1998). Respiratory distress was also seen in *P. knowlesi* malaria (Daneshvar et al., 2009).

1.4.3.7 Hypoglycaemia

Hypoglycaemia in malaria may be asymptomatic and often goes unnoticed. In severe cases, it is usually associated with severe anaemia, jaundice, lactic acidosis. The clinical diagnosis of hypoglycaemia is difficult and the usual physical signs such as sweating, gooseflesh, tachycardia, are absent; and the neurological impairment caused by hypoglycaemia cannot be distinguished from that caused by malaria. It occurs in approximately 8% of adults and 30% of children with cerebral malaria but responds well to glucose (White, 1996). It is also commonly seen in cases treated with quinine as a result of drug-induced hyperinsulinaemia. Hypoglycaemia is associated with a poor prognosis (White et al., 1983; Migasena, 1983; Krishna et al., 1994) and is particularly in children and pregnant women (Looareesuwan et al., 1985; White et al., 1987; Taylor et al., 1988). Intravenous dextrose infusion can prevent hypoglycaemia. Hypoglycaemia tends to be recurrent so that the regular monitoring of blood glucose is necessary.

1.4.3.8 Black Water Fever

Black water fever results from massive intravascular haemolysis in severe falciparum malaria. Renal biopsies from ceases of black water fever had demonstrated haemoglobin tubular casts and tubular atrophy (Rosen et al., 1968). It usually associated with severe anaemia and acute renal failure (Canfield et al., 1969; Chau et al., 1996). The haemolysis occurs rapidly, the release of haemoglobin into the circulation is increased and the urine becomes dark or black. Haematocrit and haemoglobin levels drop within a few hours.

Figure 1.4 A black water fever case (N.H.Phu)

1.4.3.9 Haematological abnormalities

Anaemia is a common manifestation of all types of malaria and poses more problems in children and pregnancy. The causes of anaemia include accelerated destruction of red blood cells, increased splenic clearance in conjunction with ineffective erythropoiesis and gastrointestinal bleeding. In non-immune individuals and in areas with unstable transmission, anaemia can develop rapidly and transfusion is often required (Phillips et al., 1986; Hien et al., 1996). Red blood cell deformability may also contribute to the development of severe anaemia (Dondorp et al., 1999). In Africa children may develop severe anaemia as a result of repeated malarial infections (Marsh et al., 1995).

Slight coagulation abnormalities are common in falciparum malaria. The fibrin degradation products are elevated (Jaroonvesama et al., 1972; Sucharit et al., 1975).

Increased coagulation cascade activity with antithrombin III depletion and prolongation of the prothrombin time can be seen in severe cases (Pukrittayakamee et al., 1989; Clemens et al., 1994). Fewer than 5% of patients with severe malaria have significant bleeding with evidence of disseminated intravascular coagulation (Phillips et al., 1986). Mild thrombocytopenia is usual but it may be profound in severe cases (Skudowitz et al., 1973; Kelton et al., 1983; Looareesuwan et al., 1992).

Haematemesis, presumably from stress ulceration (Hien et al., 1996) or corticosteroid therapy, may also occur (Warrell et al., 1982; Hoffman et al., 1988). Conjunctival haemorrhage may be seen in severe jaundice patients.

A vigorous eosinoplilic response shortly after completing antimalarial therapy predicts a good recovery from anaemia in acute malaria (Camacho et al., 1999).

Figure 1.5 Conjunctival haemorrhage in severe falciparum malaria (N.H.Phu)

1.4.4 Malaria in pregnancy

In malaria areas with intense transmission anaemia and a reduction in birth weight are common in infected pregnant women (Brabin, 1983; McGregor, 1983) but infected mothers remain asymptomatic despite intense parasitization of the placenta due to sequestration of parasitized erythrocytes in the placental microcirculation (Bray et al., 1979). In unstable malaria areas the non-immune and primi-gravidae are usually the most affected, pregnant women often develop severe malaria, particularly in the second and third trimesters (White and Ho, 1992). Hypoglycaemia, anaemia, cerebral malaria, acute pulmonary oedema and fetal loss are particular complications and consequently there is an increase of infant and childhood mortality (Menon, 1972; Brabin, 1983; Gaain et al., 1985; Looareesuwan et al., 1985; Nosten, et al., 1991). Foetal distress, premature labour and stillbirth or low birth weight are commonly seen. The mortality is approximately 50% of infected pregnant women. Congenital malaria occurs in fewer than 5% of newborns whose mothers are infected and is related to the parasite density in maternal blood and in the placenta (Quinn et al., 1982; Hulbert, 1992; Balaka et al., 2000). *P. vivax* malaria in pregnant patients is also associated with mild anaemia and a reduction in birth weight, this effect is greater in multigravidae than in primigravidae (Nosten et al., 1999). The relation between malaria and pregnancy is poorly understood.

1.5 Diagnosis of malaria

The diagnosis of malaria is based on epidemiological information, clinical symptoms and parasitological methods. The clinical diagnosis has low specificity because the symptoms of malaria are nonspecific. The WHO recommendations of clinically malaria diagnosis based on fever or history of fever and the transmission settings are still valid (WHO, 2000). The two principal parasitological methods are light microscopy and rapid diagosis tests.

1.5.1 Conventional methods

Peripheral blood smear examination for malaria with light microscopy is the gold standard in confirming the diagnosis of malaria. Thick and thin films (Figure 1.6 and 1.7) from the peripheral blood should be made on clean, grease-free glass slides. Making good thin films requires technical skills. Thick film should be dried thoroughly otherwise it may wash away during staning. Thin film should be dried then fixed in pure methanol. A number of Romanowsky stains like Giemsa's at pH 7.2, Field's, Wright's can be used. Thick films are ideally stained by Giemsa's stain for screening the parasites. Thick films also have the advantages of concentrating the parasites by 20 to 40 folds compared with thin film, thus increasing diagnostic sensitivity. Thin films are ideally stained by Field's stain, and are more accurate for detecting the malaria species and parasite counting. The test takes about 60 to 90 minutes depending on the skill of the technicians, especially the time spent on reading the smear. If there is a high degree of suspicion but the result is negative, blood smears must be repeated every 6 to 8 hours. Both thick and thin films should me examined on the microscope. Thin films are examined under oil immersion and the best area is the tail of the films. The peripheral blood smear provides information on the species, the density of parasitaemia and the stage of parasite. The number of

infected red cells per 1,000 red cells on the thin film should be counted. The parasitaemia is expressed as the number of parasitized erythrocytes among 1,000 red cells, and this figure is converted to the number of parasitized erythrocytes per microlitre. At a low parasitaemia (<1/1,000 RBC on the thin film) the thick film should be counted; the number of parasites per 400 white cells is noted and an average of 8,000 WBC/µl is taken as standard. The "plus system" is less precise as variation in the thickness of the film results in false variation in parasite count, but this system is still widely used. In severe malaria, a poor prognosis is indicated by a predominance of more mature P. falciparum parasites (>20% of parasites with visible pigment), by the presence of circulating schizonts in the peripheral blood film (Silamut and White, 1993). Pigment-containing neutrophils (Figure 1.8) are noticed in severe malaria for the prediction of diseases and prognosis (Phu et al., 1995) and are associated with cerebral malaria and with death in children with severe malaria (Lyke et al., 2003). The presence of malarial pigment in monocytes (Figure 1.9) should also be noted because it may provide a clue to recent infection if malarial parasites are not detectable (Day et al., 1996). After the peripheral blood smears become negative, malaria pigment is evident in peripheral blood leukocytes, bone marrow and intradermal smears for several days. Phagocyte pigments can be used to diagnose severe malaria in pretreated, parasite-negative patients (van der Berghe et al., 1951; Day et al., 1996). The sensitivity and specificity of the microscopic test depend on the experience of the microscopist; the quality of the slides, stains, microscopes, and the time spent examining the smears.

Alternative microscopic methods:

- Acridine orange is used as a direct staining method (Quantitative Buffy Coat system or Kawamoto technique).

- DNA and RNA of malaria parasites can be stained with fluorescent dyes and examined under ultraviolet light microscopy.

1.5.2 Rapid diagnosis tests (RDT)

The immunochromatographic tests for malaria diagnosis have been developed in the recent years. The rapid diagnosis tests (Figure 1.10) is based on the capture of parasite antigens from the peripheral blood using monoclonal or polyclonal antibodies, and target the histidine-rich protein 2 of *P. falciparum* (Pf HRP2) and the parasite lactate dehydrogenase (pLDH) of all 4 human malaria species. Different test kits have been produced like dipstick, strip, card, pad and well. A finger-prick blood sample or anticoagulant blood or plasma can be used. The comparison of peripheral blood smear examination and RDTs for malaria is shown in Table 1.5.

Table 1.5 Comparison of Peripheral Blood Smear Examination and RDTs for Malaria

	Peripheral Smear	Rapid Diagnostic Tests
Material	Slides and blood films	Test strips and fresh blood
Equipment	Microscope	Kit only
Training	Trained microscopist	'Anyone with a little training
Test duration	60 minutes or more	30 minutes
Test result	Direct visualization of the parasites	Color changes on antibody coated lines
Capability	Detects and differentiates all plasmodia at different stages	Detects malaria antigens (PfHRP2/PMA/pLDH) from asexual and/or sexual forms of the parasite
Detection threshold	20 parasites/μL of blood	100-500/μL for *P. falciparum*, higher for non-falciparum
Species differentiation	Possible	Cannot differentiate among non-falciparum species
Quantification	Possible	Not possible
Differentiation between sexual and sexual stages	Possible	Not possible
Disadvantages	Availability of equipment and skilled microscopists, particularly at remote areas and odd hours	Unpredictable efficiency at low and very high parasite; cross reactions among plasmodial species and with auto-antibodies; persistence of antigens

Figure 1.6 Peripheral blood smear of *P. falciparum* (thick film) (N.H.Phu)
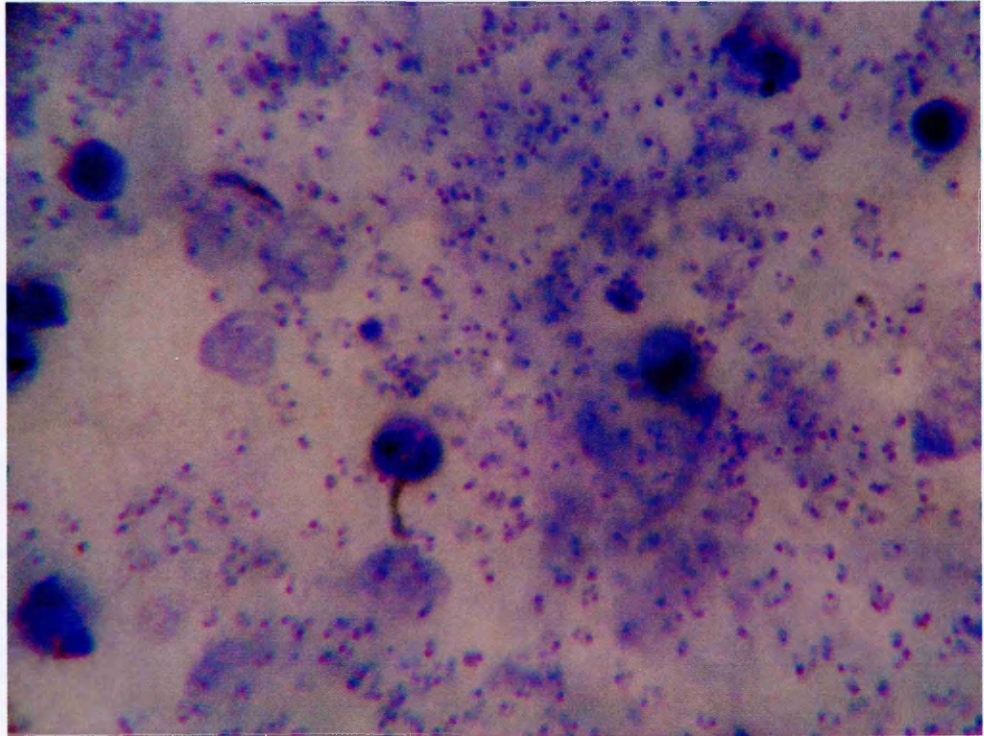
Figure 1.7 Peripheral blood smear of *P. falciparum* (thin film) (N.H.Phu)
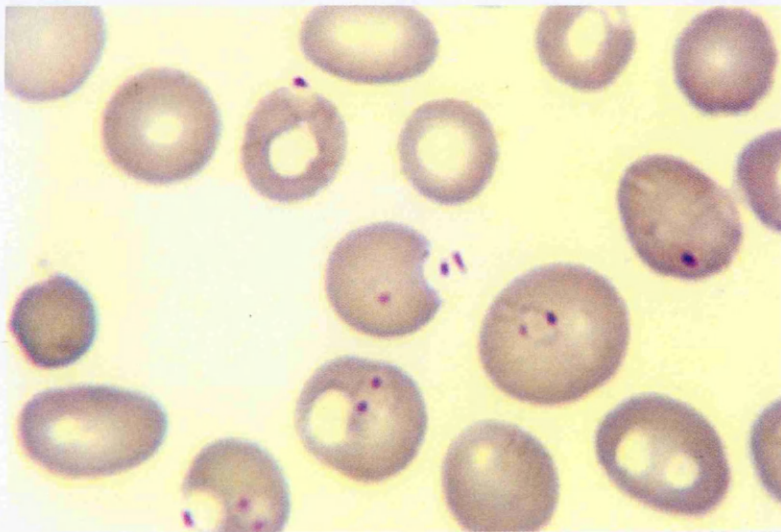
Figure 1.8 Pigment-containing neutrophil (N.H.Phu)
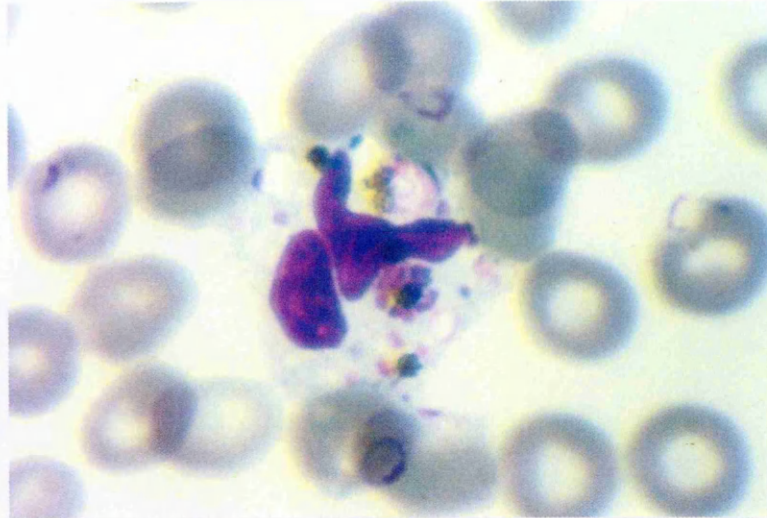
Figure 1.9 Pigment-containing monocytes (N.H.Phu)
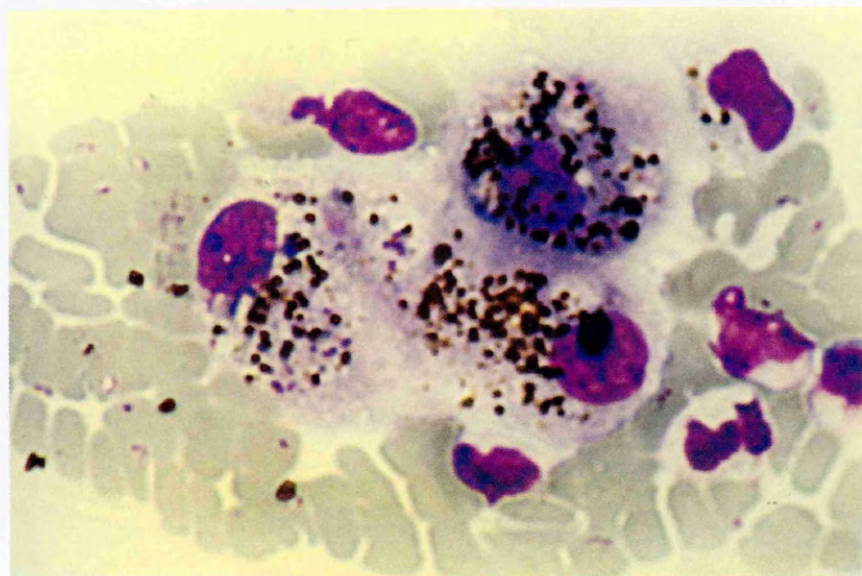
Figure 1.10 Rapid diagnosis test for malaria (N.H.Phu)
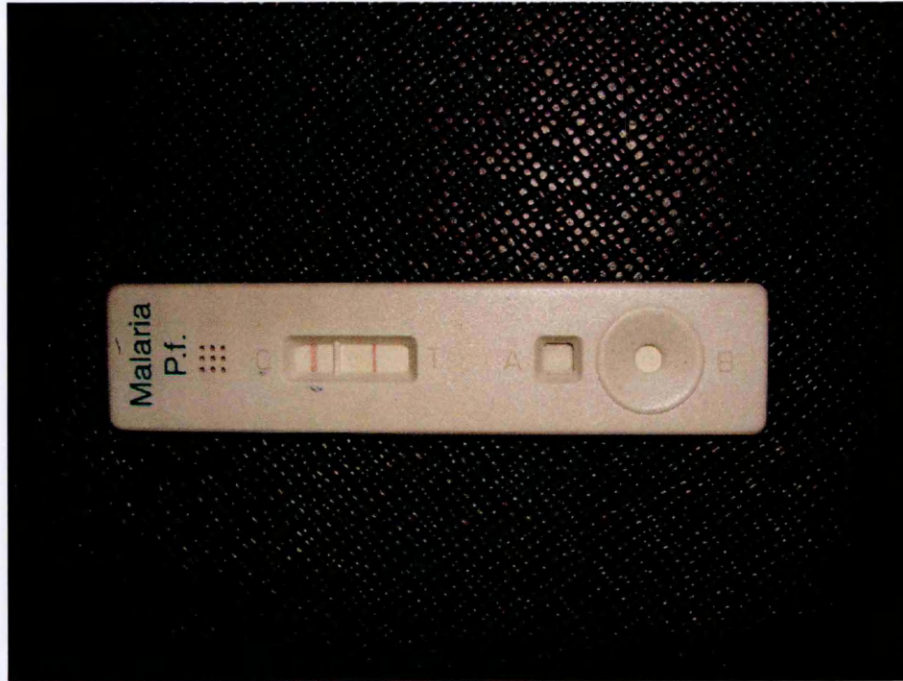
1.5.3 Other tests for malaria diagnosis

- Polymerase Chain Reaction (PCR): The morphologic characteristics provides valuable criteria for determination of malaria parasite species, but they occasionally fail to differentiate between species that share morphological characteristics (especially *P. vivax* and *P. ovale*) or in cases where parasite morphology is altered by drug treatment or improper storage of the sample. In such cases, molecular diagnostic tests using PCR technique have to be found to be highly sensitive and specific for detecting all 4 species of human malaria. It is also reportedly 10- fold more sensitive than microscopy (Snounou et al., 1993, Sidhu and Madhubala, 2000).

- Detection of antimalarial antibodies (Serology): Malarial antibodies can be detected by indirect immunofluorescence (IFA) or enzyme-linked immunosorbent assay (ELISA). These tests are useful in epidemiological surveys, do not detect current infection and may be important in assessing the response to malaria vaccines in future.

- Flowcytometry: Flowcytometry and automated haematology analyzers have been found to be useful in malaria diagnosis during routine blood count (Hanscheid et al., 2001; Kramer et al., 2001).

- Mass spectrometry: This new method can detect (in vitro) 10 parasites/μL of blood and measure many samples in a few seconds (Demirev et al., 2002; Mann, 2002)

1.6 Laboratory findings

There is a normochromic, normocytic anaemia in malaria. The white blood cell count is usually low to normal, although it may be raised in severe infections or in case of superinfection. The platelet count is usually lower than 100,000/μl, and it may be profound in severe cases (Skudowitz et al., 1973; Looareesuwan et al., 1992; Supanaranond et al., 1992). Prolonged prothrombin and partial thromboplastin times may occur in severe malaria (Pukrittayakamee et al., 1989; Clemens et al., 1994).

Metabolic acidosis with low plasma concentrations of glucose, sodium, bicarbonate, calcium, phosphate, and albumin are documented in severe malaria. There are the elevations in lactate, blood urea nitrogen, creatinine, urate, myoglobin, liver enzymes, conjugated and unconjugated bilirubin in severe infections.

In adults and children with cerebral malaria, the mean opening pressure at lumbar puncture is 160 mmH$_2$O of cerebrospinal fluid. The CSF is usually normal or has a slightly elevated total protein level (<1.0g/L) and cell count (<10/μL) with all lymphocytes. High lactate concentration in CSF indicates a poor outcome.

1.7 Antimalarial drugs

This section describes the key features of the antimalarial drugs relevant to the work of this thesis and does not attempt to provide a comphrehensive overview of all antimalarial drugs.

Quinine: Quinine is the main alkaloid of cinchona bark. This bark contains a mixture of 10 alkaloids, but only 2 of them (quinine and quinidine) have a higher potency against human malaria parasites. Quinine has a history of more than 350 years and is still widely used for severe chloroquine-resistant falciparum malaria. The French chemists Pierre Pelletier and Joseph Caventou isolated the alkaloid quinine from cinchona bark in 1820. Quinine acts principally as a blood schizonticide, on the mature trophozoites stage of parasites. It has some activity against immature falciparum gametocytes and is an effective gametocytocide in vivax, ovale and malariae parasite. It is still the drug of choice in the management of severe falciparum malaria in areas with known resistance to chloroquine and artemisinin based combination therapy (ACT) is not available. Quinine sulphate is well absorbed when given orally in healthy subjects, and in patients with malaria (Paintaud et al., 1993: Supanaranond et al., 1991; White, 1987). Peak plasma concentrations are achieved within in 1-3 hours after oral dose (Hall et al., 1973) and plasma half-life is about 11 hours. Studies of intravenous quinine in severe malaria (Looareesuwan et al., 1985) show no evidence of extensively toxic effect. The use of intramuscular quinine has been controversial for many years, but recent studies (Hien et al., 1996) suggest good bioavailability, safety profile and effectiveness even in very young children (<2 years) with severe malaria (van Hensbroek et al., 1996; Waller et al., 1990; White, 1995). Quinine is predominantly (80%) eliminated by hepatic biotransformation (White, 1985) and only 10-20% is excreted unchanged in the urine. Total systemic clearance is reduced in uncomplicated malaria, and may be reduced in

severe malaria (White et al., 1982; White, 1987). Quinine has minor adverse effects and the typical syndrome known as "cinchonism" occurs with plasma concentrations over 5 mg/L (Powell and McNamara, 1966). This consists of tinnitus, headache, vertigo, nausea, photophobia, blurred of vision and high tone deafness (Roche et al., 1990). Hypoglycaemia is the most important problem. In therapeutic doses quinine is a potent stimulus to pancreatic insulin secretion (White et al., 1983) and hyperinsulinaemic hypoglycaemia is particularly in severe infection and in pregnant women (Looareesuwan et al., 1985). Intravenous injections may cause acute cardiovascular toxicity presumably because transiently toxic blood concentrations occur before adequate distribution (Davis et al., 1988). Quinine can cause postural hypotension in acute malaria (Supanaranond et al., 1993). Many strains of *P.falciparum* resistant to chloroquine are cross-resistant to quinine (WHO, 1981). A multicentre, open-label, randomised comparison of parenteral quinine and parenteral artesunate in severe falciparum malaria showed that the mortality in the quinine group was 22% (SEAQUAMAT, 2005).

Artemisinin and its derivatives: Artemisinin or Qinghaosu is a sesquiterpene lactone with an endoperoxide bridge (C-0-0-C) extracted from the leaves of the herb *Artemisia annua*. It has been used as the treatment of fevers in China for more than 1000 years. The active antimalarial constituent of this plant was isolated in 1971. The artemisinin and its derivatives are the most rapidly effective of the antimalarial drugs and retain efficacy even against multi-drug-resistant parasites. Artemisinin and its derivatives are short-acting antimalarial drugs that kill parasites more rapidly than conventional antimalarials, and are active against both the sexual and asexual stages of the parasite cycles. Increasing evidence demonstrates that their widespread deployment leads to a reduction in transmission potential, and their early use may prevent the progression to severe falciparum malaria. Dihydroartemisinin, the reduced lactol derivative of artemisinin, is

the common biologically active metabolite of artesunate and artemether. Artesunate and artelinate are the water-soluble derivatives; artemether and arteether are both oil soluble. These four drugs are the semi-synthetic derivatives of the parent artemisinin compound. The artemisinin and its derivatives are well tolerated and are relatively safe. Rectal preparations of artemisinin derivatives appear to have acceptable therapeutic efficacy, including in severe malaria (Hien et al., 1992; Nosten et al., 1998; Karunajeewa et al., 2003 and 2007; Gomes et al., 2008). There has been no documented significant toxicity. In volunteer studies, a depression of reticulocyte counts has been noted, but increased anaemia has not been observed in clinical studies. It has not been found to have any teratogenic effects on the embryo in animal studies. The principal toxicity in animals has been an unusual dose related selective pattern of neuronal cell damage affecting certain brain stem nuclei. Neurotoxicity related to intramuscular administration of the oil based artemether and arteether was noticed in laboratory experiments (Brewer et al., 1994; Petras et al., 1997; Genovese et al., 1998).

ARTEMISININ: Artemisinin is the natural substance extracted from the leaf of *Artemisia* annua L. It has been formulated as a crystalline powder and can be administered orally as a tablet, or in a capsule, and rectally as a suppository. Initially, in China, it was also given intramuscularly as an oil suspension and as a water suspension (China Cooperative Research Group on Qinghaosu and Its derivatives as Antimalarials Metabolism and pharmacokinetics of Qinghaosu and its derivatives, 1982). Recent pharmacokinetic studies of the artemisinin derivatives using high performance liquid chromatography with electrochemical detection (HPLC-ECD) and bioassay in healthy volunteers and non-complicated malaria patients showed that the pharmacokinetic parameters of orally administered artemisinin are similar in both groups: artemisinin is rapidly absorbed with the peak concentration recorded at 1-2 hours after administration,

but the absorption is incomplete (Alin et al., 1996; De Vries and Dien, 1996; De Vries et al., 1997; Benakis et al., 1997; Ashton et al., 1998). The elimination half-life of artemisinin is of 2 to 5 hours (Alin et al., 1996; De Vries et al., 1997). The metabolites and the potential for drug interactions have not been fully characterised. The rectal bioavailability is 30% relative to the oral dose although there was large interindividual variation. Therapeutic concentrations could be achieved with artemisinin suppositories (Koopmans et al., 1998; Sidhu et al., 1998). Early studies in China suggested that an optimum treatment was 3g of artemisinin given over 3-5 days (10-20 mg/kg in the 1st day at (+0 and +4hrs) then 10mg/kg/day in subsequent days) (Li et al., 1994). However, studies using monotherapy regimens with less than a 7-day treatment course (oral or rectal), and with a 28-day follow-up period, confirmed that the true recrudescence rate was unacceptably high (20-50%) (Hien, 1994). The combination of artemisinin (20 mglkg given on one day or 3-day dose) and a long elimination half-life drug such as mefloquine would seem to overcome many of the problems of high recrudescence rates seen with artemisinin monotherapy. Several clinical trials showed that this is an effective treatment of multi-drug resistant *P. falciparum* malaria (Hien et al., 1994; Bich et al., 1996; Le et al., 1997). Artemisinin suppositories remain an effective treatment for *P. falciparum* malaria in remote areas where facilities for parenteral antimalarials maybe limited. In the early 1990s artemisinine suppositories were shown to be as effective as parenteral antimalarial drugs in clinical trials for the treatment of severe malaria (Hien et al., 1992; Phuong et al., 1997; Vinh et al., 1997). There is no evidence of neurotoxicity in patients who received multiple treatment courses of artemisinin (Kissinger et al., 2000).

ARTESUNATE: Artesunate, the water-soluble hemisuccinate derivative of dihydroartemisinin, was developed as a drug for an intravenous use in the treatment of severe malaria. It is unstable in neutral solutions and therefore only available in powder form as artesunic acid. This powder requires reconstruction with 5% sodium bicarbonate solution immediately before use. At neutral pH hydrolysis to DHA is rapid. Artesunate is the artemisinin derivative most widely used. It is administered orally, intravenously, intramuscularly or rectally. Artesunate is hydrolysed very rapidly after intravenous injection to DHA in 2 minutes (Yang et al., 1985). After oral administration artesunate is also hydrolysed to DHA and unchanged artesunate is detectable in plasma (Batty et al., 1998). The mean absolute oral bioavailability of the drug in patients with acute malaria was 61%; the absorption and elimination of oral artesunate was rapid with mean elimination half-life of 43 minutes (Newton et al., 2000). The oral antimalarial bioavailability following artesunate was significantly higher than that after artemether (Suputtamongkol et al., 2001). After repeated oral administration of artesunate a time-dependent decline of artesunate and DHA concentrations in plasma occurs, the mechanism of this decrease of concentration is unclear (Khanh et al., 1999). Despite rapid clearance of artesunate and DHA in patients with uncomplicated falciparum malaria prompt parasite and fever clearance are achieved and once-daily administration has been shown to be highly effective with similar parasite and fever clearance times to those treated with a twice-daily regimen (Nosten et al., 1994). Several clinical trials in South East Asia have shown that artesunate, given as single oral dose (2-4mg/kg) or for 3 days (4mg/kg for the 1st day then 2mg/kg for 2nd and 3rd day) in combination with mefloquine (15 mg/kg as single dose) was an effective and safe treatment for uncomplicated falciparum malaria (Nosten et al., 1994; Vinh et al., 1997; Price et al., 1997). Many randomized controlled studies in Thailand, Viet Nam, and African countries

indicate that one dose of artesunate (4mg/kg) given daily for 3 days in combination with mefloquine (25mg/kg), chloroquine, sulfadoxine/pyrimethamine or amodiaquine is better than either a single dose of artesunate with these drugs or the other antimalarials given as mono-therapies in terms of recrudescence rates. These studies assessed efficacy for more than 28 days and were supported by PCR genotyping; in addition, treatment with artesunate results in lower gametocyte rates and may reduce transsmission rates (Price et al., 1997; Doherty et al., 1999; von Seidlein et al., 2000). On the basis of pharmacologial study parenteral artesunate has considerable pharmacokinetic advantages over artemether in the treatment of severe falciparum malaria, particularly in patients who are most seriously ill (Hien et al., 2004). Studies in Viet Nam, in patients with uncomplicated falciparum malaria treated with a single rectal dose of 2mg/kg of artesunate (produced in China) confirmed that fever and parasite clearance times obtained were similar to those seen with 20mg/kg artemisinine (Hien, unpublished data). A clinical trial in children with uncomplicated falciparum malaria in Gabon using single dose of 50 mg (equivalent to 0.8-2 mg/kg) of a new pharmaceutical form of this drug (PlasmotrimB Rectocaps, MEPHA) obtained similar results (Halpaap et al., 1998). A trial in Thailand using higher rectal doses of artesunate (15mg/kg/day for 3 days) was compared with oral artesunate (6mg/kg/day) both in combination with mefloquine (25m/kg). The fever clearance time, parasite clearance time and cure rates were similar in both groups (Looareesuwan et al., 1997; Sabchareon et al., 1998). Pharmacokinetic and pharmacodynamic study found that repeated administration of artesunate thermostable suppositories in treating uncomplicated falciparum malaria could extend the duration of therapeutic plasma levels of the drug (Benakis et al., 2006). Artesunate suppositories have been used for the treatment of severe malaria in children (Karunajeewa et al., 2003 and 2006).

ARTEMETHER: Artemether is an oil soluble derivative of artemisinin. It is the methyl ether of dihydroartemisinin and has been used widely in China. It is more stable than artesunate. In healthy volunteers, artemether was absorbed rapidly after oral administration and underwent extensive first-pass metabolism to DHA with the concentration of this metabolite higher than that of the other compound. The mean blood concentrations were reached at 1.2-2.2 hours and the mean elimination half-life was 2.0-2.6 hours (Teja-Isavadharm et al., 1996; Mordi et al., 1997). There was no difference in pharmacokinetics between healthy volunteers and patients with uncomplicated malaria (Bangchang et al., 1994). Plasma antimalarial activity following oral administration is significantly greater than following intramuscular administration (Teja-Isavadharm et al., 1996) because the first-pass biotransformation is inhibited. Some studies demonstrated that grape juice significantly increased the oral bioavailability of artemether and acute renal failure significantly modified the pharmacokinetics of intramuscular artemether with better absorption and bioavailability and a reduction of systemic clearance (Karbwang et al., 1998). Artemether oral antimalarial bioavailability is reduced in acute malaria (Suputtamongkol et al., 2001). Bioavailability of intramuscular artemether was also shown to be highly variable and may be associated with an inadequate response in children with severe malaria especially in the presence of respiratory distress (Murphy et al., 1997). Monotherapy with oral or intramuscular artemether (1-4 m/kg/day for 3-5 days) resulted in rapid fever and parasite clearance but the recrudescence rate was high (25-40%). Artemether given in combination with other antimalarial drugs with long half-lives such as mefloquine have demonstrated cure rates of 95-98% (Price et al., 1995). Since 1992, 2042 patients had been recruited to a series of trials using a new combination of artemether and lumefantrine (benflumetol). Four tablets of artemether and lumefantrine (1 tablet = 20 mg of artemisinin + 120 mg of lumefantrine) given at 0, 8, 24, 48 hours, had

shown to be very well tolerated by all age groups with a cure rate of 81% (White et al., 1999) . A double-blind trial in Thailand in which patients with uncomplicated multi-drug resistant falcipanun malaria were treated with 6 doses of 4 tablets given at 0, 8, 24, 36, 48, 60 (or 72, 96) hours indicated that the 28 days cure rates were 96.9 - 99.1% (Vugt et al., 1999). A meta-analysis of nine randomised clinical trials comparing artemether and quinine for treatment of severe malaria concluded that these two drugs were equally efficacious in terms of reduction in mortality rate (Artemether-Quinine Meta-Analysis Group, 2001). No evidence of significant toxicity was found in clinical trials in Viet Nam and Thailand (Hien et al., 1996; van Hensbroek et al., 1996; Newton et al., 1999; Hien et al., 2003).

The following artemisinin based combination therapy (ACT) regimens are currently recommended by the WHO in the treatment of uncomplicated malaria (WHO, 2006):

- artemether + lumefantrine,

- artesunate + amodiaquine,

- artesunate + mefloquine,

- artesunate + sulfadoxine–pyrimethamine.

For the parenteral treatment of severe malaria two drugs are recommended by the WHO (WHO, 2006):

- the cinchona alkaloids (quinine and quinidine)

- the artemisinin derivatives (artesunate, artemether and artemotil)

CHAPTER 2

MALARIA IN VIET NAM AND THE RESEARCH SITE

2.1   Study site

2.1.1   Geography

Viet Nam is an S-shaped peninsula in South East-Asia, shares borders with Cambodia,

Laos in the west, China in the north, and faces the East Sea in the east and south (Figure

2.1). The country's land area is 335,211.6 square km, lies between North latitudes 8°02'

and 23°23', and East longitudes 102°08' and 109°28' and is completely within the tropical

zone. Viet Nam has 4,510 km inland borderline and 3,260 km coastline. Three quarters of

the land area consist of the mountainous regions (the Truong Son mountain ridge); the

two major valleys (the Red river delta and the Mekong delta) and the coastal plains.

2.1.1.1 Climate

Viet Nam is located in a tropical and temperate zone. The climate is characterized by

sunny time, monsoon influence, heavy rainfall and high humidity. The climate of

mountainous regions is temperate. The annual average temperature is 23°C in the North

of Viet Nam, 25°C in the Central and 26°-27°C in the South. The Northern provinces have

4 distinguished seasons; the winter is cold (15°-17°C) but the summer is hot and humid

(28°-29°C). However the Central and the South have only two seasons: the hot and dry

season (from November to April) and the rainy season (from May to October). The

annual average rainfall of Viet Nam is around 1600 mm and may rise up to 200-300 mm

per month in the rainy season. The two delta areas are usually flooded during the rainy

season. The two main rivers of the country are the Red River in the North and the Mekong River in the South. Parts of the Central of Viet Nam have a shorter rainy season (April-June) but endure more than 10 tropical storms per year.

Figure 2.1 Viet Nam map

2.1.1.2 Population

The Vietnamese population was reported to be 84,155,800 in 2006, 49.1% of them males. The population density was 252 people per square kilometres, with most (73%) of the population living in rural areas. Viet Nam has 54 different ethnic groups. The largest groups, Kinh, account for 87% of the total population, and most live in low land regions, speak Vietnamese (an alphabet based on the Latin script), and has one close ethnic group (Muong). The other two large minority groups are Chinese and the Khmer origins live mainly in the South, around the Mekong Delta. The Cham are concentrated in the costal area of the Central region. The other 50 ethnic groups traditionally inhabit the highland areas, and have been living in endemic regions of malaria for centuries. Population growth is very high, nearly one million per year. The total population in 1994 was 72,509,500, and in 2007 were 85,154,900. Since 1980s, the government decided to implement the liberal economic policy and there have been many controlled and uncontrolled population movements from plain to highlands.

2.1.1.3 Economy and Environment

Viet Nam is a developing country that has changed from a centrally planned to liberal economy. The GDP per capita income was estimated to be US$ 213 in 1994 and increased to US$ 722 in 2006. GDP growth was of 4% in 1998 and rose to 8.2% in 2006. Although the economy is growing in many parts of the country in the central and northern highlands where the incidence of malaria is still high the economic situation remains largely unchanged. The ethnic minority population lives in scattered villages with many difficulties. Air and water pollution threaten the life of people. Urban industrialization

and population migration are degrading the environment of big cities. Uncontrolled agricultural practices contribute to deforestation and soil degradation.

2.1.2 Health services

Viet Nam is committed to the primary health care approach. A public health system was set up to improve the health status in remote rural regions. In 2006 Viet Nam had 954 hospitals, 847 local medical centres, 10,672 commune health stations, 52,800 doctors, 5,500 pharmacists, 48,800 medical assistants, 55,400 nurses and 19,000 midwives. On average there were 4.4 doctors per 10,000 of population in 1996, and this number increased to 6.3 in 2006. The government takes the major responsibility for provision of health care although there is a growing private sector. Health services are delivered through commune health stations, district hospitals and provincial hospitals. The hospitals in three big cities (Ha Noi, Da Nang and Ho Chi Minh City) act as tertiary referral centres for severe patients. Commune health stations in malaria areas are the most peripheral unit where most of malaria patients can access the health care. Private medical services have been improved through out the country but the prescription practices and drug distribution have been uncontrolled. Almost all antimalarials are available for sale. The wide use of effective antimalarials such as chloroquine, quinine, fansidar, artesunate, mefloquine, may contribute to reduction of malaria cases by allowing quick unlimited access, but the practice risks encouraging the development of drug resistant malaria parasites.

2.1.3 National Malaria Control Programme (NMCP)

Structure:

The NMCP is directed by a national steering committee chaired by a Vice-Minister of Health. The committee manages the malaria control programme with the assistance of

three Institutes for Malariology, Parasitology and Entomology (IMPE) that are in Ha Noi, Quy Nhon and Ho Chi Minh City (HCMC). The three institutes are responsible for malaria control activities in their regions. The IMPE in Hanoi is responsible for technical advice, training, research, data analysis, and helps the peripheral health communes in case of malaria outbreaks in the Red River delta and northern Viet Nam. The IMPE in Quy Nhon manages central and southern highlands. The IMPE in HCMC manages the Mekong delta and southern provinces. At provincial level, there is a Centre for Malaria Control and Malaria Prevention that is responsible for all malaria control activities at provincial and district level of the province. This centre attaches to the provincial health service. There is a district team of hygiene, epidemiology and malaria at district level. In provinces where malaria is not a major problem, the provincial malaria centre is integrated with the provincial Centre for Preventive Medicine.

Policies:

The aims of the NMCP are:

1. To improve the capacity of early diagnosis

2. To reduce malaria deaths by early and effective treatment

3. To prevent malaria epidemics or at least detect them early and control them

4. To reduce the incidence of malaria in high-risk population by applying vector control and personnel protection programmes.

There are no specific policies for protection of pregnant women and children.

Based on the results of epidemiological and clinical data, the NMCP prepare detailed treatment guidelines periodically. In Viet Nam, resistance to anti-malarial drugs has been increasing over many years. The first clinical report of chloroquine treatment failure from

South Viet Nam appeared in 1961. Local studies conducted in 1980s-1990s showed an increase in failure rates to 65% for chloroquine, 70% for sulfadoxine-pyrimethamine and 15% for quinine (Morillon et al., 1996; Cong, 2000). Quinine was the drug of choice in the treatment of falciparum malaria in combination with pyrimethamine-sulfadoxine or tetracycline. Chloroquine and primaquine have been recommended for vivax malaria until today. *P. falciparum* resistant rate in vitro to chloroquine was 92% (61/66), to quinine was 18% (11/9) and to mefloquine was 6% (4/62) and clinical trials showed that failure rates with mono therapy of up to 60% (Hien et al., 1997). Since 1997, the artemisinin derivatives have been proved to be rapidly effective and non-toxic anti malarial drugs and the NMCP put the new drug in the second-line treatment to be used when uncomplicated falciparum malaria did not respond to the first-line recommendation that was quinine. Artemisine for 3 days in combination with mefloquine in a single dose of 15mg/kg was the best regimen. There was a report of a 34% failure rate with mefloquine monotherapy in South Viet Nam (Le et al., 1997). For severe malaria treatment, quinine IV was the only choice until artesunate/artemether was produced. In 2003, the drug policy in Viet Nam recommended the use of artesunate monotherapy for seven days or the combination of dihydroartemisinin, piperaquine, trimethoprim and primaquine (CV8) for the confirmed falciparum malaria. The second–line treatment was artesunate 8mg/kg for three days plus mefloquine 15mg/kg on day 3. Recent policy changes recommend the combination of dihydroartemisinin-piperaquine as the first-line treatment for all cases of confirmed falciparum malaria nationwide. Chemoprophylaxis is not recommended in Viet Nam. The Viet Nam malaria map is showed in Figure 2.2. The management system of the National Malaria Control Program as showed in Figure 2.3. The national guidelines for malaria treatment are shown in Table 2.1 (NMCP, 2009).

Figure 2.2 Viet Nam Malaria Map
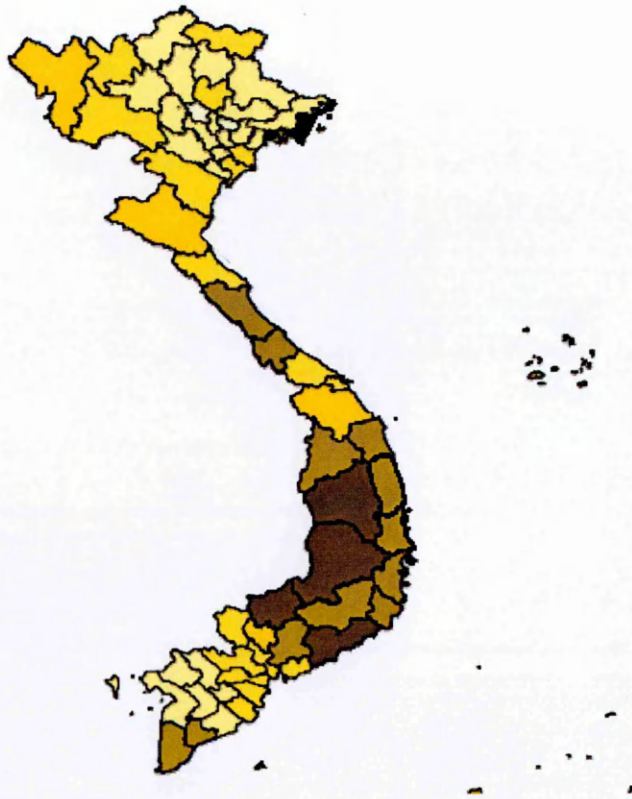
No of confirmed cases, 2000
- 1 - 10
- 11 - 100
- 101 - 500
- 501 - 1000
- 1001 - 5000
- 5001 - 10000
- Not available
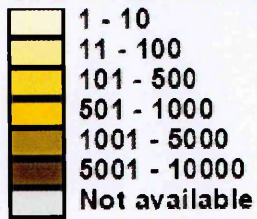
(Source: WHO, WPR, 2002)

Figure 2.3

MANAGEMENT SYSTEM OF THE NATIONAL MALARIA CONTROL PROGRAM

Government

Ministry of Planning & Investment

Ministry of Health

Ministry of Finance

National Steering Committee
- Leadership of the MOH
- Military Health Department
- Departments, Institutions
- Leading professors

Sub-Committee Treatment

Sub-Committee Vector control

Sub-Committee Epidemiology

Sub-Committee Training & Health Education

Military Health Dept.
- Department leader
- Other departments

Prov. Steering Committee
- Leader of People's Committee
- Director of PHS
- Malaria control center

Health Service of other sectors
- Leaders
- Health centres

UNITS

District Steering Committee
- Leader of People's Committee
- DHC
- District team of HEM
- others

UNITS

Communes

Table 2.1 The national guidelines for malaria treatment (NMCP, Viet Nam, 2009)

Patients group	Clinical diagnosed malaria	*P. falciparum* malaria	*P. vivax* malaria	*P. malariae* malaria	Mixed infection
<3 years of age	Dihydroartemisinin-Piperaquine	Dihydroartemisinin-Piperaquine	Chloroquine	Chloroquine	Dihydroartemisinin-Piperaquine
≥3 years of age	Dihydroartemisinin-Piperaquine	Dihydroartemisinin-Piperaquine + Primaquine (1 day)	Chloroquine + Primaquine (14 days)	Chloroquine	Dihydroartemisinin-Piperaquine + Primaquine (14 days)
Pregnant woman < 3 months	Quinine + Clindamycin	Quinine + Clindamycin	Chloroquine	Chloroquine	Quinine + Clindamycin
Pregnant woman ≥ 3 months	Dihydroartemisinin-Piperaquin	Dihydroartemisinin-Piperaquin	Chloroquine	Chloroquine	Dihydroartemisinin- Piperaquine

2.1.4 Hospital for Tropical Diseases (HTD)

The Hospital for Tropical Diseases is located in HCMC, and serves the city and the southern provinces of Viet Nam (population around 43,000,000). The hospital has 550 beds, including two (paediatric and adult) intensive care units and 16 wards for non-severe infectious diseases. HTD has laboratories for haematology, biochemistry, microbiology, parasitology and serology. The hospital acts as a primary, secondary and tertiary referral centre for patients with infectious diseases and also involves in the control of epidemics or emerging diseases of the south of Viet Nam.

2.1.5 Oxford University Clinical Research Unit (OUCRU)

The Oxford University Clinical Research Unit was opened in January 1991, funded by the Wellcome Trust of Great Britain. The unit is located in the Hospital of Tropical Diseases and serves as a collaborative centre between the HTD and the University of Oxford. The unit started as an 8-bed ward (named as Malaria Research Unit) for the treatment of all severe malaria cases. Over 18 years the OUCRU has expanded and implemented research on many infectious and emerging diseases such as malaria, dengue, tetanus, typhoid, AIDS, tuberculosis, central nervous system infection, avian and swine influenza.

2.2 Aims of this thesis

Malaria in Viet Nam was a major public health problem resulting in serious morbidity and mortality from the 1960s onwards. The contributing factors were delayed diagnosis and treatment due to lack of trained health care services at the village level, national financial difficulties, and the increase of drug resistance in falciparum malaria. The in

hospital mortality of severe falciparum malaria was between 30-50% in Viet Nam (Hien, 1994). Since the implementation of National Malaria Control Programme the malaria situation in Viet Nam has improved compared with that of the 1980s and the early 1990s. However hundreds of thousands of Vietnamese still suffer from this debilitating infection every year. In addition, worldwide malaria causes an estimated 2-3 million deaths per year, mostly among children. Recently, prolonged parasite clearance time following treatment with artemisinin monotherapy and some ACTs has been observed along the Thai-Cambodian border (WHO, 2008). These alarming figures explain the continuing need for malaria research; particularly that focuses on reducing the morbidity and mortality.

Artemisinin and its derivatives have been one of the regimens recommended by the National Malaria Control Programme of Viet Nam since 1995. Many of the early studies of these drugs were conducted in Viet Nam and showed that these antimalarials are the best in terms of clearing malaria parasites (Arnold et al., 1990; Hien and White, 1993; Hien et al., 1992 and 1996; Vinh et al., 1997; Phuong et al., 1997) but the recrudescence rate was high if these drugs are used as monotherapy for less than 7 days (Hien, 1994). The widespread and uncontrolled use of artemisinin and its derivatives in Viet Nam may lead to drug resistance and treatment failure. The incidence of cerebral malaria in Vietnamese adult patients declined from 90% in 1980 to 50% in 1992-1995 while acute renal failure and jaundice have become more common (1980: 6%; 1986-1995: 50-60%) (Hien, unpublished data). One of the largest double blind, randomised controlled trials in severe falciparum malaria compared to artemether and quinine and was conducted between 1991 and 1996 in HTD. This trial indicated that artemether is an effective alternative to quinine for the treatment of severe malaria in adults, but failed to show any

significant difference in mortality (Hien et al., 1996). Why the mortality was not reduced in hospitalised patients even though artemether was administered? The explanations could be: those patients were admitted too late, they might have developed severe complications or they did not have appropriately adjunctive treatment such as renal replacement therapy, correct fluid therapy, intensively nursing care or the pharmacological properties of this formulation of the artemisinin derivatives was not optimal. A comparative study conducted by our group of the pharmacokinetic profile of artesunate and artemether showed artesunate to be the preferred choice for the treatment of severe malaria (Hien et al., 2004).

Therefore, there were important questions that still required further investigation:

1.   Was artesunate a better clinical choice than artemether for the treatment of complicated falciparum malaria?

2.   Which appropriately ancillary treatments should be introduced to reduce the case fatality?

3.   Which markers can also be used as predictors of poor outcome?

Research objectives of this thesis:

1.   To compare the efficacy of artesunte and artemether in treatment of severe falciparum malaria.

2.   To compare two available methods of renal replacement therapy (haemofiltration and peritoneal dialysis) in acute renal failure associated with severe infection.

3.   To assess the fluid therapy in severe malaria.

4.   To evaluation the relation of parasite staging in the peripheral blood to prognosis in severe falciparum malaria.

5. To devise a Malaria Severity Score for predicting the outcome of adults with severe falciparum malaria that can be applied at remote health settings

6. To assess whether there has been prolongation of parasite clearance time after nearly 30 years of implemention of artemisinin treatment in Viet Nam.

2.3 Clinical methods

2.3.1 Scientific and Ethical approval

All study protocols were approved by the Scientific and Ethical Committee of the HTD. Informed consent was obtained from each patient or accompanying relative.

2.3.2 Patients, diagnosis and treatment

All patients with a clinical diagnosis and a positive smear for malaria were admitted to a dedicated Malaria Research Unit (WHO, 2000). Patients with laboratory confirmed malaria were regarded as having severe falciparum malaria if one or more of the following: a score on the Glasgow Coma Scale of less than 11 (indicating cerebral malaria); anaemia (haematocrit, <20 percent), with a parasite count exceeding 100,000 per cubic millimetre on a peripheral-blood smear; jaundice (serum bilirubin, >2.5mg per decilitre [50µmol per litre]), with a parasite count of more than 100,000 per cubic millimetre on a peripheral-blood smear or with serum creatinine, >3mg per decilitre [250µmol per litre]; renal impairment (urine out put, <400ml per 24 hours; and serum creatinine, >3mg per decilitre [250µmol per litre]); hypoglycaemia (blood glucose, <40mg per decilitre [2.2mmol per litre]); hyperparasitaemia (>10 percent parasitaemia) or parasite count of more than 500,000 per cubic millimetre; plasma lactate > 4mmol per litre; arterial pH < 7.34 with standard base excess < -5mmol per litre (metabolic

acidosis); and systolic blood pressure below 80 mmHg with cool extremities (indicating shock).

On admission patients were examined fully, weighed, and baseline blood samples taken for full blood count, clotting studies, biochemistry (BUN, serum creatinine, serum bilirubin, AST, ALT, plasma lactate, potassium, sodium etc.), blood culture and malaria parasite counts. Arterial pH and blood gases were also measured. Chest X-ray and electrocardiography were also performed on admission. Each patient underwent a full clinical examination that included a detailed neurological assessment. A full history was taken from either the patient or attendant relatives. Hydration status was assessed, and patients were hydrated, if necessary with pressure monitoring, via a central venous catheter. A urinary catheter was inserted if needed. All antimalarial drugs were free of charge and other patient costs were met by the study.

Blood was obtained by a finger-prick for haematocrit measurements and blood smears every 4 hours for the first 24 hours and every 6 hours until three consecutive smears were negative for asexual stages of P. falciparum. The degree of parasitaemia was determined on the basis of the number of parasitized red cells per 1,000 red cells (thin film) or the number of parasites per 400 leukocytes (thick film). Parasite counts were derived from the percentage thin film parasitaemia and an estimate of the red cell count derived from the admission haematocrit (haematocrit x 125,000/μL). Thick film counts were obtained assuming a white blood cell count of 8,000/μL.

Hypoglycaemia was corrected with a bolus injection of 50ml of 30 percent dextrose in water and a subsequent maintenance infusion of 5 to 10 percent dextrose in water. A diagnostic lumbar puncture was performed if the score on the Glasgow Coma Scale was below 14. Opening cerebrospinal fluid pressures were measured, the cerebrospinal fluid

was analyzed microscopically, and levels of protein, glucose, and lactate were determined. Renal replacement therapy was performed in patients with acute renal failure. Patients with respiratory failure were ventilated. Paracetamol was given for a temperature above 39°C. A single dose of intramuscular dose of 5mg/kg Phenobarbital was given if Glasgow Coma Scale (GCS) was below 11, and intravenous diazepam, and if necessary, intravenous phenytoin were given for convulsions. Antibiotics were prescribed for suspected cases of bacterial sepsis. Intensive nursing care was implemented. Detailed clinical and nursing observations were recorded a minimum of every 4 hours for the first 24 hours and every 6 hours thereafter.

CHAPTER 3

RANDOMIZED CONTROLLED TRIAL OF ARTESUNATE OR ARTEMETHER IN VIET NAMESE ADULTS WITH SEVERE FALCIPARUM MALARIA

INTRODUCTION

Since 1990 the artemisinin derivatives have been increasingly used for the treatment of severe malaria. They are the most rapidly acting and potent of all the antimalarial drugs. They can be given once daily and are safer and easier to administer compared to quinine. Artemisinin derivatives are now part of recommended first line combination treatment for uncomplicated falciparum malaria everywhere in the tropical world. Recently the largest ever-randomised trial in severe malaria compared intravenous artesunate with intravenous quinine. It enrolled 1,461 patients with severe falciparum malaria in South East Asia before it was stopped by the data and safety monitoring committee (Newton et al., 2003; Dondorp et al., 2005). Artesunate reduced mortality by 34.7% compared to quinine. The number need to treat to save one life ranged from 11 to 20 (International Artemisinin Study Group, 2004; Jones et al., 2007). As a result the new WHO guidelines (2006) recommend parenteral artesunate as the first choice antimalarial treatment of severe malaria in low transmission areas. But the recommendation for higher transmission areas (i.e. much of Africa) has remained artemether or artesunate or quinine (van Hensbroek at al., 1998). The reason for this continued equipoise is that there are important differences in the clinical manifestations and evolution of severe malaria and the treatment responses in

African children compared with adults in Southeast Asia. Notably, in a meta-analysis of randomised trials in severe malaria, intramuscular injection of the oil-based artemisinin derivative artemether did reduce mortality in Southeast Asian adults compared with quinine, but it did not do so in African children (Artemether-Quinine Meta-analysis Study Group, 2001). This raised the possibility that artemether was not inferior to artesunate for the treatment of severe falciparum malaria in SE Asian adults. The largest study from South East Asia in this series was a randomised comparison of artemether and quinine that enrolled 560 adults and was conducted in the Hospital for Tropical Diseases of Ho Chi Minh City (HTD), Viet Nam (Hien et al., 1996). This study reports a subsequent study from this centre in which artemether was compared with artesunate.

METHODS

This was an open label comparison of intramuscular artesunate and intramuscular artemether in patients admitted to HTD with severe falciparum malaria. This study was approved by the Ethical and Scientific Committee of HTD.

Entry criteria

Patients were included in the study if they (or an accompanying relative) gave informed consent, had asexual forms of *P. falciparum* on a peripheral-blood smear, were older than 14 years, were not in the first trimester of pregnancy, were not intravenous drug users, had received less than 3g of quinine or two doses of artemisinin or a derivative in the previous 48 hours, and had one or more of the following: a score on the Glasgow Coma Scale of less than 11 (indicating cerebral malaria); anaemia (hematocrit, <20 percent), with a parasite count exceeding 100,000 per cubic millimetre on a peripheral-blood

smear; jaundice (serum bilirubin, >2.5mg per decilitre [50μmol per litre]), with a parasite count of more than 100,000 per cubic millimetre on a peripheral-blood smear or with serum creatinine, >3mg per decilitre [250μmol per litre]; renal impairment (urine output, <400ml per 24 hours; and serum creatinine, >3mg per decilitre [250μmol per litre]); hypoglycaemia (blood glucose, <40mg per decilitre [2.2mmol per litre]); hyperparasitaemia (>10 percent parasitaemia) or parasite count of more than 500,000 per cubic millimetre; plasma lactate > 4mmol per litre; arterial pH < 7.34 with standard base excess < -5mmol per litre; and systolic blood pressure below 80 mmHg with cool extremities (indicating shock).

Clinical Procedures

On admission patients were examined fully, weighed, and baseline blood samples taken for full blood count, clotting studies, biochemistry (BUN, serum creatinine, serum bilirubin, AST, ALT, plasma lactate), blood culture and malaria parasite counts. Arterial pH and blood gases were also measured. Chest X-ray was also performed on admission. Each patient underwent a full clinical examination that included a detailed neurological assessment. A full history was taken from either the patient or attendant relatives. Hydration status was assessed, and patients were hydrated, if necessary with pressure monitoring, via a central venous catheter. A urinary catheter was inserted. Patients were not included if there was a convincing history of full treatment with quinine or an artemisinin derivative for more than 48 hours before admission. Full treatment with quinine over the 24 hours before admission was defined as 40mg salt/kg on the first day and 30mg/kg on any subsequent day. Patients were also excluded if there was known allergy to one of the artemisinin derivatives or quinine.

Management, Clinical and Parasitological Monitoring

Patients were cared for according to standard recommendations. All patients were given isotonic saline to restore fluid balance, and fluid balance was maintained with saline or 5 percent dextrose in water. When necessary, a central venous catheter was inserted and the central venous pressure maintained at 5cm of water. Blood was transfused if the hematocrit fell below 20 percent. Hypoglycaemia was corrected with a bolus injection of 50ml of 30 percent dextrose in water and a subsequent maintenance infusion of 5 to 10 percent dextrose in water. Detailed clinical and nursing observations were recorded a minimum of every 4 hours for the first 24 hours and every 6 hours thereafter.

A diagnostic lumbar puncture was performed if the Glasgow Coma Scale was below 14. Opening cerebrospinal fluid pressures were measured, the cerebrospinal fluid was analyzed microscopically, and levels of protein, glucose, and lactate were determined. Haemofiltration was started in patients with established renal failure. Patients with respiratory failure were ventilated. Paracetamol was given for a temperature above 39°C. A single dose of intramuscular Phenobarbital was given if Glasgow Coma Scale was below 11, and intravenous diazepam, and if necessary, intravenous phenytoin were given for convulsions. Antibiotics were prescribed for suspected cases of bacterial sepsis.

Blood was obtained by a finger-prick for haematocrit measurements and blood smears every 4 hours for the first 24 hours and every 6 hours until three consecutive smears were negative for asexual stages of *P. falciparum*. The degree of parasitaemia was determined on the basis of the number of parasitized red cells per 1,000 red cells (thin film) or the number of parasites per 400 leukocytes (thick film).

On discharge a detailed neurological examination were performed. A full autopsy was performed on patients who died of malaria if permission could be obtained from relatives. All information was recorded in the patients' records and then triple-entered in a computer database.

Antimalarial treatment and randomization.

Patients were randomized to treatment with either intramuscular artesunate or intramuscular artemether.

Artesunate (Guilin No 2 Pharmaceutical Factory, Guangxi, People's Republic of China) (Figure 3.2) was given in a dose of 2.4mg/kg body weight on admission, then 1.2mg/kg was given daily until oral medication could be taken reliably. Each 60mg vial contained anhydrous artesunic acid that was dissolved initially in 1ml 5% sodium bicarbonate and then mixed with 5ml of 5% dextrose before injecting as a bolus into an indwelling intravenous cannula.

Artemether (80mg per millilitre; Kunming Pharmaceutical Company, Kunming, People's Republic of China) (Figure 3.3) was given in a dose of 3.2mg/kg body weight on admission, followed by 1.6mg/kg daily until oral medication could be taken.

Both drugs were given intramuscularly to the anterior thigh. Randomly selected ampoules were checked for sterility and potency. When the patients were able to take tablets, oral artesunate was given in a dose of 2mg/kg/day to complete a total course (including parenteral treatment) of 7 days, providing a total cumulative dose between 17 and 18mg/kg over 7 days.

Treatment failure: If parasitaemia did not fall by >75 % of the admission value within 48 hrs the treatment will be changed to intravenous quinine (20mg/kg followed by 10mg/kg 8 hourly).

Randomization and Blinding

The randomization was generated from random number tables. The drugs for each patient were placed in a coded sealed envelope, and the envelopes were randomized in blocks of 20. Once a patient was enrolled in the study the envelope was opened. Subsequent analysis of efficacy was on an intention-to-treat basis. To maintain blinding, a separate team of nurses, who were not otherwise involved with the care of the study patients, drew up and gave the injections. The drugs were kept in an opaque packet in a locked cabinet during the study.

Endpoints

The primary endpoint was death during the first 28 days following recruitment to the study.

The secondary endpoints were:

1. Time to 50% reduction in parasite count

2. Time to 90% reduction in admission parasite count

3. Time to discharge from hospital

4. Incidence of complication during hospital admission

Statistical Analysis

Results were entered into a database (Microsoft Excel, Microsoft Corp, USA) and analysed with a statistical software package (Stata 10, StataCorp, Texas, USA). Chi-square or Fisher's exact test was used to compare proportions as appropriate. Risk verification was expressed with odds ratio (OR) and 95% confidence interval (CI). Assuming a 20% of mortality in the artemether arm and the study had a power of 70% to

demonstrate a 50% reduction in absolute mortality. The estimated required sample size for each group was 177 patients.

RESULTS

As a result of the fall in incidence of severe malaria in Viet Nam the trial was finished early on the recommendation of the Scientific Advisory Committee due to low recruitment. The study has recruited enough patients to detect a 50% fall in mortality from 20% to 10% with 70% power. We recruited 370 patients into the study at the time of completion. The baseline characteristics of patients on admission are shown in table 3.1. Both admission clinical parameter and laboratory investigation were similar in 2 randomised treatment groups.

Outcome

The overall mortality rate was ten percent (37 of 370 patients). The mortality rate in artesunate group (ARTS) was lower than in the artemether group (ARTM) (7 percent vs. 13 percent, unadjusted relative risk of death for artesunate = 0.54, 95% CI: 0.28 – 1.02, p = 0.052 (Table 3.2). For cerebral malaria subgroup patients, there was no significant difference in this outcome: the unadjusted mortality in the ARTS group was 12.9 percent and in the ARTM group was 13.8 percent (RR for artesunate = 0.94, 95% CI: 0.44 – 2.01, p = 0.87). On further exploration of factors associated with fatal outcome using multiple logistic regression (Table 3.3), patients who received intramuscular artesunate still had a decreased risk of death compared with those treated with artemether (adjusted OR for ARTS 0.41, 95% CI: 0.16 – 1.06, p = 0.06). Other important contributors associated with death were admission to hospital after year 2000, an increase in plasma lactate on admission, decreased Glasgow Coma score, previous treatment with artemisinin derivatives and raised white cell in the peripheral blood. No significant difference was

found in of the rate of complications between the groups except for the development of coma after receiving treatment. The ARTS group was associated with lower risk (7/94, 6.9%) of developing coma than the ARTM group (15/82, 15.5) RR=0.45 CI 95%: 0.19 – 1.05, p = 0.056. This may explain why the mortality in the former subgroup was also lower than the latter. (2/99 vs. 12/85, RR = 0.16 CI 95% 0.04 – 0.70, p = 0.004).

Causes of Death

The causes of death in severe malaria are often not confined to any specific complications, as multiple organ disorder often developed before death. Of the 37 fatal cases, we found that 25 of them had acute renal failure (24 of this group underwent dialysis); 28 cases had persistent and unrecoverable shock; 20 cases had spontaneous bleeding mostly from gastro-intestinal lumen; 12 cases had co-infection of lung and urinary tract; and 8 cases had pulmonary oedema.

Recovery

Assessment of most indicators of recovery did not reveal any significant differences between the treatment groups (Table 3.4). However, the ARTS group showed a quicker initial clearance of parasite from the peripheral blood (median time to 50% parasite clearance (IQR) (hrs): 8 (4-12) in ARTS and 8 (4-16) in ARTM). This advantage lasted until a decrease of ninety percent of the parasite load (median time to 90% parasite clearance (IQR) (hrs): 16 (12-30) in ARTS and 24 (12-36) in ARTM) (Table 3.4; Figure 3.1) and was lost thereafter.

Haematologic Recovery

There was no significant difference between two groups in fall of haematocrit after baseline (median percentage reduction of admissive haematocrit in the ARTS group: 23.1, (IQR: 14.3-35.1), range (0-70%) and in the ARTM group: 21.4, (IQR: 12.9-34.7),

range (0-70), p = 0.25) or in the number of times required blood transfusion (p = 0.19). Bleeding sites included of gastro-intestinal, skin, conjunctiva and urinary tract.

Adverse Effects

There was no significant adverse reaction between 2 groups.

DISCUSSION

The treatment of severe malaria has changed in recent years with the introduction of the artemisinin derivatives. Until recently artemether was favoured by the WHO and therefore most studies had been conducted with it. In an individual patient data meta-analysis of randomised trials in severe falciparum malaria artemether was superior to quinine in Southeast Asian adults, but not in African children. Unfortunately intramuscular artemether was probably not the best artemisinin formulation to choose; it is an oil-based formulation that releases the drug slowly and erratically from the injection site (Murphy et al., 1997; Hien et al., 2004). In contrast to parenteral artemether, intravenous artesunate is obviously instantly bioavailable, and intramuscular artesunate is absorbed reliably and rapidly with peak concentrations occurring within one hour (Nealon et al., 2002; Ilett et al., 2002: Hien et al., 2004). After i.m. injection, in cases of severe malaria, concentrations of artesunate in plasma peaked within 20 minutes of injection, and artesunate was hydrolysed rapidly and completely to the biologically active dihydroartemisinin (DHA). On the other hand maximum artemether concentrations in plasma occurred at a median of 10 hours and there was relatively little conversion to DHA (Hien et al., 2004). Moreover, artesunate is a more potent antimalarial; artesunate and its main active metabolite dihydroartemisinin have relative in vitro potencies, in comparison to artemether, of 2.9

and 4.0 respectively (Brockman et al., 2000). The pharmacokinetics of artesunate and dihydroartemisinin are not influenced by the severity of malaria (Davis et al., 2001). A recent large multicentre trial from South East Asia showed that artesunate reduced mortality by 34.7% compared with quinine (SEAQUAMAT, 2005). This left the role of artemether uncertain –was it as good as artesunate despite the pharmacokinetic drawbacks, or was it inferior? This trial set out to answer this question.

The ARTS group was associated with lower risk (7/101, 6.9%) of developing coma after initiation of therapy than the ARTM group (15/97, 15.5) RR=0.45 CI 95%: 0.19 – 1.05, p = 0.056. The parasite clearance times were also faster in the ARTS treated patients (median 90% reduction PCT (IQR) (hrs): 16 (12-30) in ARTS and 24 (12-36) in ARTM). These data may be explained by the pharmacokinetic properties of artesunate compared with artemether and the faster bioavailaibilty of the active metabolites with artesunate especially in severly ill patients with marked metabolic acidosis. It also suggests that the first few hours of effective treatment are very important and the faster the active drug can be got to the site of action (the blood) the better. As outlined above after i.m. injection concentrations of artesunate peaked within 20 minutes of injection, and artesunate was hydrolysed immediately and completely to the biologically active dihydroartemisinin (DHA). Maximum artemether concentrations occurred at a median of 10 hours and there was much less and slower conversion to DHA. There are also some severely ill patients, mostly with severe metabolic acidosis who taken even longer to obtain maximal artemether concentrations. This hypothesis would be consistent with the pharmacology work done at the Hospital for Tropical Diseases (Hien et al., 2004). Getting active drug effective against all stages of the parasites (and thereby reducing or preventing maturation and reducing sequestration) into the patient quickly seems crucial. When faced with a patient with severe malaria the first few minutes and hours may make a huge difference to

their subsequent chances of survival. Perhaps it is time to learn the lessons from the cardiologists and aim to administer the most effective, fasting acting anti-malarial drug into patients as an emergency in the same way as thrombolytic therapy is administered urgently in patients with myocardial infarction where delays of minutes can make a huge difference to mortality and preservation of myocardium. Getting patients referred through the health care systems quickly is crucial, but once in a hospital able to administer parenteral drugs speed is of the essence to prevent the further maturation of parasites, reduce sequestration and prevent consequent complications.

The trial was overtaken by events. Improved malaria control in Viet Nam led to a dramatic decline in the incidence of severe malaria. The Hospital for Tropical Diseases is a referral hospital that in the early 1990s was receiving approximately three to five patients each day with severe malaria. A decade later this had fallen to approximately one patient per month. This was insufficient to sustain this trial and so the trial was terminated before reaching the pre-defined end points. The trial was stopped with the investigators blind to the allocations and prior to any analysis of the results. The result after 370 patients had been randomised was a borderline difference in favour of artesunate (Unadjusted relative risk of death for artesunate = 0.54, 95% CI: 0.28 − 1.02, p = 0.052). The superiority of artesunate over artemether is supported by the larger differences in mortality rates when artesunate was compared with quinine (35% reduction in mortality), versus artemether (a 26% reduction) (International Artemisinin Study Group, 2004), although these trials were conducted in different sites, with different protocols and different drug dosages, so are not strictly comparable. There is probably little to choose intrinsically between these two derivatives, and so the superiority of artesunate is likely to derive mainly from its more rapid and more reliable absorption following intramuscular injection. This may explain why the rate of reduction in parasitaemia was more rapid following artesunate.

Both drugs were very well tolerated with little or no discomfort at the injection site, no serious local or systemic reactions, a low rate of hypoglycaemia (presumably disease related), and no neurological sequelae (Nontprasert et al, 1998; Hien et al, 2003). Although artemether is easier to administer than artesunate, which is an advantage in busy epidemic settings, it is probably an inferior drug. Artesunate, administered as an emergency should be the treatment of choice for severe falciparum malaria.

Table 3.1 Baseline Characteristics of Patients, According to Treatment Group

Parameters	Number in each group (ARTS/ ARTM)	Artesunate (ARTS) n = 186	Artemether (ARTM) n = 184	P value
Age (years)				0.33
Median		32	32.5	
Range		15-74	15-77	
Interquartile range		23-42	25-44	
Sex – Male/Female		133/53	142/42	0.21
Previous treated with quinine for current malaria contracted: no. of patients (%)		11(5.9)	15(8.2)	0.4
Previous treated with artemisinin derivatives for current malaria contracted: no. of patients (%)		114(61.3)	105(57.1)	0.41
Convulsions: no. of patients (%)		13(7.0)	14(7.6)	0.82
Temperature (^{o}C)				0.75
Median		38.1	38.0	
Range		36.5-40.5	36.0-41.0	
Interquartile range		37.5-39.0	37.5-39.0	
Pulse (rate/min)				0.30
Median		100	100	
Range		68-150	60-151	
Interquartile range		90-115	90-120	
Respiratory rate (rpm)				0.27
Median		28	28	
Range		12-60	18-68	
Interquartile range		24-32	24-32	
Glasgow Coma Score				0.61
Median		11	11	
Range		3-15	3-15	
Interquartile range		8-15	8-15	
Glasgow Coma Score less than 11: no. of patients (%)		85(45.7)	87(47.3)	0.76
Opening CSF pressure (cmH$_2$O)	72/80			0.61
Median		15	16	
Range		4-30	2-29	
Interquartile range		11-18.5	11-20	
Shock: no. of patients (%)		6(3.2)	10(5.4)	0.30
Hypoglycaemia: no. of patients (%)		1(0.5)	0(0)	1.0
Haematocrit (%)				0.42
Median		32	31	
Range		10-50	8-53	
Interquartile range		26-38	25-37	
Parasite count (x10^3/μL)				0.75

Median		80.7	70.5	
Range		0.02-3471	0.02-3534	
Interquartile range		10.4-255	7.1-286	
White cell count (x10^3//μL)	173/171			0.45
Median		7.93	8.40	
Range		1.1-47.6	1.7-27.0	
Interquartile range		5.5-10.9	5.7-11.2	
Serum creatinine (mg %)	179/176			0.68
Median		1.8	1.7	
Range		0.6-9.5	0.6-17.0	
Interquartile range		1.1-3.2	1.1-3.6	
Total bilirubin (mg %)	124/120			0.40
Median		5.7	5.0	
Range		0.6-39.0	0.6-38.8	
Interquartile range		2.4-13.6	2.3-12.0	
ALT (U/L)	155/161			0.45
Median		70	71	
Range		10-1900	6-414	
Interquartile range		44-129	41-119	
Lactate (mmol/L)	168/166			0.85
Median		3.2	3.6	
Range		0.5-17.7	0.5-16.8	
Interquartile range		2-5.6	1.8-5.1	

Table 3.2 Primary and secondary outcomes after treatment with intramuscular Artesunate or Artemether

Outcomes	ARTS	ARTM	Relative Risk (95% CI)	P value
Mortality rate	13/186(7.0)	24/184(13.0)	0.54(0.28-1.02)	0.052
Developing coma*	7/101(6.9)	15/97(15.5)	0.45(0.19-1.05)	0.056
Convulsions	7/186(3.8)	9/184(4.9)	0.77(0.29-2.02)	0.59
Require blood tranfusion	69/186(37.1)	69/184(37.5)	0.99(0.76-1.29)	0.94
Renal impairment	111/186(59.7)	105/184(57.1)	1.05(0.88-1.24)	0.61
Renal failure	85/186(45.7)	85/184(46.2)	0.99(0.79-1.23)	0.92
Require dialysis	52/186(28)	54/184(29.3)	0.95(0.69-1.31)	0.77
Hypoglycaemia	7/186(3.8)	9/184(4.9)	0.77(0.29-2.02)	0.59
Spontaneous bleeding	26/186(14)	35/184(19)	0.73(0.46-1.17)	0.19
Shock	22/186(11.8)	26/184(14.1)	0.84(0.49-1.42)	0.51
Concomitant infection	66/186(35.5)	75/184(40.8)	0.87(0.67-1.13)	0.30
Jaundice	144/186(77.4)	144/184(78.3)	0.99(0.89-1.1)	0.85
Pulmonary oedema	10/186(5.4)	13/184(7.1)	0.76(0.34-1.69)	0.5

* The number of patients who were not comatose on admission in ARTS group was 101, and in ARTM group were 97.

Table 3.3 Multivariate logistic regression analysis of factors associated with death in severe falciparum malaria patients

Factors	Adjusted Odds Ratio (95% CI)	P value	Notes
IM artesunate treatment	0.41 (0.16 – 1.06)	0.066	60 out of 370 were
Year of study (after 2000)	0.14 (0.03 – 0.71)	0.017	not included (30 in
Each unit higher in Glasgow Coma score	0.82 (0.72 – 0.93)	0.002	ARTS and 30 in ARTM) due to
Unit increase of admissive plasma lactate	1.28 (1.15 – 1.43)	< 0.001	missing lactate and/or WBC count
Each 10^3 WBC/mm^3 rise	1.1 (1.02 – 1.18)	0.017	values)
Pre-entry treated with artemisinin derivatives	0.29 (0.11 – 0.82)	0.019	

Forward stepwise on conditioned of likelihood change logistic regression (p to enter = 0.075, p to remove = 0.15)

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Table 3.4 Assessment of recovery after treatment with intramuscular Artesunate or Artemether

Parameters	ARTS n = 186	ARTM n = 184	P value*
	hours		
Time to parasite clearance			
Decrease to 50% of admission value			0.007
Median	8	8	
Range	4-42	2-60	
Interquartile range	4-12	4-18	
Decrease to 90% of admission value			0.004
Median	16	24	
Range	4-102	2-152	
Interquartile range	12-30	12-36	
Total clearance			0.97
Median	72	72	
Range	7-330	2-204	
Interquartile range	48-90	54-96	
Resolution of fever			
Time first temperature < 37.5°C			0.99
Median	30	20	
Range	0-768	0-648	
Interquartile range	4-66	4-60	
Time of fever clearance			0.27
Median	108	108	
Range	0-888	0-1088	
Interquartile range	54-180	60-204	
Time to recovery from coma			
Time to Glasgow Coma score of 8[†]			0.55
Median	48	54	
Range	4-2136	2-2232	
Interquartile range	20-90	20-108	
Time to Glasgow Coma score of 11[‡]			0.44
Median	42	42	
Range	4-2136	2-2232	
Interquartile range	20-80	24-78	
Time to Glasgow Coma score of 15[¶]			0.11
Median	60	72	
Range	4-2136	2-2232	
Interquartile range	36-108	48-126	
Time to physical recovery			
Time before patient able to drink			0.20
Median	30	42	
Range	0-2136	0-2232	
Interquartile range	8-66	4-84	
Time before patient able to eat			0.18
Median	42	46	
Range	0-2136	0-2232	

Interquartile range	12-78	14-96	
Time before patient able to sit			0.32
Median	78	90	
Range	0-2136	0-2232	
Interquartile range	48-156	48-174	
Time before patient able to stand			0.66
Median	96	114	
Range	0-2136	0-2232	
Interquartile range	60-180	60-198	
Time before patient able to walk			0.64
Median	102	120	
Range	0-2136	0-2232	
Interquartile range	72-192	66-216	
Time before patient able to leave hospital			0.68
Median	264	288	
Range	7-2136	2-2232	
Interquartile range	192-480	192-456	

* Log-rank test
† Only patients who decreased in Glasgow Coma score below 8 were included (130 pts, 58 in ARTS group and 72 in ARTM group)
‡ Only patients who decreased in Glasgow Coma score below 11 were included (204 pts, 93 in ARTS group and 111 in ARTM group)
¶ Only patients who decreased in Glasgow Coma score below 15 were included (274 pts, 134 in ARTS group and 140 in ARTM group)

Figure 3.1

Kaplan-Meier survival estimates

p = 0.08

Kaplan-Meier remaining parasitaemic estimates
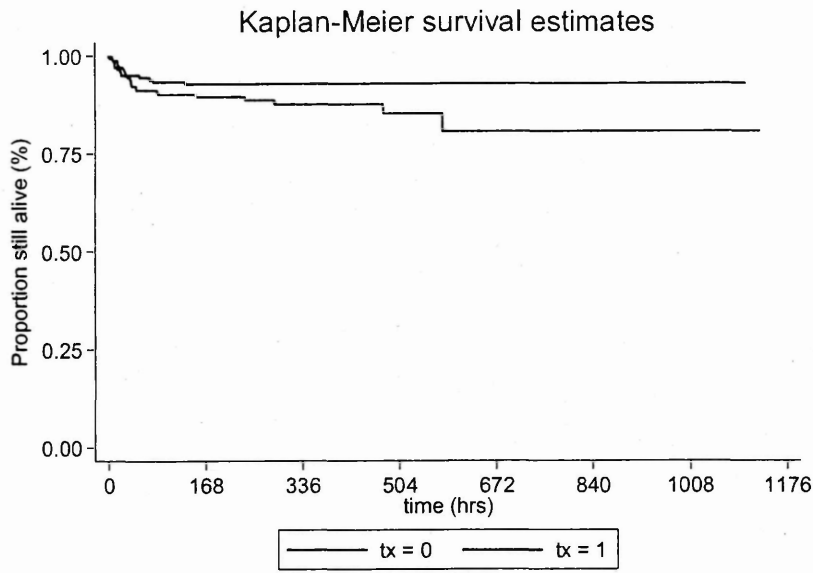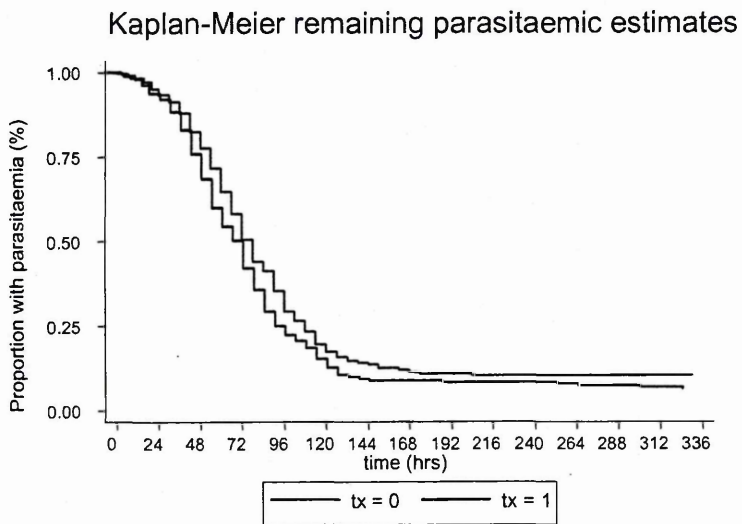
p = 0.39

tx = 0 : artemether, tx = 1 : artesunate

Figure 3.2 Artesunate (Guilin No 2 Pharmaceutical Factory, Guangxi, PR China)

(N.H.Phu)

Figure 3.3 Artemether (Kunming Pharmaceutical Company, Kunming, PR China) (N.H.Phu)

CHAPTER 4

A RANDOMIZED COMPARISON OF PUMPED VENOVENOUS HAEMOFILTRATION AND PERITONEAL DIALYSIS IN ACUTE RENAL FAILURE ASSOCIATED WITH SEVERE INFECTION

INTRODUCTION

Acute renal failure (ARF) is a major contributor to the morbidity and mortality associated with severe infections (Lombardi et al., 1998; Karnik et al., 1998). The management of ARF in severe sepsis is often difficult because the patients are haemodynamically unstable and may have multi-organ dysfunction. In the developed world both hemodialysis and peritoneal dialysis (PD) have been used in the acute phase of renal impairment, although the early use of haemodialysis was dogged by difficulties with blood pressure control (Huyghebaert et al., 1985). With the introduction of continuous haemofiltration, particularly when pump-assisted to reduce cardiovascular complications and ensure constant filtration, many of these difficulties were resolved (Voerman et al., 1990; Dunham, 2001). In recent years pumped venovenous haemofiltration, and variants such as haemodiafiltration, have become standard renal replacement therapy in acutely ill patients. However these procedures require the constant presence of highly trained staff, anticoagulation to prevent clotting in the extracorporeal circulation, large volumes of appropriate fluids, and venous access with sufficient capacity for the relatively high extracorporeal flow rates required. With a pump in the circuit there is also an added risk of air embolus. These factors and uncertainties over cost and availability cast doubt on

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the practicality, feasibility and safety of haemofiltration in less developed parts of the world.

Falciparum malaria is a major cause of acute renal failure in the tropics, and ARF in malaria carries a high mortality. There have been a number of reports of successful treatment of malaria-associated acute renal failure with acute peritoneal dialysis (Reid et al., 1967; Whelton et al., 1967; Reid et al., 1968; Canfield et al., 1968; Lowenthal et al., 1974; Indraprasit et al., 1988; Trang et al., 1992). PD has the advantage of simplicity, low cost, and relatively widespread availability. There are both theoretical advantages and disadvantages of using PD in malaria. Severe falciparum malaria is characterised by the sequestration of parasitised erythrocytes in the deep microvasculature, which may lead to compromise of the blood flow through the peritoneal membrane and reduced dialysis efficiency (Canfield et al., 1968). Malaria is particularly severe in pregnant women, and an additional limitation of PD is that it cannot be used in late pregnancy. On the other hand both malaria itself, and the most widely used treatment of severe malaria, quinine, are associated with hypoglycaemia and the use of hypertonic glucose as an osmotic agent in peritoneal dialysis fluid may reduce the risk of hypoglycaemia by providing a constant glucose infusion. Severe malaria is associated consistently with lactic acidosis, yet most haemofiltration replacement fluids use lactate as the alkali generated base. Inability to metabolise this additional lactate load could worsen the metabolic acidosis.

There has never been a randomized comparison of acute PD with haemofiltration in the treatment of acute renal failure in any context. We conducted this comparison in patients with severe infection-related acute renal failure. The setting was an infectious disease hospital in Viet Nam, where, before the start of the study, only acute peritoneal dialysis was available for the treatment of acute renal failure.

PATIENTS AND METHODS

Study Site

The study was carried out in a purpose-built intensive care unit at the Hospital for Tropical Diseases, Ho Chi Minh City, an infectious disease hospital which acts as a referral centre for much of southern Viet Nam, particularly for the management of acute renal failure. Ethical approval was obtained from Ethical and Scientific Committee of the institution.

Objectives

This was to assess, using a randomized controlled trial, the relative efficacy, safety, practicality and cost of acute peritoneal dialysis and pumped venovenous haemofiltration in the setting of a well-equipped hospital in a developing country. The primary end point was the rapidity of resolution of metabolic derangements, specifically the rates of change and times to normalization of arterial pH and venous creatinine. Mortality, requirement for further renal replacement therapy, and incidence of serious complications such as bleeding, air embolus, peritonitis, lactic acidosis and hypoglycaemia were documented as secondary endpoints.

Patients

Any patient in whom urgent renal replacement therapy (RRT) was indicated to treat acute renal failure was considered eligible for the study, with the following exceptions: patients were excluded from the study if they were pregnant, under the age of 15 years, or had already received renal replacement therapy of any type during the current illness. Informed consent was obtained either from the patient, or from their attendant relative if the patient was comatose or below the age of 18.

As this was an infectious disease hospital patient either had severe falciparum malaria or sepsis-related ARF. A decision to start RRT was based on the presence of one or more of

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the following indications: oliguria (urine output <15mls/hour despite rehydration) or anuria; metabolic acidosis (standard base excess < -10mmol/L with serum creatinine >265μmol/L); fluid overload with ARF (serum creatinine >265μmol/L); clinical uremic syndrome; and hyperkalaemia (>6mmol/L). Diagnosis and appropriate treatment of the underlying condition (malaria or sepsis) was carried out in accordance with normal clinical practice, and was not in any way compromised by the study protocol. Patients with severe malaria were assessed and managed as described previously (Donadio et al., 1968; Trang et al., 1992) and patients with sepsis were assessed using Bone's criteria (Bone, 1991), though these were not part of the inclusion criteria for the study.

Study procedure

On admission patients were examined fully, weighed, and baseline blood samples taken for full blood count, clotting studies, biochemistry, blood culture and malaria parasite counts. Arterial pH and blood gases were also measured. Hydration status was assessed, and patients were hydrated, usually with pressure monitoring, via a central venous catheter. A urinary catheter was inserted. After the decision was made by the clinician, and consent was obtained either from the patients or their relatives, patients were randomized to receive either peritoneal dialysis (PD) (Figure 4.4) or pumped venovenous haemofiltration (HF) (Figure 4.3). The randomization was generated from random number tables, and placed in consecutively numbered sealed, opaque, double-wrapped envelopes. A full history was taken from either the patient or attendant relatives. Before starting dialysis or filtration the patient was weighed, and body length recorded and, if the decision to start renal replacement was taken ≥ 24 hours after admission, the full blood sampling was repeated. Once randomized to HF or PD catheters were inserted and dialysis or filtration initiated as quickly as possible.

Peritoneal dialysis

Peritoneal dialysis was carried out as described previously (Trang et al., 1992). Briefly a rigid peritoneal dialysis catheter was inserted under local aenesthetic, and an open drainage system used. The acetate-based dialysis solution was prepared by the hospital pharmacy with the following electrolyte composition (in mmol/L): sodium, 141; chloride, 101; calcium, 3.5; magnesium, 1.5; and acetate, 45. Two solutions were available: an isotonic solution containing dextrose 1.5g/100mL, and a hypertonic solution containing dextrose 7g/100mL. The hypertonic solution was used in cases of volume overload and pulmonary oedema. Heparine (200 IU) was added to each liter for preventing catheter obstruction. The solution was heated in a water bath to 37°C before administration. Two litre exchanges were used with a 30 minutes dwell time (a total of up to ~ 70 litres/day).

Haemofiltration

Venous access was obtained through an 8.5Fr Quinton-Mahurkar double lumen catheter inserted into the femoral vein. Blood was pumped through a FH-66 haemofilter by a BMM 10-1 blood pump (Gambro, Sweden) at a flow rate of 150 mLs/min. Lactate-based haemofiltration fluid (Formula No.1, Gambro, Australia) was infused into the extracorporeal circuit before the haemofilter (predilution). Haemofiltrate and replacement fluid flow rates, and hence overall fluid balance, was controlled using a BS1 Balancing System (Gambro, Sweden). Overall haemofiltrate volume was set at ~ 25 litres/day. Heparin was infused at an initial rate of 500 IU / hour. The Activated Clotting Time was monitored every 5 hours using a Hemochron 801 bedside coagulation testing system (International Technidyne Corporation, New Jersey, US).

Monitoring

Regular clinical assessment (at least 4-hourly), and monitoring of urine output and overall fluid balance, was carried out throughout the period of renal replacement therapy. Arterial pH, blood gases, SaO_2, potassium, venous plasma lactate, glucose, and creatinine were measured 5-hourly. Glucose and lactate were determined using on-line analysers (Analox, London, UK), as was plasma creatinine (Boerhinger-Mannheim, Germany). Arterial blood gas measurement and haemoximetry were performed using a pre-calibrated ABL4 blood gas machine (Radiometer, Copenhagen, Denmark). Any complications of RRT were assessed and recorded prospectively.

A session of RRT was continued until the physicians caring for the patient considered it was no longer indicated clinically. If a complication of therapy occurred there was provision for stopping the current treatment session and restarting RRT with the other treatment modality. Peritonitis in PD was not considered an indication for stopping dialysis unless it persisted despite intraperitoneal (IP) antibiotics. First line treatment for peritonitis consisted of IP cefotaxime or ceftriaxone. If the cultures yielded a resistant organism or there was no clinical response within 24 hours the combination of IP vancomycin and IP gentamicin was substituted. If the patient was systemically septic broad-spectrum intravenous antibiotics were started or added.

Only the first RRT session (i.e. until the physician discontinued RRT) was randomized to either HF or PD. If a patient required further RRT after initial improvement, then PD was used, and was carried out on the same intensive care ward using the same PD protocol. Requirement for further RRT was a secondary end-point of the study.

Statistical analysis

An *a priori* power calculation showed that a sample size of 108 patients would be required to demonstrate a 50% increase in the rate of fall in plasma creatinine on dialysis,

with 95% confidence and 90% power on the basis of data collected during a previous study (Trang et al., 1992). An interim analysis was planned after 50 patients were recruited. A significant difference in mortality was not anticipated before the trial with these sample sizes.

The statistical packages Stata (College Station, Texas, USA) and StatView (SAS Institutes Inc., Cary, North Carolina, USA) were used for data summary and analysis. The distributions of continuous variables were tested for normality using the Shapiro-Wilks test. When distributions were skewed log transformations were applied if appropriate. Normally distributed continuous variables were compared between groups with the unpaired Student's t test, and untransformed skewed continuous variables with the Kruskal Wallis test. Nominal variables were compared between groups using Fisher's exact test. Time to event analyses were carried out using the Kaplan-Meier method and logrank test for comparison of treatment groups.

To assess the effects of underlying disease and hepatic dysfunction multivariate analysis was carried out using analysis of variance or logistic regression analysis, depending on the nature of the outcome variable.

RESULTS

A total of 70 patients were entered into the study. An interim analysis was carried out after 42 patients had been recruited, which showed an unexpected trend towards a higher mortality in the PD group ($p = 0.04$). As this was not the originally stated primary end-point of the trial, it was decided to continue the study and to conduct a second interim analysis at 70 patients. The mortality difference persisted so the study was stopped on ethical grounds.

Thirty-six patients had been randomized to PD, 34 to HF. The demographic and baseline clinical findings are given in Table 4.1. There was no significant difference in any of the baseline variables between groups. Falciparum malaria was the underlying cause of ARF in 48 patients (69%). The other 22 patients all had presumed bacterial sepsis: in 8 cases serology was positive for leptospirosis, and in 2 an organism was cultured from blood (1 *E.coli* and 1 *Klebsiella pneumoniae*); the remaining 12 cases fulfilled the criteria for sepsis syndrome though no organism was cultured (5 had definitely received antibiotics before admission, and the antibiotic drug history in the other 7 was unknown). They were presumed to have been septicemic.

The indications for dialysis are given in (Table 4.2). One patient with severe malaria had a creatinine of 0.9mg/dL on admission, though this rose quickly to 3.1mg/dL. Dialysis in this case was carried out for severe metabolic acidosis (pH 7.11, SBD 17.9mmol/L), but the patient died of refractory shock (algid malaria) 30 hours after entry. All the other patients had abnormally raised plasma creatinine levels (>1.5mg/dL). There was no significant difference between the HF and PD groups in the frequency of any of these indications, or in the number of indications per patient.

Correction of metabolic abnormalities

Plasma creatinine concentrations in the HF group declined more than twice as rapidly as in the PD group (p=0.004, Table 4.3), although there was no difference between the treatment groups in the proportion of patients with a normal creatinine at the end of the RRT session (Table 4.3)

For technical reasons independent of the conduct of the study, full arterial blood gas measurements were available only on 55 of the 70 patients (26 randomised to PD and 29 to HF, p=0.25). Recruitment continued when the blood gas analyzer was unavailable as the other major metabolic measure and primary endpoint of the study (i.e. plasma

creatinine), was still measurable. In the sub-group with full acidosis measurements, which was not selected in any way other than by the serviceability of the blood gas analyzer, the rate of resolution of acidosis was considerably faster and normalisation more complete in the HF group.

Resolution of acidosis was assessed by

i.   the rates of change of pH and base excess, calculated over the duration of the RRT session (Table 4.3),

ii.   the absolute minimum values of pH and base excess observed (Table 4.3), and

iii.   time to normal pH and base excess (Table 4.3 and Figure 4.1).

A significantly higher proportion of patients in the HF group had a normal pH and base deficit at the end of the RRT session, even though the duration of the session was significantly shorter (Table 4.3 and Figure 4.1).

Mortality

There were 17 deaths in the PD group (47%) compared with 5 (15%) in the HF group (relative risk (95% confidence intervals (CI)) 3.2 (1.3 to 7.7), p=0.005).

The median (95% CI, range) time to death in the PD group was 29.8 hours (17.5 to 92.5, 0.5 – 258), compared with 13.8 hours (3.6 to 52, 3.6 to 52) (p=0.17) in the HF group (Figure 4.2). Severe acidosis secondary to a combination of severe sepsis / malaria and renal failure was a major contributor to death in 16 patients (73%). In 9 patients (41%) prolonged hemodynamic shock was present. The ultimate mode of death was cardiorespiratory arrest in 18 patients (82%), and respiratory followed by cardiac arrest in the remaining 4 (18%). No patient died from hyperkalaemia. A complication of therapy contributed either directly or indirectly to death in one patient, who had a major gastrointestinal haemorrhage while anticoagulated for HF.

Except for two patients in the PD group, all fatal cases died during the first RRT session. In one severe malaria patient, PD was stopped after a diagnosis of brain stem death was made 213 hours into the study; he was taken off life support 38 hours later. PD was stopped after 168 hours in a second malaria patient when it was clear major brain damage had been sustained secondary to severe malaria. He died at home 5 days later.

Complications

Although cloudy dialysate was seen at some stage in 15 (48%) of the patients treated with PD, peritonitis was only confirmed in one patient (dialysate white cell count >250 cells/mL). No organism was cultured. Intraperitoneal and parenteral cefotaxime was given, and the PD fluid became clear within 24 hours.

Serious upper gastrointestinal bleeding was a major complication in 3 patients, 2 in the PD group and 1 HF. In these three patients the bleeding was thought to contribute to death, though 2 also had intractable acidosis and the other septic shock (before bleeding).

One patient randomized to haemofiltration was thought to have received an air embolus when an alarm on the blood pump was overridden in error, although it was unclear what volume of air (if any) entered the circulation. The patient's systolic blood pressure fell to 90 mmHg from 115 mmHg, though this recovered after approximately 5 minutes. The patient survived and was discharged well from hospital.

There was no difference in the incidence of hypoglycaemia between the two groups (Table 4.3).

Duration of RRT and need further dialysis.

The median duration of HF was less than half that of PD (p=0.006) (Table 4.3). Of the patients in the PD group who survived the first RRT session 70% (14/20) needed further dialysis (with 3 of these patients requiring 2 further dialysis episodes), compared with

37% (11/30) of those in the HF group (p=0.04). This reduced requirement for further dialysis in the HF group was despite a higher proportion surviving to the end of the first RRT session. Three PD patients required two further episodes of dialysis.

Multivariate analysis

Logistic regression analyses were done with death and further dialysis requirement as the dependent variables and treatment group, underlying disease (malaria or presumed bacterial sepsis) and presence or absence of jaundice as independent variables. PD was associated significantly with both death (OR (95% CI) 5.1 (1.6 to 16), p=0.006) and need for further dialysis (4.7 (1.3 to 17), p=0.017), in accordance with the univariate analyses. There was no significant effect of underlying disease or jaundice on either outcome measure.

Similar logistic regression analyses were carried out on the subgroup of 48 patients in whom full serial arterial blood gas data were available and who survived the first RRT session. HF was associated significantly with correction to a normal pH (OR 23 (3.9 to 138), p=0.001) and normal base excess (OR 244 (17 to 3500), p<0.0001) at the end of dialysis. The covariates jaundice and underlying disease had no significant effect. To explore the possibility of lactate intolerance in jaundiced patients in the HF group (who received lactate-base haemofiltration fluid) a term for the interaction of treatment and jaundice was added; this had no significant effect on either model.

On multivariate analysis of the variances of i. the rate of change of creatinine and ii. the rate of change in pH, treatment group had a significant effect in both cases (p=0.02 & p<0.0001 respectively), whereas underlying disease and presence of jaundice did not.

Impact of lactic acidosis

Although 54 /55 patients in whom admission blood gas measurements were carried out had a metabolic acidosis (SBE < -3.3 mmol/L), only 20 (36%) and 40% of all patients (28/70) had admission hyperlactataemia (plasma lactate > 4 mmol/L). It is known that other acids, primarily renal in origin, contribute to the acidosis in severe malaria patients both with and without lactic acidosis. To assess whether lactic acidosis per se was refractory to treatment with HF using lactate-based replacement fluid we entered plasma lactate and treatment modality into a logistic regression model containning outcome as the dependent variable. Plasma lactate, itself is an independent predictor of poor outcome (p < 0.05), had no significant influence on the relative efficacy of HF compared with PD. Admission hyperlactataemia also had no influence independent of admission SBE on the rate of normalization of acidosis.

Peak plasma lactate concentrations were significantly higher in the HF group than the PD group (p=0.018), reflecting infusion of lactate-based haemofiltration fluid. This was not associated with acidosis indeed mean minimum pH and base excess were significantly lower in the PD group (p=0.0001 & p=0.002 respectively, Table 4.3).

Economic implications

The mean (95% CI) cost of hospital stay (from diagnosis of ARF to discharge) for PD patients was $1,580 (1,170 to 2,000), compared with $1,150 (960 to 1,330) for HF patients. This includes the cost of dialysis, drugs, and bed charges for both the intensive care unit and the convalescent ward. The costs per survivor were $3,000 (2,210 to 3,790) for PD and $1,340 (1,130 to 1,560) for HF.

The approximate cost per life saved was $6,950 for PD and $2,080 for HF. These figures do not include the cost of staff training, though this is unlikely to be very different between the two RRT modalities. Nor do they take into account the capital costs of

equipment. Although these will be higher for HF, with reasonable patient throughput of patients these costs are likely to be rapidly defrayed by the per case cost differences outlined above, which are all substantially higher for PD. For example, assuming an outlay of $10,000 for the haemofiltration system, HF would have become less expensive overall in our centre after only 24 patients had received RRT. These calculations are based on costs of health care, drugs and consumables in Viet Nam in the late 1990s, but can probably be generalized to other developing countries.

DISCUSSION

Acute renal failure associated with severe infections such as malaria and bacterial sepsis are a major cause of death in adults in the developing world. Renal replacement therapy is one of the most expensive and difficult forms of medical treatment, and is often beyond the financial reach or logistic capability of health care systems in the tropics. However infection-related ARF carries mortality of over 70% if treated conservatively (Trang et al., 1992) and in contrast to the situation in rich countries, ARF in poorer countries often affects the young, most economically productive members of society. If temporary renal support of some sort can be provided during the acute illness, these previously fit individuals will often recover quickly and fully, and very rarely progress to chronic renal failure (Trang et al., 1992). Acute peritoneal dialysis has been used extensively in the tropics for the treatment of ARF associated with malaria and other infections precisely because it is considered relatively inexpensive and practical in a resource-poor context, and it is clinically effective. In richer countries acute peritoneal dialysis has been replaced almost everywhere (except in paediatric nephrology) by either acute haemodialysis or continuous haemofiltration. This is largely because these newer techniques are considered more efficient at restoring normal biochemical homeostasis, although there have been no

randomized comparisons in the management of ARF to determine whether this translates into clinical benefit.

As countries develop economically and health budgets increase, the problem arises of how to spend most effectively the modest sums available for new initiatives. Expensive tertiary care facilities such as dialysis and intensive care units present a particular dilemma, particularly as their benefits are difficult to quantify. If haemofiltration or haemodialysis is to replace acute peritoneal dialysis as the usual treatment for ARF in developing countries, there must be good evidence that it is clinically superior and cost beneficial.

The peritoneal dialysis technique used at this referral centre was first introduced in 1989. It was associated, at the time, with a halving of the mortality from malaria-associated ARF compared with historical controls (Trang et al., 1992). Peritoneal dialysis is affordable and practical, and sufficiently simple that, with a moderate degree of training, it can be transferred to district hospitals. However this randomized comparison demonstrates conclusively that venovenous haemofiltration is superior to acute peritoneal dialysis clinically and furthermore in the setting of this regional referral centre in Viet Nam it costs less. The large difference in mortality between the two groups was not anticipated, but it continued following the initial interim analysis, and it is significant at the 1% level even after Bonferroni adjustment to allow for the "double look" at the data. This trial is small for a mortality study, and death was not the primary outcome measure at the start of the comparison. The mortality difference is readily explained by the considerably inferior efficacy of peritoneal dialysis in normalizing the biochemical disruption that characterises ARF.

The results from analysis of protocol-specified outcome markers are all greatly in favour of HF. Patients randomized to HF were dialysis dependent for a shorter time period, and

cleared acidosis and azotaemia quicker than those receiving PD. Could this finding have resulted from physician bias? It is true that two of the outcome measures were dialysis duration and the number of dialysis episodes required, both of which were subjective decisions made by physicians who could not be blinded to treatment allocation. However despite the longer median dialysis duration in the PD group, the objective measurements of metabolic improvement were all strongly in favour of HF, suggesting that the physicians' decisions to prolong dialysis were based on objective clinical concerns rather than intrinsic bias.

The majority of patients in this study had severe falciparum malaria as a cause of ARF, the central pathophysiological feature of which is sequestration of parasitized erythrocytes in the deep microvasculature, including the splanchnic bed. Peritoneal dialysis relies on perfusion of the splanchnic bed for apposition across the peritoneal membrane of circulation and dialysis fluid. Any obstruction to peritoneal microvascular blood flow by sequestration could reduce dialysis efficiency substantially. Previous studies have suggested that dialysis efficiency may be reduced in severe malaria (Yorke et al., 1911; Canfield et al., 1968; Donadio et al., 1968). A potential benefit from PD in severe malaria is the theoretical beneficial effect on malaria and quinine-associated hypoglycaemia of the glucose used as an osmotic agent in the dialysis fluid. This is not supported by the current trial; of the 6 malaria patients who were hypoglycaemic during dialysis, 4 received PD and 2 HF.

Patients receiving HF cleared their acidosis significantly more rapidly than those receiving PD. This suggests that hepatic conversion of exogenous lactate anion into alkali was possible in the majority of cases despite the high incidence of hepatic dysfunction, manifested by jaundice and other biochemical abnormalities in this patient population. In severe infections hyperlactataemia usually reflects lactic acidosis, and in severe malaria

lactic acidosis is a major cause of death (Davenport et al., 1990; Day et al., 2000). Plasma lactate increased significantly during HF, but this rise was associated with increases in pH and base excess rather than worsening acidosis. Multivariate analysis showed that the presence of jaundice had no effect on clearance of acidosis in the HF group, and there were no patients in the HF group in who base excess fell after HF started. Other workers have described patients with lactate intolerance, but this was not seen in our study population (Davenport et al., 1990; English et al., 1997)

There is evidence that HF increases the clearance of molecules of potential pathological significance in severe malaria and severe sepsis, though the clinical significance of this remains unclear (Hoffmann et al., 2001). Raised levels of pro-inflammatory cytokines are associated with poor outcome in both diseases (Casey et al., 1993; Day et al., 1999), and molecules of this size are cleared efficiently by HF. Whether this is also true of PD is unknown.

The causes of acute renal failure in malaria are not completely understood. The presenting features and the clinical evolution suggest 'acute tubular necrosis.' Rhabdomyolysis with raised creatinine kinase and myoglobin levels is common in severe malaria (Miller et al., 1989), and the combination of myoglobinuria, sometimes haemoglobinuria, and acidosis may contribute to tubular damage. Haemodialysis and haemofiltration have been shown to be of benefit in the early treatment of rhabdomyolysis caused by the crush syndrome (Amyot et al., 1999; Splendiani et al., 2001), raising the possibility that early haemofiltration in severe malaria may actually modify the course of the renal pathological process, leading to the observed shortening of the period of dialysis dependence.

This study provides evidence that for countries at an intermediate level of development of their health care system investment in facilities for venovenous haemofiltration is a more

cost-effective alternative to the introduction or continuing use of peritoneal dialysis for the treatment of acute renal failure, and is associated with a significantly better clinical outcome.

Table 4.1 Clinical and Laboratory Characteristics at Base line[*]

Characteristic	Peritoneal Dialysis (N=36)	Haemofiltration (N=34)	P Value
Age – yr			0.87
Median (95% CI)	36 (29.6-38.4)	35 (29.5-38.2)	
Range	15-74	16-57	
Male sex – no. (%)	27 (75)	30 (88)	0.22
Malaria – no (%)	23 (64)	25 (74)	0.45
Weight – kg			0.80
Median (95% CI)	53.5 (49.7-55)	53.5 (50-55)	
Range	36-79	39-71	
Body-surface area – m^2			0.99
Mean (95% CI)	1.56 (1.49-1.60)	1.58 (1.52-1.60)	
Range	1.23-1.89	1.31-1.82	
Anuria – no. (%)	7 (19)	5 (15)	0.75
Cerebral events – no. (%)	15 (42)	13 (38)	0.81
Glasgow Coma Score[†]			0.58
Median (95% CI)	12.5 (9-15)	14.5 (9.8-15)	
Range	3-15	3-15	
Duration of illness – days			0.06
Median (95% CI)	7 (6-8)	6 (5-7)	
Range	2-35	3-13	
Time to parenteral antimalarial treatment – days			0.94
Median (95% CI)	5 (3.1-5.9)	4 (4-5)	
Range	1-8	3-8	
Hypoglycaemia – no. (%)[**]	1 (3)	3 (9)	0.35
Plasma creatinine – mg/dl[§]			0.99
Mean (95% CI)	6.3 (5.3-7.3)	6.3 (5.5-7.1)	
Range	0.9-13.4	2.1-10	
Plasma bilirubin – mg/dl[‡]			0.67
Geometric mean (95% CI)	6.10 (3.9-9.6)	5.4 (3.5-8.2)	
Range	0.6-48.5	0.7-75.2	
Base deficit – mmol/litre			0.37
Mean (95% CI)	11.9 (9.9-14.0)	10.6 (8.2-12.9)	
Range	4.6-21.7	0.7-26	
Plasma lactate – mmol/litre			0.51
Geometric mean (95% CI)	3.7 (2.6-5.1)	3.2 (2.4-4.3)	
Range	1.1-12.7	0.6-11	
Shock – no. (%)[†]	3 (8)	5 (15)	0.47
Peripheral-blood parasite count in patients with malaria –/µL			0.36
Geometric mean (95% CI)	16,600 (3,800-72,000)	6200 (1,200-32,600)	
Range	20-363,000	20-710,000	

Table 4.2 Indications for dialysis

Characteristic	Peritoneal Dialysis (N=36)	Haemofiltration (N=34)	P Value
Oliguria‡	28 (78)	28 (82)	0.77
Hyperkalaemia‖	4 (11)	2 (6)	0.67
Severe acidosis✦	18 (50)	21 (62)	0.39
Fluid overload with acute renal failure (serum creatinine > 3 mg/dl)★	6 (17)	1 (3)	0.11
Uraemic syndrome	2 (6)	1 (3)	0.99
No. of indications for dialysis per patient			0.75
Mean (95% CI)	1.6 (1.4-1.9)	1.6 (1.3-1.8)	
Range	1-3	1-3	

‡Oliguria was defined as a urine output of less than 15 ml per hour despite adequate fluid replacement.

‖Hyperkalaemia was defined as a potassium concentration of >6 mmol/litre.

✦Severe acidosis was defined as standard base deficit of more than 10 mmol per litre with a serum creatinine concentration of more than 3 mg per decilitre

★Fluid overload with acute renal failure was defined by a creatinine concentration of >3 mg per decilitre.

Table 4.3 Outcomes According to Treatment[*]

Variable	Peritoneal Dialysis (N=36)	Haemofiltration (N=34)	P Value
Plasma creatinine			
Normal at the end of dialysis – no. (%)**	4/26 (15)	6/31 (19)	0.74
Rate of decrease – mg/dl/hr**			0.004
Median (95% CI)	0.018 (0.005 to 0.030)	0.039 (0.029 to 0.061)	
Range	-0.002 to 0.18	0.001 to 0.17	
Maximal rise after the start of renal-replacement therapy - mg/dl			<0.001
Median (95% CI)	0.2 (0 to 1.2)	0 (0 to 0)	
Range	0 to 5.0	0 to 1.8	
Arterial blood pH			
Normal at the end of renal-replacement therapy – no. (% [95% CI])▲	8/21 (38 [18 to 62])	25/27 (93 [76 to 99])	<0.001
Rate of increase – X10^{-5}/hr▲			<0.001
Median (95% CI)	-26 (-63 to 10)	218 (108 to 304)	
Range	-2150 to 103	-163 to 1020	
Maximal decrease after the start of renal-replacement therapy			<0.001
Median (95% CI)	0.125 (0.068 to 0.267)	0 (0 to 0.016)	
Range	0 to 0.842	0 to 0.207	
Base deficit			
Normal at the end of renal-replacement therapy – no. (% [95% CI])▲	1/21 (5 [0.12 to 24])	24/27 (89 [71 to 98])	<0.001
Rate base decrease – mmol/litre/hr			<0.001
Median (95% CI)	0.005 (-0.051 to 0.041)	0.26 (0.20 to 0.32)	
Range	-0.17 to 0.27	0 to 0.75	
Maximal increase after the start of renal-replacement therapy – mmol/litre			<0.001
Median (95% CI)	2.8 (1.7 to 5.3)	0 (0 to 0)	
Range	0 to 15.5	0 to 7.1	
Secondary outcomes			
Death – no. (% [95% CI])	17 (47 [30 to 65])	5 (15 [5 to 31])	0.005
Duration of first session of renal-replacement therapy – hrs			0.006
Median (95% CI)	92 (30 to 128)	43.5 (27.5 to 67)	
Range	0.5 to 340	3 to 120	

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Further dialysis needed – no./total no. (% [95% CI])**	14/20 (70 [46 to 88])	11/30 (37 [20 to 56])	0.04
Hypoglycaemia during dialysis – no. (% [95% CI])	5 (14 (5 to 30])	2 (6 [0.7 to 18])	0.43
Severe bleeding during dialysis – no. (% [95% CI])§	2 (6 [0.7 to 18])	1 (3 [0.7 to 15])	0.52
Peritonitis during dialysis – no. (% [95% CI])	1 (3 [0.07 to 15])	-	
Peak plasma lactate concentration during dialysis – mmol/litre			0.05
Geometric mean (95% CI)	5.5 (4.3 to 6.0	7.6 (5.9 to 9.9	
Range	2.1 to 24.2	1.6 to 18.9	

*CI denotes confidence interval

**This variable excluded patients who died during the first session of renal-replacement therapy.
To convert values for creatinine to micromoles per litre per hour, multiply by 88.4. A normal creatinine concentration was considered to be <1.5mg per decilitre (133 μmol per litre).

⅄This variable included only patients enrolled in the study when measurements of arterial blood gases were available and excluded patients who died during the first session of renal-replacement therapy.
A normal pH was considered to be ≥7.35, and a normal base deficit ≤3.3 mmol per litre.

§Severe bleeding was defined by a need for blood transfusion.
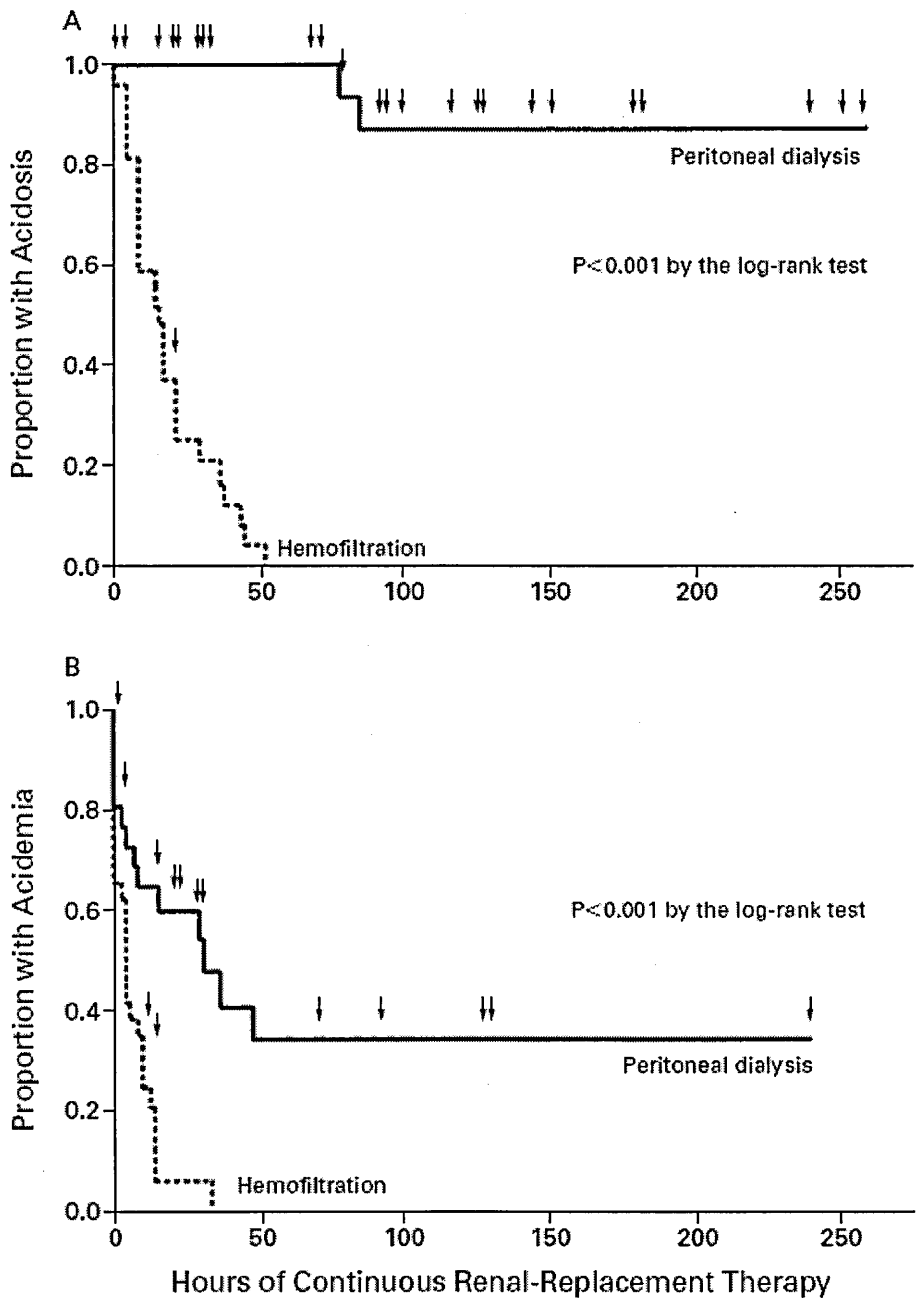
Figure 4.1 Kaplan-Meier Plots of the Time to the Resolution of Acidosis (Panel A) and Acidaemia (Panel B). Acidosis was defined as a standard base deficit of >3.3 mmol per liter, and acidaemia as pH< 7.35. Arrows indicate censoring of data on patients who died during the first session of renal replacement therapy or in whom acidosis or acidaemia failed to resolve by the end of the first session of renal replacement therapy.
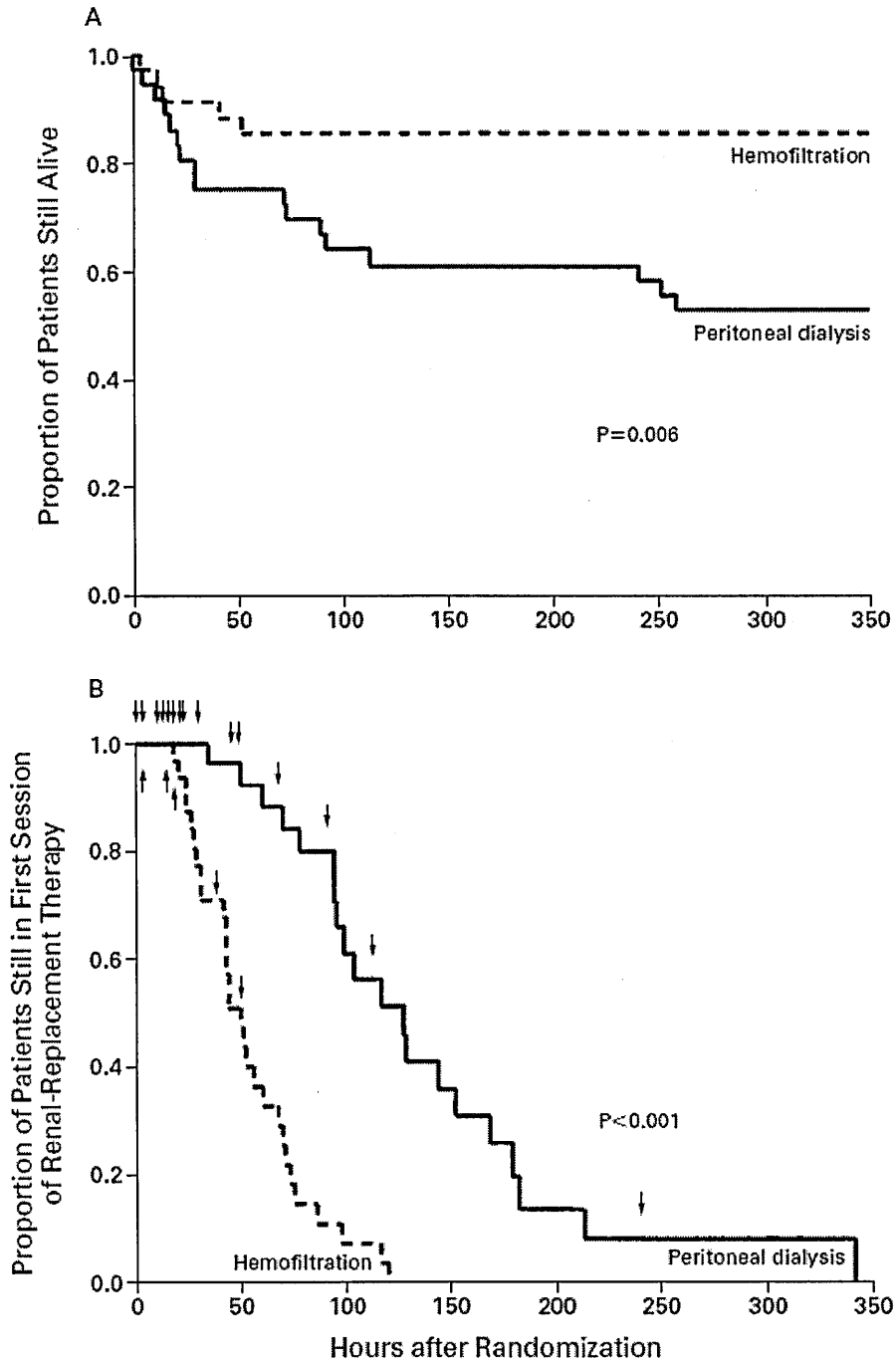
Figure 4.2 Kaplan-Meier Plots of Time to Death (Panel A) and Time to the End of the First Session of Renal replacement therapy (Panel B).
In Panel B, arrows indicate censoring of data on patients who died during the first session of renal replacement therapy. P values were derived by the log-rank test.

Figure 4.3 Haemofiltration in severe falciparum malaria (N.H.Phu)
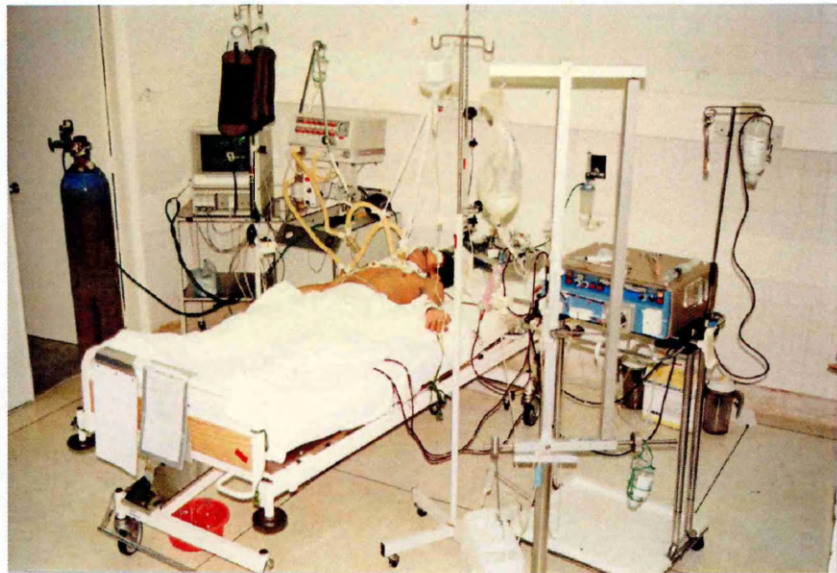
Figure 4.4 Peritoneal dialysis in severe falciparum malaria (N.H.Phu)

CHAPTER 5

FLUID MANAGEMENT IN SEVERE MALARIA

INTRODUCTION

The introduction of artemisinin based combination therapy has revolutionised the care of patients with malaria. However, even with the use of intravenous artesunate, between 7-25% of adult patients with severe malaria will die; the majority during the first 48 hours of their hospital admission (Dondorp et al., 2005). Whilst effective adjunctive chemotherapy is being sought, there is surprisingly little clinical data regarding the optimal supportive care of these patients in the early stages of their admission. This is despite the impressive improvement in mortality that has been demonstrated using early goal-directed therapy in patients with sepsis (Rivers et al., 2001) and, in resource poor settings, with simple fluid resuscitation protocols in dengue fever (Dung et al., 1999). Indeed, even using inferior quinine therapy, patients admitted to a French intensive care unit with severe malaria had a mortality rate of 11% (Bruneel et al., 2003), suggesting that improved supportive care can lead to better outcomes in malaria as well. Currently, there is debate in the paediatric literature about the extent of hypovolaemia in children with severe malaria and its contribution to the pathophysiology of the disease (Akech et al., 2006; Planche, 2007). However, even those who query its clinical relevance acknowledge that moderate dehydration can be demonstrated in this population (Planche et al., 2004). In adults, where the duration of coma is relatively prolonged and renal failure is more common, hypovolaemia might contribute more significantly to the manifestations of the disease. Limited series have confirmed that adult patients with

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severe malaria are hypovolaemic (Davis et al., 1990) or at least have a reduced effective circulating volume (Malloy et al., 1967; Sitprija et al., 1996). However, the issue of fluid replacement has been relatively ignored, with no good data to guide the selection of which fluid should be administered to these patients, nor how quickly it should be given. If too little fluid is given, there is the risk of exacerbating the renal failure and acidosis associated with severe malaria – complications associated with a poor outcome (Trang et al., 1992; Day et al., 2000). If too much is administered, there is the risk of pulmonary oedema, a condition which is the result of increased pulmonary capillary permeability with a mortality rate of 80% (WHO, 2000). In most parts of the world where malaria is seen precipitating pulmonary oedema is a disaster. Good ventilation is usually not possible and the experience of clinicians and nurses to deal with pulmonary oedema is limited.

The most recent WHO guidelines emphasise the physical examination of patients and offer only very general advice to clinicians: "If there is evidence (on physical examination) of dehydration, give only isotonic fluid (0.9% saline) by intravenous infusion, but avoid circulatory overload as it may rapidly precipitate fatal pulmonary oedema" (WHO, 2006). This is despite the fact that there is little correlation between the physical assessment of hypovolaemia and true volume status (McGee et al., 1999) and no data to recommend saline over other fluid options. The WHO guidelines also suggest that patients with severe malaria should, if possible, have a central line inserted and the central venous pressure (CVP) should be used to assist in the assessment of volume status (WHO, 2006), despite data that suggest that its role may be limited (Ausudkij et al., 1998).

As prolonged fluid therapy will be required in almost all adult patients it makes sense to better define the effect of fluid resuscitation on the manifestations of the disease, so that recommendations to physicians are based on sound pathophysiological principles. To take some early steps down this path, we retrospectively reviewed the clinical, haemodynamic and biochemical data of adult patients with severe malaria who were enrolled in previous studies (Hien et al., 1996). We aimed to determine whether there was any improvement in mortality or prognostic indicators when patients in these studies were resuscitated with normal saline or colloid. We also hoped to establish the utility of central venous pressure (CVP) and pulmonary artery occlusion pressure (PAoP) as measures of volume status in patients with severe malaria.

METHODS

The studies were carried out in a purpose-built intensive care unit at the Hospital for Tropical Diseases in Ho Chi Minh City, a hospital that acts as an infectious disease referral centre for much of southern Viet Nam. Written informed consent was obtained from each patient or, in the case of comatose patients, their attendant relative. All studies were approved by the Ethical and Scientific Committee of the Hospital for Tropical Diseases. As the fluid resuscitation of patients guided by invasive pressure measurements represented standard of care at the time, consent was not specifically sought for the collection of data for this retrospective chart review.

Patients were included in this analysis if they had invasive haemodynamic monitoring performed as part of the initial study. They were considered to have severe malaria if they had asexual forms of *P. falciparum* on their peripheral blood smear and at least one of the following criteria of severity: acute renal failure with oliguria and plasma creatinine of >3 mg/dL (265 μmol/L); hypoglycaemia (plasma glucose of <40 mg/dL [2.2 mmol/L]);

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shock with systolic blood pressure of <80 mmHg; metabolic acidosis with a base deficit of <10 mmol/L; venous plasma lactate of >4 mmol/L; or pulmonary oedema. These criteria are a stricter modification of the widely used World Health Organization criteria for severe malaria (WHO, 2000).

Depending on the study in which they were enrolled, patients received either an intramuscular quinine-loading dose regimen (20 mg/kg quinine dihydrochloride salt followed by 10 mg/kg, eight hourly), intramuscular artemether treatment (4 mg/kg stat followed by 2 mg/kg, eight hourly) or artesunate (2.4 mg/kg iv stat) followed by 1.2 mg/kg at 12 and 24 hrs daily. The requirement for fluids was assessed clinically and guided by measures the pulmonary artery occlusion pressure (PAoP). Depending on the extent of estimated hypovolaemia and tissue hypoperfusion, patients received normal saline, colloid (Gelofundin, B. Braun Medical, Metsungen, Germany) or no fluid loading. Patients who received fluid loading were resuscitated to a PAoP of 9-12 mmHg. Oxygen was given as necessary, but the patients were not routinely mechanically ventilated.

A full history was taken from either the patient or the attendant relatives and a detailed physical examination was performed. The patient was weighed, and body length was recorded. Baseline blood samples were taken for full haematology, quantitative parasite count, clotting studies, biochemistry (including lactate and glucose), and blood culture.

An arterial catheter was inserted into the femoral artery, and a flow-directed pulmonary artery catheter (Abbott Laboratories, North Chicago, IL) was introduced via the internal jugular route under fluoroscopy. Continuously monitored intravascular pressures (arterial pressure, CVP, PAP), electrocardiogram, and oximetric oxygen saturation were recorded on a multifunction monitor (Hewlett-Packard, Palo Alto, CA). PAoP was measured intermittently. Cardiac output was determined by thermodilution in triplicate using 10 mL

boluses of cooled 5% dextrose. Body temperature was recorded as the core temperature from the pulmonary artery catheter terminal thermistor. Simultaneous arterial and mixed venous plasma lactate and glucose concentrations were determined enzymatically using dedicated on-line analyzers (Analox, London, UK). Systemic vascular resistance, oxygen delivery (DO_2), and oxygen consumption (VO_2) were calculated and cardiac index, systemic vascular resistance index, DO_2 index, and VO_2 index derived using standard formulae (Edwards et al., 1993). Preparation of the patient and recording of baseline hemodynamic and metabolic measurements took 45-120 mins and did not interfere with other aspects of clinical management.

Results were entered into a database (Microsoft Excel, Microsoft Corp, USA) and analysed with a statistical software package (Stata 9.2, StataCorp, Texas, USA).

RESULTS

Overall there were 43 patients who met criteria for severe malaria and who had data available for analysis. Their management is recorded in Figure 5.1.

Figure 5.1.
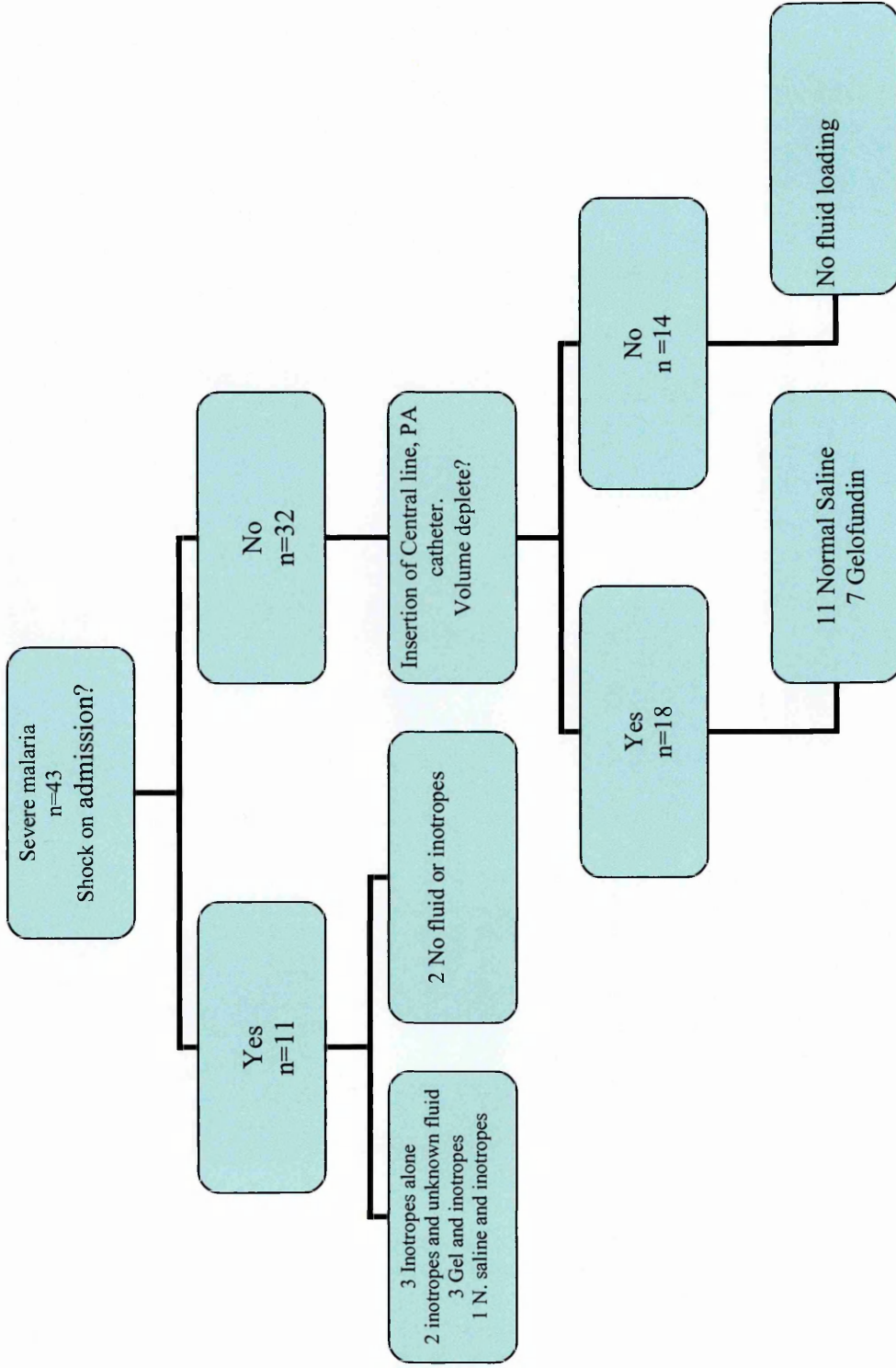
Befitting a series set in the ICU, the patients were extremely unwell on admission. Of the 43 patients, 11 were admitted in shock, 11 had pulmonary oedema and 26 met WHO criteria for malaria associated acute renal failure. The median GCS was 8 (95% CI: 7 to 10), the median base excess was -10.3 (95% CI:-11.9 to -7.9) and the median arterial lactate was 6.1 mmol/L (95% CI: 5.1 to 7.8). Thirty five percent of the patients would die during their admission. Logistic regression using purposeful selection in a model incorporating anti-malarial drug used, Glasgow coma scale, PaO_2/FiO_2 ratio, plasma creatinine, total bilirubin, peripheral parasite count, age, haematocrit, cardiac index, lactate and arterial pH confirmed that acid base status, as determined by the arterial pH, was the only significant predictor of outcome. Acid base status was a strong predictor of death: those with a base excess less than -10, had 4 times the risk of death (95%CI 1.01 - 15.86, p=0.049), and those with a bicarbonate less than 15mmol/L had 6.5 times the risk of death (95% CI 1.4 – 29.7, p=0.02).

Twenty-four (56%) of the patients received a fluid load and nineteen (44%) did not. The PaO_2/FiO_2 ratio and the CVP and PAoP were the only variables that were statistically significantly different between these two groups (the PAoP was used to determine whether a fluid load would be given, and the CVP was highly correlated with the PAoP (p <0.0001, r_s=0.75)) (Table 5.1). The median volume of fluid administered was 918mls (range 350 – 2000), and it was delivered over a median of 75 minutes (range 30 minutes to 3 hours 45 minutes). All the patients who later died were able to complete their fluid load.

Table 5.1 Baseline demographics of the patients (all values are median (95% CI))

Variable	Fluid load n=24	No fluid load n=19	P value*
Age (years)	38 (25.7 - 48.3)	34 (21 - 43.5)	0.39
Male (%)	21/24 (88%)	15/19(79%)	0.68
Parasite count (x 10^3/µl)	94.5 (33 - 305.1)	50 (8.7 - 107)	0.17
Artemisinin based therapy	15/23 (65%)^	8/17 (47%^	0.34
Glasgow Coma Score	8 (5 - 10)	10 (7 – 13)	0.15
Lactate (mmol/L)	6.1 (3.9 - 7.8)	6.2 (5.3 - 9.7)	0.52
Creatinine (µmol/L)	339 (261 - 526)	247 (203 - 444)	0.39
Total bilirubin (µmol/L)	121 (74 - 178)	103 (53 - 158)	0.48
Haematocrit (%)	27 (24 – 32)	30 (28 - 31)	0.45
Sodium (mmol/L)	134 (130 - 136)	137 (128 - 143)	0.16
Potassium (mmol/L)	4.1 (3.8 - 4.5)	4.2 (3.9 - 4.6)	0.63
Renal failure*	18/24 (75%)	12/19 (63%)	0.51
Anuric^	11/24 (46%)	8/19 (42%)	1
Shock	6/24 (25%)	5/19 (26%)	1
Pulmonary oedema	4/24 (18%)	7/19 (37%)	0.17
MABP (mmHg)	78.5 (75.3 - 82)	83 (64.5 - 93.3)	0.59
CVP (mmHg)	2 (0 – 3)	4 (3 – 6)	0.005
PAoP (mmHg)	6 (4 - 6.4)	10 (7 - 13.3)	0.001
Cardiac index (L/min/m²)	4.1 (3.3 - 4.7)	3.9 (3.2 - 4.7)	0.91
SVR (dyne/s/cm^{-5}m²)	1429 (1313 - 1907)	1375 (1243 - 1962)	0.86
Oxygen saturation (%)	96.5 (95.2 - 97.2)	95.3 (92.4 - 97.5)	0.24
PaO2/FiO2 ratio	436 (381 - 564)	207 (183 - 475)	0.02
CvO2 (%)	60.2 (55.3 - 64.3)	59.3 (49.5 - 63.1)	0.57

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pH	7.36 (7.31 - 7.39)	7.32 (7.27 - 7.36)	0.12
Bicarbonate (mmol/L)	13.9 (11.6 - 16.9)	15 (9.9 - 16.9)	0.99
Base excess	-10.5 (-12.3 - -7.1)	-9.4 (-15.3 - -7.6)	0.78
DO2 (mL/min/m^2)	454 (378 - 538)	452 (343 - 533)	0.99
VO2 (mL/min/m^2)	154.5 (144 - 185.4)	168 (151.7 - 187.7)	0.52
O2ER %	36.5 (32.7 - 42.7)	37 (32 - 47.2)	0.83

* P test by Chi squared. ^ in three patients the antimalarial used was not recorded.

* 1 patient did not have dopamine receipt recorded.

MABP: Mean arterial blood pressure, PAoP: Pulmonary artery occlusion pressure,

SVR: Systemic vascular resistance, CvO2: Mixed venous oxygen saturation.

DO2: Oxygen delivery. VO2: Oxygen consumption. O2ER: Oxygen extraction ratio.

There was a higher mortality rate in the patients who received a fluid load, although with the small sample size, this did not reach statistical significance. There was no significant difference between the two groups in the development of shock, renal failure, pulmonary oedema or neurological deterioration (Table 5.2).

Table 5.2 Outcome by fluid load

	Fluid load		No fluid load		p value
Died	9/24	38%	6/19	32%	0.8
APO developed	1/24	4.2%	1/19	5.3%	1
CM developed	0/24	0%	3/19	16%	0.08
ARF developed	3/24	13%	1/19	5.3%	0.62
Shock developed	5/24	21%	6/19	32%	0.5

P value is determined by Chi-squared.

APO: Acute pulmonary oedema. CM: Cerebral malaria. ARF: Acute renal failure

With a fluid load there was a significant median increase (95% CI) in Cardiac Index: 0.63 L/min/m^2 (0.31 - 0.94), PAoP: 5mmHg (3.7-6.7) CVP: 3mmHg (1-5) and mixed venous oxygen saturation (CvO2) 3.9% (0.5 - 5.3), and there was a significant median decrease (95%CI) in haemoglobin -0.7g/dL (-1.4 to -0.3), systemic vascular resistance (SVR) -139dyne/s/cm^{-5}m^2 (-334 to -68), oxygen consumption (VO2) -8.5 mL/min/m^2(-26 to -0.7) and the oxygen extraction ratio (O2ER) -4% (-6 to -0.7). Notably there was no significant median change in the acid base status of the patients (Table 5.3)

Table 5.3 Change in variables with fluid loading n=24

Variable	Median Δ	95% CI
ΔMABP (mmHg)	1	-0.3 to 5
ΔCVP (mmHg)	3	1 to 5
ΔPAoP (mmHg)	5	3.7 to 6.7
Δ Cardiac index (L/min/m^2)	0.63	0.31 to 0.94
Δ SVR (dyne/s/cm^{-5}m^2)	-139	-334 to -68
ΔSaO2 (%)	0	-0.2 to 0.6
ΔpO2/FiO2	0	-18 to 22
ΔPaO2 mmHg	0	-3.8 to 4.8
ΔPaCO2 mmHg	0	-0.7 to 2.1
Δ Haemoglobin (g/dL)	-0.7	-1.4 to -0.3
Δ CvO2 (%)	3.9	0.5 to 5.3
Δ pH	-0.01	-0.02 to 0.01
ΔBicarbonate (mmol/L)	-0.1	-0.9 to 0.9
ΔBase excess	-0.55	-1.6 to 0.1
ΔDO2 (mL/min/m^2)	18	-16 to 68.4
ΔVO2 (mL/min/m^2)	-8.5	-26 to -0.7
ΔO2ER (%)	-4	-6 to -0.7
ΔLactate (mmol/L)	-0.1	-0.4 to 0.3

MABP: Mean arterial blood pressure, CVP: Central venous pressure, PAoP: Pulmonary artery occlusion pressure, SVR: Systemic vascular resistance, SaO2: Oxygen saturation, PaO2: Partial pressure of oxygen, PaCO2: Partial pressure of carbon dioxide, CvO2: Mixed venous oxygen saturation, DO2: Oxygen delivery, VO2: Oxygen consumption, O2ER: Oxygen extraction ratio

Twenty-two patients (92%) had the fluid that was used as the resuscitation agent recorded; twelve of these patients received normal saline and ten received gelofundin. The group that received gelofundin were sicker with a lower MABP, pH, and base excess and a greater arterial lactate. Despite this, patients receiving gelofundin had a similar mortality rate to those receiving normal saline. (Tables 5.4 and 5.5)

Table 5.4 Baseline demographics by resuscitation agent (all values are median (95% CI))

Variable	Normal saline	Gelofundin	p value*
Age	36.5 (22.4 - 52.8)	34.5 (21 - 46.7)	0.67
Sex (male)	12/12 (100%)	7/10 (70%)	0.08
Parasite count (x 10^3/μl)	63.6 (10 - 285)	113 (26.3 - 560)	0.32
Artemisinin based therapy	9/11 (82%)	5/10 (50%)	0.18
Glasgow coma score	8 (3 – 13)	7.5 (3 - 10)	0.49
Creatinine (μmol/L)	441 (160 - 675)	309 (203 - 532)	0.4
Total bilirubin (μmol/L)	7.1 (2.6 – 14.5)	6.9 (2.5 - 10)	0.64
Haematocrit	32 (25.5 – 34)	24.5 (19.3 - 36.1)	0.11
Sodium (mmol/L)	134 (127 - 139)	133.5 (126.1-138.3)	0.84
Potassium (mmol/L)	4.4 (3.8 - 4.7)	3.8 (3.2 - 4.3)	0.07
Renal failure	9/12 (75%)	8/10 (80%)	1
Anuric on admission	6/12 (50%)	4/10 (40%)	0.69
Shock on admission	1/12 (9%)	3/10 (30%)	0.29
Cerebral malaria	10/12 (85%)	10/10 (100%)	0.48
APO on admission	2/12 (17%)	1/10 (10%)	1
MABP (mmHg)	84 (78 - 91.7)	77.5 (69.3 - 79.7)	0.009
CVP (mmHg)	2.5 (0 - 3.9)	1 (-1 - 2.7)	0.27
PaoP (mmHg)	5.5 (4 – 6)	6 (3.3 - 7.7)	0.64
Cardiac index (L/min/m^2)	4.38 (3.24 - 4.98)	4.06 (2.92 - 4.54)	0.45
SVRdyne/s/cm^{-5}m^2)	1570 (1179 – 2136)	1429 (1120 - 2142)	1
PaO2/FiO2 ratio	425 (312 - 575)	477 (389 - 620)	0.29

CvO2 (%)	62.4 (58.4 - 67.5)	54.7 (40.9 - 64.4)	0.048
pH	7.39 (7.37 - 7.41)	7.30 (7.24 - 7.38)	0.004
HCO3 (mmol/L)	16.3 (13.7 - 19.3)	10.8 (8.3 - 16.4)	0.009
Base excess	-7.4 (-10.5 - -4.9)	-13.4 (-16.7 - -8.4)	0.006
DO2 (mL/min/m^2)	518 (385 - 610)	377 (291 - 538)	0.09
VO2 (mL/min/m^2)	178 (135 - 195)	146 (142 - 209)	0.57
O2ER (%)	34 (30.1 - 40.7)	41.9 (33.5 - 58.2)	0.06
Lactate (arterial)(mmol/L)	4 (1.6 - 6.8)	8.3 (2.8 - 12)	0.03

* by Mann Whitney. APO: Acute pulmonary oedema, MABP: Mean arterial blood pressure, MPAP: Mean pulmonary artery pressure, CVP: Central venous pressure, PAoP: Pulmonary artery occlusion pressure, SVR: Systemic vascular resistance, SaO2: Oxygen saturation, CvO2: Mixed venous oxygen saturation, DO2: Oxygen delivery, VO2: Oxygen consumption, O2ER: Oxygen extraction ratio

Table 5.5 Outcomes by resuscitation agent.

Variable	Normal saline		Gelofundin		p value*
Died	5/12	42%	3/10	30%	0.68
APO during admission	0/12	0%	1/10	10%	0.46
ARF during admission	1/12	9%	2/10	20%	0.57
Shock during admission	3/12	25%	2/10	20%	1

* by Mann Whitney. APO: Acute pulmonary oedema, ARF: Acute renal failure

Patients receiving gelofundin had a significantly greater increase in the cardiac index than those patients who received normal saline as the resuscitation fluid. Patients who received the colloid had a median increase of 1.07 L/min/m^2 (95% CI 0.66- 2.22) as opposed to a median increase of 0.38L/min/m^2 (95% CI 0.01 - 0.66) in the patients who received normal saline (p=0.006), this was despite the fact that there was no difference in the volume of fluid (median (95% CI) that the two groups received: normal saline 818ml (500 - 1196), Gelofundin 1000ml (500-1000) (p=0.97). However, this did not translate into a statistically significant improvement in the DO2, CvO2, VO2, pH, base excess or lactate (Table 5.6).

There was no relationship between the volume of fluid administered and the patient's outcome or their likelihood of developing shock, renal failure or pulmonary oedema. As would be expected, there was a significant relationship between the volume of fluid infused and the changes in the cardiac index (p=0.04, r_s=0.41), haemoglobin (p=0.03, r_s =-0.44), and the SVR (p <0.001, r_s=-0.63). However, there was no relationship between the volume of fluid infused and the changes in acid - base status (ΔBE p=0.23, r_s=-0.29, Δlactate p=0.30, r_s=-0.22, ΔHCO3 p=0.24, r_s=-0.28, ΔpH p=0.63, r_s= -0.11).

There was no relationship between the CVP and PAoP on admission and cardiac output, oxygen delivery or acid/base status. After volume loading there was still no relationship between CVP and PAoP and these measures. The increase in CVP and PAoP with resuscitation was correlated with the increase in cardiac index, but there was no association between the change in CVP and PAoP and the changes in any other markers (Table 5.7).

Table 5.6 Response to resuscitation agents at 24hrs

Variable	Normal Saline	Gelofundin	P*
ΔMABP (mmHg)	1 (-4.7 - 4.9)	4.5 (-0.67 - 13.4)	0.19
ΔCVP (mmHg)	2.5 (0 – 4)	5.5 (1.3 – 7)	0.07
ΔPAoP (mmHg)	5.5 (2.2 – 7.8)	5 (3.3 – 9)	0.43
ΔCardiac index (L/min/m^2)	0.38 (0.01 – 0.66)	1.07 (0.66 - 2.22)	0.006
ΔSVRI (dyne/s/cm^{-5}m^2)	-127 (-281 – -8.4)	-365 (-712 - -30)	0.15
ΔSaO2 (%)	0 (-0.2 – 1.34)	-0.1 (-0.5 – 1)	0.22
ΔHaemoglobin (g/dL)	-0.7 (-1.6- -0.2)	-1.1 (-3 - 0)	0.37
ΔCvO2 (%)	2.8 (-0.3 – 5.5)	4.7 (-0.9- 11.1)	0.55
ΔpH	-0.02 (-0.04 - -0.01)	0 (-0.03 - 0 .03)	0.08
ΔHCO3 (mmol/L)	-0.2 (-1.6 – 1)	0 (-1.3 - 0.7^)	0.78
Δbe	-1 (-2.3- 0.3)	-0.5 (-1.7 – 1^)	0.46
ΔDO2 (mL/min/m^2)	8.5 (-21 - 30)	67.5 (-27.9 - 121)	0.11
ΔVO2 (mL/min/m^2)	-9 (-34 –6.3)	-6.2 (-45.6 - 23.3)	0.74
ΔO2ER (%)	-2.5 (-5.9 – 0.9)	-5.2 (-11.1- 0)	0.32
ΔLactate (mmol/L)	-0.1 (-0.7 – 0.9)	-0.1 (-1.5 - 0.5)	0.7

* by Kruskal Wallis. ^ upper confidence limit held at maximum of sample. MABP: Mean arterial blood pressure, CVP: Central venous pressure, PAoP: Pulmonary artery occlusion pressure, SVR: Systemic vascular resistance, SaO2: Oxygen saturation, CvO2: Mixed venous oxygen saturation, DO2: Oxygen delivery, VO2: Oxygen consumption, O2ER: Oxygen extraction ratio

Table 5.7 Correlation between ΔCVP and ΔPAoP

Variable	CVPΔ	Rho*	PAoPΔ	Rho*
LactateΔ	0.56	-0.13	0.71	-0.08
BEΔ	0.88	0.03	0.62	0.13
HCO3Δ	0.59	-0.14	0.85	-0.05
CIΔ	0.06	0.39	0.44	0.16
DO2Δ	0.98	0.01	0.74	0.07
VO2Δ	0.8	0.05	0.66	0.1
O2ERΔ	0.69	-0.08	0.66	0.1
CvO2Δ	0.58	0.12	0.76	-0.07
MABPΔ	0.13	0.32	0.5	0.14

* Spearman's rho, p value by Spearman's.

CVP: Central venous pressure, PAoP: Pulmonary artery occlusion pressure, BE: Base excess, HCO3: Bicarbonate, CI: Cardiac index, DO2: Oxygen delivery, VO2: Oxygen consumption, O2ER: Oxygen extraction ratio, CvO2: Central mixed venous oxygen saturation, MABP: Mean arterial blood pressure.

A PAoP of 9-12 was used as the target for the resuscitation, and the baseline PAoP was correlated with the volume of fluid that was required to achieve this endpoint (p=0.001, rho=-0.57). However there was a great deal of overlap in these values, for instance in the 5 patients with a PAoP of 4mmHg anything between 800ml and 2000ml of fluid was required to raise the PAoP to the target range. On the other hand, there was no relationship between the baseline CVP and the volume of fluid that was required to resuscitate the patient (Figure 5.2).

Figure 5.2   Relationship between baseline CVP and volume of fluid required to resuscitate patient
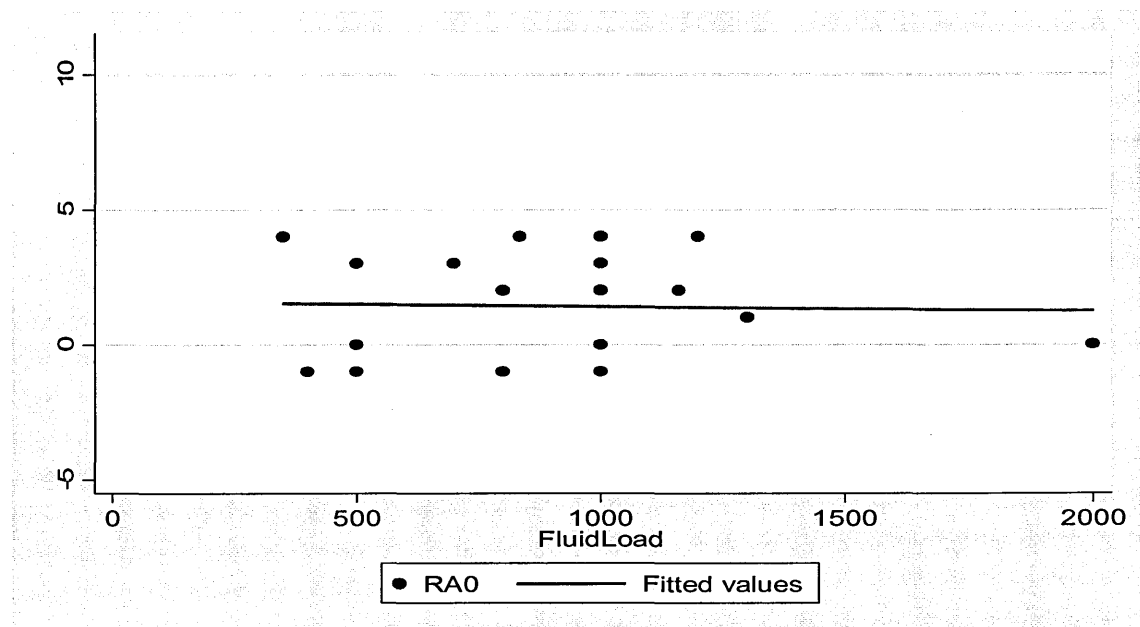
Baseline CVP v fluid load required P= 0.87, r= 0.04

There was no significant difference between the CVP of the patients who did and did not have pulmonary oedema on admission. The median CVP (95% CI) of patients both with (n=11) and without pulmonary oedema (n=32) was 3mmHg 95% (CI: 0.7-4.2 and 2-4 respectively, p=0.89 for a difference). Similarly there was no statistical difference in the PAoP of the two populations: the median PAoP (95% CI) was 9mmHg (4-11) in patients with pulmonary oedema and 7mmHg (6-8) in those without the complication (p=0.71 for a difference). There were only two patients who developed pulmonary oedema during their admission, preventing meaningful analysis of this phenomenon, however examination of the 56 time points for which there were simultaneous CVP, PAoP and PaO_2/FiO_2 measurements, revealed no relationship between either CVP (p=0.55) or PAoP (p=0.77) and gas exchange.

When all of the simultaneous assessments of macrovascular function and acid base status were pooled, there were up to 76 time points for which data were available. There was no relationship between the oxygen delivery and the prognostic markers of pH, bicarbonate or base excess. There was a correlation between the mean arterial blood pressure (MABP) and pH (p=0.03, r=0.27), bicarbonate (p=0.01, r=0.34), and base excess (p=0.02, r=0.31), although if the 8 datapoints where the MABP was less than 60 were removed, there was no longer a correlation: pH (p=0.7, r=0.05), bicarbonate (p=0.31, r=0.15), and base excess (p=0.46, r=0.11).

DISCUSSION

Although this is a small series, reviewed retrospectively and not powered to detect differences between the treatment groups, it nonetheless raises some interesting issues in

the fluid management of adults with severe malaria. Apart from the CVP and related PAoP (which was used to determine the need for a fluid load), and the PaO2/FiO2 ratio (significantly higher in those who were fluid loaded), there were no significant differences between the baseline characteristics of patients who received a fluid load and those who did not. Whilst it was anticipated that fluid loading would improve outcome and renal function in patients who were assessed as being hypovolaemic, the risk of both death and renal failure developing were absolutely, if not significantly, higher in the patients receiving a fluid load.

It has been suggested that fluid resuscitation can improve the acid base status of patients with severe malaria (Maitland, 2006), which is important, as acidosis is repeatedly noted to be a factor strongly associated with outcome (Day et al., 2000; WHO, 2000). Indeed, in our series, acid base status was the strongest predictor of death: those with a base excess less than -10, had 4 times the risk of death (95%CI 1.01 - 15.86, p=0.049), and those with a bicarbonate less than 15mmol/L had 6.5 times the risk of death (95% CI 1.4 – 29.7, p=0.02). However, in the 21 patients who had a base excess and bicarbonate measured before and after their fluid loading, the median values of these variables actually fell: -0.6 (95% CI -1.2 - 0.1) and-0.2mmol/L (95% CI -0.7-0.7mmol/L) respectively.

Patients who received a fluid load did have a rise in their cardiac index, and most had a rise in their oxygen delivery, so how do we explain the lack of improvement in their acidosis? The mechanical obstruction of the microvasculature is central to the pathophysiology of severe malaria (Dondorp et al., 2004), and has been demonstrated to link to acid base status (Dondorp et al., 2008). It may be that whilst fluid loading

improves macrovascular status, it does little to overcome the synchronous microvascular sequestration. Indeed, this series supports this hypothesis; when data were pooled there was no statistical correlation between cardiac index, oxygen delivery and the markers of acidosis.

Could it be that the patients were just given inadequate volumes of fluid to correct any hypovolaemia? It would seem unlikely. After fluid loading the cardiac index had improved a median of 15% (95% CI 5.4-21.5) and almost 90% of patients had a normal cardiac index. Even if we limit our analysis to the patients who had a low DO2 prior to resuscitation the median change in BE was 0 (95% CI -2.5-1), suggesting that fluid loading is doing little to change the marker of tissue perfusion most linked to outcome.

Another explanation for the lack of improvement in acid base status is the possibility that any improvement in the acidosis resulting from hypoperfusion has been offset by a hyperchloraemic metabolic acidosis induced by resuscitation with chloride rich saline and Gelofundin. In recent years, use of the physicochemical Stewart approach to acid base disturbance (Stewart, 1983) has increased the awareness that resuscitation with fluid of a supraphysiological chloride concentration, can lead to a metabolic acidosis through a reduction in the strong ion difference of plasma (Brill et al., 2002; Kaplan et al., 2005). Although the hyperchloraemic acidosis associated with resuscitation has not been conclusively linked to deleterious sequelae (Burdeet et al., 2003), it has been implicated in neurological dysfunction, coagulopathy, cardiac dysfunction, renal dysfunction and haemolysis (Burdeet et al., 2003). Unfortunately we lack sufficient data to make an assessment of the acidosis using the Stewart approach to further elucidate this relationship in this series.

Another concern with fluid loading is that not only might it fail to overcome the mechanical obstruction in the microcirculation, but with the increased pulmonary capillary permeability present in severe malaria (Charoenpan et al., 1990; Taylor et al., 2006), it may increase the risk of pulmonary oedema - a complication with a dismal prognosis in the resource poor setting. However in this small series only two patients developed this complication, one of whom received a fluid challenge and the other not, precluding any meaningful analysis.

The decision to fluid load patients, and the volume of fluid that was administered, was on the basis of the PAoP, a parameter strongly linked to the CVP. Since this study was performed, the weight of clinical opinion has turned against a reliance on pressure-based measures of preload to assess the patient's volume status. Neither CVP nor PaOP are good predictors of fluid responsiveness in either healthy volunteers (Kumar et al., 2006) or hypovolaemic critically ill patients (Michard et al., 2002), and current opinion in the developed world is that neither of these indices should be used to define the state of ventricular filling or the potential of patients to respond to a fluid challenge (Pinsky, 2005). In this series, there was no correlation between point measures of CVP and PAoP and cardiac index, oxygen delivery or acid base status, before or after fluid resuscitation. In addition to the observations about the potential limitations of CVP and PAoP being used as targets for resuscitation, there was no relationship between the CVP and PAoP and the presence of pulmonary oedema. There was also no relationship between the CVP/PAoP and the PaO_2/FiO_2 ratio. This accords with previous studies that have identified little correlation between pulmonary oedema and CVP (Edwards et al., 1993) or PAoP (Kumar et al., 2006), and is consistent with the hypothesis that pulmonary

oedema in severe malaria is predominantly the result of increased pulmonary capillary permeability (Charoenpan et al., 1990). Whilst central lines offer secure access and a conduit for the administration of veno-irritant drugs, they are relatively expensive and may be associated with serious complications during insertion and maintenance. In view of the limited benefit that they appear to provide the clinician in managing the patient with severe malaria in this and other series, the WHO recommendation to use them appears ill founded.

At baseline the patients who received gelofundin were sicker than the patients who received saline. They had a lower pH, MABP, base excess, a higher lactate, and would be expected to have a higher mortality rate; but there was no significant difference in mortality (Krishna et al., 1994; Day et al., 2000). The patients who received gelofundin had a significantly greater increase in their cardiac index and a trend to an increased oxygen delivery, although there were no changes in base excess and bicarbonate. A large multicenter, randomised trial showed no difference between the 28 day outcomes in a heterogeneous group of ICU patients resuscitated with 4% albumin when compared with saline (Finfer et al., 2004). However, there are possible reasons why colloid may be beneficial in the specific case of severe malaria: by increasing osmotic pressure, colloid therapy might reduce the risk of both cerebral and pulmonary oedema (Maitland et al., 2005). Furthermore, there are promising early results with the use of albumin therapy in paediatric series (Akech et al., 2006), although these results need to be replicated in prospective trials (Day, 2006; Planche, 2007). Our findings in the Gelofundin group are interesting in the light of this, though it must be noted that the possibility of a deleterious effect from normal saline loading cannot be dismissed and this has been one of the

criticisms of the work of proponents of colloid resuscitation in the paediatric malaria population (Planche, 2005).

There are several flaws in our study that prevent the generation of strong recommendations based on its findings. The first and most obvious is that the small sample size limits the power of the study to detect subtle differences between the various groups, and the patients were managed according to clinical judgement rather than via a defined protocol. Although renal failure is a common complication of severe malaria (Trang et al., 1992) the median serum creatinine in this series of 309.4μmol/L (95% CI 225.7 - 413.6) is much greater than in other series; indeed 44% of the patients were anuric on admission. Patients with renal impairment might be expected to handle a fluid load poorly and thus our observation that these patients had little benefit from fluid loading may reflect their renal impairment rather than any haemodynamic manifestations of their severe malaria. If fluid administration is guided by suboptimal markers of volume status – in this series CVP and PAoP – it may not be unsurprising that the results are poor. The number of patients with severe renal dysfunction also complicates interpretation of the acid-base data. Most patients in the series received quinine or intramuscular artemether as their anti-malarial agent – both therapies have now been superseded by intravenous artesunate - and thus the mortality rate in the series is higher than we might expect if the patients were managed with this newer agent.

Despite these caveats there are several observations worth making. The first is that while almost all adults with severe malaria will require intravenous fluids, the results of this study do not predict significant improvements in acid base status and outcomes with fluid resuscitation. The role of fluid resuscitation remains unclear, and although many will

have true or at least effective hypovolaemia on admission, a more conservative fluid regimen may turn out to be preferable, as has been demonstrated in other patients with ARDS and septic shock (Alsous et al., 2000). The results with the colloid resuscitation are interesting in light of the results with albumin-based resuscitation published in the paediatric literature. However, as in the paediatric population, there would need to be prospective randomised clinical trials to conclusively determine if there is any benefit with colloid therapy, and importantly to exclude the possibility of a deleterious effect from saline resuscitation (Molyneux et al., 2005; Kaplan et al., 2005). On the other hand, while concerns have been raised about the cost of colloid solutions, when compared with other therapies being instituted in the developing world at present such as anti-retroviral therapy for HIV/AIDS (Bradi et al., 2006), and other interventions proposed for severe malaria such as haemofiltration (Phu et al., 2002), any clear clinical benefit of colloid may be highly cost effective.

Despite malaria claiming the lives of thousands of adults every year, little is known about the optimal supportive therapies for these patients. Whilst the search for adjunctive therapies continues, much could be gained by optimising the use of the relatively cheap, widely available and easy to administer interventions already available – including intravenous fluids. High quality trials are urgently needed to determine the utility and safety of fluid resuscitation in severe malaria and the clinical features present in those who require fluid resuscitation. Such trials have already been performed in Dengue Shock Syndrome and simple, safe resuscitation protocols for this disease are now published (Wills et al., 2005). It must be remembered that the vast majority of patients with severe malaria will be managed in a resource poor setting, often by relatively inexperienced health care providers, with limited intensive care support. Resuscitation protocols would

need to be tailored to this environment to provide appropriately easy to implement, practical guidelines for these practitioners.

CHAPTER 6

PARASITE STAGING AND PREDICTING

OUTCOME IN SEVERE FALCIPARUM MALARIA

INTRODUCTION

Severe malaria is a multisystem disease requiring intensive care management. The assessment of disease severity is based on the multi-organ dysfunction, the haematological and biochemical indices, and the parasitaemia (WHO, 1986, 1990, 2000 and 2006). Admission peripheral blood smears are an important indicator of prompt treatment, and can be performed quickly (White and Silamut, 1989). The relationship between parasite counts and mortality in falciparum malaria was first described in 1937 (Field and Niven, 1937), and since then parasitaemia has been used for outcome prediction. The correlation between parasite count and prognosis varies at different levels of malaria endemicity. Patients who live in moderate or high transmission likely tolerate higher parasitaemia (WHO, 2006). Some patients had died with low parasite density (Fitz-Hugh et al., 1944). Pathophysiological and pathological features of severe falciparum include sequestration, cytoadherance, rosetting formation and reduced red cell deformability (MacPherson et al., 1985; White, 2003), thus the disease severity is related more to the number of parasites sequestered than to the number of immature ring forms that are seen in the peripheral blood (White and Ho, 1992). The morphology of parasite development in peripheral blood films can be used as a reflection of the proportion of infected red cells sequestered in the microvasculature of vital organs (White and Krishna, 1989; White et al., 1992). The stage of parasite development on the admission blood

slide provides important prognostic information in severe falciparum and previous malaria treatment with quinine did not appear to alter the stage distribution of parasites (Silamut and White, 1993). However the effects of antimalarial drugs other than quinine on the distribution of stage development were as yet unknown. In recent years, antimalarial drugs such as quinine, mefloquine, chloroquine, artemisinin and its derivatives, have been used widely in Viet Nam for the treatment of falciparum malaria. Artemisinin and its derivatives are the most rapidly acting antimalarials known to-date and are well tolerated. By acting on ring stages, they clear peripheral parasitaemia more quickly than other antimalarial drugs and prevent the development into mature sequestering blood stages (White and Olliaro, 1998). I conducted the present study to assess the stage distribution of malaria parasite on admission peripheral blood films of untreated and treated patients and its relationship with the prognosis in severe malaria.

PATIENTS AND METHODS

The study was conducted in the Malaria Research Unit, Hospital for Tropical Diseases, Ho Chi Minh City and was approved by the hospital's Scientific and Ethical Committee. Informed consents were obtained from the patients or their accompanying relatives.

Patients were included in had asexual forms of *P. falciparum* on a peripheral-blood smear, were older than 14 years, were not in the first trimester of pregnancy, were not intravenous drug users, had received less than 3g of quinine or two doses of artemisinin or a derivative in the previous 48 hours, and had one or more of the following: a score on the Glasgow Coma Score of less than 11 (indicating cerebral malaria); anaemia (haematocrit, <20 percent), with a parasite count exceeding 100,000 per microlitre on a peripheral-blood smear; jaundice (serum bilirubin, >2.5mg per deciliter [50μmol per litre]), with a

parasite count of more than 100,000 per microlitre on a peripheral-blood smear; renal impairment (urine output, <400ml per 24 hours; and serum creatinine, >3mg per decilitre [250μmol per litre]); hypoglycaemia (blood glucose, <40mg per decilitre [2.2mmol per litre]); hyperparasitaemia (>10 percent parasitaemia); and systolic blood pressure below 80mmHg with cool extremities (indicating shock). Patients were also excluded if there was known allergy to one of the artemisinin derivatives or quinine. All patients were managed with similar intensive care and by the same staff throughout the study.

Parasite counting and staging

Blood was obtained by a finger-prick for hematocrit measurements and blood smears on admission, and every 4 hours for the first 24 hours and every 6 hours until three consecutive smears were negative for asexual stages of *P. falciparum*. Thick films were stained with Giemsa's stain and thin films with Field's stain. The degree of parasitemia was determined on the basis of the number of parasitized red cells per 1,000 red cells (thin film) or the number of parasites per 400 white blood cells (thick film). Blood smears were examined microscopically under oil immersion at a magnification of x 1,000. Parasite counts were derived from the percentage thin film parasitaemia and an estimate of the red cell count derived from the admission haematocrit (haematocrit x 125,000/μL). Thick film counts were obtained assuming a white blood cell count of 8,000/μL. The slides were also divide into groups based on the basis of parasitaemia, whether < 1%, 1-5%, 5-10%, or > 10%. Each slide was examined independently by two microscopists, and the smears were re-read if there was a disagreement of assessement.

All slides were staged by two experienced microscopists who did not know the severity of the patients. One hundred randomly parasites were assessed from the tail of each slides for staging. The stage of parasite development is graded into 8 stages, using a

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modification of the method described by Silamut et al., 1999 (Figure 6.1). Thin film was used whenever possible, as morphology was easier to categorize. Parasites with bizarre forms were not staged. Gametocytes were noticed but excluded in analysis. The distribution of parasite stage from slides of survivors was compared to those of fatal cases. The comparison between untreated and treated patients was also observed.

Figure 6.1 The stage of malaria parasite development (reproduced from Silamut, 1999 with permission)

Tiny ring form (estimated age = 0-6 h): The width of cytoplasm band was less than half of the diameter of the nucleus.

Small ring form (estimated age = 6-16 h): The width of cytoplasm band was equal to, or greater than, half of the diameter; but less than of the diameter of the nucleus.

Large ring form (estimated age = 16-26 h): The width of cytoplasm band was equal to, or greater than, the diameter of the nucleus.

Early trophozoite (estimated age = 26-30 h): The cytoplasm was a blue mass containing little brown pigment first visible pigment.

Middle trophozoite (estimated age = 30-34 h): Nucleus and cytoplasm enlarged. Cytoplasm became dark with brown pigment.

Late trophozoite (estimated age = 34-38 h): Cytoplasm became spherical with brown pigment. No more than 2 irregular shaped nuclei evident.

Schizont (estimated age = 38-44 h): Dark brown pigment with 3-5 nuclei clearly visible. Red cell cytoplasm was paler.

Schizont (estimated age = 44-48 h): Dark brown pigment with more than 5 nuclei clearly visible. Red cell cytoplasm was paler.

Statistical analysis

Results were entered into a database (Microsoft Excel, Microsoft Corp, USA) and analyzed with a statistical software package (Stata 10, StataCorp, Texas, USA). Stage distributions were described by using analysis of raw data, stratified data by admission parasitaemia, and by comparison of the distribution of mode value. These distributions were compared by Kolmogorov-Smirnov equality of distribution test. Because the total parasite counts on admission were not normally distributed, we presented the geometric mean for log transformation of these variables with 95% Confident interval (CI). All significant tests were set at $p < 0.05$.

RESULTS

A total of 370 cases of severe malaria were studied. There were 333 admission slides from survivors and 37 slides from fatal cases. The geometric mean admission total parasite counts (per µL) were 69,587 (95% CI 26,155 – 185,139) in the fatal cases, 26,991 (95% CI 19,409 – 37,534) in survivors. The different between both groups was not significant (p> 0.05). There were 76 slides missing staging information because the parasite counts were too low for accurate assessment, or the stage assessment was difficult due to inappropriate staining. The total number of slides using for analysis was 294 (31 from fatal cases (10.5%)).

Parasite age distribution Overall the stage distribution of malaria parasites from survivors was shifted towards young ring forms. Tiny rings comprised > 50% of parasites in 159/263 survivors compared with 11/31 in fatal cases. Late parasite forms (trophozoites and schizonts) carried a poor outcome (Table 6.1).

Table 6.1 Stage distribution and mortality: number of patients in each group.

	Died	Survived	OR (95%CI)
Number	31	263	
Proportion in blood film			
>50% tiny rings	11 (35.5)	159 (60.1)	0.36 (0.17 - 0.78)
>10% trophozoites	16 (51.6)	85 (32.3)	2.2 (1.1 - 4.7)
>20% trophozoites	14 (45.2)	57 (21.7)	3.0 (1.4 - 6.4)
>1% schizonts	6 (19.3)	23 (8.9)	2.5 (0.93 - 6.7)

This shift towards younger forms in survivors was also stated in the distribution of modal stages (the most frequent stage), but there was not significant difference between fatal cases and survivors (Table 6.2; Figure 6.2 and 6.3).

Table 6.2 Comparison of synchronicity using means of modal stage.*

Synchronicity	N	Died Mean (SD)	N	Survived Mean (SD)	p- value
< 1%	3	42.3 (22.5)	58	60.3 (20.3)	0.14
1-5%	10	50.9 (19.6)	80	59.3 (21.6)	0.26
5-10%	8	63.4 (24.4)	45	64.6 (19.0)	0.88
>10%	9	57.3 (23.6)	60	67.7 (17.2)	0.11
	30		243		

* An additional 21 with no RBC parasitaemia information; **n for analysis = 273 (30 (11% died))**

Figure 6.2 Stage distribution of parasites, divided according to admission parasitaemia.*
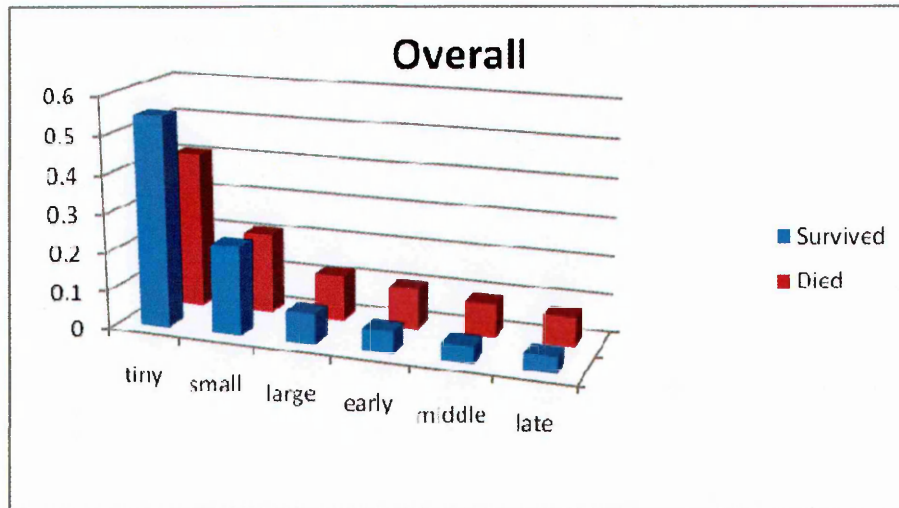
Variable	Survived (n=243)	Died (n=30)
tiny	0.54	0.40
small	0.23	0.20
large	0.07	0.11
early	0.05	0.10
middle	0.04	0.08
Late	0.04	0.07

Comparison of distributions by Kolmogorov-Smirnov test, p = 0.47

Figure 6.3 Stage distributions of parasites, divided according to admission parasitaemia
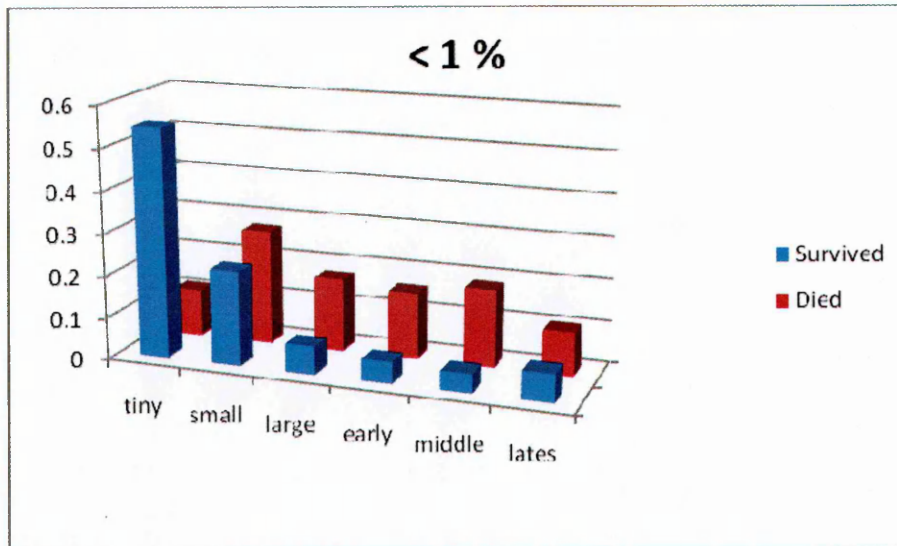
Variable	Survived (n=58)	Died (n=3)
tiny	0.54	0.11
small	0.22	0.27
large	0.06	0.17
early	0.05	0.15
middle	0.04	0.18
late	0.06	0.10

Comparison of distributions by Kolmogorov-Smirnov test, p = 0.03

Variable	Survived (n=80)	Died (n=10)
tiny	0.50	0.42
small	0.23	0.22
large	0.10	0.10
early	0.07	0.08
middle	0.04	0.07
late	0.03	0.08

Comparison of distributions by Kolmogorov-Smirnov test, p = 0.47

5 - 10 %
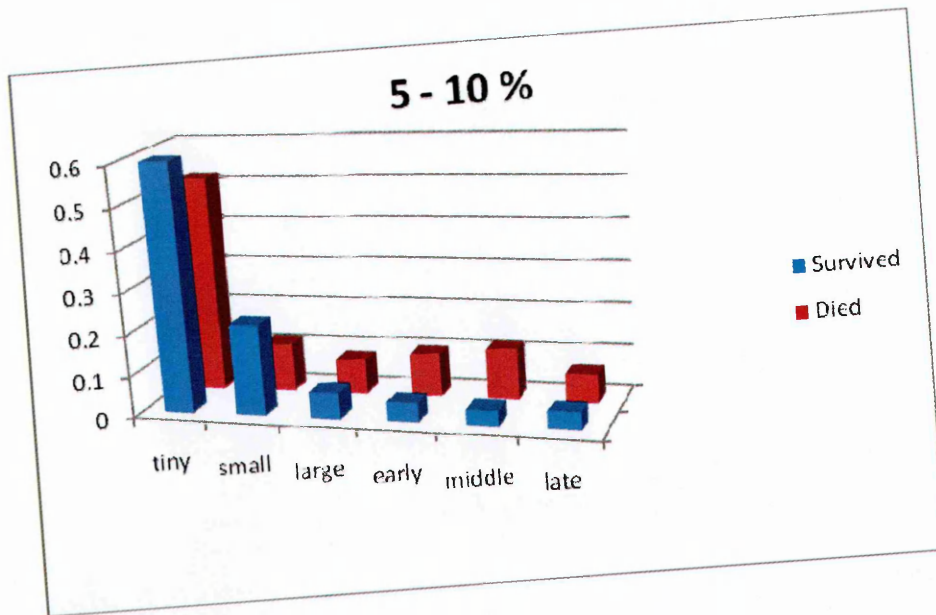
Variable	Survived (n=45)	Died (n=8)
tiny	0.59	0.51
small	0.21	0.11
large	0.06	0.08
early	0.04	0.10
middle	0.03	0.11
late	0.04	0.06

Comparison of distributions by Kolmogorov-Smirnov test, p = 0.03

> 10 %
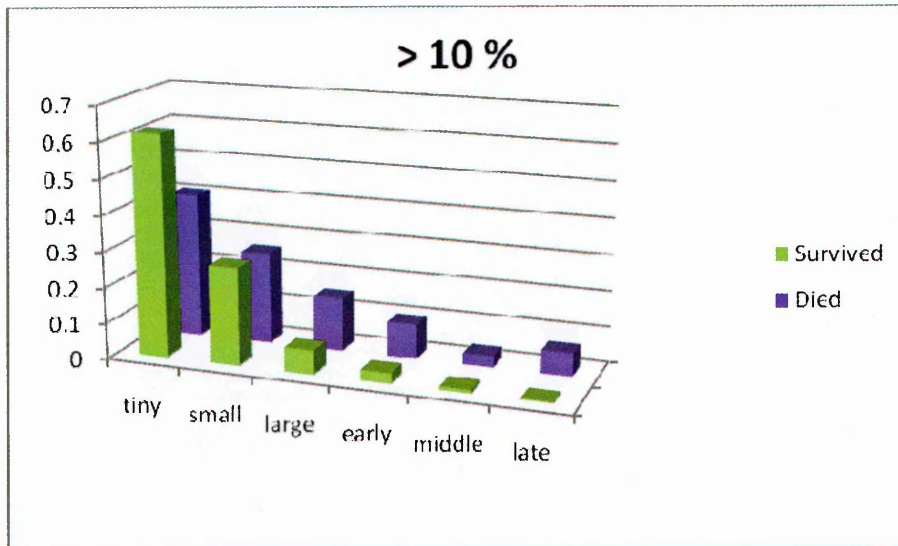
Variable	Survived (n=60)	Died (n=9)
tiny	0.61	0.404
small	0.27	0.25
large	0.06	0.14
early	0.02	0.09
middle	0.009	0.03
late	0.003	0.06

Comparison of distributions by Kolmogorov-Smirnov test, p = 0.47

* An additional 21 with no RBC parasitaemia information; **n for analysis = 273 (30 (11% died))**; category
'Late' includes late trophozoites combined with schizonts.

Effect of previous treatment on stage distribution: Since 1990 there has been widespread use of a wide range of antimalarial drugs in Viet Nam. Artesunate, quinine, chloroquine and mefloquine could be administered in any health centres and even in private sectors as well. Many patients had antimalarial drugs before admission to HTD. In our study a number of 142 (52%) patients had been treated with one or two antimalarials before admission, and 17 patients did not know which drug they had taken. I was not able to measure the admission plasma level of any antimalarial drug. The stage distribution of parasites were compared in each non pretreated and pretreated groups (Figure 6.4)

Figure 6.4   Stage distributions of parasites, divided according to pre-treatment
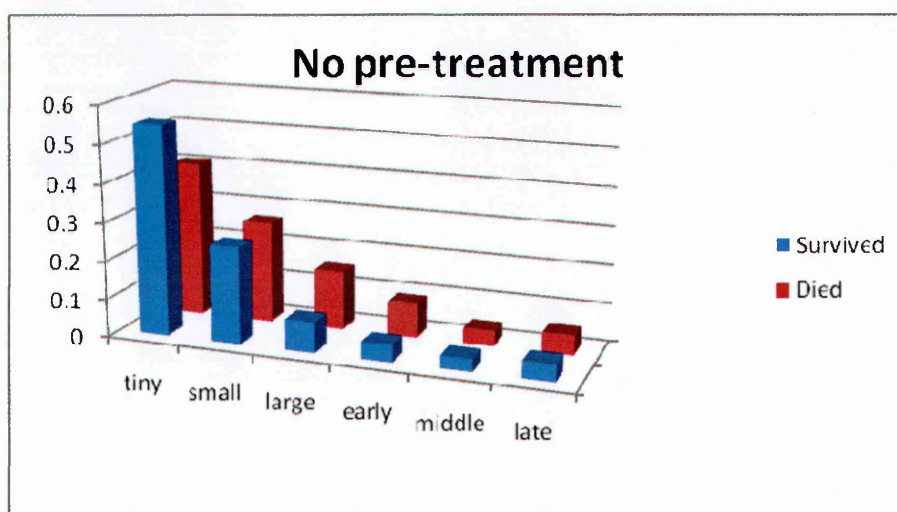
Variable	Survived (n=99)	Died (n=15)
tiny	0.54	0.40
small	0.25	0.26
large	0.07	0.15
early	0.04	0.08
middle	0.03	0.03
late	0.04	0.05

Comparison of distributions by Kolmogorov-Smirnov test, p = 0.47

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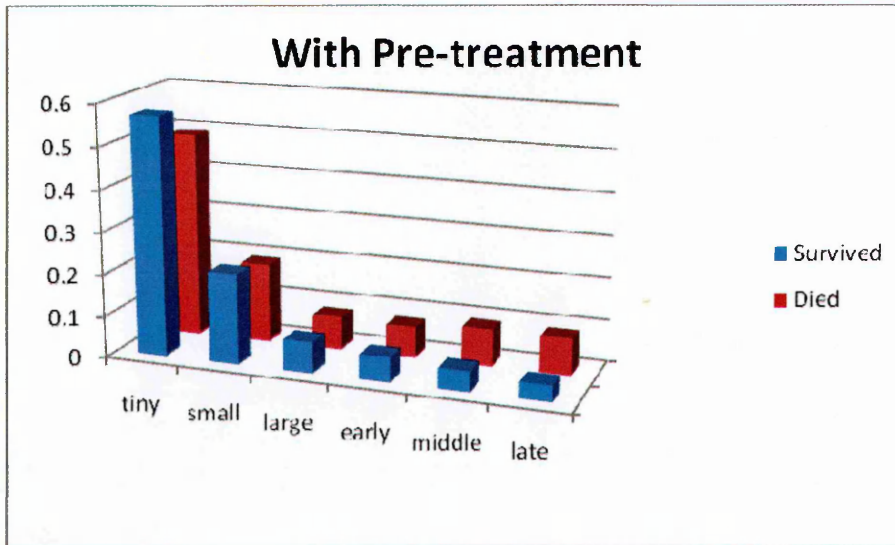
With Pre-treatment

Variable	Survived (n=132)	Died (n=10)
tiny	0.56	0.48
small	0.21	0.18
large	0.07	0.07
early	0.05	0.07
middle	0.04	0.08
late	0.03	0.08

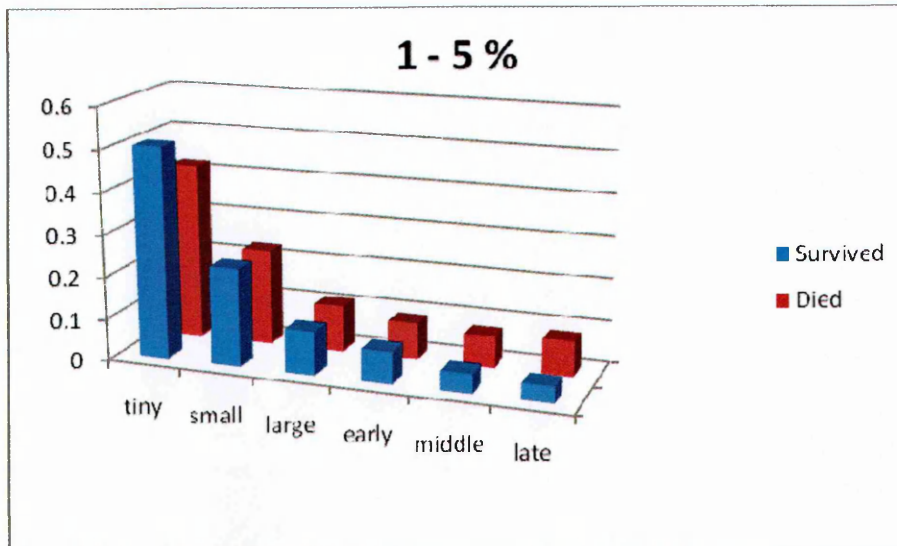Comparison of distributions by Kolmogorov-Smirnov test, p = 0.47
NOTE: there were an additional 17 pts with no pretreatment data excluded from this analysis

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DISCUSSION

In 1894 the Italian researchers noticed the difference between the number and stages of malaria parasite development in the peripheral blood and the brain of fatal cases (Marchiafava and Bignami, 1894). Only the younger forms of *P. falciparum* were seen in the blood films and the mature stages were noted in the cerebral vessels. Sequestration occurs in falciparum infection and is the patholophysiological feature of severe malaria (White and Ho, 1992). In contrast all stages of parasite development may be seen in peripheral blood smears of *P. vivax, P. ovale and P. malariae* infections (Field and Shute, 1956; Garnham, 1966) and those infections are very rarely fatal. The use of hyperparasitaemia as criteria for the diagnosis of severe malaria has been recommended by WHO (WHO, 1996, 2000, 2006), but peripheral falciparum parasitaemia alone provides limited information about the disease severity and outcome. Thick blood film examination was less sensitive and may underestimate the parasite density (Bejon et al., 2006). Hyperparasitaemia (over 100,000/µL) was correlated with increased mortality (Field and Niven, 1937) and the presence of schizonts in the peripheral smears is a poor prognosis, particularly if accompanied by hyperparasitaemia (Field, 1949), but a predominance of mature trophozoites and schizonts were also seen in peripheral blood of mildly ill immune adults (Raper et al., 1945; Schwetz, 1949). High schizont count in peripheral blood smear in uncomplicated malaria was indicative of potential deteriotation (Lwin et al., 2008). Recent study showed that the prognostic significance of schizontaemia depended largely on the overall age distribution of parasites. Schizontaemia was a specific indicator of poor outcome, but was not sensitive. In this study, we did not use the method described by Jiang et al in 1982, in which asexual parasites were graded for stage of development into 5 stages (tiny rings, small rings, large rings, mature trophozoites and schizonts). I found that using the modified method of

Silamut et al. was difficult to differentiate between early and middle trophozoites.when observing on thick films.

A predominance of older parasites carries a worse prognosis and it may reflect a relatively greater sequestration of parasitized red cells (Silamut and White, 1993). The effect of parasite age development was examined independently from parasitaemia, and the results from our study again suggest that a predominance of tiny ring forms (more than 50% tiny rings in blood film) carries a better prognosis in severe falciparum malaria than a predominance of older parasites because it reflects a relatively lower sequestration (Table 6.1). The difference was not significant at any group of parasitaemia (Table 6.2), presumably because the sample size was small.

The sequestered parasite biomass, calculated from parasite derived plasma PFHRP-2 concentrations, correlates with disease severity (Dondorp, 2008) but laboratory measurement equipment is needed. Parasite staging only needs direct microscopic observation. Moreover, the dominant clones of parasite sequestered in deep organs are ussually the same as those in peripheral circulation (Dembo et al., 2006). There are very few studies focusing on the relation of the stage of malaria parasite development in the peripheral blood to the prognosis of severe falciparum malaria. Parasite staging has not been recognised yet as a predictor of outcome. This might be because it is easier to conduct the parasite count, which takes less time to perform than the technique of parasite staging, and currently there are not many microscopists interested in this feature.

The effects of antimalarial drugs on the stage distribution: The shift to more mature forms in fatal groups was evident in both non pretreated and pretreated patients (Figure 6.3). The result suggests that previous treatment did not alter the stage distribution although most of patients had received artemisinin derivatives (39.4% 56/142 treated

patients) in 48 hours before admission, and there were 8 pretreated patients received artemisinin derivarives among 10 fatal cases of treated group. This finding should be interpreted with some caution as a high proportion of artesunate in pharmaceutical markets in Viet Nam has been found to be counterfeit.

The conclusion of this study was similar to that of the study of Silamut et al. in 1993 that there was a relationship of the stage of parasite development in the peripheral blood with the severity of malaria disease. A poor outcome should be considered if more mature parasites are detected in peripheral smears at any parasitaemia on admission. Further prospective studies are needed to determine the applicability of this finding.

CHAPTER 7

PREDICTIVE SCORE OF OUTCOME IN ADULTS WITH SEVERE

FALCIPARUM MALARIA

INTRODUCTION

Severe falciparum malaria remains one of the most important causes of death in the tropics. The increasing prevalence of multi-drug resistant falciparum parasite strains is a great problem in terms of reducing morbidity and mortality. Severe malaria results in serious complications and mortality. Among factors probably contributing factors to this high mortality are delayed diagnosis and inappropriate treatment. A reliable malaria severity score may help to predict the outcome of severe patients, and facilitate early transfer to an appropriate intensity of care. The common definition of severe malaria is that recommended by the World Health Organization and based on the presence of clinical and laboratory features (WHO, 1996, 2000 and 2006). It has been known that some vital organs such as the brain, kidneys, liver and lungs are affected in severe malaria. Even with specific antimalarial treatment, vital organ dysfunction will lead to the death of patient if these complications are not been immediately treated and resolved. The risk of death from severe malaria is very high in the first 2-3 days. In Viet Nam, as in other malaria endemic countries, most of severe malaria patients are firstly admitted in rural village or district health settings where laboratory facilities and supportive treatments are limited. Many outcome predictors have been identified in severe malaria, including hyperparasitaemia, schizontaemia, impaired consciousness associated with

repeated convulsions, respiratory distress, hypoglycaemia, lactic acidosis and acute renal failure (Field and Niven, 1937; Molyneux et al., 1989; Trang et al., 1992; Krishna et al., 1994; Marsh et al., 1995; Dondrop et al., 2004). A predictive score for the development of uncomplicated falciparum malaria into severe malaria was published recently (Tangpukdee et al., 2007). However there are few studies focusing on the scoring for predictive outcome in severe malaria. The objective of this study is to devise a simple Malaria Severity Score (MSS) that would help the clinicians to identify the adult malaria patients at high risk of death and which might be used as an indicator for referral to highest level of health care setting.

PATIENTS AND METHODS

A logistic regression model was built to assess the probability of death using data from two studies on severe falciparum malaria in the Hospital for Tropical Diseases (HTD) that compared the efficacy of antimalarial drugs. The first study was conducted to compare the effects of artemether and quinine (Hien et al., 1996) and the subsequent study in which artesunate was compared with artemether (Chapter 3). Severe malaria was defined with the same criteria in both studies (WHO, 1990). Because the clinical manifestations of severe malaria are different in children and due to the initial protocols of both studies, patients below the age of 15 were excluded from our prediction model. Both studies were approved by the Scientific and Ethical Committee of HTD. Informed consent was obtained from all patients or their accompanying relatives.

Statistical analysis

Results were entered into a database (Microsoft Excel, Microsoft Corp., USA) and analysed with a statistical software package (Stata 10, Stata Corp., Texas, USA). Using death as the dependant variable, an initial model was set up including all variables used

by the WHO to define severe malaria (WHO, 1990, 2006) as potential predictors (independent variables). A total of 19 admission clinical and laboratory parameters of patients were used for analysis. Univariate analysis was performed to examine variables associated with death, using the Mann-Whitney U-test for continuous variables. Multivariate logistic regression was used to model the probability of death, with the results of univariate analysis guiding data selection. Stepwise forward entry logistic regression was used to select variables associated with poor outcome and to find independent predictors with p-to-enter of ≤ 0.05 and p-to-reject ≥ 0.051. Continuous variables were transformed into categorical variables and entered into the model. The performance of the new score was assessed, sensitivity, specificity and the area under the Receiver Operator Characteristic (ROC) curve was calculated.

The clinical parameters associated with deaths and survivals were compared by using Chi-square or Fisher exact test; risk verification was expressed with Odds ratio (OR) and 95% Confident interval (CI).

Acidosis (as base excess) was not included because arterial blood gases were not routinely measured. Age was included because it was reported to be associated with poor outcome (Molyneux et al., 1989; Marsh et al., 1995; Dondorp et al., 2008), although most severe malaria cases were seen in Viet Namese adults. Pregnancy was also not included due to the protocols of both studies.

RESULTS

A total of the 975 adult patients were enrolled in both studies. Of these patients, 931 cases subsequently fulfilled the defined WHO criteria for severe malaria. I compared characteristics on admission using univariate analysis (Table 7.1). A review of admission

criteria confirmed that 42 patients had moderately severe malaria, and 2 patients were drug abusers. The differences in mortality rate were not significant between the artemether group and the quinine group (13% vs. 17%, p= 0.16 in the first study) (Hien et al., 1996), and between the artemether group and the artesunate group (13% vs. 7%, p= 0.52 in the second study) (see Chapter 3), thus the treatment with these artemisinin derivatives was not excluded. I compared characteristics on admission using univariate analysis. Among continuous data, age, temperature, Glasgow Coma Scale, respiratory rate, parasitaemia, serum creatinine, serum bilirubine, white blood cell count, platelet count and blood lactate were significantly different between who died and those who survived (Table 7.1). All categorical data such as pre-treatment with antimalarial drugs, previous malaria infection, convulsions, pulmonary oedema, spontaneous bleeding, except sex were also significantly different between the two groups (Table 7.2). Because of the widespread use of antimalarial drugs and the relationship between pre-treatment and outcome, the dataset was divided into two groups (pretreated or non pretreated) and the number of patients was reduced because there were 24 with missing data. The final logistic model with independent predictors of the two groups was described in Tables 7.3 and 7.4. To create the severity score of each group all significantly continuous variables were transformed to categorical variables. The final severity score of each group is calculated from the total of individual section score. For the non pretreated group, MSS 1 was from 0 to 6, with Glasgow Coma Scale have a maximum score of 2 (Tables 7.5), and a cut point of 4 was selected to divide "predicted survival" from "predicted death" (with $\geq$ 4 indicating predicted death), with the sensitivity was 70.7% (95% CI: 54.4-83.8), the specificity was 90.5% (95% CI: 85.9-94.0) and AUC = 0.84. For pre-treated group, MSS 2 was from 0 to 4 (Table 7.6), a cut point of 2 was selected with > 2 indicating predicted death, in order to optimize sensitivity (77.7%, 95% CI: 64.4-87.9), specificity (69.0%,

95% CI: 64.5-73.2) and AUC = 0.80. Each MSS had different cut-off points to optimize sensitivity and specificity.

Table 7.1 Results of univariate analysis of continuous data for symptoms present on or before admission to hospital. Values are median (interquartile range)

	n	Survived	Died	p-value
Age (years)	931	30 (22-41)	35 (26-45)	<0.0016
Temperature (°C)	925	38.5 (37.5-39)	38 (37-39)	<0.023
Systolic BP (mmHg)	929	110 (100-120)	110 (100-120)	<0.80
Glasgow Coma Scale	931	11 (8-15)	9 (7-13)	<0.0000
Respiratory Rate(/min)	931	28 (24-32)	32 (28-40)	<0.0000
Parasitaemia (/µl)	930	72094 (12057-300435)	114045 (35168-384959)	<0.0097
Haematocrit (%)	931	31 (25-37)	30 (27-37)	<0.33
Blood glucose (mg/dL)	928	94.5 (74-122)	90 (60-134.5)	<0.31
Serum creatinine (mg/dL)	915	1.85 (1.3-3.09)	3.15 (1.87-5.4)	<0.0000
Serum bilirubine (mg/dL)	791	3.6 (1.8-8.6)	8.5 (3.2-14.8)	<0.0000
White blood cell count (/µl)	893	8000 (5800-11000)	11850 (9200-16000)	<0.0000
Platelet count (/µl)	864	60000 (38000-90000)	40000 (26000-78000)	<0.0000
Blood lactate (mmol/L)	878	3.1 (1.9-4.6)	5.9 (3.4-10.2)	<0.0000

Table 7.2 Results of univariate analysis of categorical data for symptoms present on or before admission to hospital.

	Proportion dying (n=931)		
	n	%	p-value
Sex			0.27
Male	96/701	13.69	
Female	25/230	10.87	
Previously infected malaria			0.009
No	52/320	16.25	
Yes	39/400	9.75	
Pre-treated			0.021
No	49/298	16.44	
Yes	67/609	11.00	
Convulsion			0.019
No	102/840	12.14	
Yes	19/91	20.88	
Pulmonary oedema			0.000
No	108/898	12.03	
Yes	13/33	39.39	
Spontaneous bleeding			0.000
No	62/751	8.26	
Yes	59/180	32.78	

Table 7.3 Multivariate logistic regression analysis of admission data (non pretreated patients)

	OR	(95% CI)	p-value
Glasgow Coma Scale	2.503	(1.356-4.620)	0.003
High white blood cell count	2.863	(1.264-6.480)	0.012
Spontaneous bleeding	2.944	(1.279-6.773)	0.011
Renal failure	3.473	(1.385-8.706)	0.008
Hyperlactataemia	2.847	(1.273-6.370)	0.011

Table 7.4 Multivariate logistic regression analysis of admission data (pretreated patients)

	OR	(95% CI)	p-value
Jaundice	3.910	(1.456-10.495)	0.007
Spontaneous bleeding	4.724	(2.515-8.871)	0.000
Renal failure	2.605	(1.356-5.003)	0.004
Pulmonary oedema	3.014	(0.963-9.425)	0.058

Table 7.5 Malaria Severity Score 1 (MSS 1) for non pretreated patients

	Score
Glasgow Coma Scale	
= 15	0
11-14	1
≤ 10	2
High white blood cell count (> 10,000/µL)	
No	0
Yes	1
Spontaneous bleeding	
No	0
Yes	1
Renal failure (serum creatinine > 3 mg/dL)	
No	0
Yes	1
Hyperlactataemia (plasma lactate > 4 mmol/L)	
No	0
Yes	1

Total MSS 1 $\leq$ 4 = predicted survival

Total MSS 1 > 4 = predicted death

Table 7.6 Malaria Severity Score 2 (MSS 2) for pretreated patients

	Score
Jaundice (total bilirubinaemia > 2.5 mg/dL)	
No	0
Yes	1
Spontaneous bleeding	
No	0
Yes	1
Renal failure (serum creatinine > 3 mg/dL)	
No	0
Yes	1
Pulmonary oedema	
No	0
Yes	1

Total MSS 2 $\leq$ 2 = predicted survival

Total MSS 2 > 2 = predicted death

DISCUSSION

Rapid diagnosis and prompt treatment are crucial to prevent the development of complications in malaria infection. An accurate and inexpensive way of assessing levels of severity of severe malaria is also very important to reduce the mortality because it can help the physician at resource-poor settings to decide whether further supportive treatment is needed or whether the patient needs referring to the next level of health care. A Malaria Prediction Score was introduced in 2007 by using 5 variables such as age, serum creatinine, haemoglobinaemia, presence of cerebral malaria or pregnancy or ventilatory support (Mishra et al., 2007). In Viet Nam, jaundice and acute renal failure have been more often seen most of complicated malaria cases were in adults, and health care facilities at district level are limited. The mortality rate of severe falciparum malaria admitted to the Hospital for Tropical Diseases has declined markedly since early 2000, which was from 50-70% as reported in the decade of 1990 dropping to below 10%. In 2005, there were only 22 hospitalized severe malaria cases, and none of them was fatal. However, the mortality rate increased from that year to 2008. It was 2.9% in 2006, 6.6% in 2007, and 7.1% in 2008 (HTD, unpublished data). Most of the fatal cases were referred from remote health centres and had severe complications such as deep jaundice, acute renal failure and lactic acidosis which might represent some explanation for this outcome. To take some examples, the patients had arrived to the first health station for seeking antimalarial drugs too late, the junior doctors seldom thought that their patients had malaria, which resulted from the fact that malaria incidence had decreased dramatically in Viet Nam. Another reason was that the doctors delayed referring their severe patients to the higher level hospitals due to their failure to predict the severity of with an absence of

ventilators, blood gas measurement machines and dialysis capability. This tendency for the mortality rate of severe malaria tends to increase again in the recent years has made it is necessary to develop the Malaria Severity Score (MSS). The assessment indicators should be developed for the health care system where resources are limited, but the health officers can apply them in making their decisions on whether to retain their patients for further treatment at their sites or to transfer the patients to a well-equipped hospital.

I used data from two clinical studies which included detailed clinical and laboratory assessments and derived severity scores based on clinical signs and simple laboratory features. The univariate analyses showed that 15 variables were significantly associated with outcome, but only 7 independent predictors were selected due on multivariate analysis (Tables 7.5 and 7.6). The MSS 1 or MSS 2 can be easily applied in any district health settings. The new two MSS can be used in routine clinical practice to identify high-risk malaria patients to be transferred to higher levels of intensive care available, although further validation is needed in different settings.

CHAPTER 8

ASSESSMENT OF THE EFFICACY OF THE ARTEMISININ DERIVATIVES IN THE TREATMENT OF SEVERE FALCIPARUM MALARIA IN VIET NAM 1991-2008

INTRODUCTION

Since 1995 Viet Nam has been one of the first countries to decide to use artemisinin and its derivatives for the treatment of falciparum malaria, although the effect of these antimalarials had not been widely studied. Remarkably China was the only manufacturer of these drugs at that time. The reason for Viet Namese authorities making that decision was the high morbidity and mortality of malaria in the population. Although antimalarials such as quinine and mefloquine were available at all levels of the health care system, clinical trials had shown artemisinin and its derivatives to be the useful alternative to quinine; which was why artemisinin and its derivatives became the national first-line treatment of falciparum malaria since 2000. After 15 years of using these antimalarials, there was a need to know whether artemisinin resistance has emerged in Viet Nam. Recent clinical and molecular studies suggested the emergence of ACT-resistant *P. falciparum* infections along the Cambodia-Thailand border (Wongscrichalanai et al., 2006; Lim et al., 2009). Widespread and uncontrolled use of artemisinin could result in improper dose schedules and poor compliance, which in turn, might cause primary treatment failure, recrudescences and unexpected resistance to the drug. Such problems can be avoided or managed by regulating and monitoring its use. In 1989, the first preparation of artemisinin used in the Hospital for Tropical Diseases of Ho Chi Minh

City (HTD) was a suppository (Figure 8.2). Since 1995, artemisinin and its derivatives have been used as the main treatment for both uncomplicated and severe falciparum malaria, and the use of quinine has reduced. Hence, we conducted this preliminary study to investigate the parasite clearance time (PCT) from all severe malaria cases admitted to the Hospital for Tropical Disesases, Ho Chi Minh City between 1991 and 2008, and to monitor the efficacy of artemisinin derivatives over the years.

PATIENTS AND METHODS

Four therapeutic studies on severe malaria were conducted in the Hospital for Tropical Diseases, Ho Chi Minh City between 1991 and 2008: intramuscular (i.m.) artemether vs i.m. quinine (AQ study – Hien et al., 1996), i.m. artemether vs i.m. artesunate (pilot study) in 1996 (SMD – Unpublished data), i.m. artemether vs i.m. artesunate (AAV – Manuscript in preparation), intravenous artesunate between 2005 and 2008 (FK – Manuscript in preparation). All these studies took place on the same clinical ward, with essentially the same staff and followed identical procedures and so I believe the results are comparable. Patients were included in the studies if they (or an accompanying relative) gave informed consent, had asexual forms of P. falciparum on a peripheral-blood smear, were older than 14 years, were not in the first trimester of pregnancy, were not intravenous drug users, had received less than 3g of quinine or two doses of artemisinin or a derivative in the previous 48 hours, and had one or more of the following: a score on the Glasgow Coma Scale of less than 11 (indicating cerebral malaria); anaemia (hematocrit, <20 percent), with a parasite count exceeding 100,000 per cubic millimetre on a peripheral-blood smear; jaundice (serum bilirubin, >2.5mg per decilitre [50μmol per litre]), with a parasite count of more than 100,000 per cubic

millimetre on a peripheral-blood smear or with serum creatinine, >3mg per decilitre [250μmol per litre]; renal impairment (urine out put, <400ml per 24 hours; and serum creatinine, >3mg per decilitre [250μmol per litre]); hypoglycaemia (blood glucose, <40mg per decilitre [2.2mmol per litre]); hyperparasitaemia (>10 percent parasitaemia) or parasite count of more than 500,000 per cubic millimetre; plasma lactate > 4mmol per litre; arterial pH < 7.34 with standard base excess < -5mmol per litre; and systolic blood pressure below 80 mmHg with cool extremities (indicating shock).

These studies were approved by the Ethical and Scientific Committee of HTD.

Blood was obtained by a finger-prick for haematocrit measurements and blood smears every 4 hours for the first 24 hours and every 6 hours until three consecutive smears were negative for asexual stages of *P. falciparum*. The degree of parasitaemia was determined on the basis of the number of parasitized red cells per 1,000 red cells (thin film) or the number of parasites per 400 leukocytes (thick film).

Artesunate was given in a dose of 2.4mg/kg body weight on admission, and then 1.2mg/kg was given daily until oral medication could be taken reliably. Each 60mg vial contained anhydrous artesunic acid that was dissolved initially in 1ml 5% sodium bicarbonate and then mixed with 5ml of 5% dextrose before injecting.

Artemether (50mg per millilitre; Kunming Pharmaceutical Company, Kunming, P People's Republic of China) was given in a dose of 3.2mg/kg body weight on admission, followed by 1.6mg/kg daily until oral medication could be taken.

Both drugs were given intramuscularly to the anterior thigh in AQ, AAV and SMD studies. Artesunate was given intravenously in the FK study.

The blood level of any antimalarial drugs before admission was not measured.

Statistical analysis

Data were entered into a database (Microsoft Excel, Microsoft Corp., USA) and analysed with a statistical software package (Stata 10, Stata Corp., Texas, US). Chi-square or Fisher's exact test was used to compare proportions as appropriate.

RESULTS

A total of 807 patients were studied: 371 in AAV study, 271 in AQ, 43 in SMD and 122 in FK. Only patients who received artemether in the AQ study were included. There were 37 deaths in AAV, 33 in AQ, 11 in FK and none in SMD. Thirteen cases were missing an admission parasite count and were excluded, so the total number of cases using for analysis was 794 (Table 8.1).

Table 8.1 Number of patients included by study

Study	Freq.	Percent
AAV	369	46.47
AQ	266	33.50
SMD	38	4.79
FK	121	15.24
Total	794	100.00

The patients were classified into 3 groups by year (1991-1995; 1996-2000 and 2001-2008) (Table 8.2) for final analysis because the artemisinin derivatives have been deployed widely since 1996, and there has been a remarkable reduction of malaria morbidity and mortality since 2001. Because of the widespread and uncontrolled use of

antimalarial drugs in Viet Nam since 1991, the history of previous antimalarial treatment

before admission was also recorded (Table 8.2).

Table 8.2 Number (%) of patients pretreated or not pretreated before admission *

Group	Years	Not pretreated	Pretreated	Total
0	1991-1995	223 (81%)	52 (19%)	275
1	1996-2000	103 (39%)	159 (61%)	262
2	2001-2008	61 (30%)	143 (70%)	204
	Total	387 (52%)	354 (48%)	741*

* 53/794 patients were missing data on pretreatment; **n for analysis = 741**

The results of our study showed that between 1991 and 2008, 82.9% of severe malaria

cases were still parasitaemic after 48 hours in those patients who were not pretreated and

in 67.8% in patients who had been treated with any antimalarials before admission. At 72

hours, 59.1% of cases are still parasitaemic in the non pretreated group, and 41.2% in the

pretreated group (Table 8.3 and 8.4) (p= 0.15 and 0.26). In pretreated patients, the

proportions of patients in each group of year who did not cleared their parasitaemia after

48 hours were also not significantly different (Table 8.3) (p= 0.35), but there was a

significant decline in the proportion clearing after 72 hours between groups of years,

group 0: 53.8% and group 2: 32.9% (Table 8.4) (p= 0.01) throughout this period.

Table 8.3 Proportion of patients still parasitaemic on day 2

Group	Not pretreated		Pretreated		Total
	N	% (95%CI)	N	(%) (95%CI)	(N)
0	178/223	79.8 (74.0-84.5)	34/52	65.3 (51.8-76.8)	212/275
1	91/103	88.3 (80.7-93.2)	114/159	71.7 (64.2-78.1)	205/262
2	52/61	85.2 (74.3-92.0)	92/143	64.3 (56.2-71.7)	144/204
Total	321/387	82.9 (78.8-86.3)	240/354	67.8 (62.7-72.4)	561/741

Table 8.4 Proportion of patients still parasitaemic on day 3

Group	Not pretreated		Pretreated		Total
	N	% (95%CI)	(N)	(%) (95%CI)	(N)
0	124/223	55.6 (49.0-62.0)	28/52	53.8 (40.5-66.7)	152/275
1	66/103	64.1 (54.5-72.7)	71/159	44.7 (37.1-52.4)	137/262
2	39/61	63.9 (51.4-74.8)	47/143	32.9 (25.7-40.9)	86/204
Total	229/387	59.1 (54.2-63.9)	146/354	41.2 (36.2-46.4)	375/741

Figure 8.1

Kaplan-Meier remaining parasitaemic estimates

(Y-axis: Proportion still parasitaemic; X-axis: analysis time, 0 to 300 hrs)

Legend: Years 1991-1995 — Years 1996-2000 — Years 2001-onward
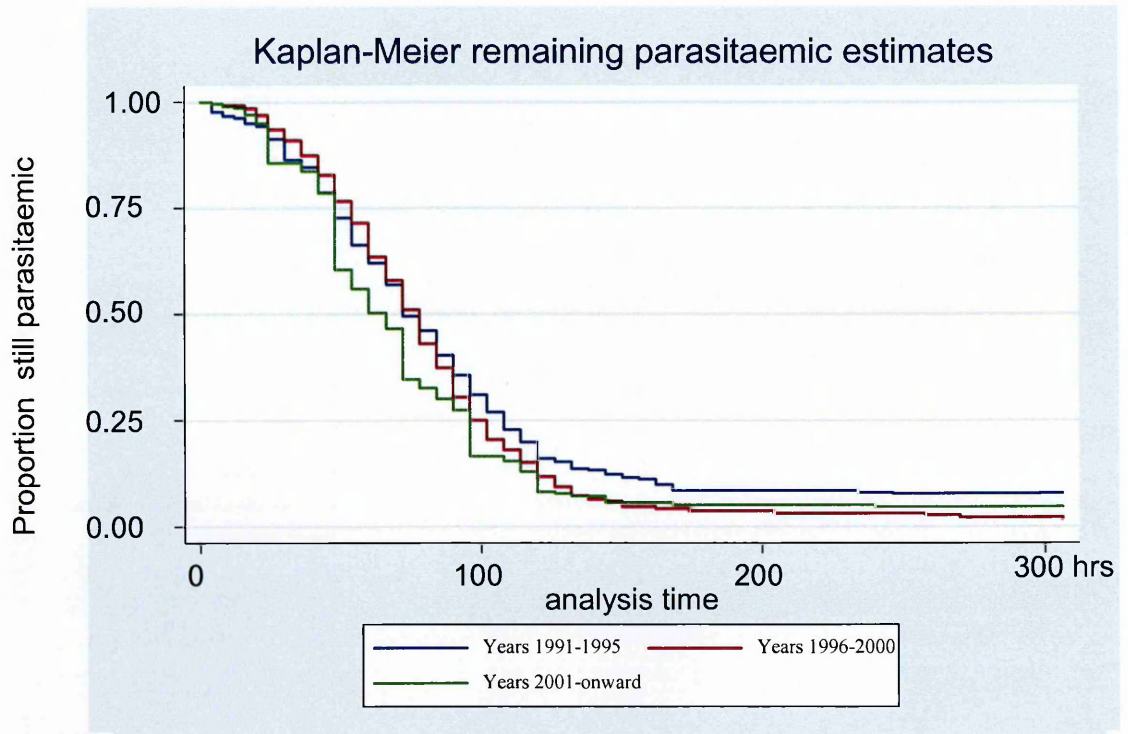
DISCUSSION

I assessed the efficacy in terms of parasite clearance of the artemisinin derivatives in the treatment of severe malaria in patients admitted to the Hospital for Tropical Diseases between 1991 and 2008.

From 1991 to 1996, artemisinin derivatives were not yet available in Viet Nam; most of pretreated patients received other antimalarials such as chloroquine, quinine or sulfadoxine-pyrimethamine. With the introduction of artemisinin derivatives in 1995, the use of chloroquine, sulfadoxine-pyrimethamine and quinine was reduced. Since 2000 quinine and other antimalarials have become unavailable for sale, therefore all of pretreated patients are likely to have taken artesunate before admission to hospital, and the continued treatment with parenteral artemisinin derivatives has affected the parasite clearance time (Table 8.4). Overall, the number of patients who had received antimalarial drugs as pretreatment increased through the years (Table 8.2), and approximately 40-60% of severe patients became aparasitaemic on day 3.

Through this parasitological observational study we could find no clear evidence of a change in the number of patients failing to clear their parasites after 72 hours. However there may be a small number of patients in the later years of this period who failed to clear their parasites (Table 8.3 and 8.4; Figure 8.1), leading to wider confidence intervals around the medians.

There are some reasonable criticisms of this study. Inevitably the study was retrospective, although the fact that all patients were seen on a single ward with a single study team following identical protocols I hope mitigates some of the retrospective nature of the

study. Despite studying 800 patients this may still not be a sufficient number of patients to see significant difference in the parasite clearance times, particularly if the number of patients with a resistant parasite was low. Obviously the time to parasite clearance is a variable controlled by many factors including age, sex, pregnancy, prior immune status, malnutrition, and the pharmacokinetic: pharmacodynamic relationship of the drugs. However in this low transmission setting most patients were non-immune, we only assessed adults (>15 years of age), very few suffered from other medical conditions including malnutrition and I believe these factors were less relevant in this relatively homogenous population anyway. Also I was unable to assess recrudescence by following up the patients for 28 days. It is recommended that the minimum duration of patient follow-up is 28 days in a study of uncomplicated malaria assessing antimalarial drugs with elimination half-lives of less than 7 days such as the artemisinin derivatives (WHO, 2003 and 2009). If there were 'resistant' parasites we might have expected to see more relapses in that group.

Southeast Asia is home to drug-resistant *P. falciparum* malaria. Both chloroquine-resistant and mefloquine-resistant falciparum parasites were firstly found in this region (Moore and Lanier, 1961; Harinasuta et al., 1965, Wongsrichanalai et al, 2004). The first clinical report of chloroquine treatment failure from South Viet Nam appeared in 1961. A standard *in vivo* test to assess the response of *P. falciparum* to chloroquine was developed in 1965 (WHO, 1965). This WHO classification of *in vivo* antimalarial drug sensitivity was used to assess the antimalarial drug efficacy, and was revised twice, in 1967 and 1972 (WHO, 1967 and 1973). In 2003, another classification for assessment of the efficacy of antimalarial drugs was introduced (WHO, 2003).

Recently, a significant increase in the proportion of patients failing to clear their parasitaemia by day 2 was noticed at the Thailand-Myanmar border (WHO Meeting report, Cambodia, 2008). Prolonged parasite clearance time following treatment with artemisinin mono or combination therapy has been observed along the Thai-Cambodian border from 2001 to 2007 (WHO Meeting report, Cambodia, 2008). In 2000, the artesunate and mefloquine combination was introduced as the first-line treatment for falciparum malaria in Cambodia, Thailand and Viet Nam. Recent clinical trials at the Thai-Cambodian border have pointed to the declining efficacy of both artesunate-mefloquine and artemether-lumefantrine combinations (Wongscrichalanai et al., 2006; Denis et al., 2006), and high dose of artesunate montherapy (Noedl et al., 2008).

Since the 1990s, artemisinin or artesunate monotherapy have been used as the main treatment for falciparum malaria in Viet Nam. It is regarded as one of the important factors leading to the reduction of malaria mortality and morbidity since then. While there were no clear documented reports of artemisinin derivative resistance *in vitro*, *in vivo* studies indicated emerging resistance with 17% failures in Dak Lak province, 18% in Binh Thuan and 8-50% in Binh Phuoc (NMCP, 2003). In the period 1991-1995 an *in vitro* drug resistance study was carried out in Yunnan, China in sites close to the border with Viet Nam. The result showed that there was no resistance to quinine or mefloquine, but there was resistance to chloroquine, amodiaquine and pyronaridine, and some unexpected resistance to artesunate, arteether and dihydroartemisinin (Yang et al., 1995). Molecular studies documented an increase in the level of mefloquine resistance in secondary isolates after artemisinin treatment and amplification of the wild-type Pfmdr1 gene, which is linked to antimalarial drug resistance, so that the monitoring for artemisinin resistance is needed (Ngo et al., 2003; Phuc et al., 2008).

Artesunate plus mefloquine is a mutually protective combination and remains highly effective in Viet Nam, but because of the lack of mefloquine supply the NMCP has switched to the combination of dihydroartemisinin and piperaquine for the treatment of uncomplicated falciparum malaria since 2007. *In vitro* study noticed that some isolates showed a diminished susceptibility to dihydroartemisinin (Cojean et al., 2006). For the moment, there is no replacement for parenteral artesunate in treatment of severe falciparum malaria. It is however required to monitor the efficacy of artemisinin derivatives routinely and precisely for early detection of artemisinin resistance, especially at the malarious regions near the Viet Nam-Cambodia border. The *in vivo* therapeutic efficacy and *in vitro* sensitive tests; molecular and genetic studies should be conducted to observe the decline of any antimalarial drug efficacy. The sale of all antimalarial drugs must be banned and their distribution should be strictly controlled by the NMCP. And all malaria patients should be treated with ACT regimen. Despite the official malaria drug policy, the injudicious use of artemisinin monotherapy and inappropriate dose used by the public health settings and private sectors may contribute to the development of resistance because of the increased drug pressure for selection of artemisinin tolerant malaria parasites. In this 18 year study we could find no clear evidence of a poorer response to artemisinin therapy. But the containment of artemisinin tolerant malaria should be considered. Otherwise, it will be very difficult to manage malaria disease, and as a result, malaria will probably re-emerge and spread in Viet Nam. Artemisinin resistance is truly a global threat! The emergence of malaria parasites that are resistant to the artemisinin derivatives will seriously undermine global malaria control.

Figure 8.2 Qinghaosu suppository, the first artemisinin used in HTD in 1989 (N.H. Phu)
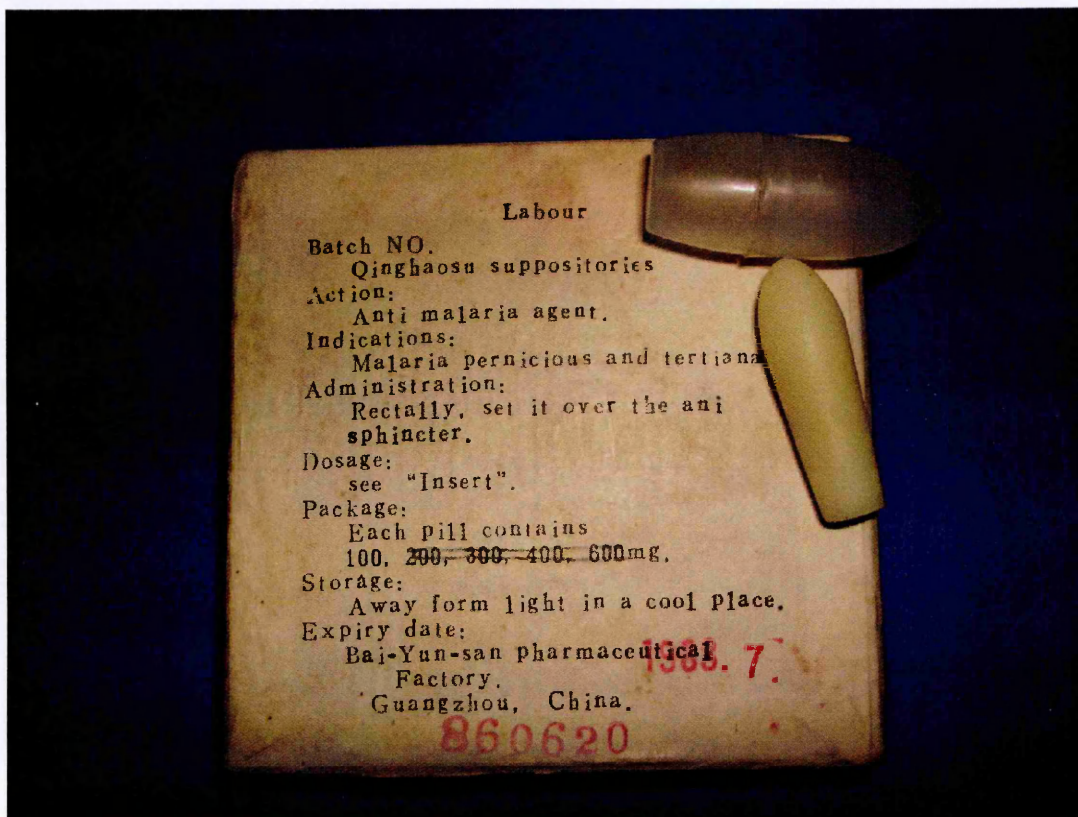
CHAPTER 9

DISCUSSION

In the last twenty years, the most important change in the management of malaria is the introduction of artemisinin based combination therapy as first line treatment. The effect of Qinghaosu (artemisinin and its derivatives) in the treatment of malaria has been studied in China since 1979 (Li et al., 1994) and as a direct result of meeting between Cho En Lai and Ho Chi Minh. Research in China showed good results after the treatment of uncomplicated and severe malaria with artemisinin and its derivatives. The first randomised controlled comparative study, to assess the efficacy of artemisinin was conducted outside of China, was done at HTD, Viet Nam in 1989 (Arnold et al., 1990). Following these initial results, many comparative trials throughout the world have confirmed the high efficacy of these antimalarial drugs that was claimed (but initially dismissed by the rest of the world) by Chinese and Vietnamese scientists. During the 1980s the mortality of severe falciparum malaria was approximately 40 per cent in HTD (almost 100% if accompanied by renal failure) (Hien, 1994), but decreased to 14.9 per cent in the period 1991-1995 (Hien, unpublished data). In 2009 the mortality rate at HTD was <5% for the same severity of disease. We would like to reach zero tolerance for malaria deaths at HTD. However as the incidence of a disease falls the mortality often rises and young clinicians in Viet Nam have much less personal experience of managing patients with severe malaria. In Viet Nam, artemisinin and its derivatives were recommended by the NMCP in 1995 and the use of artemisinin derivatives has been central to successful malaria control efforts in the country. The most important question

is whether artemisinin and its derivatives can reduce the mortality rate of severe malaria. Unexpectedly, a largest double-blind, controlled trial of artemether or quinine in adults with severe falciparum conducted in HTD between 1991 and 1996 did not show any significant difference in mortality (Hien et al., 1996). For the parenteral treatment of severe falciparum malaria artesunate and artemether are commonly recommended. Artemether is formulated in an oil base and can only be injected by the intramuscular route. Artesunate is more widely used because it is water-soluble and can be administered by either intravenous or intramuscular injection. A comparative pharmacokinetic (PK) study was performed at HTD and suggested that parenteral artesunate had considerable pharmacokinetic advantages over artemether in the treatment of severe malaria (Hien et al., 2004). In my work reported in this thesis the unadjusted relative risk of death for artesunate = 0.54, 95% CI: 0.28 – 1.02, p = 0.052 compared with treatment with artemether (Chapter 3). This data and the data from the PK studies strongly suggests that artesunate with its better pharmacological profile should be the treatment of choice among the Qinghaosu and its derivatives. The SEAQUAMAT trial provides powerful evidence that Artesunate is better than Quinine in adults in Asia and we wait for the results of the AQUAMAT trials with great interest.

In general, the number of deaths from severe malaria in Viet Nam has declined dramatically since the deployment of artemisinin derivatives. The explanation could be the contribution of artemisinin derivatives in reducing the number of severe malaria cases because artemisinins rapidly clear all stages of parasite in the peripheral blood, thus reducing the development of complications. The improvement of facilities for diagnosis and treatment may also play a role in reducing the mortality rate of severe falciparum malaria.

Severe malaria is a multisystem disease requiring intensive care management but optimum treatment is usually not available in most of the health settings where severe malaria occurs. Severe falciparum malaria results from sequestration of parasitized red blood cells in the vital organs. The evidence that immunological processes contribute to pathology is unconvincing. In Viet Nam the incidence of cerebral malaria and convulsions have declined from 90% in 1980s to 10 % in the early years of 2000 while jaundice, acute renal failure and lactic acidosis are now more common (1980: 6%, 1986-2006: 50-80%) (Hien, unpublished data). The clinician should not feel restricted by any strict definition of severe malaria. A simpler bedside assessment based on any impairment of consciousness or prostration, respiratory distress, oliguria or anuria, visible jaundice, hypoglycaemia, anaemia, high parasitaemia or shock identifies those patients in need of intensive care. A rapid clinical appraisal should be made. This includes assessment of level of central nervous system dysfunction, measurement of vital signs, paricularly respiratory pattern and rate, measurement of urine output, questioning of the patient or relatives concerning convulsions, duration of complications and earlier antimalrial treatment. A malaria parasite count, blood glucose, haematocrit, venous lactate and arterial blood gases should be measured immediately. Blood glucose should be regularly monitored in children and pregnant patients. Prompt chest X-ray on admission is helpful to exclude pulmonary oedema or pneumonia. A lumbar pucture should be done in cerebral malaria. The quantity of malaria pigmented neutrophils and parasite staging may be the measures of disease severity. Antimalarial drugs should be given on a miligram per kilogram basis. Artesunate (i.v., i.m., or rectal preparations) is the only drug of choice for the treatment of severe falciparum malaria. Therapeutic responses are assessed in terms of clinical and laboratory measures. Artemisinin based

combination therapy must be given when the patient can take oral medicine. Oral Artekin (dihydroartemisinin-piperaquine combination) now is available on prescription and free of charge in Viet Nam. If the parasitaemia has not cleared by 72 hours with the administration of artemisinin derivatives, the parasite count should be regularly monitored in order to detect any case of drug resistance. Fluid resuscitation should be carefully considered and if there is any doubt about fluid overload, a central venous line could be inserted for central venous pressure monitoring. Dialysis should be started early in severe patients with acute renal failure or lactic acidosis. Haemofiltration has been shown to be superior to peritoneal dialysis. Blood transfusion is needed if the haematocrit falls below 20%. Broad-spectrum antibiotics should be given in malaria patients with shock or in suspectedly bacterial coinfection. There is no evidence that adjunctive therapies are beneficial. Special nursing care is very important in the management of severe malaria, but is usually forgotten.

Conclusion

In summary the specific objectives set out in this thesis have been met. I hope my work (some of it included in this thesis) and that of my many colleagues at the Hospital for Tropical Diseases has made a contribution to improving the care of patients with severe malaria in my country and by extension globally.

The artesunate has become the first line treatment of severe falciparum malaria in Viet Nam. Our study on comparison of haemofiltration and peritoneal dialysis in patients with infection-associated acute renal failure, mostly severe falciparum malaria, have made an impact on the recommendation for the clinical management of severe malaria by the NCMP, Viet Nam and by the WHO (WHO, 2006). The measurement of arterial blood

gases requires expensive equipment and lactic acidosis or hyperlactataemia is an indicator of poor prognosis in severe malaria, therefore an inexpensive lactate measurement such as dipstick should be supplied for remote health stations. The technicians' skill for performing malaria parasite count, parasite staging and pigmented neutrophil count should be improved. Fluid resuscitation has not been routinely indicated, and intravenous fluid regimens are now carefully considered by clinicians. The Malaria Severity Score can be evaluated in larger clinical trials and in different places, especially at remote settings but is now in routine use at my hospital. Resistance to artemisinin drivatives has not yet been reported in Viet Nam but antimalarial drug efficacy monitoring should be urgently established. My data suggests that between 1991 and 2008 there has not been a dramatic prolongation in the parasite clearance times in patients with severe malaria treated with the artemisinin. Artemisinin based combination therapy must be restrictly prescribed in order to prevent the development of drug resistance. Most of the fatal malaria cases in the recent years were misdiagnosed therefore the regular update of the management of malaria for the health officers at the endemic areas will be helpful, aiming to remind them to guarantee an objective attitude to malaria as receiving a febrile patient in Viet Nam, a tropical country. Because of the widespread and uncontrolled use of artemisinin derivatives a negative blood smear should not excluded malaria diagnosis or delay malaria treatment, particularly in endemic or formerly endemic malaria areas.

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APPENDICES

Publications arising from this work associated with the work outlined in this thesis:

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Some of the other publications I have contributed to whilst undertaking this thesis:

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