RANDOMIZED CONTROLLED TRIAL OF ARTESUNATE PLUS TETRACYCLINE VERSUS STANDARD TREATMENT (QUININE PLUS TETRACYCLINE) FOR UNCOMPLICATED PLASMODIUM FALCIPARUM MALARIA IN BRAZIL.

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ABSTRACT

A triple blind randomized controlled trial was undertaken in a Brazilian Amazon region (Cuiabá-MT), to compare the effectiveness and side effects of oral artesunate (7 days, total dose = 0.75 g) plus tetracycline (7 days, total dose = 10.5 g) [AT] and oral quinine (3 days, total dose = 6 g) plus tetracycline (7 days, total dose = 10.5 g) [QT]. Eligible patients had uncomplicated *P. falciparum* malaria; age ≥ 14 years; no previous malaria treatment related to the present attack; and, if women, indication of absence of pregnancy. Clinical exam and blood tests were performed at baseline (day 0) and thereafter at days 2, 4, 7, 14 and 28. Effectiveness was assessed by cure rates (WHO criteria) and parasite clearance at day 2.

176 patients were randomized, 88 to each group. 95.6% of the AT group and 93.2% of the QT group completed the 7-day treatment. 81.8% of the AT group and 78.4% of the QT group completed follow-up visits to day 28. Groups had similar clinical characteristics at baseline. The incidence of side effects was much higher in the QT group (82%) than in the AT group (50%) [$p<0.0001$]. Cure rates were similar: 80% in the AT and 77% in the QT group ($p=0.68$). Parasitemia (by day 2) cleared faster in the AT group than in the QT group (98.5% versus 47.6%, respectively) [$p<0.0001$].

These results indicate that AT is effective in the treatment of uncomplicated falciparum malaria and may provide a useful alternative to other treatment regimens.

RÉSUMÉ:

Un essai randomisé, contrôlé et à triple-insu a été entrepris dans une région de l'Amazône au Brésil (Cuiaba-MT) afin de comparer l'efficacité et les effets secondaires de l'artesunate (7 jours, dose totale = 0,75 g) avec de la tétracycline (7 jours, dose totale = 10,5 g) [AT] et de la quinine orale (3 jours, dose totale = 6 g) avec de la tétracycline (7 jours, dose totale = 10,5 g) [QT]. Les patients choisis ont eu une malaria *P.falciparum* sans complications; âge ≥ 14 ans, aucun traitement anti-paludéen relié à la crise présente; en cas de femmes, aucune grossesse indiquée. Un examen clinique et des prises de sang ont été faits au départ (jour 0) puis aux jours 2, 4, 7, 14 et 28 suivants. L'efficacité a été évaluée par les fréquences de guérison (d'après l'OMS) et par la disparition des formes érythrocytaires asexuées au jour 2.

176 patients ont été randomisés, 88 par groupe. 96.6% du groupe AT et 93.2% du groupe QT ont complété le traitement de 7 jours. 81.8% du groupe AT et 78.4% du groupe QT ont complété les visites du suivi jusqu'au jour 28. Les groupes avaient des caractéristiques cliniques semblables au départ. La fréquence d'effets secondaires du groupe QT (82%) était nettement supérieure à celle du groupe AT (50%) [p⟨0.0001]. Les fréquences de guérison étaient semblables. 80% chez le groupe AT et 77% chez le groupe QT (p = 0.68). La parasitémie (au jour 2) a disparu plus rapidement chez le groupe AT que chez le groupe QT (respectivement 98.5% versus 47.6%) [p⟨0.0001].

Ces résultats indiquent que l'AT est efficace dans le traitement contre la malaria falciparum sans complications et pourrait servir d'option utile parmi d'autres protocols de traitement.

SUGGESTED SHORT TITLE:

RCT of artesunate plus tetracycline versus quinine plus tetracycline for falciparum malaria

PREFACE

The 'Fundação Nacional de Saúde' (FNS) is the Brazilian government institution responsible for the control of malaria and other endemic diseases.

Permission for the supervised use of artemisinin derivatives by the Ministry of Health was granted in 1991 to the FNS. This prompted the 'Coordenadoria Regional de Mato Grosso' (FNS-MT) to formulate research proposals for the evaluation of the effectiveness and safety of these new drugs in the treatment of malaria in the Amazon region. Most studies of these drugs to date have been carried out in non-Brazilian populations. One proposal developed by FNS-MT was aimed at providing information with regard to the safety and advantages of oral artesunate compared with the standard treatment currently in use for falciparum malaria. This project, carried out with support of FNS-MT staff members, was developed jointly by Dr. Cor J. F. Fontes and the author and is the study reported in this thesis.

Dr. Fontes and the author were fully responsible for the study design. During the initial part of data collection (January to June 92), Dr. Fontes was responsible for all medical evaluations and lab exams and the author was responsible for the treatment administration and supervision. Follow-up procedures and administrative aspects of the study were shared between both investigators. After June 1992, data collection was completed by Dr. Fontes in collaboration with FNS-MT staff members and medical students as the author was enrolled in the Master's degree program at McGill University. The complete report of the study, literature review and the data analysis were carried out by the author under the guidance of Dr. Gyorkos (thesis supervisor), Dr. Abrahamowicz (statistical advisor) and Dr. Fontes.

Throughout the period of data collection, several logistic problems emerged, including lack of motivation by some staff members and poor financial support

especially for the purchase of supplies and labour costs associated with laboratory testing. External funds were obtained to cover part of the expenses.* However, despite these obstacles, and in large part due to the enthusiasm and determination of the principal investigators, the study was successfully completed.

This study was the first research project conducted as a result of an initiative of the FNS-MT in many years. It represents more than just a contribution of knowledge to the field of falciparum malaria treatment; it also demonstrates that, contrary to current perception, rigorous epidemiologic studies, such as an RCT, may be developed in the service setting without jeopardizing the quality of the research or its validity.

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DEDICATION

It is difficult to express all my gratitude to those who, however distant, were the ones most present during my training at McGill.

To my mother and best friend, Bárbara, who gave me the motivation and self-confidence to confront most situations in life, and who taught me dedication through her own example of life, I offer my work. I also want to share this achievement with my sisters and brothers, Eliane, Elisa, Elisete, Luiz and Waldeck Júnior, my father, Waldeck, aunt and second mother, Neuza, and my grandparents Archanjo and Elisa (*in memorium*). Their unending affection was always present.

It is to my family that I dedicate this thesis.

'É difícil expressar minha gradidão à pessoas que, embora distante, se fizeram as mais presentes durante meu período na McGill.

Meu agradecimento e carinho para minha mãe e melhor amiga, Bárbara, quem me deu motivação e auto-confiança para ir em frente nas mais malucas situações em que me coloco, e me ensinou dedicação através de seu próprio exemplo de vida. Também quero dividir esta conquista com minhas irmãs e irmãos, Eliane, Elisa, Elisete, Luiz e Waldeck Júnior, tia e segunda mãe, Neuza, meu pai, Waldeck, e meus avós, Archanjo e Elisa (em memória). Pessoas que sempre foram só sorriso e carinho.

É para minha família que eu dedico esta tese.'

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My deep gratitude also goes to my sister and great friend, Eliane F. Duarte Franco (my tireless private English teacher) and my brother-in-law, Eduardo L. F. Franco, and their children, Eduardo and Fernando, for their constant, friendly and unconditional support. I am especially grateful to them for having made it possible for me to feel at home during my stay in Montreal.

I cannot express all my gratitude and admiration to Dr. Cor J. F. Fontes, professor of Infectious Diseases at 'Universidade Federal de Mato Grosso', Brazil, my like-brother friend and collaborator. He assumed sole responsibility for the data collection in Brazil while I was taking courses at McGill. His vast experience and competent performance with malaria patient management as well as our enthusiastic discussions about several aspects of this thesis helped me to better understand and interpret the study outcomes. Many of his points of view are present in this thesis.

I am profoundly indebted to Dr. Carlos Castillo-Salgado, regional advisor for

the Pan-American Health Organization in Washington, who encouraged me to take up the challenge of postgraduate studies and who has never wavered in his support He was certainly instrumental in guiding me towards McGill His competent guidance provided the pathway for my understanding of malaria epidemiology research I very much appreciated his enthusiasm and support

I am thankful for the technical support of the biochemistry technician Ana Lucia M. Ribeiro, the nurse Claudia Veras and the parasitology laboratory technicians: Ruth Guerra, Benedita Monteiro Braga and Lauremil Batista de Azevedo I would like also to thank the medical students, Gisele M. Souza and João C C. Pereira for their assistance in clinical evaluations.

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TABLE OF CONTENTS page:

Abstract		2
Résumé		3
Suggested short title		4
Preface		5
Dedication		7
Acknowledgements		8
Table of contents		11
List of tables		15
CHAPTER 1.	**Introduction**	18
	1.1. Malaria epidemiology and control	18
	1.2. Malaria in Brazil	20
	1.3. Rationale	23
CHAPTER 2.	**Literature review**	26
	2.1. General aspects of malaria infection	26
	2.2. Chemotherapy and drug resistance in falciparum malaria	30
	2.3. Artemisinin derivatives used in falciparum malaria	38
	2.4. Statement of purpose	41

page:

CHAPTER 3. **Objectives** 42

3.1. General objective 42

3.2. Specific objectives 42

CHAPTER 4. **Methods** 43

4.1. Overall design 43

4.2. Source population and study area 43

4.3. Sample size 47

4.4. Methods of patient recruitment and eligibility criteria and their rationale 47

4.5. Randomization 49

4.6. Blinding 49

4.7. Treatment procedures 51

4.8. Baseline and follow-up assessment 51

4.9. Lab diagnosis of malaria 52

4.10. Side effects: definition and assessment 53

4.11. Effectiveness: definition and assessment 53

4.12. Procedures used in the study design to ensure internal validity 55

4.13. Withdrawals and losses to follow-up 56

4.14. Data analysis 57

4.15. Ethical aspects 59

page:

CHAPTER 5. **Results** 61

5.1. Enrolment 61

5.2. Comparability of treatment groups 64

5.3. Treatment and follow-up compliance 67

5.4. Summary of univariate analysis 67

5.5. Treatment side effects 70

5.6. Treatment cure rates 77

5.7. Parasite clearance 81

5.8. Lab findings 85

CHAPTER 6. **Discussion** 88

6.1. Comparison of treatment regimens 88

6.2. Internal validity and generalizability considerations 92

CHAPTER 7. **Conclusions and recommendations** 95

7.1. Conclusions 95

7.2. Recommendations 96

page:

REFERENCES 98

APPENDICES 106

Appendix 1: *Trial organization scheme* 106

Appendix 2: *Map of the State of Mato Grosso* 107

Appendix 3:

Table I: (QT) Quinine plus tetracycline treatment schedule 108

Table II: (AT) Artesunate plus tetracycline treatment schedule 109

Appendix 4: *Baseline questionnaire (translated version)* 110

Appendix 5: *Clinical exam form (translated version)* 112

Appendix 6: *Notification of ethics review clearance* 114

Appendix 7: *Informed consent form (translated version)* 115

LIST OF TABLES

page:

TABLE 2.1:	*Recent studies of oral quinine and/or artemisinin derivatives with or without other drugs for treatment of uncomplicated P. falciparum malaria*	34
TABLE 5.1:	*Characteristics of patients enrolled into the RCT*	63
TABLE 5.2:	*Clinical characteristics of patients randomized to artesunate plus tetracycline (AT) and quinine plus tetracycline (QT) treatment groups at baseline*	65
TABLE 5.3:	*Laboratory findings of patients randomized to artesunate plus tetracycline (AT) and quinine plus tetracycline (QT) treatment groups at baseline*	66
TABLE 5.4:	*Description of the treatment and follow-up compliance during the RCT of patients given artesunate plus tetracycline (AT) and quinine plus tetracycline (QT)*	68
TABLE 5.5:	*Odds ratios (OR) and significance levels of likelihood ratio test (LRT) from univariate logistic regression for associations between independent and dependent variables*	69
TABLE 5.6:	*Comparative incidence of side effects observed in patients given artesunate plus tetracycline (AT) and quinine plus tetracycline (QT)*	71
TABLE 5.7:	*Stratified analyses of the association between one or more incident side effects and treatment group [artesunate plus tetracycline (AT) and quinine plus tetracycline (QT)]*	73

page:

TABLE 5.8: *Association between one or more incident side effects and treatment group [artesunate plus tetracycline (AT) and quinine plus tetracycline (QT)], by parasitemia level at baseline* 74

TABLE 5.9: *Estimated logistic regression coefficients, log-likelihood and the Likelihood Ratio Test statistics (LRT-G) for the association between one or more incident side effects and some selected variables* 76

TABLE 5.10: *Estimated logistic regression coefficients for the association between one or more incident side effects and treatment group accounting for the interaction with parasitemia level at baseline* 78

TABLE 5.11: *Estimated risks and relative risks for the association between one or more incident side effects and treatment group [artesunate plus tetracycline (AT) and quinine plus tetracycline (QT)] accounting for the interaction with parasitemia level at baseline* 79

TABLE 5.12: *Comparison of effectiveness of the two treatments [artesunate plus tetracycline (AT) and quinine plus tetracycline (QT)]* 80

TABLE 5.13: *Stratified analyses of the association between cure rate and treatment group [artesunate plus tetracycline (AT) and quinine plus tetracycline (QT)]* 82

page:

TABLE 5.14: *Association between parasite clearance at day 2 and treatment group [artesunate plus tetracycline (AT) and quinine plus tetracycline (QT)]* 83

TABLE 5.15: *Association between parasite clearance at day 2 and treatment group [artesunate plus tetracycline (AT) and quinine plus tetracycline (QT)], controlling for some covariates* 84

TABLE 5.16: *Stratified risk of presenting a positive smear at day 2 in each treatment group [artesunate plus tetracycline (AT) and quinine plus tetracycline (QT)]* 86

CHAPTER 1.

Introduction

1.1. Malaria epidemiology and control

Since the beginning of the 20th century, many attempts have been made to eradicate or control malaria in the world.

Antimalarial drugs such as chloroquine and residual insecticides such as DDT (Dicloro-difenil-tricloroetano) were successfully developed and used to combat malaria during and after the first and second world wars.[1, 2] Based on these results, the eradication of malaria was believed to be possible, and, in 1955, the World Health Organization (WHO) established an international protocol to achieve this end.[1] As a consequence, malaria was eradicated from Europe and North America, and decreased in prevalence in Africa, Asia and Latin America.[1]

However, the socio-political and environmental situations present in regions of Africa, Asia and Latin America, determined that interventions aiming at malaria eradication would have less impact than in Europe and North America, and malaria remained endemic in several countries. In addition, with the deterioration of the social conditions and health systems in endemic areas, during the 1960's and early 1970's, earlier gains could not be maintained and in some instances were reversed such that the eradication initiative lost its momentum and eventually its credibility. The eradication objective was supplanted by the expected more attainable objective of controlling the spread and severity of the disease. Malaria Control Programs were implemented in different regions, mainly in Asia and Latin America countries.[3-5]

Despite this reorientation, during the late 1980's, the malaria situation in some parts of the world seriously worsened.[5]

Today, malaria remains an important heath problem due to its increasing morbidity, increasing mortality and general failure of control strategies.

Although there is lack of reliable information regarding malarial morbi-mortality, it is believed to be the most common disease in the world at the present time. It has been estimated that approximately 300 million people in the world are infected with malaria parasites.[5, 6] Approximately 90% of these cases live in tropical Africa.[5, 6]

In tropical Africa, where the eradication campaign between 1955 and 1969 had never been widely implemented, no less than 0.5 to 1.2 million malaria-related deaths occurred annually.[5, 6] Moreover, the cost (direct and indirect) of malaria in Africa had been estimated at around US$ 800 million in 1987, and extrapolations to 1995 show that this cost may rise to more than US$ 1,800 million.[6] In the African savanna and forest regions, over 50% of the population are infected with malaria parasites, and the disease kills 5% of children before the age of five. In addition, chloroquine resistance has been increasing in several areas on this continent.[5]

It has been reported that malaria claims more than 100,000 lives outside Africa each year.[5] Countries in Asia and the Americas involved in past eradication campaigns, record approximately 5 million malaria cases annually, a figure which is believed to be an underestimation.[5] The Asian continent contributes approximately 80% of these cases and in all countries, except China, the situation is worsening.[5] The difficulty of malaria control in some of these countries has been associated with war situations.[5]

In Latin America and the Caribbean, malaria incidence presented a marked

increase over the last decade (1980's). Of 21 countries in this region, the "Annual Parasite Incidence" (API = number of malaria positive smears/ population at risk) had a two-fold increase from 1975 to 1989. In 1990, 1.04 million malaria cases were reported in these countries.[7]

More than 60% of malaria cases in the Americas occur in areas of the Amazon. Colonization and environmental disturbances have been associated with the spread and establishment of high levels of malaria transmission in the Amazon basin.[5]

1.2. Malaria in Brazil

In 1941, when the 'Serviço Nacional de Malária' was implemented in Brazil, more than 6 million malaria cases (1/7 of the total population) were reported.[2, 8] Thereafter, malaria incidence decreased and it was almost completely eliminated from Centre-South and North-East regions.[2, 9] By 1981 in Brazil, 14 million inhabitants living in areas that originally had malaria were no longer exposed to the disease.[9] Another 17 million inhabitants were living in areas where malaria transmission had been considerably reduced or interrupted.[9] It is not well understood if this success was achieved as a result of the malaria control program itself or as a natural consequence of development, with improvement in general health status, education level, sanitation and housing.[2]

However, as did the majority of countries in endemic Latin America and the Caribbean, Brazil presented an important increase in the number of reported malaria cases over the last two decades.[3, 4, 6, 7]

Around 560 thousand malaria positive smears were reported in 1990 in Brazil.[7] The overall API was estimated to be about 3.7 malaria cases per 1000

inhabitants, however this figure is misleading as it does not reflect the variability of transmission between and within geographical areas.[7] A large proportion of the Brazilian population is exposed to virtually no risk of malaria infection, compared to a smaller proportion exposed to a very intense and constant risk. For instance, in 1989, the Brazilian southeast region presented an API of 0.21 positive smears per 1000 habitants, two hundred times lower than that observed in the northern region (API = 46.1 per 1000 inhabitants) [7] During 1990, the Amazon region reported 97% of all Brazilian malaria cases identified, with three States reporting the majority (around 77%) of positive smears: Rondônia with 45% of reported cases, Pará with 21% and Mato Grosso with 11%.[7]

The causes of the dramatic malaria situation in Brazil are not well understood, although some general explanations have been proposed.

During the 1970's an intense colonization process of resettlement of non-immune populations and radical environmental disturbances in the Amazon area were observed, due to the so-called "expansion of the agricultural Brazilian frontier".[10] At the same time, several highly productive gold mines were discovered in Amazon regions and "gold fever" started attracting a large amount of migrant people. As a consequence, villages of miners, sales people and farmers were rapidly created However, the urban infra-structure, required to support this growth, such as health services, water supply and sanitation, was seriously lacking. This contributed to a constant contact between non-immune human populations and malaria vectors resulting in the intensification and spread of malaria and increasing morbidity and mortality rates.[10]

Moreover, in the Brazilian Amazon environment, malaria is relatively invulnerable to the conventional tools for disease control (*i.e.* house-spraying with insecticides, early diagnosis and radical treatment of patients) because of the following circumstances:[2, 10]

- the population is dispersed and access to health care services is difficult limiting early malaria diagnosis;

- migrants have, frequently, a low economic status and government support is insufficient; determinants of a general low standard of living, including inadequate housing (houses are often not completely enclosed in several urban and rural areas) facilitating contact with vectors;

- internal migration occurs constantly for work-related reasons, making assessment of treatment responses difficult;

- the rain forest environment is a natural habitat for several species of *Anopheles*, the malaria vectors.

These factors contribute to the persistence of malaria as a serious endemic disease in the Amazon basin.

During the last 20 years, the problem of malaria invulnerability to conventional strategies of disease management in the Brazilian Amazon region has again been highlighted with the appearance and spread of resistance of *P. falciparum* strains to 4-aminoquinolines and other previously efficacious antimalarial drugs.[8, 11] *P. falciparum* infection accounts for the majority of severe disease and mortality rates.[1, 6] The absence of early and effective treatment in falciparum malaria generally determines the clinical evolution of infection to severe disease, with high levels of parasitemia and disfunction in several body organs (see section 2.1.). Such disease progression may be irreversible, and patient death often occurs rapidly under these circumstances Therefore, specific and effective treatment, as well as early identification of infected patients, are vital to achieve the major objective of malaria control programs: to prevent mortality due to malaria.

1.3. Rationale:

The WHO global malaria control strategy states that early diagnosis and prompt treatment are basic rights of affected populations and these services need to be available wherever malaria occurs.[5] It is added that "no universal formula for the management of malarial disease can be offered that could be applied in all countries of the world".[5] It is because specific geographical and socio-cultural factors affect the effectiveness of malaria programs that control strategies have to be focussed on precisely targeted population groups.

These control strategies imply that affected populations have access to adequate diagnostic facilities and effective drugs. In addition, local areas must consider and be responsible for the management of persons who have malarial disease. This implies identification of local 'formulas' for malaria management, which should include local research into specific diagnostic and treatment options, among other aspects of disease control.

Treatment for falciparum malaria is one of the highest global research priorities, largely because of the alarming development and spread of resistance of *P. falciparum* strains to the commonly used antimalarial drugs, the association of some current treatment with side effects and low compliance to completing the course of therapy, the absence of an effective vaccine and the continuous migration of non-immune human populations into areas favourable to malaria transmission.[6, 9]

Today's current antimalarial drugs (chloroquine, sulfadoxine-pyrimethamine, quinine and mefloquine) are increasingly demonstrating losses in efficacy in falciparum malaria treatments.[12-19] Although in Brazil the standard treatment for falciparum malaria, quinine (3 days) plus tetracycline (7 days), is still efficacious, concerns have arisen regarding side effects that may lead to poor compliance in at-home treatments.[20-22]

The search for new malaria drugs, shorter and practical treatment schedules and efficacious and well tolerated drug combinations is intensifying.[23, 14]

Artemisinin derivatives are promising drugs against falciparum malaria, however recrudescences are often observed when they are used alone for short treatment periods (see section 2.3.). This is because, although artemisinin derivatives such as oral artesunate are fast acting drugs (parasite clearance in 2 days), their half lives seem to be insufficient to kill all blood parasites.[24, 25] The remaining parasites are able to multiply again and generate recrudescences. Therefore, the combination of oral artesunate with tetracycline, an antibiotic with a known blood schizontocidal action, seems to offer the possibility of reducing recrudescence rates.[12, 26]

The present study was formulated to assess the effectiveness and side effects of oral artesunate plus tetracycline and to compare this drug combination with the standard treatment (quinine plus tetracycline) currently used to treat falciparum malaria in the area.

Although the efficacy of oral artesunate plus tetracycline to treat falciparum malaria is not known, an effectiveness analysis was chosen for several reasons:[27]

- Efficacy studies of artesunate and quinine alone, and quinine plus tetracycline, have already been conducted in different populations (see sections 2.2. and 2.3.);

- It was expected that there would be loss to follow-up for work-related reasons;

- The Brazilian National Malaria Control Program's strategies emphasize the need to cover a large malaria endemic area. Drug evaluations incorporating a diversified population is recognized as an essential component of this program and are particularly needed. It was therefore important that this study evaluate treatments under the conditions in which they would be eventually used in practice. The

pragmatic or effectiveness procedure was chosen as the most appropriate approach.

CHAPTER 2

Literature review

2.1. General aspects of malaria infection

Malaria is a parasite disease caused by protozoans of the genus *Plasmodium* in the family Plamodiidae within the order Haemosporididia. There are nearly 100 species of *Plasmodium* found in a wide range of vertebrates, such as birds, reptiles and monkeys. The plasmodia found in monkeys are very similar morphologically to human plasmodia. For instance, the *P. rodhaini* from monkeys has been recognized to be identical with *P. malariae*, one of human malaria parasites.[1] However, in general, the plasmodia of animals are rarely transmitted to man.

There are four recognized species of *Plasmodium* that cause malaria infection in man: *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*. The first two species are the causes of more than 95% of malaria cases in Brazil and also worldwide.[28] *P. malariae* is common on the African continent, but rare in Brazil, and *P. ovale* is limited to Africa and Asia, and not found in Brazil.[29]

All human malaria parasites undergo two types of multiplication. Their life cycle comprises an exogenous sexual phase (sporogony) in *Anopheles* mosquitos (*Anopheles darlingi* is the main malaria vector in Brazil), and an endogenous asexual phase (schizogony) in man.[1, 29]

The female vector is hematophageous and becomes infected by ingesting infected blood containing gametocytes (the mature sexual cells of malaria parasites) of

a human host .[1, 28, 29] In the mosquito stomach, the fusion of male and female gametes occurs. The resultant cell, called the zygote, acquires mobility (now called an ookinete) and penetrates the stomach wall, where it becomes rounded up into a small sphere called an oocyst. Oocysts gradually develop and release thousands of mobile forms (sporozoite) into the hemocoel (body cavity) of the mosquito, from where they will reach the salivary glands. The female mosquito is now infective, and during the next blood meal, sporozoites will be injected together with saliva into the new host's bloodstream.[1, 28, 29]

Wheather conditions (high temperatures and high humidities), the susceptibility of the mosquito to the parasite, the feeding habits of female mosquitos and also the availability of infected human hosts in the area influence the duration of the sporogony cycle. In ideal conditions, this phase of the life cycle is completed in about 15 days.[29]

The asexual cycle (schizogony) starts soon after (about half an hour) sporozoites are introduced into the human bloodstream. Later, these forms disappear from the blood; many are destroyed by phagocytes, and some enter into hepatocytes (parenchymal liver cells) where they develop and multiply by schizogony. This process is called exoerythrocytic schizogony (or pre-erythrocytic schizogony or the tissue phase).[1, 28, 29]

The exoerythrocytic phase is also influenced by several factors: the species of *Plasmodium* injected, the number of infective mosquito bites, the number of sporozoites injected and the immune status of the host.[1, 29] In general, the mean duration of the exoerythrocytic phase is between 6 and 15 days.[28-30]

From each infected hepatocyte thousands of merozoites are set free into the surrounding tissue and, from these, into the bloodstream where the erythrocytic phase starts.[1, 29, 30] Some merozoites are phagocytized, but most of them invade the

erythrocytes. These early erythrocytic parasite forms, now called trophozoites, absorb the hemoglobin of the red blood cell and, after asexual division and a schizont stage, generate a second generation of merozoites. The infected erythrocyte ruptures, releasing merozoites into the bloodstream. The reinfection of red blood cells by merozoites occurs over and over again in the course of infection, leading to a gradual increase in the level of parasitemia. This process can be slowed, interrupted or terminated by action of antimalarial drugs, an adequate host immune response or the death of either host or parasites

After several generations, some merozoites differentiate into gametocytes, the sexual cells of the parasites, and the entire life cycle is completed.

Of all the species of human malaria parasites, *P. falciparum* is the most pathogenic, and if the infection is not promptly and adequately treated, can easily lead to death.

Falciparum malaria is characterized by a shorter pre-erythrocytic period (from 5.5 to 7 days) and by a larger number of merozoites released by each hepatic schizont (about 40,000 merozoites) than all other human malaria parasites.[30] Also, distinct from other malaria parasites, *P. falciparum* merozoites invade erythrocytes of any age.[30] These aspects explain the high levels of parasitemia frequently found in falciparum malaria infections.

In addition to hyperparasitemia (levels higher than 250,000 asexual parasites per mm^3 blood), falciparum malaria infection can cause clinical complications and involvement of several organs.[31, 32] Although the physiopathology of the severe malaria caused by *P. falciparum* is not completely understood, several consequences of infection are known. These include:[2, 31, 33]

- Mechanic obstruction of capillaries by parasitized erythrocytes, mainly in the brain;

- Increase in the permeability of capillaries followed by perivascular infiltrates and demyelination;
- Activation of host immune response mechanisms.

Human malaria caused by any parasite species may relapse. In *P. vivax* and *P.ovale* infections, relapses occur because some sporozoites become 'dormant' and persist as hepatic forms called hypnozoites. From these forms, merozoites can eventually be released into the bloodstream and initiate a relapse of infection. These long-term relapses are called recurrences.[28] In *P. malariae* and *P. falciparum* infections, such persistent tissue forms are absent.[28] Evidence indicates that relapses observed in *P. malariae* may originate from erythrocytic forms remaining in the body for a considerable time (as long as 20 or more years).[30]

In falciparum malaria, it is unusual for relapses of infection to occur later than 3 months after the primary attack, although short-term relapses, termed recrudescences, may occur.[30] Recrudescences are different from recurrences in that they are caused by the survival of erythrocytic parasite forms and not liver forms. This results when not enough of the antimalarial drug or its active metabolite has reached the parasite (for example, in poor compliance with treatment or if there are problems of drug absorption), or the drug has reached the parasite but the parasite has become resistant to the action of the drug.[30]

The World Health Organization has defined drug resistance in malaria as the 'ability of a parasite strain to survive and/or to multiply despite the administration and absorption of a drug given in doses equal or higher than those usually recommended but within the limits of tolerance of the subjects' [13, 30] Although this definition includes all malaria parasites, it is most commonly interpreted as relating to the effect of blood schizontocides (drugs acting on the erythrocytic stage of malaria parasites) in falciparum malaria.[30]

The development of resistance of *P. falciparum* to different antimalarial drugs (*eg.* chloroquine, proguanil and pyrimethamine) is attributed to the selection of parasite mutants resistant under drug pressure.[30] The selection of mutants is more likely if large numbers of infected patients are treated, if treatment continues over a long time and if many parasites are exposed to the drug in each patient.[30, 13] For instance, for a drug such as chloroquine, widespread use of a low dose of the drug is likely to promote the selection of resistant forms.[13] Some biochemical and biological mechanisms of drug resistance in malaria parasites have been identified. For example, resistance to chloroquine and quinine apparently relates to less effective total uptake of the drug due to permeability changes in the parasite.[13] Some mutants are also able to use alternative metabolic pathways to those blocked by a particular drug.[30]

2.2. Chemotherapy and drug resistance in falciparum malaria

Malaria is one of the oldest infections mentioned in early writings in Egypt, India and China. Also mentioned are the attempts to treat this disease using roots, leaves and flowers.[30] Although ch'ang shan (*Dichroa febrifuga*) and qinghao (*Artemisia annua*) have been used in China for at least 2000 years, it is uncertain when the first potent treatment for malaria was discovered.[30]

It is not known when and where the bark of the 'fever trees' (plants growing originally in the Andes mountains of Peru) was first used in malaria treatment.[30] Juan Lopez, a Jesuit missionary, first recorded the use of the 'fever tree bark' by Peruvian Indians in 1600.[1] Its curative properties were increasingly recognized throughout the Americas and Europe during the 1600's, and by 1677 it was included in the London Pharmacopoeia as *Cortex peruanus*. By 1749, Linnaeus in Sweden described the tree and named it as *Cinchona*.[1, 30]

In the early nineteenth century four basic alkaloids of cinchona - quinine,

cinchonine, quinidine and cinchonidine - were isolated in France.[30] Thereafter, the demand for the new drug was so great that the native forests of cinchona in South America quickly became overexploited. With the subsequent cultivation of cinchona in other parts of the world (Java, India and Indonesia), drug availability became less problematic.[1, 30]

Several attempts to synthesize quinine failed. During World War I, the use of animal models increased the interest for testing alternative compounds.[30] Such research procedures were possible because of two momentous discoveries: Laveran identified the malaria parasite in human blood in 1880 and, 10 years later, Danilevsky identified similar parasites in birds.[30]

The first identified synthetic antimalarial compound from the 8-aminoquinoline series, plasmochin (pamaquine - 1928), had only limited action on asexual forms of falciparum malaria, but greatly reduced the occurrences of relapse in vivax malaria. However, its toxicity was not negligible.[30] Later (1952), a less toxic compound from this series was synthesized - primaquine. It continues to be the best drug for the radical cure of vivax malaria.[1, 30]

Atabrin (now called mepacrine), synthesized in 1932 by Germans, proved to have considerable activity on the asexual forms of falciparum malaria.[30] Studies regarding the safety of mepacrine, as well as studies of new antimalarial compounds, greatly increased in Germany during the Second World War (both Germany and the Allies were cut off from the main source of quinine which was Indonesia, at that time occupied by the Japanese Army). Mepacrine was soon introduced and successfully used in areas of war, which is believed to have probably changed the course of modern history.[30]

In 1934, the 4-aminoquinoline series, also discovered by the Germans and tested in Tunisia and USA after the Second World War (now using human volunteers

rather than animal models), proved to be superior to any other antimalarial therapeutic drug. Among the approximately 17,000 compounds tested, two antimalarial compounds were found promising: chloroquine and amodiaquine.[30] These two drugs remained the best therapeutic and suppressive malarial drugs in the world for more than 25 years.[30] (Suppressive malaria drugs prevent or eliminate clinical symptoms and/or parasitemia by the early destruction of erythrocytic parasites.)[30]

A similar research advance in malaria treatment was also observed in the United Kingdom during Second World War, when proguanil, a chloroguanil series derivative, was discovered in 1944. An Australian team conducted clinical trials using this drug and described its activity as a prophylactic agent in falciparum malaria and as a suppressive drug in vivax malaria.[30] Pyrimethamine, developed in 1951 by an American and British team, was found to perform better than proguanil.[30]

Because of the arsenal of new compounds identified as antimalarial drugs and the successful uses of chloroquine and primaquine in military forces, it was believed that problems related to the chemotherapy of malaria had almost been solved by the late 1950's.[30]

However, at a time when malaria eradication seemed imminent, a precipitous event occurred: evidence was obtained that several strains of *Plasmodium falciparum* showed resistance to chloroquine and amodiaquine and to other antimalarial drugs in many endemic areas around the world.[12-19, 30]

The spread of chloroquine resistance from its original foci - probably along the Thai-Kampuchean border in 1957 - to most malaria endemic areas was and continues to be alarming.[12, 13] Chloroquine resistance can now be found in almost all falciparum malaria endemic countries of the world.[14]

Following the identification of resistance of *P. falciparum* to chloroquine,

there was increased interest in the development of long-acting sulfonamides.[30] Thereafter, sulfadoxine became to be used in combination with pyrimethamine for falciparum malaria resistant to chloroquine.[30] As expected, sulfadoxine-pyrimethamine resistance has spread from Myanmar to Vietnam and has also been reported from the Brazilian Amazon region.[14]

As a consequence of the comprehensive research program in USA, which screened a total of 250,000 compounds over 12 years, mefloquine, a 4-quinolinemethanol derivative, was identified in the late 1970's.[30] Although mefloquine is still a very useful fast acting drug against falciparum malaria, some level of resistance has also been reported in Thailand's border areas and in western Kampuchea.[14]

P. falciparum strains moderately resistant to 4-aminoquinoline drugs are generally susceptible to quinine. As a consequence, quinine is the most widely used drug for treating chloroquine-resistant falciparum malaria.

Table 2.1 presents a summary of recent studies on the action of oral quinine (as well as artemisinin derivatives), used singly or in combination with other drugs, for the treatment of uncomplicated *P. falciparum* malaria.

When oral quinine was used alone over 10 or 14 days, cure rates of 42% and 40% were achieved, based on studies along the Kampuchea-Thai border and in Vietnam, respectively (Table 2.1).[18, 34]

Quinine has been combined with other drugs, such as tetracycline, clindamycin or sulfadoxine-pyrimethamine, as a means of preserving its efficacy and improving its effectiveness while decreasing its administration period. Drug combinations have been extensively studied and used by national malaria control programs in many endemic areas.[17, 20, 35-38]

TABLE 2.1.: Recent studies of oral quinine and/or artemisinin derivatives with or without other drugs for treatment of uncomplicated *P. falciparum* malaria.

DESIGN				RESULTS[a]		
Type of study [area, year]	Treatment group: drug (days) = total dose[b]	N	F/U (D)	PC (D)	FC (D)	% CURED
Controlled trial[22] [Brazil, 1990]	1: quinine (5D)=10g	15*	>28	-	-	80
	2: quinine (7D)=14g	9*	>28	-	-	78
	3 quinine (10D)=20g	23*	>28	-	-	100
	4 quinine (10D)=15g	15*	>28	-	-	87
Controlled trial[26] [Kampuchea-Thai border, 1988]	1: quinine (10D)=15g	43*	10	5.6	3.4	42[c]
	2: quinine (10D)=15g + tetracycline (7D)=10.5g	22*	10	5.9	3.8	100[c]
Observational retrospective study (records review)[42] [Germany, 1992]	1 quinine (7D)=10.5g + optional doxycycline (10D)=1g	99	-	3.7	3.6	100
	2: choroquine (2D)=1.5g	144	-	4.0	3.5	90
	3: sulfadoxine+pyrimethamine (1D)[c]	11	-	4.1	3.5	73
	4: mefloquine (1D)=1.25-1.5g	45	-	3.8	3.6	94
Descriptive study[20] [Brazil, 1986]	1 quinine (3-4D)=4.5-8g + tetracycline (7D)=7g	75	28	-	-	95
Observational prospective study (monitoring program)[44] [Brazil, 1992]	1: quinine (3D)=6g + tetracycline (7D)=10.5	164	28?	-	-	58-67[e]
	2: quinine (3D)=6g + sulfadoxine+pyrimethamine (1D)[c]	267	28?	-	-	16-19[e]
	3 quinine (10D)=15g	116	28?	-	-	25-44[e]
Controlled trial (non-concurrent groups)[47] [NE Thailand, 1992]	1: [1987] quinine (7D)=13.65g + tetracycline (7D)=7g	50	f	4.0	-	100[d]
	2: [1990] quinine (7D)=13.65g + tetracycline (7D)=7g	50	f	3.4	-	100[d]
Controlled trial (non-concurrent groups)[47] [SE Thailand, 1992]	1. [1981] quinine (7D)=13.65g + tetracycline (6D)=7g	42	f	3.9[g]	2.7	-
	2: [1990] quinine (7D)=13.65g + tetracycline (7D)=7g	26	f	4.0[g]	3.1	-
Descriptive study[28] [Thailand, 1992]	1: quinine (7D)=12.6g + tetracycline (7D)=7g	70	28	3.7	2.8	90

TABLE 2.1. (continuation): Recent studies of oral quinine and/or artemisinin derivatives with or without other drugs for treatment of uncomplicated P. falciparum malaria.

DESIGN			RESULTS[a]			
Type of study [area, year]	Treatment group: drug (days) = total dose[b]	N	F/U (D)	PC (D)	FC (D)	% CURED
Controlled trial[37] [Thai-Kampuchea, 1986]	1 quinine (3D)=5.4g + tetracycline (7D)=10.5g	42*	>28	3.5	1.0	76
	2 quinine (7D)=12.6g + tetracycline (7D)=10.5g	36*	>28	3.3	2.0	92
	3 mefloquine(1D)=0.75g + sufadoxine+pyrimethamine (1D)[c]	41*	>28	2.4	1.0	98
Controlled trial (induced infection using Cambodian, Panama, Burma (Thai) strains)[25] [USA, 1974]	1 quinine (3D)=5.85g + clindamycin (7D)=12.6g(in sequence)	4	>28	2.6	-	100
	2 quinine (3D)=5.85g + clindamycin (3D)=5.4g (in sequence)	8	>28	1.8	-	100
	3 quinine (3D)=5.85g + clindamycin (3D)=5.4	5	>28	1.7	-	100
	4 quinine (2,3,5 or 7D)=3.9-13.65g	7	?	-	-	14
Controlled trial (induced infection using Vietnam, Cambodia strains])[25] [USA, 1972]	1 amodiaquine (3D)=1.5g + tetracycline (7D)=7g	9	>28	1.5-3.7	1.3-3.7	33-83
	2 amodiaquine (3D)=1.5g + tetracycline (10D)=10g	18	>28	4.7-5.0	3.5-3.8	87-100
	3 quinine (3D)=4.86g + tetracycline (10D)=10g (in sequence)	4	>28	5.7	6.7	100
RCT[39] [Vietnam, 1990]	1 quinine (14D)=21g	30	28	2.8	1.6	40
	2 artemisinin (3D)=2.2g (suppositories)	32	28	1.7	1.2	34
Descriptive study[40] [Thailand, 1992]	1 artesunate (5D)=0.6g+ mefloquine (1D)1.25g (in sequence)	24*	28	1.7	1.4	100
RCT[41] [Thailand, 1991]	1 artesunate (7D)=0.6g	40	28	1.6	0.8	92.5
	2 artesunate (5D)=0.6g	40	28	1.7	1.2	85
Observational retrospective study (multi-centre)[42] [Vietnam, 1993]	1 artemisinin (2-5D)=1-5g	36	14-28	-	-	50
	2 artemisinin (9D)=2.5g	38	14-28	2.0	-	89
	3 artemisinin (5D)=2.5g	102	14-28	1.5	1.8	85
	4 artemisinin (7D)=2g	111	14-28	1.9	1.9	86
	5 artemisinin (3D)=1.5g + tetracycline (5D)=12.5g	21	14-28	1.4	-	90
RCT[41] [Thailand, 1991]	1 artesunate (5D)=0.6g (one daily dose)	25	28	1.6	0.8	72
	2 artesunate (5D)=0.6g (bid dose)	25	28	1.7	1.2	76

TABLE 2.1. (continuation): Recent studies of oral quinine and/or artemisinin derivatives with or without other drugs for treatment of uncomplicated P. falciparum malaria

DESIGN			RESULTS[a]			
Type of study [area, year]	Treatment group: drug (days) = total dose[b]	N	F/U (D)	PC (D)	FC (D)	% CURED
RCT [45] [Thailand, 1991]	1 artesunate (5D)=1.2g	5*	28	1.3	1.7	100
	2 artesunate (5D)=0.6g	10*	28	1.1	1.0	90
	3. artemether IM (5D)=0.48g	5*	28	1.2	1.3	100
	4· artesunate IV (3D)=0.3g	5*	28	1.4	1.1	20
	5 artesunate (2D)=0.6g	5*	28	1.1	1.1	0
	6 artesunate (1D)=0.6g	5*	28	1.2	1.6	0
	7 artesunate (5D)=0.65g	20*	28	1.1	1.0	95
	8 artesunate (1D)=0.2g + cloroquine (2D)=1.5g	5*	28	1.3	0.9	0
	9 artesunate (1D)=0.2g + sulfadoxine + pyrimethamine (1D)[c]	5*	28	1.3	1.0	0
Controlled trial [46] [China, 1982]	1. mefloquine (1D)=0.5-1.0g	10	>28	4.3	1.3	92?
	2· quinine IM (3D)=4.5g	10	>28	4.3	1.7	?
	3: artemisinin IM (2D)=1.2g	10	>28	3.3	1.4	?
	4 artemisinin (1D)=2g	10	>28	2.8	0.9	?
RCT [48] [Thailand, 1992]	1: artesunate (5D)=0.6g	40	28	1.5	1.5	88
	2 mefloquine (1D)=1.25g	37	28	2.6	2.9	81
	3: artesunate (5D)=0.6g +mefloquine(1D)=1.25g (in sequence)	39	28	1.6	1.6	100
RCT [19] [Thailand, 1992]	1 artemether (5D)=0.48g	33	28	1.4	1.6	84
	2 artemether (5D)=0.6g	28	28	1.4	1.4	92

a N = sample size in each group at the end of follow-up period,
F/U = follow-up period, D = days,
PC = parasite clearance time (mean or median),
PF = fever clearance time (mean or median)
b When not reported, the total doses were calculated based on an average adult weight of 50kg
c 1 5g sulfadoxine + 75 mg pyrimethamine
d S or RI (short follow-up period)
e: Reinfections were probably not completely excluded from treatment failures
f Patients were followed up to two consecutive negative blood smears
g median time
? = not clear
- = not reported
* Recrudescent or previously treated patients were included
RCT = Randomized controlled trial

The usefulness of some antibiotics, particularly tetracycline, in malaria treatment is well known, although its pharmacokinetic properties against *Plasmodium* are not completely understood. It appears that tetracycline may exert its action on parasite mitochondrial protein synthesis.[12, 26] In addition, when administered in combination with quinine, it helps maintain high plasma quinine levels throughout the treatment period.[39] Similar results were found with tetracycline in combination with mefloquine.[40] In a controlled trial with Thai and Kampuchean patients it has been shown that the efficacy of quinine (10 days) increases when administered in combination with tetracycline (7 days) (cure rate of 100%), compared to quinine administered alone during 10 days (cure rate of 42%) [Table 2.1].[18]

In one study in Brazil, 95% of 110 falciparum malaria patients from the Amazon region were successfully treated with the combination of quinine plus tetracycline.[20] Since 1990, the combination of quinine (3 days) and tetracycline (7 days) has been used in Brazil as the standard treatment for uncomplicated resistant falciparum malaria in several States, including Mato Grosso [Table 2.1].[21]

However, even though highly efficacious, quinine therapies are far from a definitive solution for falciparum malaria treatment. As a consequence of the widespread use of this drug, *P falciparum* sensitivity to quinine is decreasing in some areas of Thailand, Myanmar, Kampuchea and South Vietnam.[14-16, 18] Loss of sensitivity to quinine as well as to mefloquine has also been observed in *in vitro* studies in some endemic areas.[14, 17-19] An *in vitro* study of isolates from Thailand showed that the susceptibility of *P. falciparum* to quinine decreased two-fold between 1983 and 1990.[17] It was pointed out that the reported gradual decreasing susceptibility in *in vitro* studies suggested that deterioration of quinine therapies - with or without tetracycline - may be expected in the future.[17, 38]

In addition, between 50 and 100% of patients receiving quinine have complained of side effects, mainly cinchonism (headache, tinnitus, nausea and

disturbed vision).[16, 22, 34, 37, 38, 41, 42] This may result in poor compliance with regimens involving this drug for at-home treatment.[9] It has been demonstrated that the consequences of poor compliance - incomplete or sub-dose treatments - are associated with selection of parasite strains less sensitive to quinine, increasing the potential for resistance.[13, 42, 43]

Generally, patients with *P. falciparum* malaria receiving quinine treatment show a parasite response and clinical improvement only after relatively long time periods. Kampuchean patients receiving quinine alone over 10 days (total dose = 15 g) had a mean parasite clearance time of around 6 days and a mean time to fever remission of around 3 days (Table 2.1).[18] The concurrent controls who received quinine (10 days; total dose = 15 g) plus tetracycline (7 days; total dose = 10.5 g) showed no improvement in clearance time or fever remission time.[18] A similar study in Vietnam showed a shorter time to parasite response when quinine (14 days; total dose = 21 g) was used alone: mean parasite clearance time around 3 days and mean fever remission time around 2 days (Table 2.1).[34] However, long term quinine therapies are unpractical because of the population dynamics in some endemic areas (e.g. work-related migration and no definitive residence) and the presence of non-negligible side effects.[9] Therefore, compliance is problematic.

Patients who have residual levels of *P. falciparum* parasites in their blood following treatment (either due to a lengthy parasite clearance time or due to poor compliance) are at increased risk for developing severe malaria and serve as the potential sources of malaria transmission in the community.[1, 9]

2.3. Artemisinin derivatives used in falciparum malaria

Artemisinin (also called artemisinine or ginghaosu) is an active antimalarial compound extracted from leaves of the plant "ginghao" (*Artemisia annua L.*), a

chinese herb used for centuries in the treatment of febrile illnesses.[24, 44-46] The antimalarial properties of artemisinin which were evaluated in the People's Republic of China in the early 70's have now been more recently re-evaluated elsewhere.[24, 44-46]

Artemisinin derivatives, such as artesunate, artemether and arteether have been extensively studied in China, Thailand and Vietnam.[12, 24, 44-47] They show high activity against *P. falciparum* malaria - and low toxicity.[19, 23, 25, 44, 46, 48-51]

As do all other artemisinin derivatives, oral artesunate is highly efficacious in clearing parasites and fever, even in severe *P. falciparum* malaria, with virtually no side effects.[12, 24, 44-47] However, treatment failures caused by an RI type response (*ie.* parasite clearance after treatment but reappearing before day 28 of follow-up) have been a problem, with recrudescence rates rising as high as 100% when oral artesunate is used alone in short term regimens (1 - 3 days).[13, 52] To minimize recrudescence rates, different doses of oral artemisinin derivatives and combinations of this drug with other antimalarial drugs have recently been studied (see Table 2.1).[19, 23, 25, 44, 48-51, 53, 54]

In Thai studies, falciparum malaria patients treated with oral artesunate alone over 5 days (total dose = 0.6 g) presented cure rates between 72% and 90%, a mean time for parasite clearance occurring between 26 and 41 hours after treatment initiation and a mean fever remission time between 19 and 36 hours (Table 2.1).[25, 48-50]

The efficacy of 7-day and 5-day courses of oral artesunate (total dose = 0.6 g) to treat uncomplicated falciparum malaria was assessed in 80 Thai patients.[49] In the 5-day regimen, the mean times for parasite and fever clearance were 41 and 29 hours, respectively and the cure rate was 85% (Table 2.1). These treatment responses slightly improved with a 7-day artesunate regimen (mean time for parasite and fever

clearance of 38 and 19 hours, and a cure rate of 92.5%) but no statistically significant difference in overall improvement was reported.[49]

Both *in vitro* and animal (mice) studies have demonstrated that tetracycline as well as mefloquine show marked potentiative synergism with artemisinin against *P. falciparum* (*in vitro*) and *P. berghei* (animal model) strains.[53, 54]

Based on these studies, combinations of artemisinin derivatives with mefloquine and tetracycline have been evaluated.[23, 51] The combination of artemisinin derivatives with tetracycline is expected to be successful because the first is a fast acting drug (parasite clearance in 2 days) but with a short half-life, and the second is a slow acting antibiotic (effect manifested in about 48h) but with a known blood schizontocidal action.[12, 24, 25]

In a descriptive study, one group of 24 Thai patients with recrudescent *P. falciparum* malaria was treated with a sequential combination of oral artesunate (5 days; total dose = 0.6 g) and mefloquine (1 day; total dose = 1.25 g) [Table 2.1]. All patients were cured, and the mean time for parasite and fever clearance were 41 hours and 33.6 hours, respectively.[23]

Also in Thailand, 127 patients with acute *P. falciparum* malaria were randomly assigned to oral artesunate (5 days; total dose = 0.6 g) alone, mefloquine (1 day; total dose = 1.25 g) alone or artesunate followed by mefloquine (Table 2.1).[48] Fever and parasite clearance times were significantly shorter in the two groups treated with artesunate (with or without mefloquine) than in the group receiving mefloquine alone. Cure rates improved from 81% in the mefloquine group, to 88% in the artesunate group and 100% in the group receiving the drug combination.[48] A study evaluating oral artesunate administration concomitantly with mefloquine is currently underway in Thailand.[55]

In Vietnam, patients with *P. falciparum* or *P vivax* malaria were treated with a combination of oral artemisinin (3 days, total dose = 1.5 g) and tetracycline (5 days; total dose = 12.5 g), and compared with artemisinin alone, used in 5 different regimens (over 2 to 9 days; total dose between 1 and 2.5 g) [Table 2.1].[51] Among 308 patients presenting with *P. falciparum* malaria, 21 patients received the drug combination and 287 received artemisinin alone. Good improvement in the malaria cure rate was observed when oral artemisinin was used in combination with tetracycline, decreasing the recrudescence rates from 50% (artemisinin alone over 2 to 5 days) to around 10% (artemisinin for 3 days plus tetracycline for 5 days). Patients treated with higher doses of artemisinin (2 to 2.5 g) and over longer treatment periods (5 to 9 days) presented cure rates between 85 and 89%.[51]

Although several clinical trials involving artemisinin derivatives for malaria treatment have been conducted, no comparative controlled trials using the combination of oral artesunate plus tetracycline have been reported.

2.4. Statement of purpose

Based on current knowledge, the present study was carried out to assess the effectiveness of artesunate plus tetracycline for falciparum malaria treatment compared with the standard treatment (quinine plus tetracycline) currently used in Brazil. Under an intention-to-treat analysis, the new drug combination is expected to present less side effects, faster parasite clearance and equivalent cure rates when compared with the standard treatment.

CHAPTER 3

Objectives

3.1. General objective

- To compare the effectiveness and side effects of oral artesunate (7 days) plus tetracycline (7 days) [AT] with the standard treatment, quinine (3 days) plus tetracycline (7 days) [QT], for the treatment of adult patients with acute uncomplicated *P. falciparum* malaria in Cuiabá - MT - Brazil.

3.2. Specific objectives

- To compare cure rates and the incidence of recrudescences, over the 28 day follow-up period, between adult patients taking AT and those taking QT for the treatment of acute uncomplicated *P. falciparum* malaria.

- To compare the two treatments with respect to the proportion of negative blood smears for *P. falciparum* malaria at 48 hours after treatment initiation (day 2 of follow-up).

- To compare the two treatments with respect to the incidence of side effects.

CHAPTER 4

Methods

4.1. Overall Design

A triple blind randomized controlled trial was undertaken to compare the effectiveness and side effects of oral artesunate plus tetracycline and oral quinine plus tetracycline in the treatment of uncomplicated *P. falciparum* malaria in Cuiabá-MT, Brazil. Eligible patients were randomized to treatment group and followed up for 28 days. Incidence of side effects, parasite clearance and cure rates were assessed through systematic lab tests and clinical examinations undertaken during the follow-up period. Organization of the trial is summarized in Appendix 1.

4.2. Source population and study area

This study was carried out in a health service for malaria at 'Fundação Nacional de Saúde - Coordenadoria Regional de Mato Grosso, located in Cuiabá, Mato Grosso State - Brazil.

Mato Grosso is an Amazon state located in the Brazilian Centre-West region, populated by around 2 million inhabitants (Demographic census, 1991).[56] The northern regions of this state developed rapidly during the expansion process of the Brazilian agricultural frontier in the 1970's, as did several Amazon areas. Migrants from the Brazilian Northeast and Southwest regions, motivated by opportunity for land ownership and employment, settled in the Amazon area.[3, 10] The primary

occupations are mining, farming, construction, transportation and local small business enterprise.[10] A large proportion of this population is semi-literate with a low income and standard of living.[10]

In many situations, migrants families opt for geographical separation. Males work in farming or mining activities, where the risk of disease is high, and the remaining family members stay in neighbouring urban areas, where the standard of living is better.[10]

The Amazon colonization process clearly has not been successful in the creation of stable employment, in increasing agricultural production or in absorbing the population excess from developed areas.[57] Endemic diseases have largely contributed to this failure. An increase in malaria endemicity in the Northern region of Mato Grosso, as in other Amazon areas, occurred concurrently with this migration process because of the environmental disturbance, lack of sanitation, poor water supply, primitive urban structure and lack of immunity among immigrants.[3]

The Ministry of Health sector responsible for the malaria control program is the 'Fundação Nacional de Saúde' (FNS), represented by regional administrative units in each state ('Coordenadorias Regionais' - CR). These CRs are composed of one central office in the state capital with sub-administrative units in strategically located cities and a net of smaller malaria service units distributed in urban and rural malaria endemic areas. In addition, several mobile health units of workers and volunteers are responsible for active case detection and case management in areas where access is difficult as well as activities related to vector control (house-spraying with insecticides and antilarval measures).

Malaria service units are staffed with laboratory technicians and health care workers trained for malaria case management. These units are responsible for malaria diagnosis and administration of treatment in uncomplicated cases. Patients requiring

parenteral treatment must be reported to the nearest government or private hospital. Physicians, nurses, biologists or biochemists normally employed at the central or state level, may visit service units occasionally. Their major involvement is in training, research and administrative activities.

The FNS - CR of Mato Grosso, is the central administrative unit in the city of Cuiabá. In addition, there are four sub-administrative units (Cáceres, Nortelândia, Rondonópolis and Sinop regions - Appendix 2) and 98 malaria service units. Half of them (44 units) are located in the Sinop region (387,900 inhabitants = 19% of Mato Grosso State population), where the largest number of malaria cases occurs each year. One malaria service unit is located in Cuiabá, where the present study was carried out.

Cuiabá, the capital of Mato Grosso state, is a city almost three centuries old that developed with other areas during the Brazilian agricultural frontier expansion. In 1991 it had a population of 401,300 inhabitants (Demographic census 1991).[56] This population is about 2 times larger than that reported in 1980 (213,000 inhabitants) (Demographic census 1980).[58] Cuiabá, located in the south of Mato Grosso state (Appendix 2), is characterized by low malaria transmission, with few remaining foci of localized transmission in sub-urban areas and some sporadic outbreaks due to the constant pressure of imported malaria. In 1993, among the 129,700 cases reported in Mato Grosso State only 0.4% (API = 1.4 per 1000 hab.) had Cuiabá as the probable place of transmission. It was, therefore, assumed for the period of the study that exposure to new malaria infections in Cuiabá by participants was negligible.

Although several malaria service units are available in Mato Grosso, patients sometimes travel hundreds of kilometres to be treated in Cuiabá (Appendix 2). Reasons for this are not well understood, but may be associated with the following:

- Several workers from the northern region of Mato Grosso have family members in

Cuiabá, where the standard of living is better with respect to schooling, housing, environmental conditions and general health care services. Once they become sick and unable to work, they return to their homes;

- Inhabitants of the northern region of Mato Grosso are generally originally from the Brazilian Northeast or Southeast regions. Cuiabá is a strategically located city which provides transportation to other states (interstate bus);

- Cuiabá provides health services which are unavailable in remote areas of the State (e.g. specialized medical services). It also has a larger number of governmental general health services than in other areas;

- Cuiabá presents the attraction of a large city with diverse stores and services, goods and other provisions.

It is unknown to what extent malaria patients who travel to Cuiabá differ from those who remain to be treated in the local malaria service.

They may differ slightly in economic status (costs for the trip may not be affordable by everyone), and in perceptions and beliefs related to health. In addition, they may differ due to demographic, educational and other characteristics.

Such considerations do not affect the internal validity of the study results. On the other hand, it is possible that the study population may experience differential (probably better) treatment outcomes than the malaria patients who choose local treatment. Generalizability (external validity) of the study results may be affected, and inferences have to be made accordingly (see section 6.2).[59]

4.3. Sample size

The study was designed to ensure an 80% power of detecting a significant difference (at α=0.05) in cure rates between two treatment groups of equal size. Under the assumption that the cure rate in the QT group would be 95% (based on data from Barata et al, 1986)[20], and that a minimum clinically important difference would correspond to a 15% decrease in the cure rate (i.e. 80% cure rate in AT group) it was determined that a total sample size of 176 patients, 88 in each group, was required.[60]

This sample size also offered a power of 90% of detecting a significant difference (at α=0.05) in the incidence of side effects between the two treatment groups, assuming that the incidence of side effects in the QT group would be 50% (based on data from Souza et al, 1990)[22], and that a minimum clinically important difference would correspond to a 25% decrease in the incidence of side effects (i.e. that only 25% or less patients in the AT group would complain of one or more side effects).[60]

4.4. Methods of patient recruitment and eligibility criteria and their rationale

Patients who sought out the malaria service of the FNS - CR (MT) in Cuiabá, Mato Grosso (Amazon region - Brazil), between January 1992 and October 1993, were eligible to participate in the trial if they met the following criteria:

1- positive diagnosis of *P. falciparum* (demonstration of asexual forms in thick blood smears);

2- age equal to or greater than 14 years (tetracycline is not indicated for children under 8 years old and artesunate has not been well tested in children; moreover, the

use of age-specific dosages would complicate the execution of the study and interpretation of results);

3- no reported previous malaria treatment related to the present attack (a pre-treatment could interfere with the effectiveness of the trial treatments);

4- if women, indication of absence of pregnancy (primaquine and tetracycline are contra-indicated for pregnant woman. Artesunate has not been previously tested in pregnant women and animal studies have shown that therapeutic doses of artemisinin induce fetal reabsorption);[24]

5- intent to reside in the city of Cuiabá or in the neighbouring town of Várzea Grande for at least the next 30 days (to optimize compliance over the 28 day follow-up period and to minimize exposure to new malaria infection);

6- diagnosis of uncomplicated form of disease - clinical criteria[1] (for ethical reasons and to avoid withdrawals if hospitalization and/or parenteral treatment would be required);

7- minor or no symptoms of vomiting (patients should be able to receive oral treatment avoiding withdrawals if hospitalizations and/or parenteral treatment would be required);

8- agreement to participate in the study and signing a consent form (for ethical reasons and to avoid withdrawals).

Following confirmation of the eligibility criteria the treatment supervisor enrolled the patients into the trial. A diagnosis of *Plasmodium falciparum* malaria was confirmed by demonstration of asexual parasites in 100 microscopic fields under oil immersion in thick blood smears.

Enrolment was interrupted at times over the study period for reasons related to lack of manpower. These interruptions were judged to have negligible impact on the study validity and generalizability (see section 6.2.).

4.5. Randomization

A method of simple randomization was used in this study.

A table of 176 consecutive numbers, with 88 numbers distributed randomly into each of two groups, was generated by a computer program. Patients were assigned to one of the two treatment groups depending on which group was designated the number corresponding to their rank of entry order.

4.6. Blinding

A triple blind approach was followed in which patients, physicians, laboratory technicians and investigators had no knowledge about the group to which subjects had been assigned.

The eligibility criteria assessment was done without knowledge about the patient assignment to treatment group.

The physician who evaluated the outcome measurements of side effects and clinical improvement did not have access to information on the treatment drug or regimen of patients.

The assessment of effectiveness was done through standardized thick blood smears identified only by the numerical code of the patient.

The data analysis entry person coded the treatment group and blinded data analysis was carried out by the investigators on the coded data.

The treatment supervisor was the only unblinded individual in the trial and was not involved in any outcome measurement.

Drugs were distributed in standard unidentified envelopes. There were two small differences between treatments: 1) quinine and artesunate were both white tablets, but artesunate tablets were slightly smaller in size than the quinine tablets; 2) although tetracycline was used in both groups over 7 days making the complete time for treatment identical, artesunate was prescribed over 7 days and quinine over only 3 days.

However, there was no evidence indicating that the difference in tablet size or schedule caused unblinding. Although the standard treatment for *P. falciparum* malaria is quinine (3 days) plus tetracycline (7 days), several other drugs and schedules are available and used (e.g. quinine over 10 days, one dose of mefloquine, chloroquine over 3 days). Therefore, it was expected that patients would have difficulty in identifying the standard treatment between the trial treatments based only on tablet sizes and/or schedules.

On the other hand, specific well-known side effects associated with quinine therapies may be easily identified by patients who have had previous experience with this drug. Such side effects have not been previously reported for artesunate treatments. This may cause some suspicion on the part of patients and, perhaps also on the part of physicians. The use of 'hard measurements' in the assessment of effectiveness provided reassurance against the subjectivity of the patient's reports. However, a similar level of reassurance could not be expected for the assessment of side effects. No specific attempt was made to evaluate the level of treatment blinding *a posteriori*.

4.7. Treatment procedures

Patients were assigned to one of the following treatment groups: Group QT received oral quinine (2 g / day / 3 days) plus oral tetracycline (1.5 g / day / 7 days); Group AT received oral artesunate (150 mg on the first day followed by 100 mg / day / 6 days) plus oral tetracycline (1.5 g / day / 7 days) [See appendix 3: Tables I and II].

Both groups received a single dose of primaquine on the seventh day as a gametocytocide (drug acting on sexual forms - gametocytes - of malaria parasites.[30] This is routine procedure in the Brazilian Malaria Control Program.

The first dose of the treatment was administered by the treatment supervisor. Thereafter, patients received enough tablets to last only until the next follow-up appointment (see section 4.8. for follow-up schedule).

Drugs were separated into several small envelopes by daily dose and time. On each envelope the administration date and time was clearly indicated. A colour system (*ie.* blue for mornings, red for afternoons and black for nights) was used to help patients because a large proportion of participants was expected to be semi-literate.

All drugs were provided by the Brazilian Ministry of Health. Oral artesunate was originally manufactured in the People's Republic of China (Guilin Pharmaceutical Works), and donated to the Brazilian Ministry of Health.

4.8. Baseline and follow-up assessment

All patients were administered a baseline questionnaire at day 0 to obtain

socio-demographic information (Appendix 4). A clinical exam, including the reporting of signs and symptoms was undertaken at this visit, and at follow-up thereafter on days 2, 4, 7, 14 and 28 If one of these days fell on a holiday or weekend, the follow-up exam was performed on the closest weekday (usually Friday or Monday).

Blood tests (parasitemia, packed cell volume, white blood cell count, serum urea, creatinine) were performed at baseline and thereafter at all follow-up visits.

A minimum of 3 attempts, including home visits and telephone calls, was done to maximize compliance and minimize missing data. One member from the research team or from the health care service would try to locate the patient's address and to establish an appointment for the next evaluation or, if necessary, carry out the evaluations at the patient's home.

If a patient reported interruption of the treatment schedule, the same treatment and schedule was re-initiated from day 0. Treatment interruption was defined as a patient skipping a daily dose more than once.

4.9. Lab diagnosis of malaria

A standardized thick blood smear (technique of preparation and staining thick blood films using Giemsa stain)[1] was the basis for all parasitologic evaluations during the study period, inclusive of eligibility assessment.

This exam is used routinely by the Brazilian Malaria Control Program. It has been shown that a single thick blood smear presents a sensitivity of 92% for detecting malaria parasites when used for mass screening in endemic areas and has the ability to detect as few as one parasite-infected red blood cell per one million uninfected red blood cells.[61, 62] Moreover, 100% specificity has been reported.[61] Standardized thick

smears examined by trained laboratory technicians have been recognized as the gold standard for malaria parasite detection.

The lab technicians involved in the trial were trained and had long-term work experiences in the laboratory diagnosis of malaria. They had worked in the FNS - CR of Mato Grosso in both the accreditation of field malaria lab technicians and in the daily diagnosis of patients seen at the malaria health service.

Thick blood smears were considered negative if no parasites were seen in 100 oil immersion microscopic fields. Otherwise, the number of asexual *P. falciparum* parasites was counted per 500 white blood cells (WBC). Parasite density, expressed as parasites per mm^3, was calculated based on the number of parasites per 500 WBC and the number of WBC per cubic millimetre blood for each patient.

4.10. Side effects: definition and assessment

An incident side effect was defined as a sign or symptom arising after day 0 up to day 14 that had not been observed or reported at baseline (day 0).

Side effects were assessed through a standardized clinical examination by the study physician. Signs were assessed by a conventional physical exam. Symptoms were described by the patient in response to an open question (*eg*. Have you experienced any symptoms since the last visit ?). Identified symptoms were recorded on the clinical evaluation form (Appendix 5).

4.11. Effectiveness: definition and assessment

Two measures of effectiveness were assessed: cure rates and parasite clearance

at day 2 (*ie.* proportion of negative blood smears on day 2). When the day 2 measurement was obtained on day 1 or day 3 because of a holiday or weekend, they were excluded from day 2 analyses.

The cure/recrudescence rates were evaluated according to the WHO criteria for sensitivity (S) and resistance (RI, RII and RIII types) of *P. falciparum* to schizontocidal drugs.[9, 13]

The WHO criteria are based on the resistance of *P. falciparum* to 4-aminoquinolines (chloroquine or amodiaquine). Resistance is characterized by identification of recrudescence up to 28 days after treatment initiation in the absence of new infection (*e.g.* in areas free from transmission).[9] The classification of the resistance levels is dependent on the time of identification of the recrudescence and/or changes in parasite density.[9]

As oral quinine (half life = 8 to 12 hours), oral artesunate (half life = 10 to 12 hours), tetracycline (half life about 10 hours) and primaquine (half life about 6 hours) present elimination half lives much shorter than chloroquine (half life about 7 days), a 28-day follow-up period was expected to be sufficient to detect recrudescent patients in the present study.[21, 25, 63]

Cure (S) was defined as clearance of *P. falciparum* asexual parasitemia within 7 days of initiation of treatment and no recrudescence over the 28-day follow-up period. A resistance type I (RI) response was defined as clearance of *P. falciparum* asexual parasitemia within 7 days of initiation of treatment, with recrudescence occurring at some time over the 28-day follow-up period. A resistance type II (RII) response was defined as a marked reduction in *P. falciparum* asexual parasitemia (below 25% of the pre-treatment level) but no clearance over the 28-day follow-up period. A resistance type III (RIII) response was defined as no marked reduction of *P. falciparum* asexual parasitemia (not falling below 25% of pre-treatment level, or

increasing) and no clearance over the 28-day follow-up period.[9, 13]

Since there is virtually no urban and very low rural malaria transmission in Cuiabá and Várzea Grande, recrudescence was defined as reappearance of parasitemia after a previous negative result, unless the patient reported a trip to an endemic area.

4.12. Procedures used in the study design to ensure internal validity

Certain procedures to prevent bias and ensure internal validity of the treatment comparison were specifically incorporated into the study design:[64-66]

a. Randomization:

- randomizing patients to treatment group protects against conscious or unconscious manipulation of treatment assignment by patients or investigators and decreases the likelihood of selection bias;
- randomization in a relatively large sample size (N=176) tends to provide ar adequate balance of covariates (even if not completely perfect). It is expected to increase comparability of the groups for known and unknown characteristics, including potential confounders, and to decrease the likelihood for selection bias;
- randomization increases the validity of the statistical tests of significance used to assess the difference in treatment response. This is because these tests are based on the probability distribution of the differences in outcome between randomly defined groups receiving equally effective treatments - null hypothesis;

b. Blinding procedures:

- blinding procedures in the assessment of eligibility criteria decreases the likelihood of selection bias;

- study physician, patients and laboratory technicians were blinded to the patient's treatment group thus decreasing the likelihood of observation bias in the measurement of outcomes (however, some degree of imperfect blinding of subjects and physician due to specific and well known side effects related with the use of quinine was expected);
- blinding procedures in the data analysis decreases the potential of interpretation bias

c. 'hard measurements':

- standardized and objective questionnaires, medical evaluation procedures and forms and lab exams decreased the potential of observation bias.

4.13. Withdrawals and losses to follow-up

Withdrawal was defined as a patient who, following initiation of treatment, required a change of treatment for clinical reasons (*eg.* a treatment-related side effect, deterioration in health, inability to receive oral treatment).

Loss to follow-up was defined as a patient who could not be contacted by study personnel. This normally referred to patients who moved out of the area or who could not be traced.

Patients who withdrew before day 7 (before end of the treatment) were considered treatment failures. Patients lost to follow-up before the end of the treatment (poor compliance group) were also considered treatment failures because it was not possible to assess the reasons for these losses, and treatment-related effects could not be ruled out.

Patients who were lost to follow-up after day 7 (after completion of treatment)

were considered S/RI (ie. Sensitivity or resistance type I) if they had previously presented a negative blood smear.

4.14.Data analysis

Epi Info[67] and SAS[68] software were used to analyze the data. Cure rates were compared between the treatment groups based on an intention-to-treat analysis (effectiveness or pragmatic approach). Therefore withdrawals and losses to follow-up during treatment (poor compliance) and losses to follow-up after treatment [undefined treatment response (S/RI)] were considered treatment failures.

Chi-square tests were performed and relative risks with 95% confidence intervals (Taylor series or test based 95% CI for relative risks)[69] were calculated to compare treatment groups with respect to the incidence of side effects, cure rates and p rasite clearance at day 2. To ensure an overall type I error rate of 0.05, the Bonferoni correction was used in the side effects analysis because 12 variables were tested from the same data set.[70] Therefore, the significance of individual tests was set to the $\alpha = 0.004$ (0.05/12) level, for this stage of analysis. The significance level for all other statistical tests was set at $\alpha = 0.05$.

Continuous covariates were categorized based on their frequency distribution and/or substantive knowledge regarding expected changes in treatment responses. For instance, an effective acquired immune response in malaria is achieved after continuous exposure to malaria over approximately 6 years of residence in an endemic area. Persons presenting this characteristic have a better treatment response than others. A similar rationale was used regarding the number of self-reported life-time malaria attacks.

Stratified analyses based on these selected biologically meaningful covariates

were performed to examine relatively homogeneous subgroups and to identify potential confounding variables and effect modifiers.

Whenever the necessity for adjustments was identified, logistic regression analysis was performed when testing differences between treatment groups.

Modelling of relative risks was, thus, performed in the following sequence:

a. Assessment of results of the stratified analysis:

- overall relative risk versus stratum-specific relative risks to identify potential confounders;
- chi square test for homogeneity of the odds ratio across strata to identify potential effect modifiers.

b. Assessment of linearity in the logit for ordinal independent variables and categorized continuous variables (> 2 categories):

- plot of logit [log(p/1-p), where p = probability of the event] of the dependent variable versus categories of independent variables;[71]
- definition of dummy variables or transformation of a continuous independent variable if there was evidence of non-linearity; otherwise, the independent ordinal or continuous variables were entered into the model as continuous data.

c. Assessment of logistic regression models including potential confounders and/or interaction terms and identification of the best final model based on:

- Model log likelihood and significance level of the likelihood ratio test;
- Chi-square likelihood ratio tests for inclusion of specific variables;
- Wald chi-square statistic and significance levels for regression parameter estimates;

- Standardized changes in the parameter estimates for the main independent variable (treatment group) after adding one or more potential confounders;
- When comparing different logistic models, the data set was reduced to patients with full information on all independent variables, in order to make log likelihoods comparable.

d. Interpretation of the most appropriate final model based on relative risk estimates for associations between treatment group and incidence of one or more side effects, cure rates or parasite clearance at day 2.

4.15. Ethical aspects

This study was approved by the 'Fundação Nacional de Saúde - Mato Grosso sector - in its ethical and methodological aspects (Appendix 6).

Details of the nature, objectives and methods of the study were explained orally to all eligible patients. It was emphasized that the decision not to participate in the study would not, in any way, affect a patient's access to, or use of, the routine health or malaria care services.

Those who agreed to participate in this study signed an informed consent form. This form confirmed that the patients had received an oral explanation of the study objectives, methods and procedures. It was read to all patients before their signature (see appendix 7 for English translation of the informed consent form). This procedure was chosen because of the large proportion of semi-literate patients expected in the study.

The treatment supervisor who explained the study procedures and objectives to patients had a long work experience with this population and she communicated well

with them.

All identification numbers and codes were kept confidential and decoded individual data were not made available to anyone.

Bus tickets were offered to participants as a means of facilitating transportation.

The use of the combination of oral artesunate plus tetracycline has not been reported before. Oral artesunate with or without other antimalarial drugs has been studied in several animal and *in vitro* studies as well as in human populations in large sample trials.[23, 25, 48-50] Tetracycline has been used for decades in malaria treatments mainly combined with quinine.[20, 21, 37, 38] No important adverse reactions or side effects have been associated with the use of these drugs - oral artesunate or tetracycline - in malaria therapies. In addition, *in vitro* studies and animal (mice) studies have demonstrated that tetracycline shows marked potentiative synergism with artemisinin against *P. falciparum* (*in vitro*) and *P. berghei* (mice) strains.[53, 54] This has also been shown for mefloquine, another antimalarial drug.[53, 54] Based on these results, a study of the combination of mefloquine plus oral artesunate is currently underway in Thailand.[55] The combination of oral artemisinin and tetracycline has also been studied in Vietnam with improvement of cure rates and no important side effects.[51]

Based on the above, no important adverse reactions or side effects resulting from the association of oral artesunate plus tetracycline was expected *a priori*; nonetheless, frequent and systematic lab and medical evaluations were performed as a means of monitoring such events, as well as overall patient improvement.

CHAPTER 5.

Results

5.1. Enrolment

The characteristics of patients enrolled into the trial are presented in Table 5.1.

Among the 176 patients enrolled, the majority were males (82.4%) older than 20 years (81.8%). Thirty-two (18.2%) were between 14 and 20 years old and 41 (23.3%) were older than 40 years old (Table 5.1).

On admission, about half of the patients (88/172, 51.2%) reported having had symptoms during at least the last 4 days. Thirty-four patients (19.8%) reported symptom duration of between 4 and 7 days, and 50 patients (29.1%) of more than 7 days.

Information on the number of life-time malaria attacks was collected from 136 patients. Only 14 patients (10.3%) reported never having had malaria in the past. Among 122 patients who reported previous malaria attacks, 58 (42.7%) reported between 1 and 9 previous attacks and 64 (47.1%), more than 9 attacks.

142 patients (80.7%) provided information on length of residence in malaria endemic areas. Seventy-two (50.7%) reported time periods shorter than 2 years. Twenty-nine patients (16.5%) had never resided in an endemic area. Lengths of time of between 2 and 6 years, and more than 6 years were reported by 40 (28.2%) and 30

(21.1%) patients, respectively.

TABLE 5.1
Characteristics of patients enrolled into the RCT

Characteristics[1]	Category	n	%	Total with available data[2]
age	(14-20)	32	18.2	
(years)	(21-40)	103	58.5	
	(41 +)	41	23.3	
				176
sex	(male)	145	82.4	
	(female)	31	17.6	
				176
symptom	(0 - 3)	88	51.2	
duration	(4 - 7)	34	19.8	
(days)	(> 7)	50	29.1	
				172
previous	(0)	14	10.3	
malaria	(1 - 9)	58	42.7	
(# attacks)	(> 9)	64	47.1	
				136
residence time	(0 - 24)	72	50.7	
(months)	(25- 72)	40	28.2	
	(> 72)	30	21.1	
				142
parasitemia	(< 5000)	77	45.8	
(/mm^3)	(5000 - 10000)	43	25.6	
	(10001- 50000)	41	24.4	
	(> 50000)	7	4.2	
				168

[1] - symptom duration: Days presenting symptoms before admission
previous malaria: Number of self-reported life-time malaria attacks
residence time: Months living in the endemic area
parasitemia: Asexual *P. falciparum* parasites per mm^3 at baseline

[2] - sample size variation is due to missing information

Among the 168 patients who had parasitemia measured at baseline, 120 (71.4%) had less than or equal to 10,000 parasites/mm^3 and 41 (24.4%) had a parasitemia level between 10,000 and 50,000 parasites/mm^3. Among the remaining seven (4.2%) patients, six had a parasitemia level between 50,000 and 100,000 parasites/mm^3, and one patient had a level of 170,300 parasites/mm^3.

Proportions of patients presenting abnormal laboratory test results at baseline were noted. Fifty-four patients (31.2%) were leucopenic (WBC count lower than 4500 cells /L). Most of the patients (71%) had a packed cell volume lower than 40%. 38.4% of patients (63/164) had a serum urea result higher than 38 mg/dl and 11.8% (20/169) had a serum creatinine level higher than 1.5 mg/dl.

5.2. Comparability of treatment groups

176 patients were randomized into two treatment groups (88 patients each). The two treatment groups were similar at baseline with regard to clinical data, except for the number of previous malaria attacks (Table 5.2). The QT group had fewer patients reporting no previous malaria attacks than did the AT group (5.8% vs 14.9%, respectively) and a higher proportion of patients reporting 10 or more malaria attacks (55.1% in the QT vs 38.8% in the AT group). The QT group also had a slightly higher proportion of patients reporting a positive history of residence in endemic areas (82.9%) than did the AT group (76.4%).

No important differences in the distribution of laboratory measurements were observed between the groups (Table 5.3). Mean and median parasitemia at baseline were slightly lower in the QT group than in the AT group. The QT group presented a slightly greater variability (mean, SD and range) in parasitemia at baseline than did the AT group.

TABLE 5.2
Clinical characteristics of patients randomized to artesunate plus tetracycline (AT) and quinine plus tetracycline (QT) treatment groups at baseline

Characteristics	*Treatment Groups*[1]	
	AT	*QT*
sex		
-male	72/88 (81.8%)	73/88 (82.9%)
age (years)		
-mean ± SD	32.2 ± 11.5	30.9 ± 11.8
-range	15-57	14-70
symptom duration		
-duration < 8 days	63/86 (73.3%)	59/86 (68.6%)
-median duration (days)	3	3
residence in an endemic area	55/72 (76.4%)	58/70 (82.9%)
-median time (months)	36	48
previous malaria (# attacks)		
0	10/67 (14.9%)	4/69 (5.8%)
1 - 9	31/67 (46.3%)	27/69 (39.1%)
>= 10	26/67 (38.8%)	38/69 (55.1%)
weight (kg)		
-mean ± SD	62.8 ± 9.6	64.2 ± 11.2
self-reported fever	77/88 (87.5%)	78/88 (88.6%)
fever at baseline (>= 37.5°C)	36/86 (41.9%)	41/84 (48.8%)

[1] - number of affirmative responses / number responding (%)
denominators differ due to missing information

TABLE 5.3
Laboratory findings of patients randomized to artesunate plus tetracycline (AT) and quinine plus tetracycline (QT) treatment groups at baseline

Laboratory data[1]	*Treatment groups mean ± SD*	
	AT n=88	*QT n=88*
Parasitemia		
parasites/mm^3	11791 ± 15889	10370 ± 21099
median parasites/mm^3	6670	5174
range	204-85082	175-170285
Packed cell volume (%)	35.6 ± 5.7	36.8 ± 5.9
% with < 40%	72.7	64.8
WBC count (cells per μl)	6121 ± 3656	5760 ± 2226
% with < 4.5 cells per μl	30.7	29.5
Blood urea (mg/dl)	39.1 ± 15.1	36.3 ± 15.3
% with > 38 mg/dl	37.5	34.1
Serum creatinine (mg/dl)	1.1 ± 0.3	1.2 ± 0.3
% with > 1.5 mg/dl	9.0	13.6

[1] - Critical values based on Stein JH, 1987[72]

5.3. Treatment and follow-up compliance

Table 5.4 describes the treatment and follow-up compliance during the trial. The seven-day treatment regimen was completed by 167 patients (94.9%). Reasons for which nine (5.1%) patients did not complete the treatment regimen included: withdrawals due to severe vomiting at the beginning of the treatment period (n = 5) and loss to follow-up before the end of the treatment (n = 4). Follow-up to day 28 was completed by 141 (80.1%) patients. Twenty-five patients (14.2%) were lost to follow-up between day 7 and day 28 because they could not be traced or they had moved outside the study area for work-related reasons. Only one patient (0.5%) refused to continue with the follow-up visits.

Frequencies of withdrawals and losses to follow-up were similar in both treatment groups.

Five (2.8%) patients (3/88 from the AT and 2/88 from the QT group) interrupted their treatment but subsequently had it re-initiated. The interruptions occurred between day 0 and 2 in three patients, and between day 2 and 4 and between day 4 and 7 in the remaining two patients, respectively. All patients agreed to re-initiate the same oral treatment regimen which had been interrupted. These patients were not excluded from the analyses (see section 6.2.).

5.4. Summary of univariate analyses

Univariate analyses of associations between treatment response (incidence of one or more side effects, cure rate and parasite clearance at day 2) and independent variables are presented in Table 5.5.

TABLE 5.4
Description of the treatment and follow-up compliance during the RCT of patients given artesunate plus tetracycline (AT) and quinine plus tetracycline (QT)

Compliance description	*Treatment groups*		*Total*	*%*
	AT	*QT*		
- Initial enrolment	88	88	176	100
Treatment period (day 0-7):				
-withdrawal due to vomiting*	2	3		
-loss to follow-up**	1	3		
- Number remaining at day 7			167	94.9
Post-treatment period (day 7-28):				
-loss to follow-up***	13	13		
- Number remaining at day 28			141	80.1

* Patients with severe vomiting were hospitalized or given parenteral (IM) treatment
** Moved/missing address (n=4)
*** Refused (n=1) or moved/missing address (n=25)

TABLE 5.5
Odds ratios (OR) and significance levels of likelihood ratio test (LRT) from univariate logistic regression for associations between independent and dependent variables

Independent variables[1]	*Odds ratio (p-value for LRT) for dependent variables*[2]		
	One or more side effects	Cure	No clearance at day 2
treatment group (QT/AT)	4.66 (0.0001)	0.87 (0.71)	73.7 (0.0001)
age (1 year increases)	1.02 (0.17)	1.01 (0.50)	0.99 (0.71)
sex (female/male)	0.91 (0.83)	0.61 (0.28)	2.68 (0.10)
log parasitemia (100 parasites increases)	0.71 (0.65)	0.82 (0.82)	0 40 (0.33)
residence ..ne (12 month increases)	1.02 (0.53)	1.09 (0.13)	1.03 (0.54)
symptom duration (1 day increases)	0.99 (0.27)	1.01 (0.50)	1.01 (0.33)
previous malaria (1 attack increases)	1.02 (0.07)	1.03 (0.14)	0.98 (0.27)

[1] - treatment group: [QT = quinine + tetracycline, AT = artesunate + tetracycline]
parasitemia: Asexual *P. falciparum* parasites per mm^3 at baseline (log transformed)
residence time: Months living in an endemic area
symptom duration: Days presenting symptoms before admission
previous malaria: Number of self-reported life-time malaria attacks

[2] - one or more side effects: Side effects arising between days 1 and 14 [1 = no, 2 = yes]
cure: Negative smear after treatment, up to day 28 [1 = no (RI, S/RI response or withdrawal/lost before end of treatment), 2 = yes (sensitive response)]
no clearance at day 2: positive smear result at day 2 [1 = negative, 2 = positive]

A statistically significant association was identified between treatment group and incidence of one or more side effects with odds being 5 times lower in the AT group ($p = 0.0001$).

No evidence of association was observed between treatment group and cure rate.

The association between treatment group and parasite clearance at day 2 was extremely strong with the AT group having a greater proportion of patients achieving parasite clearance at day 2 then the QT group.

No statistically significant association (at $\alpha = 0.05$) was identified between covariates (age, sex, parasitemia, length of residence in the endemic area, duration of symptoms before admission and number of life-time previous malaria attacks) and outcome measures (incidence of one or more side effects, cure rate or parasite clearance at day 2).

5.5. Treatment side effects

The majority (82%) of patients receiving QT complained of one or more side effects (Table 5.6). The most frequent side effects identified in this group were dizziness (34.1%), tinnitus (24.4%) and abdominal pain (23.8%). Among patients receiving AT, 50% complained of one or more side effects. The most frequent side effect in this group was abdominal pain (16.5%), while the incidence of all others was below 10%. After correcting for multiple comparisons using the Bonferoni method, the risk of dizziness and the risk of tinnitus remained significant. However, all 12 incident side effects were less frequently reported in the AT group than in the QT group. [The binomial probability of observing, by chance alone, a relative risk (RR) greater than 1 in all 12 associations is 0.00025 (0.5^{12})].

TABLE 5.6
Comparative incidence of side effects observed in patients given artesunate plus tetracycline (AT) and quinine plus tetracycline (QT)

Side effects[1]	*Treatment Groups*[2]		*Relative Risk (95% CI)*[3] *QT/AT*	
	AT	*QT*		
dizziness	7/85 (8.2)	28/82 (34.1)	4.15	(1.92-8.96)**
abdominal pain	14/85 (16.5)	20/84 (23.8)	1.44	(0.78-2.67)
nausea	7/86 (8.1)	15/82 (18.3)	2.25	(0.97-5.23)
weakness	7/85 (8.2)	14/82 (17.1)	2.07	(0.81-6.72)
tinnitus	0/85 (0.0)	20/82 (24.4)	20.73[4]	(2.85-150.96)**
anorexia	4/85 (4.7)	14/82 (17.1)	3.63	(1.25-10.57)*
vomiting	6/86 (7.0)	10/84 (11.9)	1.71	(0.65-4.49)
myalgia	6/85 (7.1)	10/83 (12.0)	1.71	(0.65-4.48)
sweating	3/85 (3.5)	12/82 (14.6)	4.15	(1.21-14.16)*
headache	4/85 (4.7)	9/82 (11.0)	2.33	(0.75-7.28)
bitter taste	5/85 (5.9)	8/82 (9.8)	1.66	(0.57-4.86)
diarrhea	2/85 (2.3)	7/82 (8.5)	3.63	(0.78-16.96)
one or more side effects	43/86 (50.0)	70/85 (82.3)	1.65	(1.30-2.08)**

[1] - side effects arising between day 1 and day 14
[2] - number of affirmative responses / number responding (%)
denominators differ due to missing information
[3] - Taylor series 95% confidence intervals for relative risks
[4] - approximate relative risk was calculated where cell containing zero was replaced by 1
* Chi-square: $0.05 \geq p > 0.01$
** Chi-square: $p < 0.0001$

Although no severe side effects were reported by patients in either group, in general, patients receiving quinine plus tetracycline had a significantly higher risk of one or more side effects than those receiving artesunate plus tetracycline (RR=1.65, 95% confidence interval: 1.30-2.08). The risk of presenting one or more incident side effects remained significantly higher in the QT group than in the AT group in all stratum-specific groups of age, sex, duration of symptoms before admission, number of self-reported life-time malaria attacks, length of residence in the endemic area or parasitemia at baseline (Table 5.7). Separate stratified analyses using these variables yielded pooled Mantel-Haenszel relative risk estimates (QT vs AT) ranging from 1.46 to 1.66, and all corresponding 95% confidence intervals excluded 1.

All stratum-specific relative risks (RRs ranging from 1.40 to 1.50) for the stratification by number of life-time malaria attacks are slightly lower than the crude RR (1.65) for the association between one or more side effects and treatment group. Therefore, this variable was identified as a potential confounder. This was expected based on the slightly different distribution of number of life-time malaria attacks between treatment groups (Table 5.2), and its marginally significant association with incidence of one or more side effects in the univariate logistic regression [LRT p-value = 0.07 (Table 5.5)].

Among two-way interactions between treatment group and covariates, only the interaction with parasitemia level at baseline was statistically significant (p-value for the test of homogeneity of odds ratio = 0.047) [Table 5.7]. This indicates that the strength of the association between the incidence of one or more side effects and treatment group may change depending on the level of parasitemia. In fact, as the parasitemia level increases from $\leq$ 7000 to $>$ 7000 parasites per mm^3 the proportion of patients presenting one or more side effects increases in the QT group and decreases in the AT group (Table 5.8). The protective effect of AT against the incidence of one or more side effects becomes stronger among patients who have a

TABLE 5.7
Stratified analyses of the association between one or more incident side effects and treatment group [artesunate plus tetracycline (AT) and quinine plus tetracycline (QT)][1]

Stratification factor[2]	*Stratum*	*n*	*Estimated relative risk (QT/AT)*		*p-value (chi-square test for homogeneity of OR)*
			RR	*Pooled RR_{MH} (95%CI)*[3]	
age	(14-20)	31	2.67		
(years)	(21-40)	100	1.48	1.63	0.96
	(41 +)	40	1.67	(1.32 - 2.02)	
sex	(male)	143	1.62	1.65	
	(female)	28	1.81	(1.32 - 2.05)	0.72
symptom	(0 - 3)	87	1.65		
duration	(4 - 7)	32	1.67	1.66	0.61
(days)	(> 7)	48	1.69	(1.33 - 2.08)	
previous	(0)	14	1.50		
malaria	(1 - 9)	57	1.40	1.46	0.80
(# attacks)	(> 9)	64	1.50	(1.15 - 1.85)	
residence	(0 - 24)	71	1.69		
time	(25- 72)	40	1.56	1.51	0.70
(months)	(> 72)	30	1.19	(1.20 - 1.91)	
parasitemia	(0 - 7000)	93	1.38	1.66	
($/mm^3$)	(> 7000)	71	2.12	(1.32 - 2.09)	0.047

Crude relative risk (95% CI) = 1.65 (1.30 - 2.08)

[1] - one or more incident side effects arising between days 1 and 14

[2] - symptom duration: Days presenting symptoms before admission
previous malaria: Number of self-reported life-time malaria attacks
residence time: Months living in an endemic area
parasitemia: Asexual *P. falciparum* parasites per mm^3 at baseline

[3] - test-based 95% confidence intervals for Mantel-Haenszel relative risks (RR_{MH})

TABLE 5.8
Association between one or more incident side effects and treatment group [artesunate plus tetracycline (AT) and quinine plus tetracycline (QT)], by parasitemia level at baseline[1]

Parasitemia[2]	*proportion of patients with one or more side effects (%)*		*Estimated relative risk (QT/AT)*	*95% CI*[3]
	AT	*QT*		
0 - 7000	24/44 (54.5)	37/49 (75)	1.38	1.01 - 1.89
> 7000	17/40 (42)	28/31 (90)	2.12	1.46 - 3.10

[1] - one or more incident side effects arising between days 1 and 14
[2] - parasitemia: Asexual *P. falciparum* parasites per mm^3 at baseline
[3] - test-based 95% confidence intervals for relative risks

higher level of parasitemia at baseline than among those presenting a lower baseline parasitemia level. However, the 95% confidence interval for the relative risk excludes 1 even at the lower parasitemia level.

The influence of the number of life-time malaria attacks and parasitemia levels at baseline on the association between treatment group and incidence of one or more side effects was assessed using multiple logistic regression (Table 5.9).

The strength of the association between the incidence of one or more side effects and treatment group was confirmed by the significance of the parameter estimates for treatment group (p-value for LRT < 0.001) in the univariate logistic regression (Model 2, Table 5.9). As previously reported, the risk of one or more side effects in the AT group was 50%, while the risk of one or more side effects in the QT group was 82%. Therefore, the risk of presenting one or more side effects in the QT group was 65% higher than in the AT group [RR (QT/AT) = 1.65].

After adding the variable 'number of life-time malaria attacks' into the logistic model, no important changes were observed in the treatment group coefficient (estimated ß changed from -1.33 to -1.27) nor in its relative risk [RR (QT/AT) changed from 1.49 to 1.62] (models 3 and 4). The standardized change in the coefficient (ß) for treatment group was 0.16 SE. Therefore, the impact of the number of life-time malaria attacks as a confounder of the association between the incidence of one or more side effects and treatment group was considered negligible.

The role of parasitemia level at baseline as a potential modifier of the effect of treatment on the incidence of one or more side effects was then assessed. Because parasitemia level at baseline (as continuous variable) presents a skewed distribution (skewness = 4.785) and its logit was non-linear when categorized, a logarithm transformation was used to perform logistic regression analyses.

TABLE 5.9

Estimated logistic regression coefficients, log-likelihood and the Likelihood Ratio Test Statistics (LRT-G) for the association between one or more incident side effects and some selected variables[1]

model	*N*	*Variable coefficients (β's)* [2]					*model log-likelihood*	*LRT-G (p-value)*
		Constant	treatment group[3]	previous malaria	log (parasitemia)	log(par) * gr		
1	171	0 67	-	-	-	-	-109 52	-
2		1 54	1 54*	-	-	-	-99 22	20 60 (0 0001)
3	135	1 54	-1 33	-	-	-	-77 76	-
4		1.14	-1.27	0 27*	-	-	-77 33	0.85 (>0 10)
5	164	1 47	-1 51	-	-	-	-96 81	-
6		1 57	-1 51	-	-0 013*	-	-96 80	0 02 (>0 10)
7		-1 88	4 22	-	0 42	-0 70*	-92 70	8 20 (<0 01)

[1] - one or more side effects: Side effects arising between days 1 and 14 [1=no, 2=yes]

[2] - previous malaria: Self-reported life-time malaria attacks as a categorical variable [(0) = 0; (1) = 1-9; (2) = >9] was entered into the model as a continuous variable because its logit distribution was linear

log(parasitemia): Asexual *P. falciparum* parasites per mm^3 at baseline (log transformed)

log(par) * gr: Interaction term [log(parasitemia) * treatment group]

[3] - treatment group: [0 = quinine + tetracycline, 1 = artesunate + tetracycline]

* variable tested using LRT (G) statistics; previous model as reference

As in the previous stratified analyses, the significance of the interaction term [log(parasitemia) * treatment group] (LRT p-value < 0 01) suggested the presence of a significant change in the strength of the association between the incidence of one or more side effects and treatment group depending on the parasitemia level (Model 7). Coefficients estimated for the model including the interaction term (Model 7) are presented in Table 5.10. The predicted relative risks for different levels of parasitemia were reconstructed in Table 5.11. It is observed that the protective effect of the AT treatment against the incidence of one or more side effects becomes stronger with an increasing level of parasitemia at baseline. However, as this interaction was not expected *a priori*, its significance must be cautiously interpreted and the presence of effect modification should be confirmed in an independent study.

5.6. Treatment cure rates

The treatments of artesunate plus tetracycline and quinine plus tetracycline were effective in 80% (71/88) and 77% (68/88) of patients, respectively (Table 5.12). The difference of 3% (95% confidence interval: -9% to +15%) in cure rates was not statistically significant (p=0.68) For the 26 patients who completed the treatment schedule and cleared parasitemia sometime before they were lost to follow-up, it was not possible to distinguish between an S or an RI type response. Therefore, the failure rates of 20% in the AT group and 23% in the QT group may be regarded as conservative estimates as they include both true recrudescences (RI) and undefined treatment responses [withdrawals and losses to follow-up during treatment (poor compliance) and losses to follow-up after treatment (S/RI response)].

Poor compliance with the treatment schedule was observed in 3.4% (3/88) and 6.8% (6/88) in the AT and QT groups, respectively. This difference was not statistically significant (p-value for Fisher exact test = 0.49).

TABLE 5.10
Estimated logistic regression coefficients for the association between one or more incident side effects and treatment group accounting for the interaction with parasitemia level at baseline[1]

Variable[2]	*Regression coefficient*		
	Coefficient	*Standard error*	*p-value (Wald chi-square)*
constant	-1.8842	1.5618	0.228
treatment group	4.2204	2.0537	0.040
log(parasitemia)	0.4211	0.1996	0.035
log(par) * gr	-0.7018	0.2525	0.005

[1] - one or more side effects: Side effects arising between days 1 and 14 [1 = no, 2 = yes]
[2] - treatment group. [0 = quinine plus tetracycline, 1 = artesunate plus tetracycline]
log(parasitemia): Asexual *P. falciparum* parasites per mm^3 at baseline (log transformed)
log(par) * gr: Interaction term [log(parasitemia) * treatment group]

TABLE 5.11

Estimated risks and relative risks for the association between one or more incident side effects and treatment group [artesunate plus tetracycline (AT) and quinine plus tetracycline (QT)] accounting for the interaction with parasitemia level at baseline[1]

Parasitemia level	*Risk of presenting one or more side effects (%)*		*Relative Risk QT/AT*
	AT	*QT*	
3000	52.3	81.6	1.56
9000	44.5	87.5	1.97
15000	41.0	89.7	2.19
21000	38.8	90.9	2.35

[1] - one or more side effects: Side effects arising between days 1 and 14
parasitemia level: Asexual *P. falciparum* parasites per mm^3 at baseline

TABLE 5.12
Comparison of effectiveness of the two treatments [artesunate plus tetracycline (AT) and quinine plus tetracycline (QT)]

Treatment response	*Treatment groups*[1]		*Chi-square statistic (p-value)*
	AT n=88	*QT n=88*	
Cure (S)	70 (80%)	68 (77%)	0.16 (p=0.68)
Treatment failure (Total)	18 (20%)	20 (23%)	
S/RI[2]	13	13	
RI	2	1	
RII	0	0	
RIII	0	0	
poor compliance[3]	3	6	

[1] - number of patients (%)
[2] - patients lost to follow-up post-treatment period. All patients had had a negative smear on day 7
[3] - withdrawal due to vomiting or loss to follow-up before completion of the treatment (day 7)

There was no evidence of confounding or effect modification by any of the following variables: age group, sex, duration of symptoms before admission, number of self reported malaria attacks, months living in an endemic area and parasitemia levels at baseline (Table 5.13). Under separate stratified analyses pooled Mantel-Haenszel relative risks for the association between cure rates and treatment groups ranged from 0.62 to 1.02 and all 95% confidence intervals included RR = 1. The crude relative risk estimated at 0 97, was within all ranges of stratified relative risks. All tests for homogeneity of odds ratios across strata yielded non-significant results.

Among the 141 patients who fully complied during both treatment and follow-up periods only 3 recrudescences occurred (all type RI), two in the AT group and one in the QT group (Table 5.12). Therefore, cure rates of 97% and 98% would have been obtained in the AT and QT groups respectively, if the analysis would have been limited to compliant patients alone (n=141).

5.7. Parasite clearance

Among the 131 patients who had a smear on day 2 (exactly), the proportion of patients clearing their infection was much higher in the AT group (98.5%) than in the QT group (47.6%) [$p<0.0000001$] (Table 5.14). All but 1 among 68 patients in the AT group had parasite clearance on day 2, whereas in the QT group only 47.6% of patients had a negative smear at that time.

Given that only one patient in the AT group had a positive smear on day 2, several cell sizes would be equal to zero. To avoid infinite relative risks the assessment of confounders and/or interaction effects (Table 5.15) focused on the ratio of the probability for presenting a negative smear at day 2 in the QT versus this probability in the AT group stratified by covariates (rather than using the risk for presenting a positive smear as presented in previous analyses).

TABLE 5.13
Stratified analyses of the association between cure rate and treatment group [artesunate plus tetracycline (AT) and quinine plus tetracycline (QT)][1]

Stratification factor[2]	*(stratum)*	*n*	*Estimated relative risks (QT/AT)*		*p-value (chi-square test for homogeneity of OR)*
			RR	*Pooled RR_{MH} (95%CI)*[3]	
age	(14-20)	30	1.71		
(years)	(21-40)	98	0.78	0.95	0.87
	(> 40)	39	1.17	(0.49 - 1.84)	
sex	(male)	140	1.00	0.97	
	(female)	27	0.83	(0.50 - 1.89)	0.84
symptom	(0 - 3)	85	1.32		
duration	(4 - 7)	31	0.28	0.98	0.30
(days)	(> 7)	47	1.17	(0.50 - 1.93)	
previous	(0)	14	1.11		
malaria	(1 -9)	55	0.65	0.62	0.87
(# attacks)	(>9)	62	0.68	(0.23 - 1.63)	
residence time	(0 - 24)	68	1.29		
(months)	(25- 72)	39	0.23	0.69	0.31
	(> 72)	30	0.38	(0.28 - 1.70)	
parasitemia	(0- 7000)	90	0.96	1.02	0.83
($/mm^3$)	(> 7000)	71	1.11	(0.51 - 2.05)	

Crude relative risk (95% CI) = 0.97 (0.50 - 1.90)

[1] - cure (negative smear after treatment, up to day 28) as binary variable: 0 = no (RI or RI/S response), 1 = yes (sensitive response)

[2] - symptom duration: Days presenting symptoms before admission
previous malaria: Number of self-reported life-time malaria attacks
residence time: Months living in an endemic area
parasitemia: Asexual *P. falciparum* parasites per mm^3 at baseline

[3] - test-based 95% confidence intervals for relative risks

TABLE 5.14
Association between parasite clearance at day 2 and treatment group [artesunate plus tetracycline (AT) and quinine plus tetracycline (QT)]

Treatment Group	*Positive smear result at day 2 / # examined patients (%)*	*Relative Risk (95% CI)[1] QT/AT*
AT	1/68 (1.5%)	
		35.62 (5.02 - 252.80)*
QT	33/63 (52.4%)	

[1] - Taylor Series 95% confidence intervals for relative risks
* Chi-square = 44.10 ($p < 0.0000001$), N=131

TABLE 5.15
Association between parasite clearance at day 2 and treatment group [artesunate plus tetracycline (AT) and quinine plus tetracycline (QT)], controlling for some covariates

Stratification factor[1]	*Stratum*	*n*	*Estimated relative risks (QT/AT)*[3]		*p-value (chi-square test for homogeneity of OR)*
			RR	*Pooled RR_{MH} (95%CI)*[4]	
age	(14-20)	25	0.45		
(years)	(21-40)	74	0.57	0.48	0.47
	(> 40)	32	0.29	(0.38- 0.59)	
sex	(male)	108	0.45	0.49	
	(female)	23	0.67	(0.39- 0.61)	0.74
symptom duration	(0 - 3)	64	0.48		
(days)	(4 - 7)	25	0.40	0.49	0.64
	(> 7)	39	0.39	(0.40- 0.61)	
previous malaria	(0)	11	0.50		
(# attacks)	(1 - 9)	48	0.30	0.48	1.00
	(> 9)	48	0.67	(0.38-0.61)	
residence time	(0 -24)	53	0.32		
(months)	(25-72)	35	0.65	0.47	1.00
	(> 72)	23	0.54	(0.37-0.60)	
parasitemia	(0 -7000)	74	0.58	0.46	0.16
($/mm^3$)	(> 7000)	54	0.28	(0.36-0.57)	

Crude relative risk (95% CI) = 0.48 (0.37 - 0.63)

[1] - symptom duration: Days presenting symptoms before admission
previous malaria: Number of self-reported life-time malaria attacks
residence time: Months living in an endemic area
parasitemia: Asexual *P. falciparum* parasites per mm^3 at baseline

[3] - relative risk = ratio of the probability of presenting a negative smear at day 2 in the QT group vs in the AT group

[4] - test-based 95% confidence intervals for relative risks

There was no evidence of confounding or effect modification by any of the following variables: age group, sex, duration of symptoms before admission, number of self reported malaria attacks, months living in an endemic area and parasitemia levels at baseline (Table 5.15). In separate stratified analyses pooled Mantel-Haenszel relative risks for the association between parasite clearance at day 2 and treatment groups ranged from 0.46 to 0.49 and all included the crude relative risk (0.48). In addition, all tests for homogeneity of odds ratio across strata yielded non-significant results.

To confirm the above results, QT and AT groups were compared with respect to the risk for presenting a positive smear at day 2 in particular strata defined by: age group, sex, duration of symptoms before admission, number of self reported malaria attacks, months living in an endemic area and parasitemia levels at baseline (Table 5.16). All strata-specific risks for presenting a positive smear at day 2 in the QT group were higher than 33%, while in the AT group, risks varied between 0% and 3%.

The following analysis is based on the proportion of patients with a negative blood smear at each follow-up visit (all patients included).

An overall faster parasite clearance was observed in the AT group than in the QT group. Parasite clearance on day 2 was achieved in 96.3% (78/81) of the patients receiving AT treatment and in 100% by day 4. Among patients receiving QT treatment, only 52.5% (42/80) had a negative blood smear by day 2 and 91.5% (65/71) by day 4. Only by day 7 was there complete clearance in the QT group.

5.8. Lab findings

Twenty one patients (11.9%) showed patent *P. vivax* infection during the

TABLE 5.16
Stratified risk of presenting a positive smear at day 2 in each treatment group [artesunate plus tetracycline (AT) and quinine plus tetracycline (QT)]

Stratification factor[1]	*Stratum*	*n*	*Estimate risks (%)*	
			QT	*AT*
age	(14-20)	25	6/11 (54.5)	0/14 (0.0)
(years)	(21-40)	74	17/38 (44.7)	1/36 (2.8)
	(> 40)	32	10/14 (71.4)	0/18 (0.0)
sex	(male)	108	30/54 (55.6)	1/54 (1.8)
	(female)	23	3/9 (33.3)	0/14 (0.0)
symptom duration	(0 - 3)	64	16/30 (53.3)	1/34 (2.9)
(days)	(4 - 7)	25	6/10 (60.0)	0/15 (0.0)
	(> 7)	39	9/21 (42.9)	0/18 (0.0)
previous malaria	(0)	11	1/2 (50.0)	0/9 (0.0)
(# attacks)	(1 - 9)	48	16/23 (69.6)	0/25 (0.0)
	(> 9)	48	9/27 (33.3)	0/21 (0.0)
residence time	(0 -24)	53	13/19 (68.4)	0/34 (0.0)
(months)	(25-72)	35	7/20 (35.0)	0/15 (0.0)
	(> 72)	23	6/13 (46.1)	0/10 (0.0)
parasitemia	(0 -7000)	74	17/39 (43.6)	1/35 (2.9)
(/mm^3)	(> 7000)	54	15/21 (71.4)	0/33 (0.0)

Crude risk: QT group = 33/63 (52.4%); AT group = 1/67 (1.5%)

[1] - symptom duration: Days presenting symptoms before admission
previous malaria: Number of self-reported life-time malaria attacks
residence time: Months living in an endemic area
parasitemia: Asexual *P. falciparum* parasites per mm^3 at baseline visit

follow-up period. One patient was identified on day 1 and twenty patients between day 20 and day 28. Another ten patients voluntarily returned to the trial after day 28 presenting *P. vivax* infection. There was no statistically significant difference in the occurrence of *P. vivax* malaria during the 28-day follow-up period between treatment groups (AT=13/88 and QT=8/88; chi-square statistic=1.35, p=0.25).

As expected, among 121 patients presenting packed cell volumes lower than 40%, only 4.1% [AT=4/64 and QT=1/57) recovered within 7 days presenting, at least, 2 sequential lab tests within normal levels.[72]

Approximately half of the patients [AT=14/27 (51.8%) and QT=14/26 (53.8%)] presenting a WBC count lower than 4.5 cells per μl at baseline recovered by day 7 and remained within normal levels on following lab tests.[72]

Eight [AT=3/88 (3.4%) and QT=5/88 (5.7%)] patients who initially had normal levels of serum creatinine and urea levels, increased both exam results to abnormal levels by day 2 or 4 (higher than 1.5 mg/dl and 38 mg/dl, respectively).[72] However, no concurrent clinical manifestation was identified and all clinical exams during follow-up visits were within normal parameters.

There was no evidence of important treatment-related toxicity in any patient based on laboratory and clinical observation. In addition, there was no evidence of differential laboratory exam changes between patients receiving QT or AT treatment.

CHAPTER 6.

Discussion

6.1. Comparison of treatment regimens

Oral artesunate (7 days, total dose = 0.75 g) plus tetracycline (7 days, total dose = 10.5 g) in this study population quickly cleared *P. falciparum* asexual parasitemia (98.5% in 2 days) and radically cured 80% of patients. Only two RI resistance responses were observed among patients who fully complied with the treatment schedule and follow-up visits. Moreover, the drug combination was very well tolerated with a relatively low incidence of minor side effects. Poor compliance with the treatment schedule was low (3/88 = 3.4%) under the trial situation.

These results concur with other similarly encouraging reports regarding oral artesunate. Cure rates between 72% [(N = 25) daily dose during 5 days, total dose = 0.6 g] and 92.5% [(N = 40) 7 days, total dose = 0.6 g] and a parasite clearance in about 2 days with no important side effects were obtained when oral artesunate was used in Thai patients.[49, 50] In another trial undertaken by this research group, oral artesunate given during 5 days in different doses achieved cure rates equal to or greater than 90% (Ns of 5, 10 and 20 patients).[25] However, when this drug was administered for only 1 or 2 days (with or without chloroquine or sulfadoxine-pyrimethamine) the cure rates were 0%.[25] Oral artesunate (5 days, total dose = 0.6 g) when followed by mefloquine (1.25 g) achieved a cure rate of 100% (N=39), higher than that observed when artesunate or mefloquine were used alone (88% and 81%, respectively) in similar populations.[48]

Artemisinin derivatives have been effective when used with other known antimalarial drugs. Antibiotics have also been used successfully in malaria therapy. As an example, quinine plus tetracycline has had much higher cure rates than quinine alone.[18, 38] This provided the rationale for combining artesunate: a fast acting drug (parasite clearance in 2 days) but with a short half-life; with tetracycline: a slow acting antibiotic (effect manifested in about 48h) but with a known blood schizontocidal action.[12, 24, 25] A cure rate of 90% was reported in Vietnamese patients when oral artemisinin (3 days, total dose = 1.5 g) was administered together with tetracycline (5 days, total dose = 12.5 g) compared to a 50% cure rate when artemisinin was administered alone (during 2 to 5 days).[51] Our study also provides evidence in favour of the combination of oral artesunate and tetracycline.

Quinine (3 days, total dose = 6 g) plus tetracycline (7 days, total dose = 10.5 g) has been used successfully as the standard treatment for uncomplicated *P. falciparum* malaria in Brazil since 1990, with continued positive results [11] In fact, in our study, we found that the combination of quinine plus tetracycline was still highly effective, curing 77% of patients. However, one or more side effects were frequently reported.

Despite the efficacy of treatment using quinine plus tetracycline for 7 days, in countries where this drug combination has been extensively used for more than 10 years, some resistance to shorter term treatment with quinine (3 days, total dose = 5.4 g) plus tetracycline (7 days, total dose = 10.5 g) has been reported (24% in Thai patients).[17, 18, 37, 38] The observed decreasing *in vitro* susceptibility to quinine suggests that we may expect a deterioration in the treatment response to quinine plus tetracycline in the future.[17]

The above considerations and the high incidence of side effects with quinine plus tetracycline observed in our study and others is worrisome.[16, 22, 34, 37, 38, 41, 42]

The incidence of side effects was significantly reduced when artesunate plus tetracycline was used in our study than when quinine plus tetracycline was used. Moreover, a slightly higher (not statistically significant) proportion of poor compliance with treatment schedule in the QT (6.9%) than in the AT (3.4%) group was observed.

An increased incidence of side effects has been associated with poor compliance in quinine treatments.[37] Therefore, better compliance may be expected with AT regimens for home-treatment than with QT regimens. However, because follow-up studies tend to increase compliance to treatment compared to the 'real world'(*eg.* not an RCT environment with systematic follow-up visits and constant questions about treatment administration and schedule), the degree to which the expected true compliance rates between the two regimens would differ could not be determined in this study.

The association between side effects and treatment group in this study was modified by parasitemia level at baseline. A clear interpretation of this finding is difficult. Previous studies are not helpful because either side effects have not been defined clearly, or they have failed to include a multivariate approach in their data analysis. Further studies need to be undertaken to clarify this observation.

Improvement of mean parasite clearance time was reported in Vietnam, when artemisinin suppositories (3 days, total dose = 2.2g) were compared with quinine (14 days, total dose = 21g).[34] Mean parasite clearance time decreased from around 3 days (quinine alone) to 2 days (artemisinin alone); however cure rates were low in both groups (40% and 34%, respectively).

In the present study, the AT regimen also resulted in a shorter parasite clearance time (96.3% of patients by day 2) than did the QT regimen (52.5% of patients by day 2 and 91.5% by day 4). Consequently, a decreased number of blood

parasite cycles would be expected. Patients treated with AT would also be expected to have a reduced risk of developing severe disease.

The observation of 21 (11.9%) *P. vivax* malaria cases during the trial period, all but one occurring after day 20 (ie. approximately 2 weeks after the end of both 7-day-treatment schedules) in the non-endemic study area is an indicator that neither drug combination interfered effectively with the hepatic cycle of this malaria parasite, as indicated previously by other authors [37, 49, 50] However, tetracycline has been reported as having some activity against the growing stages of the malaria parasites in the liver, more specifically against *P falciparum* pre-erythrocytic tissue forms.[12, 26] If so, it seems that at current dosages tetracycline is not effective against latent exoerythrocytic *P. vivax* forms (hypnozoites), and therefore infections by this parasite were not prevented. Moreover, the time over which the combination drugs remained at an inhibitory concentration for *P. vivax* blood stages seems to be at least 2 weeks after the last day of drug administration, and a prolonged follow-up period may be advised to identify patent *P. vivax* infection.[37, 49, 50]

The two treatment responses were evaluated on an intention-to-treat (effectiveness) basis. It was observed, *a posteriori*, that withdrawals and losses to follow-up were not extremely high (19.9%), and that they occurred at similar frequencies in both treatment groups. Therefore, it is possible to consider what could have been obtained if an efficacy approach had been used in the data analysis, under the assumption that withdrawals and losses to follow-up were a random sample of the study sample, completely independent of treatment groups and treatment outcomes.

Under this assumption, oral artesunate plus tetracycline in the study population radically cured 97.2% (70/72) of patients who fully complied with the treatment and follow-up visits. The combination of quinine plus tetracycline was also highly efficacious, curing 98.5% (68/69) of compliers. This difference in treatment 'efficacy' was not statistically significant.

These analyses must be interpreted with caution because withdrawals and losses to follow-up even though similarly distributed in the treatment groups, is not an assurance of comparability of outcome response within each group, and differential reasons for withdrawals/losses to follow-up could still have introduced a selection bias.

6.2. Internal validity and generalizability considerations

a. Internal validity

Although, several features of a randomized clinical trial strongly improve validity for comparisons between treatment responses, some potential limitations in this study are noted:

- Patient recruitment for the trial was interrupted at times during the study period. Sequential randomization may provide an adequate balance of any possible changes in individual characteristics occurring over time that could affect treatment response independent of the treatment itself. Moreover, adjusting for possible differences in the distribution of covariates in the analysis would remove any potential bias. Therefore, it was considered unlikely that the internal validity of the study would have been affected by interruptions in patient recruitment;

- Block randomization was not used in this RCT and there was a potential for an unbalanced number of patients to be assigned to each treatment group across the study period. However, there is no evidence that responses to treatment (parasite clearance, cure rates or side effects) would have changed over the relatively short time period (22 months) of the study and no impact on internal validity was expected;

- Considering that the standard treatment is known to have important and well known

side effects and that many patients will have had previous experience with this traditional drug combination, the blinding of the patients, and sometimes of the physician who investigated the side effects, may not have held. Where possible, hard outcome measurements were used. Under a pragmatic (effectiveness) approach, which was adopted in this trial, any additional psychosomatic treatment effect due to imperfect blinding of the patient (for instance, the potential for amplification of side effects reported by unblinded patients in the QT group due to a psychological predisposition to repeat the well known quinine-related symptoms) may be included within the 'true' effect of the treatment.[27]

- Five patients who interrupted their treatment and had it reinitiated were not excluded from the analyses. Potentially, it is possible that previously interrupted treatment would modify the response to re-initiated treatment. However, these effects seem to be randomly distributed in both treatment groups and no bias was expected.

b.Generalizability (external validity):

- Eligibility criteria used to select patients for the trial were not too restrictive. There was variation in the study patients' characteristics (for instance: age ranged from 14 to 70 years old, both sexes were represented, parasitemia levels at baseline ranged from 175 to 170,300 parasites/mm^3, the number of days presenting symptoms before admission varied from 0 to more than 7 days and different immune response levels were inferred by the variability in the number of self-reported life-time malaria attacks and the length of time living in an endemic area). These characteristics are well known prognostic factors in malaria, and could influence the treatment response Therefore, the study results are generalizable to a significant proportion of the malaria-affected population;

- Potentially, the external validity of the study results could be affected by selective participation (time-related) in the trial due to the interruption of patient recruitment

over the study period. Factors determining interruption in patient recruitment were completely independent of patient characteristics. It was assumed that the study patients constituted a random sample from eligible patients in the source population (malaria health service demand) over the study period (1992 - 1993). In addition, there were no important changes in the standard procedures for disease management or population dynamic patterns over the study period. Therefore, the study interruptions were judged to have a negligible impact on the generalizability of study results. Unfortunately, characteristics of eligible patients not included into the trial during the study period were not recorded;

- In general, treatment compliance in randomized controlled trials may differ from that observed in the 'real world' for several reasons.[65] In our study, there might have been a potential for an improved treatment compliance because: patients who agreed to participate in the trial may be naturally more motivated than others in the eligible source population, follow-up visits and questions may improve commitment and periodic clinical exams performed by a physician (not a common practice in malaria control programs) may also improve commitment and motivation. Moreover, it is expected that an improved treatment compliance may be associated a with better outcome (cure rates and parasite clearance). Under a pragmatic (effectiveness) approach, circumstantial improvement of treatment compliance (as well as treatment outcomes) have to be considered as a limitation in generalization considerations and inferences should be made accordingly,

- Our study specifically excluded certain groups: children under 14 years old, severe malaria cases and pregnant women. Moreover, malaria patients who remained in the local endemic area to be treated could be quite different from those who traveled to Cuiabá to be treated. Extrapolation of study results to these groups is not directly possible. Independent studies to determine the effectiveness of alternative drug regimens in these different populations are needed.

CHAPTER 7.

Conclusions and recommendations

7.1. Conclusions

In conclusion, although further studies are needed before oral artesunate plus tetracycline can be considered as an alternative treatment for *P. falciparum* malaria in Brazil, results are promising.

The present study shows that a 7-day treatment of oral artesunate plus tetracycline has an overall better performance than the standard treatment of oral quinine plus tetracycline for adults (age >= 14 years old) who have uncomplicated *P. falciparum* malaria in Cuiabá-MT (Amazon region, Brazil). There is a lower incidence of one or more side effects and a faster time to parasite clearance with an equally high cure rate - aspects which may contribute to improved effectiveness of malaria control programs due to the following factors:

- An expected better treatment compliance due to lower incidence of one or more side effects;
- A shorter time period in which the patient is 'infectious', due to a faster parasite clearance and an expected shorter time for gametocyte development;
- Also, as a consequence of a faster parasite clearance, there is a decreased risk for hyperparasitemia and, consequently, a decreased risk of severe malaria - which may contribute to decreasing mortality rates due to falciparum malaria;

In the 'real world' (not in an RCT environment), cure rates in both regimens

may be lower than those observed in this study, because of the expected higher compliance rates in RCTs and, consequently, better treatment responses. However, treatments with a lower incidence of side effects are expected to have better compliance rates in at-home therapies than those with a higher incidence of side effects. Therefore, an AT regimen would be favoured.

6.7. Recommendations

- An assessment of the efficacy of artesunate plus tetracycline in falciparum malaria treatment has not yet been reported. The present study assessed the effectiveness of this regimen, where treatment failure was a result of both poor treatment compliance and poor efficacy. Efficacy studies should still be carried out to identify to which degree treatment failure can be attributed to a lack of sensitivity of *P. falciparum* parasites to the artesunate plus tetracycline regimen;

- An assessment of the cost-effectiveness of artesunate plus tetracycline should be carried out in order that issues such as access, availability and cost be addressed;

- It is important to assess the efficacy and effectiveness of oral artesunate (without tetracycline) in children (under 14 years old) with falciparum malaria; in addition, the effectiveness of this drug should be investigated in rural endemic areas;

- Most previous studies used artesunate over a 5 day period or less. In our study, artesunate was used over 7 days and in combination with tetracycline. It may be important to further assess whether the success of the AT regimen is due to the 2-day increased administration of artesunate or due to the addition of tetracycline;

- The reasons for the occurrence of 3 RI type responses were not closely assessed. Problems related with drug absorption as well as resistance of the parasite to drug

action could have explained these recrudescences. Therefore, further studies are still needed in order to identify recrudescence-related problems as well as to optimize the dosage for oral artesunate therapies;

- To identify rare, but sometimes important, adverse effects of any treatment, it is often necessary to conduct several controlled trials. Repeated trials in different populations using oral artesunate are advised in order to improve our knowledge of its safety and to prevent inappropriate use.

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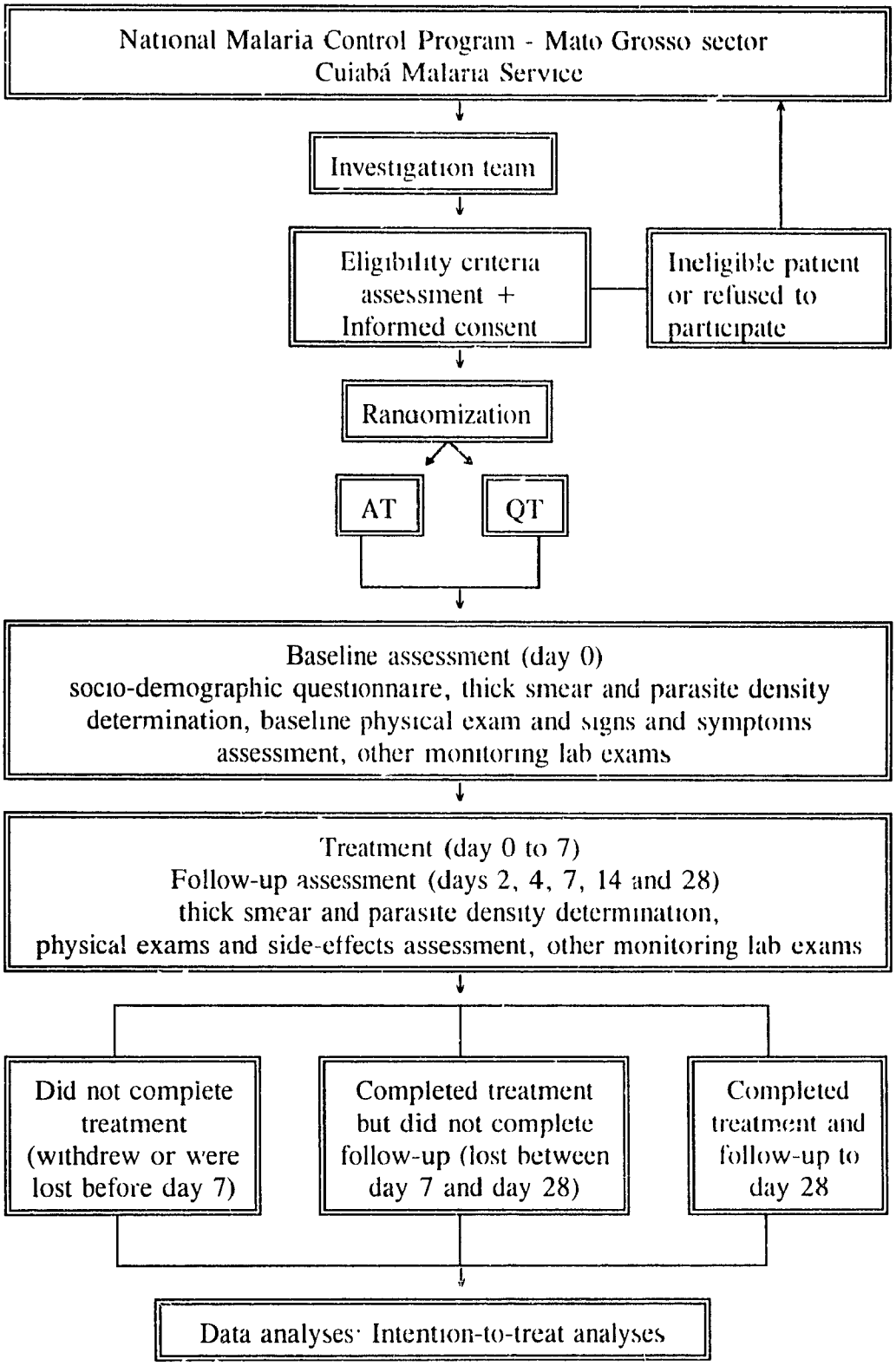
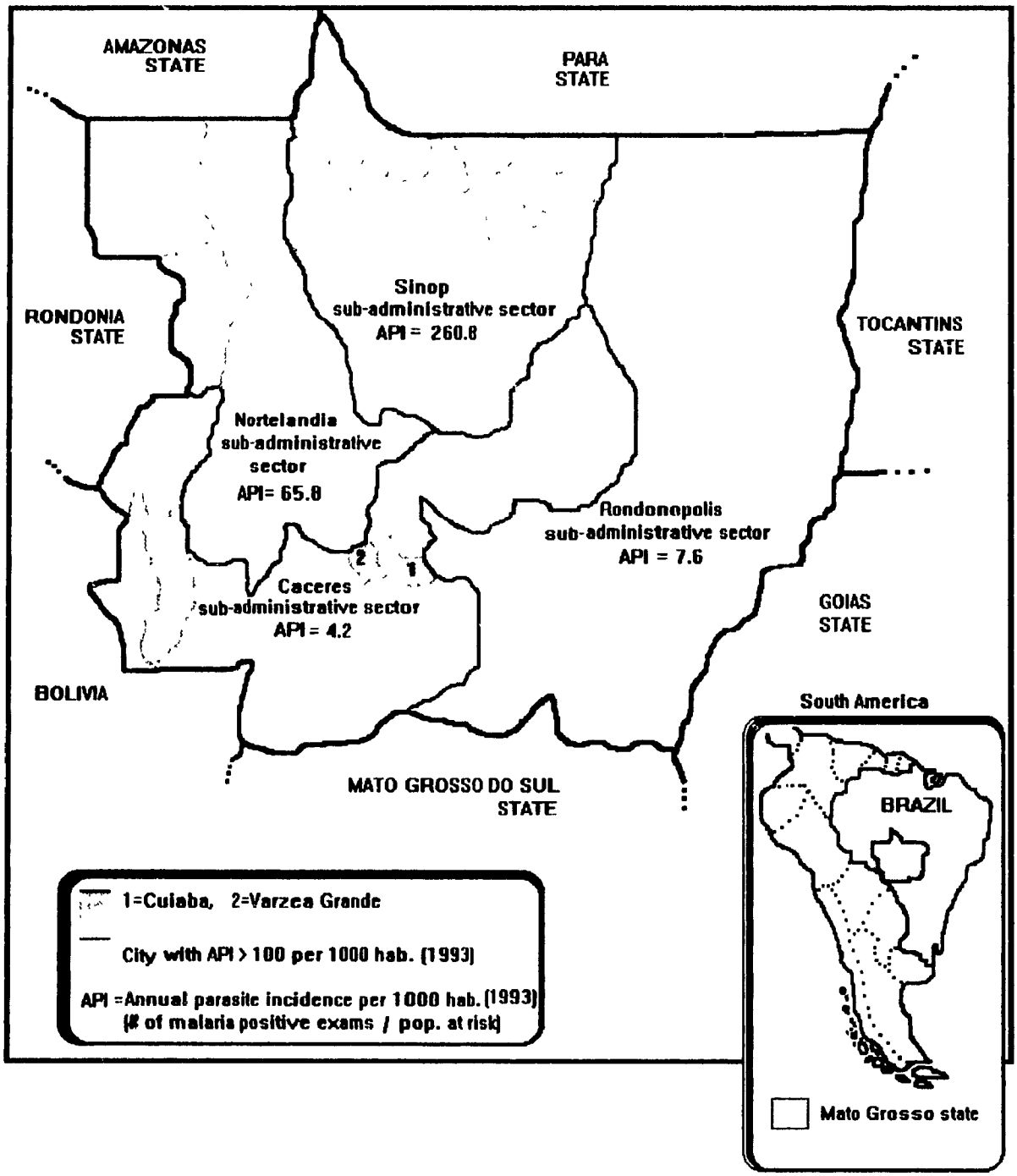
APPENDICES:

Appendix 1: *Trial organization scheme*



Appendix 2: *Map of the State of Mato Grosso*



Appendix 3:

Table I - Quinine plus tetracycline treatment schedule (QT)[1]

DAY	DRUG SPECIFICATION	DOSAGE
0	quinine sulphate 500 mg tetracycline 250 mg	4 tablets (2 g) 6 tablets (1.5 g)
1	quinine sulphate 500 mg tetracycline 250 mg	4 tablets (2 g) 6 tablets (1.5 g)
2	quinine sulphate 500 mg tetracycline 250 mg	4 tablets (2 g) 6 tablets (1.5 g)
3	tetracycline 250 mg	6 tablets (1.5 g)
4	tetracycline 250 mg	6 tablets (1.5 g)
5	tetracycline 250 mg	6 tablets (1.5 g)
6	tetracycline 250 mg primaquine 15 mg*	6 tablets (1.5 g) 3 tablets (45 mg)

1. Quinine was administered every 12 hours (1000 mg quinine twice daily); Tetracycline was administered every 8 hours (500 mg tetracycline three times daily); Tetracycline would not be taken with milk.

* Primaquine was administered in a single dose concomitant with the first drug taken on the last day of treatment.

Recommended schedule for days 0, 1 and 2

6 a.m.	2 tablets of tetracycline
10 a.m.	2 tablets of quinine
2 p.m.	2 tablets of tetracycline
10 p.m.	2 tablets of tetracycline and 2 tablets of quinine

For days 3 to 6 patients would take tetracycline alone as recommended above.

Appendix 3:

Table II - Artesunate plus tetracycline treatment schedule (AT)[1]

DAY	DRUG SPECIFICATION	DOSAGE
0	artesunate 50 mg	3 tablets (150 mg)
	tetracycline 250 mg	6 tablets (1.5 g)
1	artesunate 50 mg	2 tablets (100 mg)
	tetracycline 250 mg	6 tablets (1.5 g)
2	artesunate 50 mg	2 tablets (100 mg)
	tetracycline 250 mg	6 tablets (1.5 g)
3	artesunate 50 mg	2 tablets (100 mg)
	tetracycline 250 mg	6 tablets (1.5 g)
4	artesunate 50 mg	2 tablets (100 mg)
	tetracycline 250 mg	6 tablets (1.5 g)
5	artesunate 50 mg	2 tablets (100 mg)
	tetracycline 250 mg	6 tablets (1.5 g)
6	artesunate 50 mg	2 tablets (100 mg)
	tetracycline 250 mg	6 tablets (1.5 g)
	primaquine 15 mg*	3 tablets (45 mg)

1. Artesunate was administered every 12 hours (50 mg artesunate twice daily), except for the first dose on day 0, which would be double (2 tablets or 100 mg), as recommended by the health ministry and drug company;
   Tetracycline was administered every 8 hours (500 mg tetracycline three times daily);
   Tetracycline would not be taken with milk.

* Primaquine was administered in a single dose concomitant with the first drug taken on the last day of treatment.

Recommended schedule:

6 a.m.	2 tablets of tetracycline
10 a.m.	1 tablet of artesunate
2 p.m.	2 tablets of tetracycline
10 p.m.	2 tablets of tetracycline and 1 tablet of artesunate

For day zero the first dose of artesunate is doubled.

Appendix 4: *Baseline questionnaire (translated version)*

RANDOMIZED CONTROLLED TRIAL OF ARTESUNATE PLUS TETRACYCLINE VERSUS STANDARD TREATMENT (QUININE PLUS TETRACYCLINE) FOR UNCOMPLICATED PLASMODIUM FALCIPARUM MALARIA IN BRAZIL.

1. PATIENT IDENTIFICATION No. |_|_|_|

1.1. Name: _____
1.2. Age: _____
1.3. Sex: |_| Male |_| Female
1.4. Occupation (main current occupation): _____

2. PERMANENT RESIDENCE

Address: _____
Sector: _____
City: _____ State: _____

3. TEMPORARY RESIDENCE (current)

Address: _____
Sector: _____
City: _____ State: _____
Points of reference: _____
_____

4. PROBABLE PLACE OF TRANSMISSION

4.1. Date of initial symptoms: |day___|month___|19__|
4.2. Places where stayed in the last 30 days:

	Area	City	State
4.2.1.	_____	_____	|_|_|
4.2.2.	_____	_____	|_|_|
4.2.3.	_____	_____	|_|_|

5. RESIDENCE IN ENDEMIC AREA

5.1. Do you live in an area with malaria transmission?

|_| No |_| Yes, Where?: _____

Since when?: _____

6. HISTORY OF PREVIOUS MALARIA ATTACKS

6.1. Have you ever had malaria before today?

|_| No |_| Yes, How many times? _____

Appendix 5: *Clinical exam form (translated version)*

RANDOMIZED CONTROLLED TRIAL OF ARTESUNATE PLUS TETRACYCLINE VERSUS STANDARD TREATMENT (QUININE PLUS TETRACYCLINE) FOR UNCOMPLICATED PLASMODIUM FALCIPARUM MALARIA IN BRAZIL.

1. PATIENT IDENTIFICATION date: |day__|month__|19__|

Name: _____

No. |_|_|_| day of evaluation: |__|__| [0 to 28]

2. SYMPTOMS [P]=positive [N]=negative

Have you experienced any symptoms since the last visit?
[If baseline visit]: Have you experienced any symptoms?

[P] [N] Fever
[P] [N] Diarrhea
[P] [N] Heart burn
[P] [N] Myalgia
[P] [N] Headache
[P] [N] Itching
[P] [N] Dizziness
[P] [N] Weakness
[P] [N] Behavioural changes

Other:

[P] [N] Nausea
[P] [N] Vomiting
[P] [N] Tinnitus
[P] [N] Anorexia
[P] [N] Sweating
[P] [N] Bitter taste
[P] [N] Abdominal Pain
[P] [N] Tremors
[P] [N] Fainting
[P] [N] Motor system changes

Comments:

3. CLINICAL EXAM

Weight: ____kg BP:___x___ mmHg Pulse:____bpm Temp.:_____oC

[N]=normal [A]=abnormal Comments

[N] [A] Consciousness/alertness _____
[N] [A] Walking _____
[N] [A] Pupils (size/reflexes) _____
[N] [A] Muscle power _____
[N] [A] Romberg _____
[N] [A] Jaundice _____
[N] [A] Oropharynx _____
[N] [A] Cutaneous lesions _____
[N] [A] Lesions of the mucosa _____
[N] [A] Enlargement of lymph nodes _____
[N] [A] Heart exam _____
[N] [A] Lung exam _____
[N] [A] Abdominal exam _____

Other: _____

3. Did you use other medication since the last visit?
[If baseline visit]: Are you using any medication?

|_|No |_|Yes, Specify: _____

4. Did you travel to an area with malaria transmission since the last visit? [If baseline, please to omit this question]

|_|No |_|Yes, Specify when and where: _____

Appendix 7: *Informed consent form (translated version)*

INFORMED CONSENT FORM

I, _____

have received detailed information about the objectives, methods and procedures that will be used in the "**RANDOMIZED CONTROLLED TRIAL OF ARTESUNATE PLUS TETRACYCLINE VERSUS STANDARD TREATMENT (QUININE PLUS TETRACYCLINE) FOR UNCOMPLICATED PLASMODIUM FALCIPARUM MALARIA IN BRAZIL**" and agree to participate in this study. I also agree to comply with follow-up visits.

Cuiabá, _____ 199____

_____

Patient signature