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The Effect of Nutritional Status on the Post-treatment Prophylactic Effect of Two Artemisinin-based Combination Therapies (ACTs) in Ugandan Children Treated for Malaria

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The Effect of Nutritional Status on the Post-treatment Prophylactic Effect of Two Artemisinin-based Combination Therapies (ACTs) in Ugandan Children Treated for Malaria

by

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ABSTRACT

The Effect of Nutritional Status on the Post-treatment Prophylactic Effect of Two Artemisinin-based Combination Therapies (ACTs) in Ugandan Children Treated for Malaria

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Doctor of Philosophy in Epidemiology

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Malaria and malnutrition are major causes of morbidity and mortality in children worldwide. Malnourished children may be at higher risk of malaria due to impaired immune response. Malnutrition and young age may alter the pharmacokinetics (PK) of antimalarial treatment thus potentially impacting treatment efficacy. Though malaria and malnutrition frequently coexist, results from previous studies that have investigated the association between these two co-morbidities are conflicting. No previous studies have evaluated the effect of malnutrition on response to treatment with artemisinin-based combination therapies (ACTs). Moreover, there are no other studies that have evaluated the effect of malnutrition on the PK of ACT regimens. This dissertation examines the following: 1) the magnitude of the difference between efficacy estimates derived from 3 analytical methods and discusses the optimal statistical approach for monitoring in vivo efficacy (chapter 2); 2) the effect of nutritional status on the response to treatment with artemisinin-combination therapy (ACT) in young Ugandan children with malaria (Chapter 3); and 3) the effect of nutritional status on the pharmacokinetics (PK) of two ACT treatment regimens (chapter 4).

Chapter 2 utilizes data from 29 clinical trials conducted in Africa and Thailand to compare the risk estimates of treatment failure, adjusted and unadjusted by genotyping, derived by 3 analytical methods; intention to treat (ITT), modified intention to treat (mITT) and per protocol (PP) analysis. Estimates of treatment failure were consistently higher when derived from the ITT or PP analyses compared to the mITT approach in both unadjusted and adjusted analyses. Poor patient adherence to follow-up, higher incidence of P. vivax relapse and high incidence of P. falciparum new infections were all factors contributing to differences in failure estimates. Because estimates of antimalarial clinical efficacy vary significantly depending on the analytical methodology from which they are derived, standardized analytical tools should be

used to monitor temporal and spatial trends in antimalarial efficacy. Survival analysis is the preferred approach to monitor in vivo efficacy of malaria treatment.

Chapter 3 and 4 utilize data from the Tororo Child Cohort (TCC) Study conducted in Tororo, Uganda. In chapter 3, children aged 4 to 12 months diagnosed with uncomplicated malaria were randomized to either dihydroartemisinin-piperaquine (DP) or artemether-lumefantrine (AL) and followed for up to 2 years for repeated episodes of malaria. The primary exposure variables of interest were height-for-age (HAZ score) and weight-for-age (WAZ score) z-scores and outcomes included parasite clearance at day 2 and 3 and risk of recurrent parasitemia after 42 days of follow-up. HAZ and WAZ scores were not associated with a positive blood smear two days following treatment with DP or AL. In children treated with DP not on trimethoprim-sulfamethoxazole (TS) prophylaxis, a decreasing HAZ score was independently associated with a higher risk of recurrent parasitemia. However, statistical significance was reached only when comparing HAZ scores <-1 with those $\geq$ 0 (HAZ $\geq$ -2 - <-1: HR=2.89, p=0.039; HAZ <-2: HR=3.18, p=0.022). Overall, DP and AL are effective antimalarial therapies in chronically malnourished children in a high transmission setting however, children taking DP with signs of mild to moderate chronic malnutrition not taking TS prophylaxis are at higher risk of recurrent parasitemia.

In chapter 4, PK samples were collected from a subset of patients ages 6 months to 2 years who were randomized to DP or AL and followed prospectively for multiple episodes of malaria providing a total of 214 treatments for DP and 243 treatments for AL for PK analysis. Primary exposure variables included stunting and underweight, (HAZ score of <-2 and WAZ score of <-2, respectively). Chronic malnutrition appeared to be associated with day 3 piperaquine concentrations in adjusted analyses with stunted children having lower concentrations than non-stunted children (OR=0.78, p=0.007). Stunting was associated with apparent clearance (CL/f_{pip}) (OR=1.32, p=0.001) with stunted children having higher CL/f_{pip} than non-stunted children which may be the consequence of a lower overall exposure to drug and is consistent with the lower piperaquine concentrations measured on day 3. Chronic malnutrition does not have an effect of piperaquine or lumefantrine concentrations at day 7 – an important determinant for treatment response.

Overall, our results indicate that DP and AL are effective antimalarial treatments in very young chronically malnourished children. This is supported by the finding that chronic malnutrition does not have an effect on of piperaquine or lumefantrine day 7 concentrations, an indicator for treatment response. However, children taking DP not on TS prophylaxis may be at higher risk of recurrent parasitemia. Further studies should be conducted to justify these results and provide a definitive understanding of the causal relationship between malnutrition and malaria.

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CHAPTER 1

A Review of Malaria, Malnutrition and the Pharmacokinetics of Artemisinin-Based Combination Therapies in Malnourished Children with Malaria

MALARIA

Approximately 2 billion people are at risk for malaria [1]. The World Health Organization (WHO) estimates that 243 million clinical cases of malaria reported in 2008 resulted in 863,000 deaths [2]. Eight-five percent of the cases occurred in Africa followed by 10% in South-East Asia and 4% in the Eastern Mediterranean region. 89% of the deaths occurred in Africa primarily in children under 5 years of age (Table 1). Malaria accounts for one-fifth of child mortality in Africa, equivalent to up to 3000 deaths per day. An accurate assessment of malaria morbidity and mortality is difficult to obtain as it requires the use of health facilities or community health surveys, thus reported estimates of malaria burden are conservative at best [1, 3]. Malaria primarily affects poor populations in tropical and sub-tropical regions where conditions are most suitable for the *Anopheles* mosquito and the *Plasmodium* parasites which cause malaria to thrive. In Africa, countries with the highest malaria burden spend up to 40% of healthcare costs on malaria which account for 30-50% of all hospital admissions and up to 50% of all outpatient visits. Malaria burden tends to be highest in countries with the lowest gross domestic product (GDP) and the slowest rate of growth [4].

Table 1. Estimated numbers of malaria cases (in millions) and deaths (in thousands), by WHO region, 2008.

WHO Region	Cases				Deaths			
		Percentile				Percentile		
	Estimate	5th	95th	P. falciparum (%)	Estimate	5th	95th	Under 5's (%)
Africa	208	155	276	98	767	621	902	88
Americas	1	1	1	32	1	1	2	30
E. Mediterranean	9	7	11	75	52	32	73	77
Europe	0	0	0	4	0	0	0	3
SE Asia	24	20	29	56	40	27	55	34
Western Pacific	2	1	2	79	3	2	5	41
Total	**243**	**190**	**311**	**93**	**863**	**708**	**1003**	**85**

Note: Adopted from WHO World Malaria Report 2009 [2].

Life Cycle of Plasmodium Parasites

There are 4 types of human malaria including *P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale*. Most recently a fifth human malaria has been identified, *P. knowlesi* [5]. *P. Falciparum* is the most virulent strain of malaria causing the majority of malaria deaths. It predominates in Africa with 71% of the *P. falciparum* cases reported accounting for the disproportionate morbidity and mortality reported in this region [2]. *P. vivax*, prevailing in tropical areas outside of Africa, though the most widespread, rarely causes death. The ability of *P. vivax* and *P. ovale* to remain dormant within the liver makes these parasites difficult to eradicate and relapse can occur months to years after the initial infection.

There are numerous stages to the Plasmodium life cycle. Once an *Anopheles* mosquito infects a person, sporozoites enter the blood stream and evade antibodies by migrating to the liver within 30 minutes of infection. Once in the liver, the sporozoites infect liver cells via asexual multiplication and amplification and differentiate into merozoites. In the liver, the sporozoites utilize the circumsporozoite (CS) antigen, a protein coat that helps the sporozoites bind to hepatocytes. The CS antigens constantly change, making it difficult for the immune system to mount a response. After approximately one week, the hepatocytes rupture, and merozoites enter the blood stream and invade red blood cells where they develop into ring forms, then trophozoites (a feeding stage), then schizonts (a reproductive stage), then back to merozoites which rupture the red blood cell and again enter the blood stream to invade other red blood cells. This cyclical invasion, multiplication, invasion pattern manifests itself as periodic fevers cycles. *P. falciparum* is distinguished from other strains by the display of an adhesive protein called erythrocyte membrane protein 1 (PfEMP1) on the surface of red blood cells which cause red blood cells to stick to blood vessels and avoid destruction by the spleen. This "stickiness" is a major cause for hemorrhagic complications and the blockage of small blood vessels is a primary symptom of cerebral malaria. During the erythrocytic life cycle, gametocytes are also produced, which if taken up by the bite of the next mosquito, will fertilize and via sexual recombination, will differentiate into gametes then sporozoites. These sporozoites then travel to the salivary glands to be transmitted during the next blood meal [1, 6].

Clinical Features and Epidemiology

Clinical signs and symptoms of malaria include fever with chills, anthralgia, nausea, dizziness, fatigue, splenomegaly, and anemia. The classic presentation of malaria, particularly if left untreated, is described as cyclical periods of high fever and chills lasting four to six hours and occurring every 48 hours with *P. vivax* and *P. ovale*, every 72 hours with *P. malariae*, and every 36-48 hours with *P. falciparum* [6]. Symptoms often seen in children include hepatosplenomegaly, hypoglycemia, jaundice and pallor, and high levels of parasitemia, particularly with *P. falciparum* infection. Severe infection with *P. falciparum* in children is characterized by abnormal posturing, respiratory distress, severe anemia, and cerebral malaria, which can impair brain development, and commonly results in death [1].

The epidemiology of malaria is complex and the distribution of morbidity and mortality within communities is largely dependent on transmission intensity. Transmission intensity, or extent of endemnicity, can be directly measured by the entomological inoculation rate (EIR) defined as the number of infectious mosquito bites received per person per year. In areas where the EIR is below 10, malaria transmission is unstable and considered to be low in intensity. In these areas, almost all people, regardless of age, are considered to be at risk of moderate to severe malaria due to slow acquisition of immunity. In areas where the EIR is above 10, individuals receive multiple infective bites per year and transmission intensity is considered to be moderate to high and relatively stable throughout the year. In these areas, infants, young children, and pregnant women are at the highest risk of disease and malaria risk is linked to both age and naturally acquired immunity.

As children age and antiparasite immunity develops the density of asexual parasitemia and thus, malaria morbidity, declines. In endemic, high transmission areas, parasitemia peaks at 5 years of age then declines and age is significantly correlated with parasite density in children 6 months to 6 years of age [7]. Parasite density is correlated with extent of clinical disease in children age 3 to 4 months to 2 years. Children age 2 to 4 years are at increased risk of cerebral malaria. Typically after approximately 5 years of age, the risk of clinical malaria and death decreases though the age of onset of protection is directly correlated with transmission intensity. In adolescence and beyond, in areas of high transmission, severe disease rarely develops. Naturally acquired immunity to malaria is most likely due to maturational changes in the immune system that occur with age as well as a development of the adaptive immune response resulting from multiple parasite infections over time [8]. Interestingly, the prevalence of clinical disease in infants is low, likely due to retention of maternal antibodies such as IgG acquired in utero or because of lactoferrin, a parasite growth inhibitor, and IgA, both acquired through breast milk [8, 9].

Current Malaria Treatments

The emergence and spread of malaria resistance against previously effective treatments has proven to be a significant barrier to malaria control. Resistance to antimalarials is defined as the ability of a parasite strain (i.e., *P. falciparum*) to survive and/or multiply despite the administration and absorption of the medicine given in doses equal to or higher than those recommended and despite adequate exposure [10]. In nearly all malarious regions of the world, *P. falciparum* and *P. vivax* is resistant to chloroquine. *P. falciparum* resistance to mefloquine is reported in most countries in South-East Asia as well as in the Amazon basin. Resistance has also occurred in most countries where sulfadoxine-pyrimethamine (SP) has been used as intermittent preventive treatment (IPT) for malaria [6, 10, 11].

Artemisinin, a peroxide derived from the *Artemisia annua* plant and used as a herbal remedy to treat fevers for thousands of years, was used widely to treat malaria in China and other Asian countries by the 1990s. Artemisinins are extremely potent offering an advantage over other antimalarials in that they both kill the circulating ring stage of the parasites, thus rapidly reducing parasitemia, as well as reduce the number of parasites that are sequestering in blood vessels [12, 13]. In addition, artemisinins are fast acting, reaching a maximal concentration in the blood (C_{max}) in less than 2 hours. The potential for resistance is low because it rapidly leaves the body with a elimination half-life ($t_{½}$) of 1 to 3 hours (Table 2) [14]. Due to the short half-life, when used as monotherapy, artemisinin is given over a period of 7 days to avoid late recrudescence (recurrent infection from incomplete treatment or ineffective host immune response) (Table 2).

Table 2. Glossary of Terms

ACT:	Artemisinin-based combination treatment- a combination of artemisinin or derivative given for three days with a more slowly eliminated antimalarial drug.
AUC:	Area under the whole-blood, serum or plasma concentration–time curve.
C_{max}:	Maximal plasma concentration
Elimination Phase:	Period during which the drug is eliminated following distribution. This might have one or more phases. The last is the terminal elimination phase, which is a first-order process for all antimalarial drugs, and therefore has a half-life (the terminal elimination half-life $t_{½}$).
MPC:	Minimum parasiticidal concentration is the lowest concentration of antimalarial drug in the blood that provides maximal inhibition of parasite multiplication.
PRR:	Parasite reduction ratio is the fractional reduction in parasite numbers per asexual cycle. Values typically vary between 10 and 10 000 per cycle.
Recrudescence:	Recurrent infection from incomplete treatment or ineffective host immune response (mostly drug resistant P. falciparum)
Relapse:	Recurrent infection from dormant liver stage (P. vivax, P. ovale)
Re-infection:	Recurrent infection with a different parasite or multiple (polyclonal) infection (mostly P. falciparum in Africa and P. vivax in SE Asia and Latin America)

Although artemisinin and its derivatives (artesunate, dihydroartemisinin, and artemether) were initially used as monotherapy, it became generally accepted that malaria treatments should be used in combination, particularly in the context of widespread malarial resistance to other monotherapies. Combining antimalarials with different mechanisms of action can help prevent or slow the onset of resistance. In addition, the requirement that artemisinin monotherapy be provided over a period of 7 days to avoid recrudescence was expensive and increased the risk of noncompliance. Artemisinin combination therapies (ACT) are three-day treatments which combine a rapidly eliminated artemisinin (or derivative) component with a slowly eliminated partner drug. ACTs allow for the short-acting and rapidly eliminated artemisinin derivative to quickly reduce parasite burden by 10,000-fold per reproductive cycle while the longer-acting partner drug removes any residual parasites [10]. The benefits of these regimens are two-fold: 1) the use of two efficacious antimalarial treatments confers mutual protection against parasite resistance and 2) ACTs reduce gametocyte carriage translating to a reduction of transmissibility and overall burden of malaria [10, 15].

The WHO now recommends ACTs as first-line treatment for uncomplicated falciparum malaria. A listing of recommended ACTs is provided in Table 3. Artemether-lumefantrine (AL) and dihydroartemisinin-piperaquine (DP) are two of the most important ACTs for the treatment

of uncomplicated falciparum malaria. AL is highly efficacious and well-tolerated, and has been recommended by the WHO as first line treatment for malaria since 2004. Lumefantrine is absorbed and eliminated more slowly with a peak concentration of 3-4 hours postdose and with a $t_{½}$, of approximately 4 days in patients with malaria. The oral bioavailability of lumefantrine is highly variable and the co-administration with food increases the bioavailability by 16-fold [14, 16]. Therefore it is recommended that AL be given with a small amount of fat such as in the form of milk or a biscuit [10].

DP is a newer ACT that has proven to be equivalent to or more effective than other ACT regimens in clinical trials [17-20]. DP consists of dihydroartemisinin and the partner drug, piperaquine, which reaches a peak concentration in approximately 3 hours. Though co-administration with food has been shown to increase the bioavailability of piperquine by 41 to 121% [21, 22], administration with food is not part of the current dosing guidelines [10].

Antimalarial Pharmacokinetics and Prevention of Resistance Using ACTs

The pharmacokinetics (PK) of antimalarials is an important determinant for cure. PK is defined as the process, by which a drug is absorbed, distributed, metabolized, and excreted as it passes through the body. The dose, as well as the absorption, and distribution of a drug determine the drug concentrations (in this case, in blood or plasma) required to produce maximal effects. In malaria, the drug concentration producing maximal effect is referred to as the minimum parasiticidal concentration (MPC) which is the lowest concentration of antimalarial drug in the blood that provides maximal inhibition of parasite multiplication [23]. Cure is reliant on both the MPC as well as the parasite reduction ratio (PRR) which is the factional reduction in parasite numbers per asexual cycle. For example, the PRR of artemisinin is 10,000. Provided the MPC is exceeded for greater than 4-asexual life cycles (for *P. falciparum*) and the PRR is greater than 1000 times per cycle at the drug concentrations achieved, then a patient is effectively cured [24].

Table 3. Known PK Properties of ACTs recommended by the WHO for treatment of uncomplicated falciparum malaria [10]

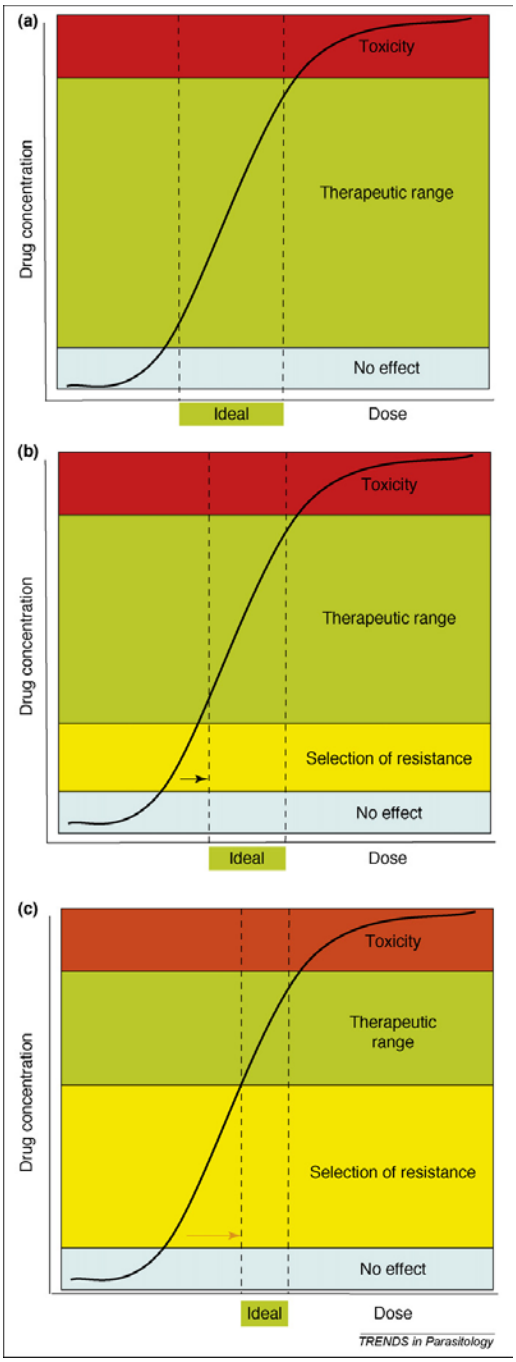
ACT (Partner Drug Highlighted)	Partner Drug Family	Absorption/Distribution	Metabolism	Excretion
Artemether-**lumefantrine** (AL)	Aryl aminoalcohol similar to quinine, mefloquine, and halofantrine	Lipid soluble T_{max} = 10 h >99% protein-bound	Metabolized in liver via cytochrome P450 enzyme CYP3A4	Via bile $t_{½}$= 3-4 days
Artesunate plus **amodiaquine** (AS+AQ)	4- aminoquinoline similar to chloroquine.	Via in GI tract	metabolized to desethylamodiaquine in liver	Via kidney $t_{½}$ = data insufficient
Artesunate plus **mefloquine** (AS+MQ)	4-methanolquinoline related to quinine	Via in GI tract Tmax is variable 98% protein bound Slightly soluble in water	Metabolized in liver	Via bile and feces $t_{½}$= 14 days
Artesunate plus **sulfadoxine-pyrimethamine** (AS+SP)	Sulfadozine: A sulfadoxine	Absorbed in GI tract T_{max} = 4 hrs Widely distributed	Metabolized in the liver via glucuronidation	Via kidney $t_{½}$ =4-9 days.
	Pyrimethamine: A diaminopyrimidine	Via GI tract T_{max} = 2-6 hr 80-90% protein-bound	Metabolized in liver	Via kidney $t_{½}$ =4 days
Dihydroartemisinin – **piperaquine** (DP)	Bisquinoline related to chloroquine	T_{max} = 3 hr	Metabolized in liver via cytochrome P450 enzymes	$t_{½}$ =3-4 weeks
Artemisinins				
Artemisinin Derivative	**Family**	**Absorption/Distribution**	**Metabolism**	**Excretion**
Artemether	Methyl ether of dihydroartemisinin	T_{max} = 2-3 hr 95% protein bound	Metabolized in liver to dihydroartemisinin Biotransformed via cytochrome P450 enzyme CYP3A4	$t_{½}$ =1 hour
Artesunate	Derivative of artemisinin	T_{max} = 1.5 hr	Metabolized in liver to dihydroartemisinin	$t_{½}$ = 45 minutes
Dihydroartemisinin	Derivative of artemisinin	T_{max} = 2.5 hr 55% protein bound	Metabolized in liver Biotransformed via cytochrome P450 enzyme CYP3A4	$t_{½}$ = 45 minutes

Resistance to antimalarials occurs as result of genetic mutations which provide a survival advantage. Partially resistance parasites are killed by high drug concentrations that occur after adequate dosing but are not killed by suboptimal doses or by drug concentrations generally present toward the end of the elimination phase of the drug. Initially, with the introduction of a new drug, all parasites are sensitive and there is no drug concentration that will increase the risk of treatment failure. As genetic mutations arise and parasites develop resistance to a drug, the drug concentrations required must be higher and less variable. As levels of resistance to a treatment regimen increase, the therapeutic range for drug concentrations to remain adequate for cure narrows (Figure 1) [24]. Ensuring drug concentrations are adequate and optimizing compliance is crucial to decrease the potential for resistance and treatment failure.

ACTs provide ideal PK properties to deter resistance. For drugs that are metabolized and eliminated slowly, such as those used in combination with artemisinins, the maximal drug concentration is considered less important than the area under the concentration time curve (AUC) for predicting therapeutic response. The AUC is determined by the absorption of a drug and the subsequent drug concentrations achieved as well as the duration of exposure (Table 2) [24]. With artemisinin, due its potency, the majority of exposed parasites are cleared from the body typically during the first two asexual cycles, or within 48 hours after the first dose. However, even though the artemisinin can reduce blood parasite numbers by 10,000 fold per asexual cycle and exposure to the artemisinin is for 4 asexual cycles (corresponding to a 3-day dose), for patients with a high parasite burden of up to 10^{13} parasites, this still does not eradicate all parasites from the body. Therefore, the partner drug contributes to the therapeutic response after the artemisinin is eliminated from the body, from the third asexual cycle and beyond. If the AUC of this partner drug falls below the MPC before the immune response can eradicate any of the remaining infective parasites, then recrudescence, or recurrent infection from the same parasite will occur (Figure 2) [12, 23, 24].

In ACT clinical trials, day 7 plasma levels of the partner drug, including piperaquine and lumefantrine have been shown to be a useful correlate to AUC for determining treatment failure [25-29]. In fact, a day 7 level may be a more reliable predictor of treatment response than AUC because of the wide inter-individual absorption variability seen with certain partner medications (i.e., lumefantrine) (Figure 3) [23] A reduction in day 7 levels could impact cure rates as well as shorten the interval between malaria episodes. Apparent clearance (*CL/f*) can also serves as a proxy for drug exposure and is may be an important determinant for treatment failure [23].

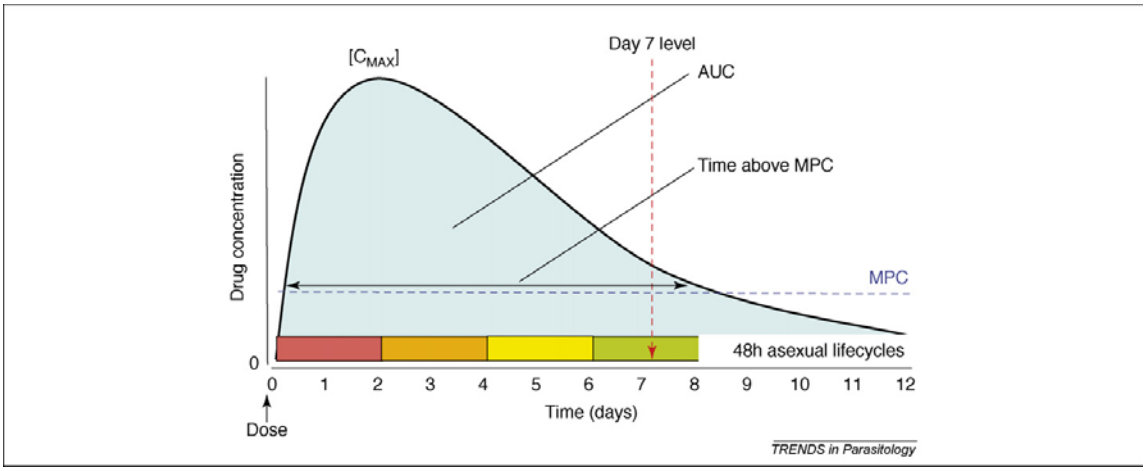
Figure 1. The association between dose and treatment response.



The ideal treatment dose changes over time with the spread of resistance. (a) Initially, because all parasites are drug sensitive, lower drug levels might not increase the risk of treatment failure. (b) With the development of resistance, higher drug concentrations are required to achieve a cure against more resistant parasites. (c) With higher levels of resistance, the ideal dose is squeezed between the minimum effective dose and that associated with toxicity.
Source: Barnes et al. [24]

In addition to deterring resistance, a second and equally important benefit of ACT regimens with slowly eliminated partner drugs is the post-treatment prophylactic effect. This is the period after which the initial infection has been cleared, where subsequent reinfection is suppressed or delayed by the remaining residual levels of drug. The duration of the post-treatment prophylactic effect is entirely dependent on how quickly a drug is eliminated [30]. The post-treatment prophylactic effect can not only reduce the incidence of infection, but it can also allow for a longer disease free interval allowing more time for recovery, particular from anemia. This has been shown in studies comparing DP with other ACTs that are eliminated more quickly [17, 31]. The benefits of a longer post-treatment prophylactic period must be balanced by the potential for more slowly eliminated partner drugs to select for resistant parasites [10, 32]. The WHO has stated that the curative efficacy of antimalarials (i.e., the prevention of recrudescence) takes precedence over providing an extended post-treatment prophylactic effect [10].

Figure 2. The pharmacokinetic determinants of outcome in uncomplicated malaria.



Drug concentrations must exceed minimum parasiticidal concentrations (MPCs) until all parasites are eliminated; this usually takes at least four 48-h asexual life cycles in *P. falciparum*. The area under the blood or plasma concentration time curve (AUC) predicts the therapeutic response. The maximum concentration (C_{MAX}) is considered less important in the treatment of uncomplicated malaria. For slowly eliminated antimalarial drugs, the blood or plasma concentration on day 7 is a good correlate of the AUC and thus treatment response.
Source: Barnes et al. [24]

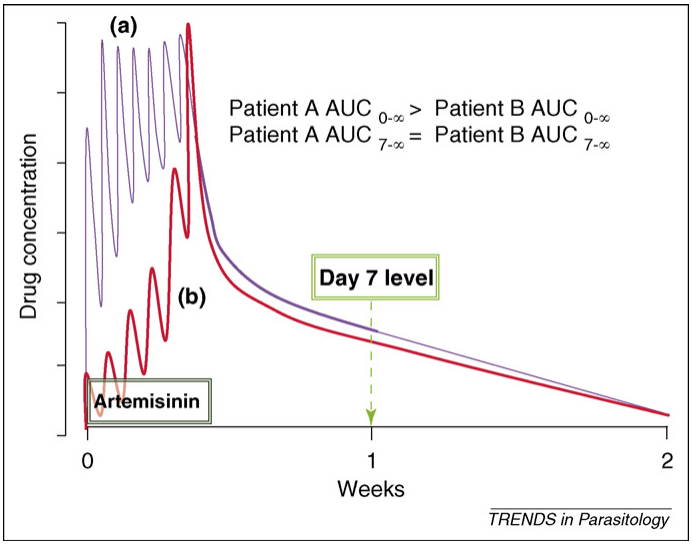
ACT Dosing Considerations in Children

Although the WHO recognizes that the absorption and disposition of ACTs [10] in children may be different from that of adults, the dosing guidelines, deduced from adult-based regimens and adjusted for body weight, are the same. Vulnerable populations, such as very young children, HIV-infected and the malnourished are typically excluded from efficacy and PK studies despite the fact that incorrect dosing in these populations could impact the risk of treatment failure, selection of drug resistance, and adverse drug reactions [33]. The few studies conducted in children have indicated that children may be receiving suboptimal doses of antimalarials, presumably due to differences in drug disposition [25, 28, 34, 35]. For instance, in a study conducted in Papua,

Indonesia evaluating DP, mean day 7 piperaquine concentrations in adults were lower in children compared to adults (37.1 vs. 50.4 ng/ml, P<0.001) and low concentrations were associated with treatment failure [28]. Likewise, in a study evaluating AL conducted in Mbarara, Uganda, the median day 7 lumefantrine levels in children less than 5 years of age was 156 ng/ml compared to 249 ng/ml in children 5-14 years and 281 ng/ml in adults [25]. Day 7 lumefantrine levels below 175 ng/ml were associated with recrudescence and higher risk of reinfection [25, 29].

The absorption, distribution, and metabolism of drugs also differ between infants and young children. The gastric pH in infants is increased which can impair absorption of drug requiring an acidic environment (i.e., lumefantrine). Total body water, which leads to larger apparent volumes of distribution, reduced plasma concentrations and increased clearance is also increased in infants. Full function of the CYP450 enzymatic system, the most important biotransformation system involved in drug metabolism, is thought to be dependent on age. Indeed, full maturation of the cytochrome P450 enzymes CYP1A2 and CYP2B6 are not achieved until peri- or post-puberty [36]. Therefore, drugs metabolized via this complex may be cleared and eliminated more quickly than in older children and adults. Despite these differences, there is limited antimalarial PK data and until now, no PK information regarding ACTs in the first year of life (see Chapter 4).

Figure 3. The Day 7 levels of ACT partner medication varies less than AUC and may be a more useful predictor for treatment failure.



This example illustrates that, for a variably absorbed drug (e.g. lumefantrine), the AUC can vary considerably –mainly because of absorption variability – yet the elimination phases are similar. Two hypothetical patient profiles are shown, A and B, with different drug absorption profiles but similar elimination profiles. The therapeutic response in these two cases should be similar because the three-day course of artemisinin (or derivative) shown in the box would determine the reduction in parasite numbers in the first two cycles (four days).
Source: White et al.[23]

Criteria for Assessing Treatment Efficacy

The WHO has recommended that a change in a national antimalarial treatment policy occur if the total treatment failure proportion equals or exceeds 10% as assessed through in-patient monitoring of efficacy. In addition, the introduction of a new or alternative treatment strategy should be based on a cure rate exceeding 95% as assessed through clinical trials. Patients enrolled in these trials should be followed for at least 28 days following the start of treatment to allow for adequate time to monitor for reappearance of parasites in the blood but follow up times of longer than 28 days should be considered for drugs with an extended elimination phase. The WHO also recommends blood levels be taken at specific time periods for genotyping purposes, in order to distinguish recrudescent species from new infections [10].

Unfortunately, in practice, researchers often utilize different statistical methods to estimate treatment failure and duration of follow up varies. Studies which follow patients over a longer period will likely reports higher rates of reinfection. These factors make it difficult to monitor and compare cure rates between locations and over time [37]. When monitoring for antimalarial drug resistance, the objective is to determine the risk of failure, with failure limited to those with a clear inadequate response to therapy. In antimalarial clinical trials, patients often do not complete the follow-up period for a wide range of reasons yet contribute important information up until the last day they are observed [38]. Importantly, if these patients do not fail therapy, and they are recorded as failures (as occurs with intention-to treat analysis) or dropped from the analysis altogether (as occurs with per protocol analysis) the outcome is biased. The appropriate method to analyze such data in order to incorporate each patient's outcome up through their last day observed is to use survival analysis. The WHO currently recommends survival analysis as the preferred method of analysis of drug efficacy [39], although accepts the option of per protocol analysis. This dissertation will analyze the degree to which employing different statistical strategies will alter reported treatment efficacy results and discuss the implications on antimalarial treatment guidelines (Chapter 2).

MALNUTRITION

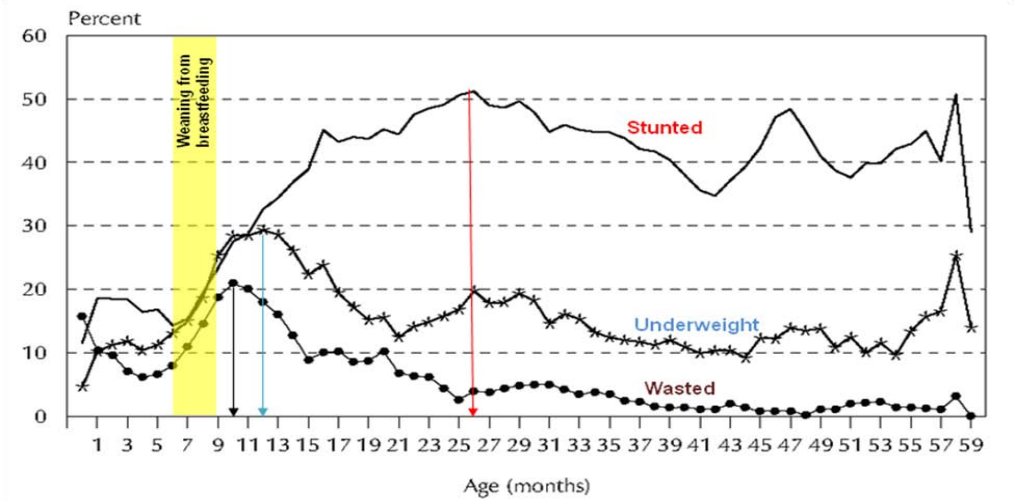
Definitions and Epidemiology

Malnutrition is the insufficient or imbalanced consumption and utilization of nutrients. The nutritional status of an individual is assessed through anthropomorphic indicators. Generally, children are considered malnourished if growth measurements, subsequently translated to z-scores, fall below 2 standard deviations (SD) under the normal height – for –age (stunted), weight, for-height (wasted), and weight-for-age (underweight) based on universally acceptable growth standards. Low height-for-age is an indicator of linear growth retardation and cumulative growth deficits. It is not sensitive to short-term changes in dietary intake but is a reflection of chronic malnutrition and is affected by recurrent and chronic illness. Low weight-for-height is an indicator of acute malnutrition and current nutritional status. It reflects inadequate food

intake immediately prior to the measurement and may be the result of a recent illness causing weight loss. Low weight-for-age is a composite of height-for-age and weight-for-height taking into account both acute and chronic malnutrition.

Malnutrition is a major public health problem in the developing world and is strongly associated with poverty. The WHO estimates that 35% of deaths in children under 5 years are a result, either directly or indirectly, of malnutrition [40]. Others have suggested that malnutrition is associated with one-half of all deaths in young children [41-44]. In a secondary analysis of 28 studies comparing anthropomorphic indicators to mortality risk, Pelletier and colleagues determined that mortality risk was inversely proportional to anthropomorphic indicators and that malnutrition had a multiplicative effect on mortality with the largest impact seen in populations with the highest baseline mortality levels [43] In developing countries, 38% of children under 5 years are stunted, 9% are wasted, and 31% are underweight [40, 42] . In Africa, 39% of children are stunted, 28% are underweight, and 8% are wasted [42]. Uganda specifically, 38% of children under 5 are stunted, 6% are wasted, and 16% are underweight. Stunting manifests itself early in infancy (below 6 months of age), increases through the first 3 years and begins to decline at 4 to 5 years of age. The decline in height-for-age z-score (HAZ score) is rapid in the first 2 years of life. Low birth weight, male gender, and living in a rural area are all predictors for stunting. Wasting varies by age but generally weight-for-height (WHZ score) peaks at 9-11 months and is associated with birth weight and male gender. Weight-for-age z-scores (WAZ score) increase rapidly from age 6 months to 11 months. Underweight is associated with low birth weight, male gender, and living in a rural area (Figure 4) [45]. Malnutrition in young children is also associated with early weaning, a diet low in protein and exposure to infection [42, 45].

Figure 4. Nutritional Status of Children under five in Uganda.



Note: Includes children below -2 standard deviations from the WHO Child Growth Standards. Arrows indicate peaks. Adopted from UDHS 2006 [45]

Growth Standards for Assessing Nutritional Status

In 2006, the WHO released revised growth standards (WHO standards) for assessing growth and development in children under 5. These standards were designed to replace the previous National Center for Health Statistics (NCHS)/WHO international growth reference (NCIS standards). The WHO standards are based on data collected from 6 countries; Brazil, Ghana, India, Norway, Oman, and the USA from 1997 to 2003 in children age 18 to 71 months. Only healthy breast-fed infants were included in the analysis [46]. The NCHS standards, first adopted in the 1970s, are based on data collected from healthy, breast-fed and non-breast-fed children from the United States only [47]. The revised WHO standards offer the following advantages; 1) they are based on data from children around the world, offering a better comparison for monitoring growth across populations; and 2) consistent with current WHO recommendations, they establish the breast-fed infant as the norm, providing a better tool for monitoring the growth of breast-fed infants. In a systematic comparison of the NCHS growth reference to the WHO standards, stunting rates were higher for all age groups, underweight rates were higher in the first 6 months of infancy and lower thereafter, and wasting rates were higher during infancy using the WHO standards [48]. Though the majority of publications cited in this dissertation utilize the NCIS growth standards, the analysis conducted in Chapters 3 and 4 utilize the new WHO growth standard as it provides a more accurate comparator for the growth of this study population against a sample of healthy breast-fed infants from around the world.

The Effect of Malnutrition on Immunity

Malnutrition and accompanying micronutrient deficiencies (e.g., zinc, magnesium, iron, selenium, and vitamin) are associated with impaired cellular and humoral response which leads to an increased susceptibility to infection [49]. Chronic malnutrition in childhood specifically affects thymic development, impairs T cell differentiation, expansion, and memory, disrupts IgA and IgG antibody response, and compromises macrophage activation. These affects on both acquired and innate immunity have been associated with an increase in infection by opportunistic pathogens including malaria [50, 51]. The relationship of malnutrition on immunity is further complicated by the effect of infection on nutritional status. For instance, malaria causes iron deficiency as well as a decrease in nutrient intake and absorption due to vomiting and lack of appetite both of which can further impair immune function and lead to acute as well as chronic malnutrition [51, 52]. Because infectious disease and malnutrition are intertwined- with malnutrition contributing to infectious disease which in turn contributes to malnutrition- it is difficult to tease apart the cause-effect relationship in epidemiological studies and generally only claims of associations can be made. With regards to malaria, although malaria and malnutrition frequently coexist [10], the results of studies evaluating the association between 1) malnutrition and malaria risk and 2) between malnutrition and immune response in children with malaria have been conflicting (Table 4).

Table 4. Selected Studies (post- 1990) evaluating the association of malnutrition with malaria.

Reference	Country/N/Ages	Study Design	Indicator for Malnutrition	Results	Interaction
Friedman et al. [53]	Kenya N=1862 Age 0 to 36 months	Cross-sectional survey	Height-for-age, weight-for-height, weight-for-age using NCHS standards	Stunted children had more parasitemia (OR 1.98), clinical malaria (OR 1.77), and severe anemia (OR 2.65).	Synergistic
Muller et al. [54]	Burkina Faso N=685 Age 6 to 30 months	Longitudinal surveillance of malaria attacks	Height-for-age, weight-for-height, weight-for-age using NCHS standards	Found no difference in P. falciparum incidence between malnourished and non-malnourished	Neutral
Danquah et al. [55]	Northern Ghana N=1200 Age 3-24 months	RCT of SP intermittent preventive therapy (IPT) given at 3, 9, and 15 months of age	Height-for-age, weight-for-height, weight-for-age using 2006 WHO standards	Malnutrition associated with increased severe anemia but not of malaria. Protective efficacy of IPT was ½ that observed in non-malnourished children	Neutral and synergistic
Deen et al. [56]	Gambia N=487 Age < 5 years	Longitudinal surveillance of malaria attacks for 4 months	Height-for-age, weight-for-height, weight-for-age using NCHS standards	Stunted children have a higher risk of malaria (RR 1.35). Underweight and wasted not at higher risk. Malaria did not have effect on follow-up anthropometry	Synergistic and neutral
Genton et al. [57]	Papua New Guinea N=136 children Age 10 months to 10 yrs	Longitudinal surveillance of malaria attacks over 1 year	Height-for-age, weight-for-height using NCHS standards	Stunted children at lower risk for malaria attack. Incident rate increased with increasing HAZ. No difference in WHZ. Increased production of cytokines in undernourished and increase in IgG	Antagonistic with some synergisms

Fillol et al. [58]	Senegal N=874 Age 2 to 59 months	Longitudinal surveillance	Height-for-age, weight-for-height, weight-for-age using NCHS standards	Stunting and underweight not associated with risk of malaria. Wasting independently associated with clinical malaria (OR=.33).	Neutral and antagonistic
Snow et al. [59]	Gambia N=138 1 to 4 years of age	Longitudinal surveillance of malaria attacks for 4 months	Height-for-age, weight-for-height, weight-for-age using NCHS standards	No impact of nutritional status on clinical malaria. Nonsignificant tendency to higher parasite density in non-malnourished children	Neutral
Renaudin et al. [60]	Chad N=144 Age birth to 1 year	Cross-sectional survey	Weight-for-age using NCHS standards	Underweight children 1.54 times more likely to have malaria	Synergistic
Tshikuka et al. [61]	Zaire N=558 Age 4 months to 10 years	Cross-sectional survey	Height-for-age, weight-for-height using NCHS standards	Stunted children 1.2 times more likely to be infected. Wasted children 1.2 times more likely to be infected	Synergistic
Toglet et al. [62]	Congo N=842 Age birth to 2 years	Longitudinal surveillance	Weight-for-age, height-for-age, arm circumference using NCHS standards	Malnourished children had more malaria attacks under age 9 months	Synergistic

The Effect of Malnutrition on Drug Pharmacokinetics in Children

Malnutrition can affect drug pharmacokinetics at every stage- absorption, distribution, metabolism, and elimination and some of these effects are similar to those that occur in young children without evidence of malnutrition. Vomiting and diarrhea associated with malnutrition will reduce the oral bioavailability of a drug. Malnutrition is also associated with atrophy of the jejunal mucosa, reduced gastric acidity, and prolonged emptying time [63-65], all of which can impair drug absorption [65]. Because total body water increases in proportion to the degree of malnutrition[66], the distribution into adipose tissue of lipid soluble drugs (i.e., lumefantrine) is reduced [64] which could potentially lead to lower plasma concentrations. For drugs that are protein bound, malnutrition can reduce protein binding due to reduced albumin levels. This increases the amount of unbound drug which may increase the rate of elimination or increase toxicity [64]. Malnourished children, may exhibit altered metabolism due to altered hepatic oxidative drug biotransformation via the cytochrome P450 (CYP) enzymatic system [67, 68]. Finally, malnutrition may slow the glomular filtration rate leading to elevated plasma levels and subsequent potential for toxicity [65, 69]. This is most relevant for drugs excreted by the kidneys such as antibiotics and aminoglycosides. Chapter 4 more closely examines the effects of malnutrition on the pharmacokinetics of piperaquine and lumefantrine.

CONCLUSION

Malaria and malnutrition are major causes of morbidity and mortality in children worldwide. Malnutrition is associated with impaired immune function of both the humoral and innate immune system which in turn, increases susceptibility to infection. While several studies have shown that malnutrition increases the risk for malaria, others have shown that malnutrition has no impact on malaria risk while still others have reported a protective effect of malnutrition on malaria risk (Table 4). In terms of treatment, the WHO recommends ACTs as first-line treatment for malaria in children. Achieving the optimal dose and therefore optimal drug concentrations to effectively eradicate malaria parasites is crucial for staving off resistance to these drug regimens. Though both age and nutritional status may adversely affect the pharmacokinetics of antimalarial treatment and therefore impact treatment efficacy, there are very few pharmacokinetics studies conducted in children and no pharmacokinetic studies conducted in malnourished children with malaria. Consequently, there is limited evidence to alter the dosing recommendations in children. The studies summarized in this dissertation are the first to examine the impact of malnutrition on risk of new malaria infection in children treated with ACTs as well as the first to examine the impact of malnutrition on the pharmacokinetics of ACTs.

DISSERTATION AIMS AND RATIONALE

The aims of this dissertation include the following:

Aim 1:

To quantify the magnitude of the difference between efficacy estimates from survival analysis using the modified intention-to-treat approach (mITT) with that of simple proportions using per protocol (PP) and intention-to-treat (ITT) approaches, and to identify factors that influence these differences.

Chapter 2 involves an aggregated analysis of 14 comparative clinical trials conducted in Thailand between 1993 and 2005 and 15 comparative clinical trials conducted in Uganda and Burkina Faso between 2003 and 2007 to quantify the magnitude of the differences between three statistical approaches. The advantages of survival analysis using the mITT approach are described and the results of this analysis provide the justification for the statistical methods utilized in Chapter 3.

Aim 2:

To evaluate the associations between measures of malnutrition and response to antimalarial therapy with the ACT regimens, artemether-lumefantrine (AL) and dihydroartemisinin-piperaquine (DP) in Ugandan children with malaria.

The longitudinal nature of the study described in Chapter 3, which provides multiple anthropometric measurements and multiple malaria episodes in each patient will help to inform clinicians if malnourished children are at increased risk of recurrent parasitemia and if alternative dosing strategies should be considered for this vulnerable population. This is the largest study to date conducted in malnourished children and is the first to evaluate the effect of malnutrition on the post-treatment prophylactic effect of ACTs.

Aim 3:

To assess the impact of two indicators of malnutrition, stunting and underweight, on the pharmacokinetic exposure of lumefantrine and piperaquine, the partner drugs for the antimalarial treatment regimens, dihydroartemisinin-piperaquine (DP) and artemether-lumefantrine (AL).

Chapter 4 will provide new understanding on the effect of malnutrition on the pharmacokinetics of ACTs by evaluating the widely accepted surrogate for drug exposure, day 7 blood levels. This is the first study assessing the effect of malnutrition on the pharmacokinetics of ACTs and one of the few studies, as well as the largest, evaluating PK in very young children with malaria. The results of this study may provide potential understanding as to why we may see differences in treatment outcome in malnourished children.

The data used in Chapters 3 and 4 are from the Tororo Child Cohort (TCC) Study, a University of California, San Francisco (UCSF)/Makerere University/US Centers for Disease Control and Prevention (CDC) collaboration sponsored by the Doris Duke Charitable Foundation and CDC and funded through DDCF 20060058, PREFA. The TCC study was approved by the Uganda National Council of Science and Technology and the institutional review boards of Makerere University, UCSF (CHR Number 07030255), the CDC, and the University of Washington.

For all studies, the code linking participants' identities was previously removed and the participants are therefore identified by study identification numbers only. In addition, the private information of the study participants was not collected specifically for this research through [my] personal interaction with the participants. Therefore, the University of California, Berkeley Committee for the Protection of Human Subjects (CPHS) confirmed these projects did not meet the definition of "human subjects" research set forth in Federal Regulations at 45 CFR 46.102(f) and did not require IRB approval by the University of California, Berkeley CPHS.

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CHAPTER 2

The Effect of Varying Analytical Methods on Estimates of Treatment Efficacy in Antimalarial Clinical Trials

ABSTRACT

Background. Analytical approaches for the interpretation of antimalarial clinical trials vary considerably. The aim of this study was to quantify the magnitude of the difference between efficacy estimates and identify the factors underlying these differences.

Methods. Data from studies conducted in Africa and Thailand were compiled and the risk estimates of treatment failure, adjusted and unadjusted by genotyping, were derived by 3 methods (intention to treat (ITT), modified intention to treat (mITT) and per protocol (PP)) and then compared.

Results. 29 clinical trials with a total of 65 treatment arms were included in the analysis: 38 from Africa and 27 from Thailand. Of the 15,409 patients enrolled, 2,637 (17.1%) had incomplete follow up for the unadjusted analysis and 4,489 (33.4%) for the adjusted analysis. Estimates of treatment failure were consistently higher when derived from the ITT or PP analyses compared to the mITT approach. In the unadjusted analyses the median difference between the ITT and mITT estimates was greater in Thai studies (11.4 [range 2.1-31.8]) compared to African Studies (1.8% [range 0-11.7]; $p<0.001$). In the adjusted analyses the median difference between PP and mITT estimates was 1.7%, but ranged from 0 to 30.9%. The discrepancy between estimates was correlated significantly with the proportion of patients with incomplete follow-up; $r_s = 0.740$, $p<0.0001$. The proportion of studies with a major difference (>5%) between adjusted PP and mITT, was 28% (16/57), with the risk difference greater in African (37% 14/38) compared to Thai studies (11% 2/19). In the African studies a major difference in the adjusted estimates was significantly more likely in high transmission sites (62% 8/13) compared to moderate transmission sites (24% 6/25); $p=0.035$.

Conclusions. Estimates of antimalarial clinical efficacy vary significantly depending on the analytical methodology from which they are derived. In order to monitor temporal and spatial trends in antimalarial efficacy, standardised analytical tools need to be applied in a transparent and systematic manner.

BACKGROUND

In the past decade, the number of antimalarial clinical trials has increased significantly. In Africa alone, the number of studies published between 2001 and 2005 increased three-fold compared to the number published in the preceding five years [1]. This is primarily due to the greater awareness of the emergence of multidrug resistant strains of *P. falciparum* and the introduction of new treatment regimens such as the artemisinin combination therapies (ACTs). In addition, study designs have evolved to include a longer duration of follow-up and the inclusion of genotyping to distinguish recrudescence from new infection [1].

Antimalarial clinical trials are usually conducted either to compare two or more treatment regimens (comparative trials) or to monitor for the emergence of antimalarial resistance over time and in different geographical areas. The World Health Organisation (WHO) currently recommends that countries change their antimalarial treatment policy when the cure rate for the current recommended therapy falls below 90% and that a new antimalarial treatment policy be adopted only when a therapy has an average cure rate$\geq$ 95% [2]. The WHO also recommends the use of survival analysis to generate efficacy estimates, however in practice researchers adopt a variety of statistical methods tailored to the specific rationale of the study in question [3-5]. The derived estimates are confounded further by variations in the PCR correction methods used to distinguish recrudescent infections from new infections [1, 6]. These core methodological issues undermine attempts to monitor and compare cure rates between locations and over time and significantly limit the utility of clinical trials to guide policy [7].

In general, the derivation of antimalarial efficacy can be generated by three approaches: per protocol, intention–to–treat and modified intention–to–treat. In the per protocol analysis (PP) the evaluable population includes only those patients who are followed throughout the protocol defined follow-up period and in whom a clear treatment outcome can be determined. In this approach patients deviating from the protocol, such as those who do not complete follow-up, are excluded from analysis. Intention-to-treat analysis (ITT) adopts a conservative approach often advocated for comparative drug trials, in which all patients randomized to treatment are included in the analysis and patients with incomplete follow-up who do not reach the primary outcome of interest are generally considered treatment failures. In the third approach, the modified intention-to-treat analysis (mITT), survival analysis is used and patients with incomplete follow-up who do not reach the primary outcome of interest are included in the analysis as non-failures but censored on the last day of follow-up. WHO guidelines and several recent consensus papers currently advocate modified ITT survival analysis as the most appropriate method for monitoring antimalarial efficacy [3, 5, 7, 8].

To quantify the magnitude of the difference between efficacy estimates from the mITT approach with that of the PP and ITT approaches and to identify factors that influence these differences, we compiled data from 29 comparative antimalarial clinical trials conducted in Africa and Thailand and compared the derived estimates of treatment failure.

METHODS

Data Sources for Analysis Comparisons

Individual patient data were available from 14 comparative clinical trials conducted in Thailand between 1993 and 2005 and from 15 comparative clinical trials conducted in Uganda and Burkina Faso between 2003 and 2007 (Table 1). Data were only included for patients enrolled with uncomplicated malaria due to *P. falciparum*. Drug treatment was supervised in all patients, with daily observation until at least day 3 followed by weekly visits up to 28, 42, or 63 days.

Thai Studies

The studies in Thailand were carried out in a camp for displaced persons of the Karen ethnic minority on the western border of Thailand [9]. Transmission of malaria here is unstable and seasonal, with peaks in May through July and December through January [10]. The estimated entomological inoculation rate (EIR) and corresponding incidence of malaria is low (approximately 0.5 to 1.5 cases/person/year) with prevalence rates of 1-4% for *P. falciparum*. Overall, *P. falciparum* accounts for 37% of malaria infections, with the remainder due to *P. vivax*. All *P. falciparum* infections and approximately 90% of *P. vivax* infections are symptomatic. In Thailand, patients of all ages were enrolled providing that they weighed more than five kilograms. Pregnant women and patients with severe disease were excluded.

African Studies

The studies in Africa were conducted in Bobo-Dioulasso, Burkina Faso and in several study sites in Uganda. Patients recruited were 6 months of age or older with no evidence of severe disease. *P. falciparum* accounts for nearly 100% of all malaria cases in these regions. In Burkina Faso, malaria is seasonal with transmission peaking during the rainy season from May to October. All patients were recruited from governmental health clinics. Studies in Uganda were conducted in areas of moderate to high transmission intensity with peaks during two rainy seasons from March to May and then from August to September. Three studies in Kampala, Apac, and Tororo were conducted in children only. Patients were recruited from district health clinics participating in the Ugandan Malaria Surveillance Project, household sampling, or from other outpatient clinics.

Malaria Outcome Classification

The key parameters for deriving the efficacy estimates were coded identically for all studies, as described previously [7]. Outcomes were classified according to the 2006 WHO guidelines as adequate clinical and parasitological response (ACPR), early treatment failure (ETF), late clinical failure (LCF), late parasitological failure (LPF), or follow-up interrupted (Table 2). For 24 of the 29 (83%) studies included, parasites were genotyped to distinguish recrudescent and new infections due to *P. falciparum* as previously described [1, 11]. All ETFs were considered to be due to recrudescence. Patients meeting the criteria for LCF or LPF in whom genotyping

was done but results were inconclusive or unavailable were classified as unsuccessfully genotyped.

Statistical Analyses

The risks of failure unadjusted and adjusted by genotyping for each treatment arm of the individual studies were derived and compared using three analytical methods; per protocol (PP), intention-to-treat (ITT), and modified intention-to-treat (mITT). Although the general principles behind these analytical approaches are well described, in practise subtle differences arise in the way in which the outcome measures may be classified. For the purpose of our analysis we chose to classify treatment outcomes as summarized in Table 3. In the ITT analyses, the evaluable population for both the unadjusted and adjusted calculations included all patients enrolled in the study. In the PP analysis, the evaluable population included only patients classified as ACPR or recurrent parasitemia with *P. falciparum* (ETF, LPF, LCF) in the unadjusted calculations and only patients classified as ACPR, ETF or LCF/LPF due to recrudescence in the adjusted calculations. In the mITT analyses, the evaluable population for both the unadjusted and adjusted calculations included all patients enrolled in the study with the exception that LCF/LPF outcomes with unsuccessful genotyping outcomes were excluded from the adjusted calculations. In the PP and ITT analyses, the risks of failure for each treatment group were calculated as the proportion of patient classified as failure (the numerator) divided by the number of patients in the evaluable population (the denominator). In the mITT analyses, the risks of failure were calculated using the Kaplan-Meier product limit formula with data censored for patients who were not classified as failures and with interrupted follow-up. For the unadjusted calculations, patients with follow-up interrupted and non-falciparum new infections were censored on the last day of observation. For the adjusted calculations, censored patients also included those with *P. falciparum* new infections.

We examined the relationship between the proportion of patients with incomplete follow-up and the risk difference when comparing two different methods for estimating the risk of failure. Incomplete follow-up included any outcome category (listed in Table 3), where the classification of success/failure/censored/excluded differed between any of the three analytical methods. In the unadjusted analyses, incomplete follow-up was defined as any patient in whom follow-up was interrupted and those with non-falciparum new infections. In the adjusted analyses, incomplete follow-up was defined as any patient in whom follow-up was interrupted, those with non-falciparum new infections, those with *P. falciparum* new infections, and those with unsuccessful genotyping.

Stratified analyses were used to evaluate factors that contribute to the pairwise differences in the risk of failure between the three analytical methods for both the adjusted and unadjusted calculations. Factors considered included the location of study (Africa or Thailand), the duration of follow-up (28, 42, or 63 days), and malaria transmission intensity. Transmission intensity was classified as low (EIR<1), moderate (EIR 1 to 100) and high (EIR >100). All

analyses were performed with Stata, version 10 (Stata-Corp, College Station, Texas). A p-value < 0.05 was considered statistically significant.

RESULTS

In total 29 drug studies were included in the analysis, with 65 treatment arms which enrolled 15,409 patients. Five (17%) trials in Thailand which included 8 treatment arms were conducted prior to the introduction of genotyping and thus were not included in the adjusted analyses. Of the 15 studies conducted in Africa, the duration of follow-up was 28 days in 12 (80%) studies and 42 days in 3 (20%) studies; table 1. Ten (66%) trials conducted in Africa were conducted in areas of moderate transmission intensity and the remainder were conducted in areas of high transmission. Of the 14 studies conducted in Thailand, the duration of follow-up was 28 days for 1 (7%) study, 42 days for 5 (36%) studies, and 63 days for 8 (57%) studies. All Thai studies were conducted in an area of low intensity transmission. Clinical outcomes for each location (Africa and Thailand) are summarized in Table 2.

Incomplete follow-up

For analyses unadjusted by genotyping, incomplete follow can be divided into two categories: patients whose follow-up is interrupted prior to reaching a defined endpoint (i.e. lost to follow-up) and recurrent malaria due to non-falciparum infections (Table 4). In total 29% (2237/7790) of Thai patients had incomplete follow-up for the unadjusted risk estimates, of whom 50% (1120/2237) had follow-up terminated early due to recurrence with *P. vivax.* Incomplete follow-up was significantly lower in African studies (5.3% 400/7619, p<0.001), with only 10% (40/400) of patients with incomplete follow-up having recurrence with a different species. For the adjusted analyses, patients with *P. falciparum* new infections and recurrent infections which could not be genotyped, were also classified as having incomplete follow-up. These outcomes occurred in 26% (1971/7619) of African patients, but only 7.3% (422/5813) of Thai patients; p<0.0001.

In Thailand, studies with a longer duration (42 days or more) had a greater proportion of patients with incomplete follow-up compared to studies with 28 day follow-up. This was apparent for both the unadjusted (median 22% vs 27%; p=0.028) and adjusted analyses (median 22% vs 35%; p=0.004). In Africa, where there was variation in transmission intensity, incomplete follow-up in the adjusted analyses was significantly higher in areas of high transmission (median 47% [range: 32.2-68.6]) compared to studies in moderate transmission areas (19% [9.9-48.2]; p<0.001). Patients with *P. falciparum* new infections accounted for 91% (1101/1204) of the patients with incomplete follow-up in high transmission areas, compared to 65% (763/1167) in moderate transmission areas; p<0.0001. Conversely in the unadjusted analyses, the proportion of patients with incomplete follow-up was low in both high transmission sites (median 1.9% [range 0.6-6.2]) and moderate transmission sites (median 4.5% [range 2.2-12.8]).

Comparison of ITT and mITT analyses

The unadjusted risk of treatment failure derived by ITT analysis was consistently higher than that derived by mITT analysis (median difference = 4.7% [-0.3 – 31.8%]) (Table 5). The difference in risk estimates (ITT-mITT) was greater in Thai studies (median = 11.4 [range 2.1-31.8]) compared to African Studies (median 1.8% [range 0-11.7]; p<0.001). The discrepancy between the unadjusted risk estimates was correlated with the proportion of patients with incomplete follow-up in African studies (r_s= 0.721, p<0.0001), although this does not reach significance in the Thai studies (r_s= 0.272, p=0.169); figure 1. The ITT-mITT risk difference was significantly higher for the adjusted estimates compared to the unadjusted estimates and this was apparent for both the African studies (median 3.5% vs 1.8%; p = 0.032) and Thai studies (median 12.3% vs. 11.4%; p <0.001). In Africa 18% (7/38) of treatment arms had a difference in the unadjusted risk estimates (ITT-mITT) greater than 5%, compared to 85% (23/27) of the studies in Thailand; p<0.001. The corresponding figures for the difference in the adjusted estimates were 29% (11/38) in and 95% (18/19) respectively, p<0.001.

Comparison of PP and mITT analyses

The unadjusted risk of treatment failure derived from the PP analyses was consistently higher than that derived from the mITT analyses (Table 5). The median difference (PP-mITT) in Thailand was 1.9% (range 0-10.6) and was correlated with both the proportion of patients with incomplete follow-up (r_s= 0.437, p=0.02) and the duration of the study (r_s= 0.419, p=0.03). The difference in estimates was significantly lower in African studies (median = 0.1% [range 0 to 2.1%]; p<0.001), and was correlated with the study duration (r_s= 0.445, p=0.005).

For the adjusted analyses the median difference between estimates was 1.7% (range 0-30.9) and was correlated significantly with the proportion of patients with incomplete follow-up (r_s =0.740, p<0.0001; Figure 2) in both Africa and Thailand. The difference was greater in Africa (median 3.2% [range 0-30.9]) compared to Thailand (median 1.0% [range 0-6.9]; p=0.033).

In total, 7.7% (5/65) of the treatment arms had a difference in the unadjusted risk estimates (PP-mITT) of greater than 5%; these studies were all from Thailand (19% 5/27), with none (0/38) conducted in Africa; p=0.01. In the adjusted analyses the proportion with a major difference (>5%), rose to 28% (16/57), with the risk greater in African (37% 14/38) compared to Thai studies (11% 2/19). In Africa the risk of a major difference in adjusted estimates was significantly greater in studies conductd in high transmission sites (62% 8/13) compared to moderate transmission sites (24% 6/25); p=0.035.

DISCUSSION

Antimalarial drug clinical trials are conducted both to monitor antimalarial drug resistance and to compare treatment regimens. As in all clinical trials, protocol violations and incomplete patient follow-up challenge the analysis and interpretation of the results. Malaria studies are by their nature logistically difficult, often being conducted in poorly resourced communities and prone to varying patient adherence to protocols. In addition to issues related to protocol adherence, antimalarial clinical trials are also confounded by interrupted follow-up resulting from recurrent infections, either by the same or different species. The statistical approach in dealing with these issues can vary according to the rationale of the study [3, 12]. For instance in comparative studies a conservative approach (intention to treat, ITT) is often advocated in which all patients are included in the analysis, but those with incomplete follow-up are classified as a treatment failure. In contrast, when monitoring antimalarial drug resistance, the objective is to determine the risk of failure, with failure limited to those with a clear inadequate response to therapy. Patients with incomplete follow-up can be either dropped from the analysis (e.g. per protocol, PP), or included in a survival analysis censoring as "non-failures" on the last day of follow-up (modified intention to treat mITT). The WHO currently recommends the latter as the preferred method of analysis of drug efficacy [8], although accepts the option of per protocol analysis. In this paper we compare these three analytical methods from drug trials conducted in Thailand, Uganda, and Burkina Faso to determine the degree of variation in the derived estimates of efficacy and factors underlying this.

Our findings show that in studies from two highly experienced research groups, the proportion of patients with interrupted follow-up (i.e. incomplete follow-up due to reasons other than recurrent infections) was generally low, but rose as high as 36%. Interrupted follow-up was greater in the Thai studies compared to those conducted in Africa, in part explained by the longer duration of study follow-up in Thailand. The occurrence of new infections with *P. falciparum* or relapse of *P. vivax*, now generally require retreatment and termination of the primary study. Even in the most adherent populations these proportions can often exceed a third of all patients enrolled (table 1), reducing considerably the per protocol population. Predictably incomplete follow-up was higher for the adjusted estimates, which distinguishes recurrent infections, and in the African studies this was more apparent in studies conducted in areas of high transmission.

The proportion of patients with incomplete follow-up has significant implications for the derived estimates of treatment efficacy. Our results highlight that both the ITT and PP methods consistently overestimated the risk of failure when compared to the preferred mITT method, the discrepancy in risk estimates varying from trivial to highly significant. For example in the comparison of the unadjusted ITT and mITT failure estimates, 46% (30/65) of the difference in estimates exceeded 5%, with one study having a difference of 31.8%. The bias was most pronounced in Thailand due to the high percentage of patients with incomplete follow-up. Our findings highlight that although the ITT method of analysis has utility for conservatively comparing treatment arms within a comparative drug trial, it is significantly biased when deriving point estimates of efficacy, for comparison over time or geographical location.

New infection with *P. falciparum* constituted an additional confounding factor for the adjusted analyses (PP-mITT), particularly in areas of high transmission in Africa, since these are removed from the PP analysis and censored in the mITT analysis. The consequences were that 28% (16/57) of derived adjusted estimates by the PP method exceeded the mITT estimate by an absolute value of 5% or greater. This discrepancy was particularly apparent in the high transmission sites. The difference in risk estimates was lower for the unadjusted analysis, although in Thailand, high relapses rates with *P. vivax* and greater loss to follow-up resulted in 18.5% (5/27) of PP estimates deviating by more than 5% from the mITT estimate.

Survival analysis is being used increasingly to derive estimates of antimalarial treatment efficacy, however the ease in calculating the simple proportions of the PP analysis retains its appeal and these estimates continue to be reported frequently in the literature. Our findings highlight that caution is needed when generating temporal and geographical trends using different analytical methods, and that this is particularly apparent for studies with poorer patient adherence to follow-up, higher incidence of *P. vivax* relapse, and high incidence of *P. falciparum* new infections. Given the variations in study methods, survival analysis remains the preferred approach for monitoring *in vivo* efficacy. First, it allows for all available data to contribute to the analysis, thus increasing the precision of the derived estimates. Second, it avoids systematic biases introduced by dropping patients from the analysis that do not complete follow-up (PP) or classifying patients as failures who do not complete follow-up (ITT). Finally, it allows for data from patients with different follow-up periods to be combined to generate efficacy estimates at different time points thus enabling direct comparison between studies with different lengths of follow-up [7].

Over the last decade it has become evident that the provision of highly effective and widely available antimalarial regimens must be an integral part of any realistic hope of achieving a global elimination of malaria [13]. Current international guidelines advocate that new antimalarial treatments should only be introduced if they have cure rates greater than 90%. The sustained efficacy of such novel regimens needs to be monitored regularly, with careful vigilance for early signs of declining efficacy. Even small fluctuations in risk estimates can have huge implications for policy makers. In order to monitor temporal and spatial trends in antimalarial efficacy, *in vivo* efficacy data needs to be collated at an individual patient level and standardized analytical tools applied in a transparent and systematic manner [7]. The recently launched WorldWide Antimalarial Resistance Network (WARN - http://www.wwarn.org), aims to do precisely that; acting as a global resource of antimalarial efficacy data, and providing open access to its uniform interpretation.

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TABLES

Table 1. Characteristics and treatment outcomes of the clinical trials.

Location	Duration	Transmission intensity	Study Drugs	Total Enrolled	Follow-up Interrupted	ACPR	ETF	LCF/LPF			
								Recrudescence	*P. falciparum* New Infection	Genotyping Unsuccessful	Non-falciparum New infection
Burkina Faso [14]	28	Moderate	AQ, AQ+SP, SP	944	113	735	9	34	40	13	0
Burkina Faso [15]	28	Moderate	AL, AQ+SP	521	43	430	1	4	43	0	0
Apac, Uganda [16]	28	High	AQ+AS, AQ+SP, CQ+SP	542	10	252	0	61	206	13	0
Arua, Uganda [16]	28	High	AQ+AS, AQ+SP, CQ+SP	534	10	188	17	75	232	12	0
Jinja, Uganda [16]	28	Moderate	AQ+AS, AQ+SP, CQ+SP	543	27	328	6	77	95	10	0
Kampala, Uganda [17]	28	Moderate	AQ+AS, AQ+SP, CQ+SP	400	11	266	5	56	52	10	0
Kampala, Uganda [18]	28	Moderate	AL, AQ+AS, AQ+SP	687	24	544	11	35	69	1	3
Kanungo, Uganda [19]	28	Moderate	AQ+SP, CQ+SP	367	10	108	18	129	100	2	0
Kyenjojo, Uganda [19]	28	Moderate	AQ+SP, CQ+SP	365	14	147	14	61	115	14	0
Mubende, Uganda [19]	28	Moderate	AQ+SP, CQ+SP	373	19	135	11	64	133	11	0
Tororo, Uganda [16]	28	High	AQ+AS, AQ+SP, CQ+SP	541	22	135	8	77	290	9	0
Tororo, Uganda [20]	28	High	AL, AQ+AS	408	5	165	1	16	217	4	0
Burkina Faso [21]	42	Moderate	AL, AQ+SP, DP	559	42	429	9	9	70	0	0
Apac, Uganda [22]	42	High	AL, DP	421	4	206	0	41	156	1	13
Kanungo, Uganda [23]	42	Moderate	AL, DP	414	6	317	1	13	46	7	24
Mae Sod, Thailand [24]	28	Low	AL	358	46	261	0	22	3	1	25
Mae Sod, Thailand [25]	42	Low	AP+AS, AP, MQ+AS	1586	173	1118	0	20	42	11	222
Mae Sod, [26]	42	Low	AL	592	68	277	0	14	40	24	169
Mae Sod, Thailand [27]	42	Low	AL, MQ+AS	170	42	97	0	3	1	0	27
Mae Sod, Thailand [26, 28]	42	Low	AL, MQ+AS	795	48	481	0	23	51	8	184

Mae Sod, Thailand [29]	42	Low	MQ+AS	1019	227	651	0	116 (genotyping not done)			25
Mae Sod, Thailand [30]	63	Low	DP, MQ+AS	1026	102	663	2	20	86	9	144
Mae Sod, Thailand [31]	63	Low	AL, MQ+AS	606	82	308	0	41	22	17	136
Mae Sod, Thailand [32]	63	Low	MQ+AS	493	67	228	0	33	61	13	91
Mae Sod, Thailand [28]	63	Low	MQ+AS	187	45	74	0	10	17	16	25
Mae Sod, Thailand [10]	63	Low	MQ+AS	34	1	22	0	9 (genotyping not done)			2
Mae Sod, Thailand [33]	63	Low	MQ, MQ+AM, MQ+AS	548	137	239	5	127 (genotyping not done)			40
Mae Sod, Thailand [34]	63	Low	MQ, MQ+AS	346	73	172	6	67 (genotyping not done)			28
Mae Sod, Thailand [35]	63	Low	MQ+AS	30	6	13	0	9 (genotyping not done)			2

AL = artemether-lumefantrine; AM = artemether; AP: atovaquone-proguanil; AQ = amodiaquine; AS = artesunate; CQ = chlorquine; DP = dihydroartemisinin-piperaquine; MQ = mefloquine; SP = sulfadoxine-pyramethamine; ACPR = adequate clinical and parasitological response; ETF = early treatment failure; LCF = late clinical failure; LPF= late parasitological failureTable 2. Treatment outcome classification system using standardised criteria [7].

Table 2. Treatment outcome classification system using standardized criteria [7].

Outcome Category	Outcome Code	Outcome Definition	Africa	Thailand	Total
Follow-up completed	0	ACPR	4385	4604	8989
	1	ETF with death	0	1	1
	2	ETF with severe malaria	5	0	5
	3	ETF with danger signs	39	0	39
	4	ETF with parasitological criteria	58	1	59
	5	ETF with clinical criteria	9	0	9
	6	ETF not otherwise specified	0	11	11
	7	LCF with death	0	0	0
	8	LCF with severe malaria	0	0	0
	9	LCF with danger signs	4	0	4
	10	LCF with fever	1033	654	1687
	11	LPF	1726	665	2391
	12	LPF/LCF indistinguishable	0	737	737
Follow-up interrupted	13	Adverse event requiring change in antimalarial therapy	0	2	2
	14	Treatment protocol violation	4	138	142
	15	Death not due to malaria	0	3	3
	16	Lost to follow-up	175	955	1130
	17	Use of other antimalarials outside of study protocol	48	6	54
	18	Withdrawal of consent prohibiting further follow-up	126	1	127
	19	Investigator initiated withdrawal from further follow-up	7	0	7
	20	Patient who does not complete follow-up for any other reason	0	12	12

ACPR = adequate clinical and parasitological response; ETF = early treatment failure; LCF = late clinical failure; LPF= late parasitological failure

Table 3. Analytical methods used to generate estimates of drug efficacy.

Outcome Category		Unadjusted by genotyping			Adjusted by genotyping		
		ITT	**mITT**	**PP**	**ITT**	**mITT**	**PP**
Follow-up interrupted		Failure	Censored	Excluded	Failure	Censored	Excluded
ACPR		Success	Success	Success	Success	Success	Success
ETF		Failure	Failure	Failure	Failure	Failure	Failure
LCF/LPF	**Recrudescence**	Failure	Failure	Failure	Failure	Failure	Failure
	P. falciparum new infection	Failure	Failure	Failure	Success	Censored	Excluded
	Genotyping unsuccessful	Failure	Failure	Failure	Failure	Excluded	Excluded
	Non-falciparum new infection	Success	Censored	Excluded	Success	Censored	Excluded

ITT = intention-to-treat; mITT = modified intention-to-treat; PP = per protocol

ACPR = adequate clinical and parasitological response; ETF = early treatment failure; LCF = late clinical failure; LPF= late parasitological failure

Table 4. Proportion of patients with incomplete follow-up.

	Africa	Thailand	Overall
Unadjusted Analysis	7619	7790	15,409
Interrupted follow-up [a]	360 (4.7%)	1117 (14.3%)	1477 (9.6%)
Non-falciparum new infections	40 (0.5%)	1120 (14.4%)	1160 (7.5%)
Overall	**400 (5.3%)**	**2237 (28.7%)**	**2637 (17.1%)**
Adjusted Analysis [b]	7619	5813	13,432
Interrupted follow-up [a]	360 (4.7%)	673 (11.6%)	1477 (9.6%)
Non-falciparum new infections	40 (0.5%)	1023 (17.6%)	1160 (7.5%)
***P. falciparum* new infections**	1864 (24.5%)	323 (5.6%)	2637 (17.1%)
Unsuccessful genotyping	107 (1.4%)	99 (1.7%)	206 (1.5%)
Overall	**2371 (31.1%)**	**2118 (36.4%)**	**4489 (33.4%)**

[a] Includes all patients with with interrupted follow-up (outcomes 13-20); see table 2
[b] Excludes 1977 Thai patients (from 5 studies with 8 treatment arms) in which genotyping not attempted

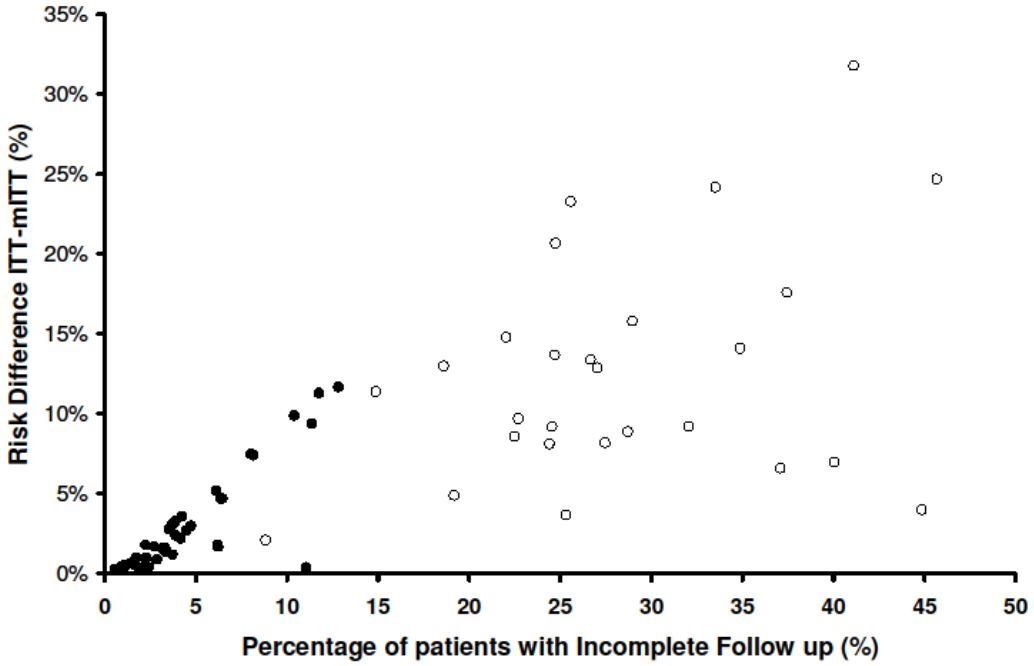
Table 5. The difference in risk estimates derived by intention to treat (ITT), modified Intention to Treat (mITT) and per protocol (PP).

	Africa	Thailand	Overall
ITT – mITT			
Unadjusted	1.8% [-0.3 – 11.7] IQR: 0.7-3.9	11.4% [2.1-31.8] [a] IQR: 8.1-15.8	4.7% [-0.3 – 31.8] IQR: 1.6-10.6
Adjusted	3.5% [-13.7 – 14.4] IQR: 0.9-6.0	12.3% [4.1 – 31.8] [a] IQR: 10.2-16.0	5.4% [-13.7 – 31.8] IQR: 1.9-11.6
PP – mITT			
Unadjusted	0.1% [0.0 – 2.1] IQR: 0-0.23	1.9% [0.0 – 10.6] [a] IQR: 0.9-4.3	0.3% [0.0 – 10.6] IQR: 0.1-1.9
Adjusted	3.2% [0.0 –0.9] IQR: 0.7-3.9	1.0% [0.0 – 6.9] [b] IQR: 0.3-1.8	1.7% [0.0 – 30.9] IQR: 0.5-5.6

Values represent Median [Range], and InterQuartile Range (IQR)
Comparison between Africa and Thailand: [a] $p<0.001$; [b] $p=0.033$

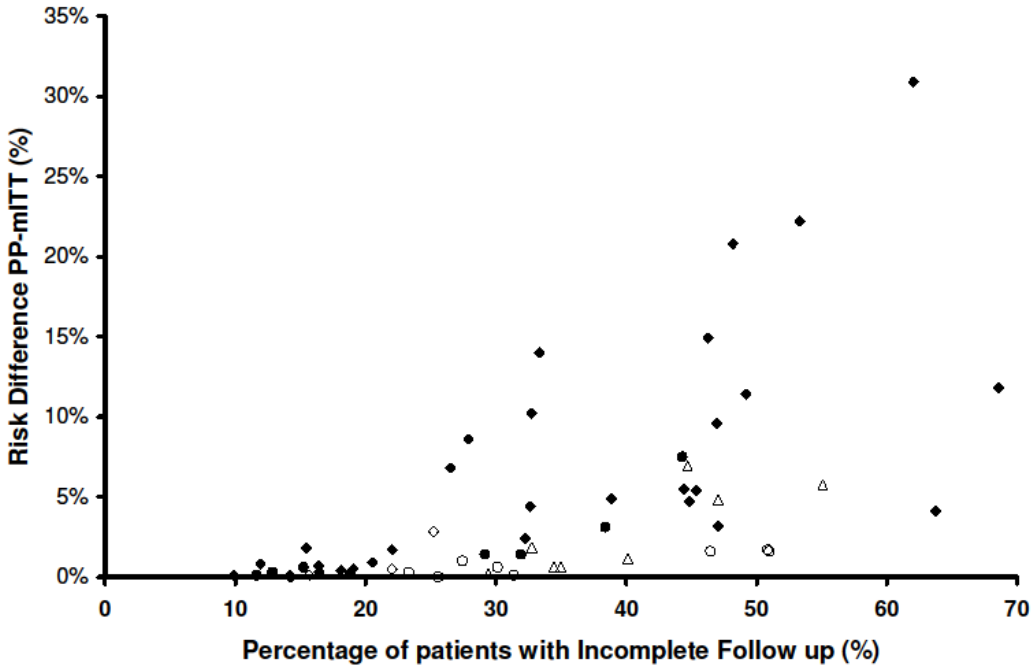
FIGURES

Figure 1. Relationship between incomplete follow up and the risk difference between unadjusted estimates from ITT and. mITT analysis.



Closed circles for African studies (38 treatment arms) and open circles for Thai studies (27 treatment arms).

Figure 2. Relationship between incomplete follow up and the risk difference between adjusted estimates from PP and. mITT analysis.



Closed markers for African studies (38 treatment arms) and open for Thai studies (19 treatment arms). Diamonds = 28 day studies, Circles = 42 days studies and triangles 63 days studies.

CHAPTER 3

Effect of Nutritional Status on Response to Treatment with Artemisinin-based Combination Therapy in Young Ugandan Children with Malaria

ABSTRACT

The relationship between malnutrition and malaria in children under 5 years is a matter of debate and there are no published studies evaluating the association between malnutrition and response to artemisinin-based combination therapies (ACTs). We evaluated the association between malnutrition and response to antimalarial therapy in Ugandan children treated with dihydroartemisinin-piperaquine (DP) or artemether-lumefantrine (AL) for repeated episodes of malaria.

Children aged 4 to 12 months diagnosed with uncomplicated malaria were randomized to either DP or AL and followed for up to 2 years. All HIV-exposed and HIV-infected children received trimethoprim-sulfamethoxazole (TS) prophylaxis. The primary exposure variables of interest were height-for-age and weight-for-age z-scores. Outcomes included parasite clearance at day 2 and 3 and risk of recurrent parasitemia after 42 days of follow-up.

292 children were randomized to DP or AL, resulting in 2013 treatments for uncomplicated falciparum malaria. Less than 1% of patients had a positive blood smear by day 3 (DP 0.2%, AL 0.6%, p=0.18). There was no significant association between height-for-age or weight-for –age z-scores and a positive blood smear 2 days following treatment. In children treated with DP not on TS prophylaxis, height-for-age z-scores <-1 were associated with a higher risk of recurrent parasitemia compared to height-for-age z-score $\geq$ 0 (HR=2.89, p=0.039; HR=3.18, p=0.022).

DP and AL are effective antimalarial therapies in chronically malnourished children in a high transmission setting. However, children with signs of mild to moderate chronic malnutrition not taking TS prophylaxis are at higher risk of recurrent parasitemia and may be considered a target for TS prophylaxis.

INTRODUCTION

Malaria and malnutrition are major causes of morbidity and mortality in children in sub-Saharan Africa. Malaria, predominantly caused by *P. falciparum*, is estimated to cause 880,000 deaths each year, with the majority of deaths occurring in children under 5 years of age sub-Saharan Africa (25). At the same time, malnutrition is a major public health problem in developing countries. Approximately one half of the 10.6 million children under 5 who die in low- and middle- income countries are malnourished (24). Common anthropometric indices used to assess the extent of malnutrition include height-for-age, a measurement for linear growth and an indicator of long-term growth deficits; weight-for-height, a measurement of body proportion and an indicator of acute growth disturbances; and weight-for-age, which represents a synthesis of linear growth and body proportion (5). In Africa, malnutrition is highly prevalent; 39%, 8%, and 28% of children under 5 are stunted (height for age z-score<-2), wasted (weight-for-height z-score<-2), or underweight (weight-for-age z-score<-2), respectively (20).

Although malaria and malnutrition frequently coexist (22), there have been few studies evaluating the effect of malnutrition on malaria and results of such studies have been conflicting Some studies have reported that children with evidence of malnutrition as characterized by either stunting, underweight, or wasting, have a higher risk of malaria, others have reported a lower risk, and still other studies have reported no association. (7, 9, 11, 12) However, in these studies, the anthropometric growth references, age ranges, transmission intensities, and definitions of malaria differed. To our knowledge, no studies have evaluated the effects of malnutrition on the risk of recurrent parasitemia.

Data are also lacking on the effect of malnutrition on response to antimalarial therapy (22). Vulnerable populations, such as very young children, the HIV-infected and the malnourished are typically excluded from or under-represented in studies of antimalarial drug efficacy (2). The World Health Organization (WHO) currently recommends artemisinin-based combination therapies (ACTs) for the treatment of uncomplicated falciparum malaria. Although WHO recognizes that malnutrition may affect the response to antimalarial therapy (22), there are no published studies examining the association between malnutrition and the response to antimalarial therapy with ACTs.

Artemether-lumefantrine (AL) and dihydroartemisinin-piperaquine (DP) are two of the most important ACTs for the treatment of uncomplicated falciparum malaria. AL is highly efficacious and well-tolerated, and has been recommended by WHO as first line treatment for malaria since 2004. DP is a newer ACT that has proven to be equivalent to or more effective than other ACT regimens in clinical trials (1, 3, 28, 32) and is now recommended by WHO for use as a first line-treatment for *P. falciparum* malaria (22). The potential advantages of DP over AL are convenient once-a day dosing and a longer half-life (3-4 weeks) of the partner drug, piperaquine, compared with lumefantrine (~4 days), leading to a prolonged post-treatment prophylactic effect thus, reducing the risk of new infection. In this study, we evaluated the associations between measures of malnutrition and response to antimalarial therapy in a cohort

of young Ugandan children treated with DP or AL for repeated episodes of uncomplicated falciparum malaria.

MATERIALS AND METHODS

Study Area and Population

This study was conducted in rural eastern Uganda in the district of Tororo. Malaria transmission in this area is holoendemic, occurring perennially and with an entomological inoculation rate (EIR) estimated to be 562 infective bites per person-year (21). Study participants were part of a clinical trial designed to compare the efficacy of two ACT regimens, AL and DP, for the treatment of uncomplicated malaria in very young children. The clinical trial was part of a larger cohort study. The study protocol was approved by the Uganda National Council of Science and Technology and the institutional review boards of Makerere University, the University of California San Francisco, the US Centers for Disease Control and Prevention, and the University of Washington.

A full description of the study design has been presented elsewhere (1). Briefly, convenience sampling was used to enroll 100 HIV-unexposed children (born to HIV-uninfected mothers), 48 HIV-infected children, and 203 HIV-exposed children (HIV-uninfected born to HIV-infected mothers) between August 2007 and April 2008. Eligibility criteria included the following: (1) age 6 weeks to 12 months, (2) documented HIV status of mother and child, (3) agreement to return to the study clinic for any febrile episode or other illness, (4) agreement to avoid medications administered outside of the study protocol, (5) residence within a 30 km radius of the study clinic, (6) currently breastfeeding if HIV-exposed, and (7) parent/guardian provision of informed consent. All mother-child pairs received two long lasting insecticide treated bednets (ITNs), a safe water vessel, multivitamins, and condoms at the beginning of the study. All HIV-infected children received antiretroviral therapy (ART) consisting of nevirapine plus lamivudine plus zidovudine or stavudine, if eligible according to WHO criteria. All HIV-exposed children and HIV-infected children received daily trimethoprim-sulfamethoxazole (TS) prophylaxis. Following cessation of breastfeeding, HIV-exposed children who remained HIV-uninfected were randomized to continue or discontinue TS through 24 months of age. Children who were HIV-exposed and subsequently seroconverted continued TS prophylaxis.

Malaria diagnosis and treatment

Subjects were followed for all medical problems at a dedicated study clinic open 7 days a week. After hours care was available at the Tororo District Hospital, which provides service for the entire Tororo district area. Subjects who presented to the clinic with a fever (tympanic temperature $\geq$ 38.0˚C) or reported history of fever in the past 24 hours provided blood obtained by finger prick for a thick blood smear. If the thick blood smear was positive, the patient was diagnosed with malaria regardless of parasite density. All episodes of malaria were classified as

uncomplicated if the following criteria were met: fever ($\geq$ 38.0ºC tympanic) or history of fever in the previous 24 hours; positive thick blood smear; and absence of complicated malaria.

At the first diagnosis of uncomplicated malaria, study participants 4 months of age or older and at least 5 kg in weight were randomized to open-label treatment with AL or DP and received the same antimalarial treatment regimen for all subsequent episodes of uncomplicated malaria. A nurse administered study drugs according to weight-based guidelines as follows: AL (tablets of 20 mg of artemether and 120 mg of lumefantrine; Coartem; Novartis), administered as 1 (5-14 kg) or 2 (15-24 kg) tablets given twice daily for 3 days; and DP (tablets of 40 mg of dihydroartemisinin and 320 mg of piperaquine; Duocotecxin: Holley Pharm) targeting a total dose of 6.4 and 51.2 mg/kg of dihydroartemisinin and piperaquine, respectively, given as 3 equally divided doses to the nearest one-quarter tablet. Each dose was given once (for DP) or twice (for AL) a day over 3 days (days 0, 1 and 2). Patients were given a glass of milk or asked to breast-feed after each dose of study medication to optimize drug absorption. The first daily dose of study medication was administered in clinic and directly observed by a study nurse. Any patient who vomited the medication within 30 minutes of administration was retreated with a second dose.

Malaria Follow-up and Outcome Classification

Study participants diagnosed with malaria were asked to return to the clinic on days 1, 2, 3, 7, 14, 21, 28 or on any other day the parents thought the child was ill. Study participants who did not return for a scheduled visit were visited at home and, if necessary, transported to the study clinic. At these visits and on any unscheduled day when a fever was documented or reported in the previous 24 hours, blood was obtained by finger prick for thick blood smears and filter paper collection. Study participants were actively followed through day 28 and treatment outcomes were classified according to the 2006 WHO treatment guidelines (23). Study participants who took antimalarials outside of the protocol, were lost to follow up, or whose parent/guardian withdrew consent were not assigned a treatment outcome. Recurrent episodes of malaria recurring within 14 days of previous treatment were treated with quinine and recurrent episodes occurring more than 14 days after therapy were treated as a new episode. After 28 days of active follow-up, study participants were followed passively until their next episode of malaria or to the end of the observation period. This study includes all episodes of malaria diagnosed from the time of enrollment through August 2009.

Anthropometric Measurements

Anthropomorphic measurements were collected in accordance with internationally accepted practices on the day malaria was diagnosed. Weight was taken using a spring scale for younger children (up to approximately 1 year of age) or with a standing scale for older children (Seca, Hamburg, Germany), both precise to the nearest 100 grams. Recumbent length measurements were taken using a steidiometer for children up to approximately 1 year of age. After that age,

standing height measurements were taken. All length and height measurements were precise to the nearest 1 centimeter. Age was calculated using the date of birth of the child.

Laboratory Methods

Malaria Diagnosis: Thick and thin blood smears were stained with 2% Giemsa for 30 minutes and read by experienced laboratory technologists who were not involved in direct patient care. Parasite densities were calculated by counting the number of asexual parasites per 200 leukocytes (or per 500 leukocytes, if the count is <10 asexual parasites/200 leukocytes), assuming a leukocyte count of 8,000/μl. A blood smear was considered negative when the examination of 100 high power fields did not reveal asexual parasites. Thin smears were used for parasite species identification. For quality control, all slides were read by a second microscopist and a third reviewer settled any discrepant readings. Microscopists were blinded to the study participants' treatment assignments.

Molecular Genotyping: Parasite species on the day malaria was diagnosed were determined using nested polymerase chain reaction as described elsewhere (30). For recurrent episodes of parasitemia, molecular genotyping was used to distinguish new infections from recrudescent infections. DNA was recovered from blood spots, and samples were genotyped in a step-wise fashion with use of six polymorphic markers as described elsewhere (13). For any of the six loci, if an allele was not shared between consecutive episodes of parasitemia, the episode was classified as a new infection. If at least one allele was shared at all six loci, the episode was classified as a recrudescence.

Statistical Analysis

All analyses included patients with uncomplicated falciparum malaria and were stratified according to the treatment arm (AL or DP). The primary exposure variables of interest were measures of malnutrition classified according to height-for-age, and weight-for-age z-scores, using the 2006 WHO child growth standards. Because the thresholds for classifying nutritional status have not been universally defined, for the purpose of this analysis, height-for-age (HAZ) and weight-for-age (WAZ) z-scores were divided into four categories with the following cut-offs; ≥ 0, <0 and ≥ -1, <-1 and ≥ -2, and <-2. Comparisons of baseline characteristics were made using generalized estimating equations with adjustment for repeated measures in the same patient by using exchangeable correlation, binomial (for categorical variables) or Gaussian (for continuous variables) distribution, and robust standard errors.

Associations were evaluated between measures of malnutrition and two treatment outcomes: 1) parasite clearance at day 2 and day 3 and 2) the risk of recurrent parasitemia. Parasite clearance was defined as the proportion of patients with a positive blood slide 2 or 3 days following initiation of therapy and comparisons made using generalized estimating equations with adjustment for repeated measures in the same patient by using exchangeable correlation, binomial distribution, and robust standard errors. Recurrent parasitemia was defined

as any early treatment failure, a positive blood smear between 4 to 28 days of active follow-up, or malaria diagnosed between days 29-42 of passive follow-up. The risk of recurrent parasitemia was estimated using the Kaplan-Meier product limit formula with censoring for patients with incomplete follow-up. The risk of recrudescence after adjustment by genotyping was not evaluated because previously published data showed that this risk was less than 3% for both treatment arms (1). Measures of association between categories of malnutrition and the risk of recurrent parasitemia were made using Cox proportional hazards models, with inference adjusted for repeated measures (14) in the same patient and adjustment for potential confounders, including age, cumulative piperaquine or lumefantrine dose (dose provided over 3 days of dosing and based on mg/kg of body weight), place of residence, breastfeeding status, and ART use. In addition, Cox proportional hazard models were stratified by TS use because of the presence of significant interaction.

Data were double entered in ACCESS (Microsoft Corporation, Redmond, WA). Statistical analysis was performed using STATA, version 9.0 (Stata Corporation, College Station, TX). For all analyses, a *P* value (two-sided) of less than 0.05 was considered statistically significant.

RESULTS

Of the 351 participants enrolled in the study, 292 (83%) were diagnosed with at least one episode of uncomplicated malaria and randomized to therapy. Of these, 145 were randomized to DP and 147 were randomized to AL resulting in 981 and 1032 treatments for uncomplicated falciparum malaria, respectively, that were included in this study (Figure 1).

Demographic and anthropomorphic baseline characteristics of all episodes of uncomplicated falciparum malaria stratified by treatment are presented in Table 1. At the time of treatment, 90% of study participants resided in a rural area, approximately one-third of all participants were breastfeeding, 30 % were taking TS prophylaxis, 8.5% were HIV-infected and 92% of these were taking ARTs. Forty-three percent of the study participants had an HAZ z-score <-2 and 13% had a WAZ z-score of <-2, consistent with rates reported across Uganda (15). Baseline characteristics of all episodes of uncomplicated falciparum malaria stratified by nutritional status are presented in Tables 2 and 3. At the time of treatment with DP, age, breastfeeding status, use of TS prophylaxis, and ART use differed significantly between HAZ categories, and place of residence, HIV status, and cumulative piperaquine dose differed significantly between WAZ categories. At the time of treatment with AL, children with decreasing levels of HAZ and WAZ scores differed significantly in terms of age, breastfeeding status (HAZ score only), use of TS prophylaxis (WAZ only), and cumulative lumefantrine dose.

Effect of nutritional status on parasite clearance for AL and DP. The proportion of patients with a positive blood smear two days following initiation of therapy was lower in patients treated with DP compared to those treated with AL (5.0% vs. 10.0%, p<0.001). There were very few

patients with a positive blood smear three days following the initiation of therapy in either the DP or AL treatment arms (0.2% vs. 0.6%, p=0.18). There was no significant association between HAZ and WAZ scores and a positive blood smear two days following treatment with DP or AL (Table 4).

Effect of nutritional status on risk of recurrent parasitemia. The overall risk of recurrent parasitemia after 42 days of follow-up was 29% (95% CI 27 - 32%) and 54% (95% CI 51-57%) in study participants treated with DP and AL, respectively. During model fitting, concomitant use of TS prophylaxis was associated with a significantly lower risk of recurrent parasitemia (HR =0.57, p=0.001 and HR=0.66, p=0.002 for patients receiving DP and AL, respectively) and there was significant interaction between TS use and associations between measures of malnutrition and the risk of recurrent parasitemia. Therefore, each model was stratified by TS use.

In study participants not on TS prophylaxis treated with DP, the risk of recurrent parasitemia after 42 days of follow-up increased as HAZ score decreased (log rank test p=0.03, Figure 2). After controlling for age, place of residence, breastfeeding status, cumulative piperaquine dose received, and ART use, a decreasing HAZ score was independently associated with a higher risk of recurrent parasitemia (Table 5). However, statistical significance was reached only when comparing HAZ scores <-1 with those $\geq$ 0. There were no significant associations between HAZ scores and the risk of recurrent parasitemia among patients treated with DP and taking TS prophylaxis (Table 5). Similarly, there were no significant associations between WAZ scores and the risk of recurrent parasitemia among patients treated with DP, regardless of whether or not the patient was taking TS prophylaxis (Table 5).

In study participants not on TS prophylaxis treated with AL, the unadjusted risk of recurrent parasitemia after 42 days of follow up increased as HAZ scores decreased (log rank test p=0.05, Figure 2). After controlling for age, place of residence, breastfeeding status, cumulative lumefantrine dose received, and ART use, a decreasing HAZ score was independently associated with a higher risk of recurrent parasitemia, although statistical significance was not achieved. There were no significant associations between HAZ scores and the risk of recurrent parasitemia among patients taking TS prophylaxis and treated with AL (Table 6). As with DP, the WAZ score was not associated with recurrent parasitemia in those not taking TS prophylaxis. In study participants taking TS prophylaxis, there was an association of WAZ scores and recurrent parasitemia. However, this was only significant when comparing those with the lowest (<-2) and highest WAZ score (<0 and $\geq$ -1) to a WAZ score $\geq$ 0 (Table 6).

DISCUSSION

To our knowledge, this is the first longitudinal study assessing the effect of malnutrition on the post-treatment prophylactic effect of ACTs; thus, no direct comparisons to previous studies can be made. We evaluated patients prospectively, taking advantage of a comprehensive clinic infrastructure which provided assurance that all episodes of malaria were captured and followed

and compliance with the treatment regimen was high. Compared to other studies which evaluated this vulnerable patient population, our sample size of over 2000 malarial episodes is one of the largest published. In addition, this study utilized the new 2006 WHO growth standards which provide a more accurate tool for monitoring growth differences as they evaluate growth patterns from healthy breast-fed children from around the world (6). Our results indicate that in a high transmission setting, both AL and DP are efficacious antimalarial treatments for treatment in children under three years of age, regardless of nutritional status. Parasite clearance overall was excellent, with more than 99% of study participants clearing all primary parasites by day three. Recrudescence could not be directly evaluated as an outcome of this study due to low numbers (less than 3%), though the lack of recrudescence is further support of the efficacy of these two drug regimens. Children with signs of mild to moderate chronic malnutrition not taking TS prophylaxis were at higher risk of recurrent parasitemia. However, this was only significant in the DP group.

Although there are no published studies evaluating the relationship of malnutrition and recurrent parasitemia, a few studies have assessed the association between malnutrition and malaria risk. In a cross-sectional study in Kenya of 1862 children under 36 months of age, stunted children were more likely to have more parasitemia (OR=1.98) and clinical malaria (OR=2.65) than non-stunted children (11). Likewise, a prospective cohort study of 487 children under 5 in the Gambia found that stunted children were at a higher risk of malaria (RR=1.35) than non-stunted children (7). Contrary to our findings, a prospective cohort study of 136 children 4 months to 10 years of age in Papua New Guinea found the incidence rate of malaria (of any type, as well as *P. falciparum* alone) increased with increasing HAZ (12), indicating that lower HAZ was protective against an attack of clinical malaria. Two longitudinal studies, one in Senegal in children 12 months to 5 years of age and the other in Burkina Faso in children 10 months to 10 years of age, found stunting and underweight were not associated with an increased risk of *P. falciparum* malaria (9, 19). There may be several explanations for the conflicting findings. The study conducted in Kenya was conducted in children of a similar age range to the children in the Tororo study, while the studies conducted in Papua New Guinea and Burkina Faso were conducted in older children. Moreover, the study in Kenya was conducted in an area of high transmission (60 to 300 infective bites per person per year) whereas the studies which found compromised nutritional status to be protective or to have no effect on malaria risk were conducted in areas where malaria transmission occurred seasonally with lower transmission rates than in the Tororo district. Both the differences in age and transmission intensity may lead to differences in acquired immunity and thus differences in malaria risk.

The mechanism behind the increased risk of recurrent parasitemia in children with signs of mild to moderate chronic malnutrition is unclear, but is likely due in part to the impact of chronic malnutrition on the immune system. Chronic malnutrition and accompanying micronutrient deficiencies (e.g., zinc, magnesium, iron, selenium, and vitamin A) can lead to immune dysfunction and increased infection in children by impairing both the innate and adaptive immune system, affecting thymic activity and cytokine production; impairing T cell response and macrophage activation; and disrupting IgA and IgG antibody response (4, 29).

Results from the few studies evaluating the relationship between malnutrition and immune response in children with malaria have been conflicting. A cross-sectional study in preschool aged children conducted in Senegal found that IgG antibody levels were significantly lower in stunted children compared to controls, regardless of differences in parasite density (10). In contrast, a study of children through 10 years of age conducted in Papua New Guinea found an increase in cytokine production in response to stimulation by specific antimalarial antigens in undernourished (stunted and wasted) children and a decrease in antibody response in wasted children (12). Future studies evaluating the effect of malnutrition on immune response are warranted. In addition to altering immune function, malnutrition may have an impact on the pharmacokinetics of antimalarial treatment. Total body water has been shown to be increased in malnourished children, leading to a greater volume of distribution of drugs, which in turn would result in lower blood concentrations of drug. In addition, malnutrition is associated with intestinal malabsorption and villous atrophy of the jejunal mucosa which can impair drug absorption (26). The few pharmacokinetic studies conducted in children have indicated that because of differences in drug metabolism and elimination, children may be receiving suboptimal doses of antimalarial drugs (27, 31). Additional analysis of data from a subset of this patient population, including complete pharmacokinetic profiles, is currently underway.

Interestingly, the association of increased risk of recurrent parasitemia with decreasing HAZ was evident only for children not taking TS prophylaxis. TS is an antifolate which has been associated with reduced morbidity and mortality in HIV-infected children and adults. It also has antimalarial properties and has been shown to reduce the incidence of malaria, both alone and in combination with ARTs and ITNs, even in areas of high parasite resistance to antifolates (16-18). Moreover, TS has been shown to be as effective as sulfadoxine-pyrimethamine for treatment of *P. falciparum* malaria (8). Perhaps the chronic use of TS, a moderately effective antimalarial, acts synergistically with the administration of a relatively more potent artemisinin-based treatment to override the deleterious effect of chronic malnutrition on the immune system. TS is easy to administer, with treatment once a day or thrice weekly for prophylaxis, is widely available, and relatively inexpensive. The results from this study indicate that children with mild to moderate signs of chronic malnutrition at risk for malaria may benefit from TS prophylaxis.

Limitations of this study should be considered. First, we may have not controlled for all potential confounders that may be involved in the complex relationship between malnutrition and malaria. In addition, we made multiple comparisons evaluating the relationship between malnutrition and the risk of recurrent parasitemia, which could potentially lead to spurious findings. Only by comparing the lowest levels of malnutrition to the baseline group was statistical significance achieved. Second, as with any study which utilizes cut-points for a continuous exposure (HAZ and WAZ scores), children may have been placed into the wrong exposure category, thus biasing the outcome. However, because the direction of bias could be either towards or away from the null, if incorrect categorization did occur, it is probable that it occurred at equal rates between categories and is unlikely to affect the outcome. Third, the causal effect of malnutrition on risk of recurrent parasitemia could not be determined. This study

was observational in nature; children cannot be randomized to their exposure, which in this case was level of malnutrition. Where the conduct of an RCT is not possible, causal methods can be employed to overcome the lack of exchangeability inherent in observational studies. We plan to use causal methods in a future analysis. Finally, the mechanisms underlying the differences in risk of recurrent parasitemia in children with mild to moderate chronic malnutrition could not be elucidated.

Conclusion: AL and DP are effective antimalarial therapies for clearing primary infection in chronically malnourished young children in a high transmission setting. However, young children with signs of mild to moderate chronic malnutrition not taking TS prophylaxis are at increased risk of recurrent parasitemia. Further studies are warranted to evaluate if this risk is mediated by altered drug metabolism in chronically malnourished children or through differences in immune response. Although WHO currently recommends the use of TS prophylaxis in HIV-infected adults and children in resource limited settings, TS prophylaxis could be considered in chronically malnourished children at high risk of malaria, regardless of HIV status.

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TABLES

Table 1. Demographic and anthropomorphic characteristics of all episodes of uncomplicated falciparum malaria treated with DP and AL.

	DP **N=981**	**AL** **N=1032**
Age in months, mean (SD)	18.2 (6.3)	18.4 (6.3)
Age categories		
Age ≤ 12 months, n (%)	197 (20%)	198 (19%)
Age > 12 months ≤ 18 months, n (%)	278 (28%)	286 (28%)
Age > 18 months ≤ 24 months, n (%)	298 (30%)	330 (32%)
Age > 24 months, n (%)	208 (21%)	218 (21%)
Rural (v. urban) residence, n (%)	869 (89%)	950 (92%)
Breastfeeding, n (%)	334 (34%)	314 (30%)
HIV-infected, n (%)	71 (7.2%)	101 (9.8%)
Taking TS prophylaxis, n (%)	276 (28%)	333 (32%)
Taking ARTs, n (%)	64 (6.5%)	94 (9.1%)
Cumulative piperaquine dose (mg), mean (SD)	57.8 (8.1)	
Cumulative lumefantrine dose (mg), mean (SD)		149.0 (27.3)
Height-for-age (HAZ) z-score categories, n (%)		
HAZ score ≥ 0	50 (5.0%)	57 (5.5%)
HAZ score <0 and ≥ -1	176 (18%)	208 (20%)
HAZ score <-1 and ≥ -2	290 (30%)	376 (36%)
HAZ score <-2	465 (47%)	391 (38%)
Weight-for-age (WAZ) z-score categories, n (%)		
WAZ score ≥ 0	229 (23%)	319 (31%)
WAZ score <0 and ≥ -1	318 (32%)	362 (35%)
WAZ score <-1 and ≥ -2	260 (27%)	256 (25%)
WAZ score <-2	174 (18%)	95 (9.0%)

Table 2. Baseline characteristics of all episodes of uncomplicated falciparum malaria treated with DP stratified by nutritional status.

	HAZ Score				
	HAZ ≥ 0 (n=50)	**HAZ <0 and ≥ -1 (n=176)**	**HAZ <-1 and ≥ -2 (n=290)**	**HAZ <-2 (n=465)**	**p-value**
Age in months, mean (SD)	15.0 (8.2)	17.2 (7.0)	17.9 (6.3)	19.2 (6.0)	<0.001
Rural (v. urban) residence, n (%)	46 (92%)	147 (84%)	249 (86%)	427 (92%)	0.227
Breastfeeding, n (%)	22 (44%)	69 (39%)	101 (35%)	142 (31%)	0.002
HIV-infected, n (%)	3 (6.0%)	8 (4.6%)	12 (4.1%)	48 (10%)	0.151
Taking TS prophylaxis, n (%)	19 (38%)	54 (31%)	74 (26%)	129 (28%)	0.016
Taking ARTs, n (%)	2 (4.0%)	7 (4.0%)	9 (3%)	45 (9.7%)	0.023
Cumulative piperaquine dose (mg), mean (SD)	58.1 (6.6)	57.6 (6.7)	57.9 (7.6)	57.8 (8.9)	0.131
	WAZ Score				
	WAZ ≥ 0 (n=229)	**WAZ <0 and ≥ -1 (n=318)**	**WAZ <-1 and ≥ -2 (n=260)**	**WAZ <-2 (n=174)**	**p-value**
Age in months, mean (SD)	19.2 (6.3)	18.0 (18.2)	17.7 (5.9)	18.2 (6.8)	0.062
Rural (v. urban) residence, n (%)	179 (78%)	283 (89%)	250 (96%)	157 (90%)	0.007
Breastfeeding, n (%)	79 (35%)	100 (31%)	92 (35%)	63 (36%)	0.590
HIV-infected, n (%)	11 (4.8%)	16 (5.0%)	17 (6.5%)	27 (16%)	0.041
Taking TS prophylaxis, n (%)	66 (29%)	98 (31%)	70 (27%)	42 (24%)	0.885
Taking ARTs, n (%)	10 (4.4%)	16 (5.0%)	14 (5.4%)	23 (13%)	0.626
Cumulative lumefantrine dose (mg), mean (SD)	57.0 (6.0)	56.6 (7.2)	56.3 (7.0)	63.3 (10.8)	<0.001

Table 3. Baseline characteristics of all episodes of uncomplicated falciparum malaria treated with AL stratified by nutritional status.

	HAZ Score				
	HAZ ≥ 0 (n=57)	**HAZ <0 and ≥ -1 (n=208)**	**HAZ <-1 and ≥ -2 (n=376)**	**HAZ <-2 (n=391)**	**p-value**
Age in months, mean (SD)	14.4 (7.3)	18.6 (7.3)	18.3 (6.2)	19.1 (5.4)	<0.001
Rural (v. urban) residence, n (%)	48 (84%)	193 (93%)	350 (93%)	359 (92%)	0.238
Breastfeeding, n (%)	24 (42%)	70 (34%)	129 (34%)	91 (23%)	0.001
HIV-infected, n (%)	11 (19%)	35 (17%)	27 (7.2%)	28 (7.2%)	0.102
Taking TS prophylaxis, n (%)	29 (51%)	71 (34%)	94 (25%)	139 (36%)	0.075
Taking ARTs, n (%)	9 (16%)	31 (15%)	27 (7.2%)	27 (6.9%)	0.357
Cumulative piperaquine dose (mg), mean (SD)	148.3 (35.2)	139.5 (28.4)	144.4 (23.8)	158.4 (25.8)	0.048
	WAZ Score				
	WAZ ≥ 0 (n=319)	**WAZ <0 and ≥ -1 (n=362)**	**WAZ <-1 and ≥ -2 (n=256)**	**WAZ <-2 (n=95)**	**p-value**
Age in months, mean (SD)	19.2 (6.4)	18.0 (6.6)	18.7 (5.9)	17.0 (5.2)	0.006
Rural (v. urban) residence, n (%)	298 (93%)	332 (92%)	238 (93%)	82 (86%)	0.406
Breastfeeding, n (%)	106 (33%)	117 (32%)	71 (28%)	20 (21%)	0.695
HIV-infected, n (%)	30 (9.4%)	50 (14%)	13 (5.1%)	8 (8.4%)	0.219
Taking TS prophylaxis, n (%)	89 (28%)	120 (33%)	74 (29%)	50 (53%)	0.030
Taking ARTs, n (%)	30 (9.4%)	44 (12%)	12 (4.7%)	8 (8.4%)	0.516
Cumulative lumefantrine dose (mg), mean (SD)	128.4 (20.1)	148.4 (21.8)	161.3 (20.2)	186.8 (24.4)	<0.001

Table 4. Associations between measures of malnutrition and parasite clearance at day 2 following therapy with DP or AL.

HAZ Score	Proportion with Positive Blood Smear (%)	Odds Ratio (95% CI)[1]	p-value	WAZ Score	Proportion with Positive Blood Smear (%)	Odds Ratio (95% CI)[1]	p-value
Treatment with DP							
HAZ ≥ 0 (n=50)	8.0%	1.00 (ref)	-	WAZ ≥ 0 (n=229)	6.1%	1.00 (ref)	-
HAZ <0 and ≥ -1 (n=176)	5.1%	0.62 (0.21-1.83)	0.386	WAZ <0 and ≥ -1 (n=318)	6.0%	0.97 (0.50-1.87)	0.934
HAZ <-1 and ≥ -2 (n=290)	4.1%	0.50 (0.15-1.64)	0.253	WAZ <-1 and ≥ -2 (n=260)	3.9%	0.61 (0.30-1.24)	0.171
HAZ <-2 (n=465)	5.2%	0.64 (0.22-1.80)	0.395	WAZ <-2 (n=174)	3.5%	0.54 (0.21-1.41)	0.211
Treatment with AL							
HAZ ≥ 0 (n=57)	5.3%	1.00 (ref)		WAZ ≥ 0 (n=319)	8.5%	1.00 (ref)	
HAZ <0 and ≥ -1 (n=208)	13%	4.19 (1.08-16.20)	0.038	WAZ <0 and ≥ -1 (n=362)	12%	1.31 (0.70-2.44)	0.404
HAZ <-1 and ≥ -2 (n=376)	7.5%	2.42 (0.64-9.20)	0.195	WAZ <-1 and ≥ -2 (n=256)	11%	1.20 (0.65-2.21)	0.557
HAZ <-2 (n=391)	13%	4.70 (1.25-17.64)	0.022	WAZ <-2 (n=95)	14%	1.76 (0.85-3.65)	0.128

[1] Adjusted for repeated measures in same patient

Table 5. Associations between measures of malnutrition and recurrent parasitemia following therapy with DP after 42 days of follow-up

Measure of Malnutrition	Not on TS Hazard Ratio[1] (95% CI)	p-value	Cumulative Risk of Recurrent Parasitemia[2]	On TS Hazard Ratio[1] (95% CI)	p-value	Cumulative Risk of Recurrent Parasitemia[2]
Height-for-Age Z-scores						
HAZ ≥ 0	1.00	-	13%	1.00	-	22%
HAZ ≥ -1 - < 0	2.35 (0.85-6.48)	0.099	28%	1.15 (0.46-2.91)	0.765	23%
HAZ ≥ -2 - < -1	2.89 (1.06-7.89)	0.039	33%	0.58 (0.20-1.68)	0.319	12%
HAZ <-2	3.18 (1.18-8.56)	0.022	36%	1.01 (0.30-3.40)	0.993	23%
Weight-for-Age Z-scores						
WAZ ≥ 0	1.00	-	36%	1.00	-	17%
WAZ ≥ -1 - < 0	0.65 (0.37-1.15)	0.137	27%	1.46 (0.83-2.58)	0.192	24%
WAZ ≥ -2 - < -1	0.86 (0.45-1.62)	0.636	35%	1.05 (0.46-2.37)	0.908	21%
WAZ <-2	1.01 (0.54-1.89)	0.969	35%	1.13 (0.33-3.89)	0.844	15%

[1] Adjusted for age, residence, cumulative piperaquine dose (mg), breastfeeding status, ART status (on TS only), and for repeated measures in same patient

[2] Unadjusted

Table 6. Associations between measures of malnutrition and recurrent parasitemia following therapy with AL after 42 days of follow-up.

Measure of Malnutrition	Not on TS Hazard Ratio[1] (95% CI)	p-value	Cumulative Risk of Recurrent Parasitemia[2]	On TS Hazard Ratio[1] (95% CI)	p-value	Cumulative Risk of Recurrent Parasitemia[2]
Height-for-Age Z-scores						
HAZ ≥ 0	1.00	-	37%	1.00	-	50%
HAZ ≥ -1 - < 0	1.55 (0.76-3.17)	0.232	55%	1.15 (0.69-1.90)	0.588	61%
HAZ ≥ -2 - < -1	1.68 (0.77-3.66)	0.194	58%	0.62 (0.33-1.17)	0.143	37%
HAZ <-2	1.98 (0.93-4.22)	0.077	64%	0.71 (0.41-1.22)	0.209	39%
Weight-for-Age Z-scores						
WAZ ≥ 0	1.00	-	59%	1.00	-	47%
WAZ ≥ -1 - < 0	0.97 (0.69-1.34)	0.837	54%	1.62 (1.03-2.54)	0.038	48%
WAZ ≥ -2 - < -1	1.47 (0.89-2.43)	0.131	69%	1.61 (0.82-3.13)	0.164	35%
WAZ <-2	0.94 (0.43-2.06)	0.873	44%	3.60 (1.26-10.27)	0.017	46%

[1] Adjusted for age, residence, cumulative piperaquine dose (mg), breastfeeding status, ART status (on TS only), and for repeated measures in same patient

[2] Unadjusted

FIGURES

Figure 1. Trial Profile.

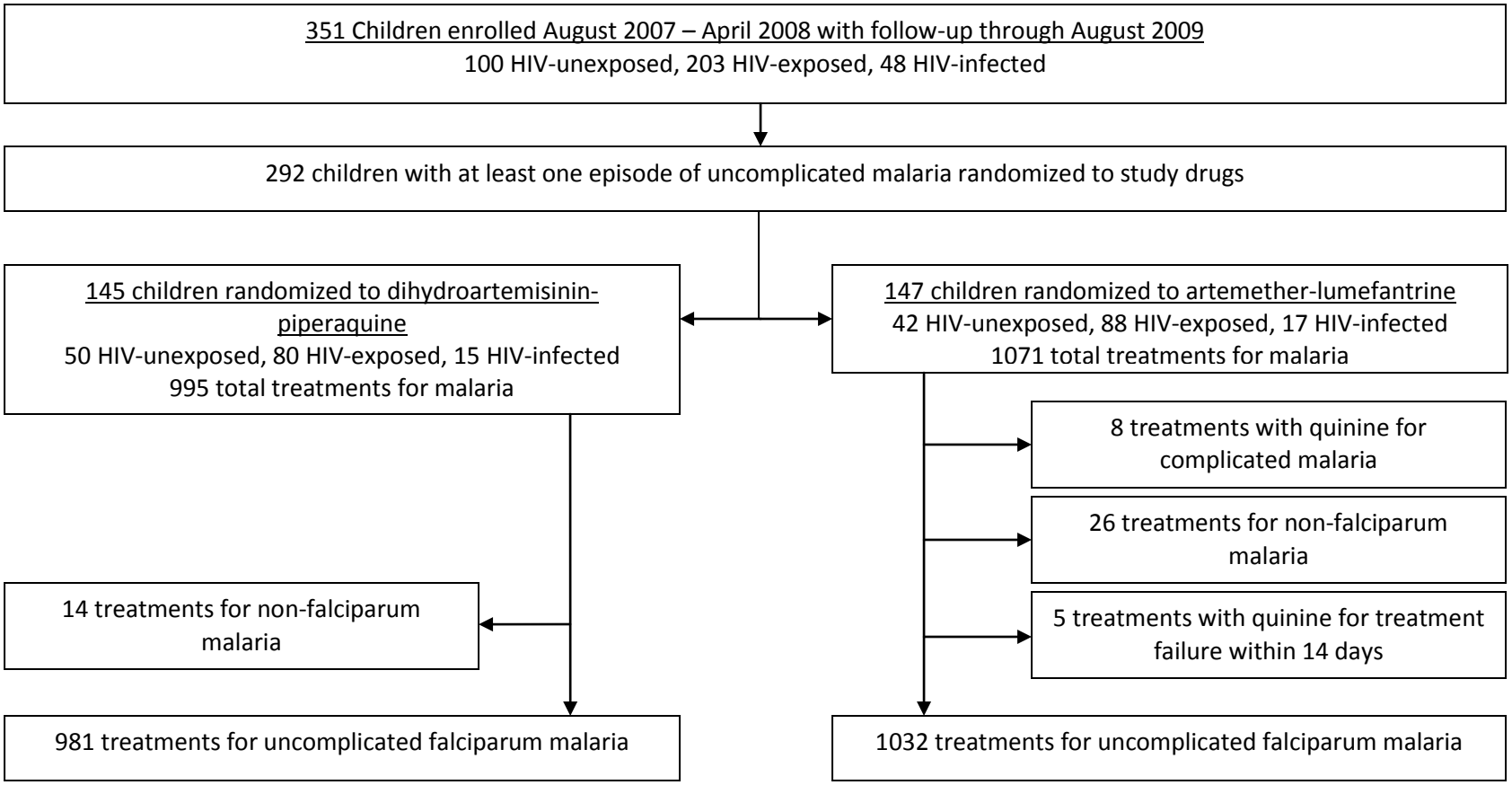
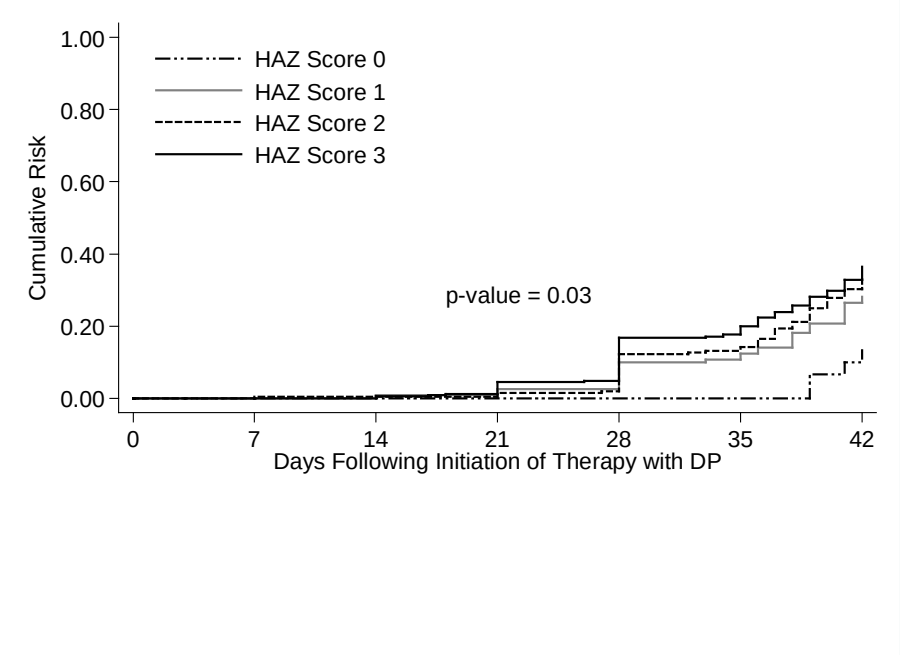
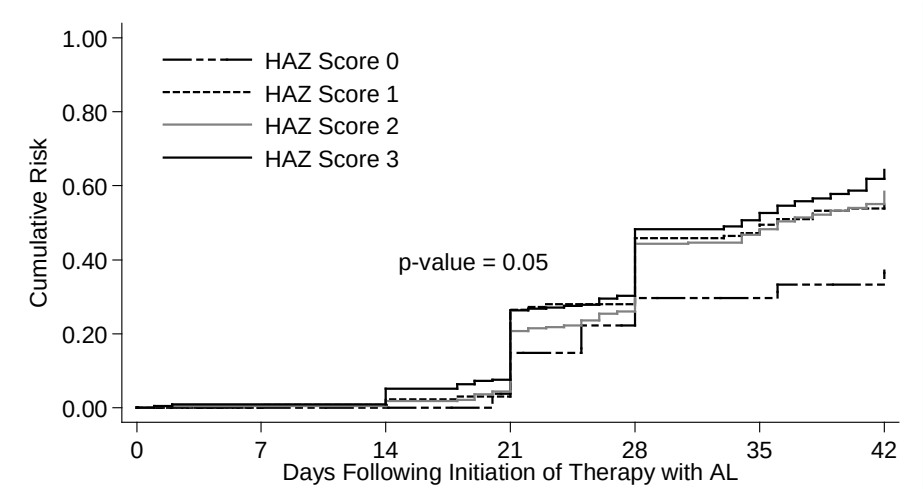


Figure 2. Cumulative risks of recurrent parasitemia stratified by HAZ following treatment with AL or DP using the Kaplan-Meier product limit formula.





HAZ Score 0 = HAZ score ≥ 0, HAZ score 1 = HAZ score <0 and ≥ -1;
HAZ score 2 = HAZ score <-1 and ≥ -2; HAZ score 3 = HAZ score <-2

CHAPTER 4

Effect of Nutritional Status on the Pharmacokinetics of Piperaquine and Lumefantrine Following Treatment with Dihydroartemisinin-Piperaquine or Artemether-Lumefantrine in Young Ugandan Children with Malaria

ABSTRACT

Background: Artemisinin-combination therapies **(**ACTs) are recommended as first line treatment for falciparum malaria in both adults and children. Although the pharmacokinetics (PK) of ACTs in the very young and in the malnourished may be different from that of adults, dosages are deduced from adult-based regimens without consideration for age or nutritional status. We evaluated the impact of stunting and underweight on the PK of lumefantrine and piperaquine, the partner drugs for the ACTs dihydroartemisinin-piperaquine (DP) and artemether-lumefantrine (AL).

Methods: Children randomized to DP or AL were followed prospectively for multiple episodes of malaria. PK samples were collected from a subset of patients ages 6 months to 2 years. Primary exposure variables included stunting, defined as height-for-age z-score of <-2 and underweight defined as a weight-for-age z-score of <-2. Associations were evaluated between these measures of malnutrition and differences in piperaquine concentrations at day 3, 7, and 14, differences in lumefantrine concentrations at day 3 and 7, and differences in apparent clearance of piperaquine (CL/f_{pip}) using generalized estimating equations.

Results: PK samples were collected from 106 patients treated with DP and 101 patients treated with AL providing a total of 214 treatments for DP and 243 treatments for AL for PK analysis. Stunting was significantly associated with day 3 piperaquine levels with stunted children more likely to have lower median day 3 levels than non-stunted children (OR=0.78, p=0.007). Stunting was not associated with day 7 or 14 piperaquine levels or with day 3 or 7 lumefantrine levels. Underweight was not associated with piperaquine or lumefantrine levels on any sampling day. Stunting was associated with CL/f_{pip} (OR=1.32, p=0.001) with stunted children having higher CL/f_{pip} than non-stunted children (1.63 l/hr/kg vs 1.34 liters/hr, p<0.001).

Conclusion: Stunting or underweight do not effect piperaquine or lumefantrine levels at day 7 – an important determinant for treatment response. Stunting does have an impact on day 3 piperaquine levels and stunted children have higher clearance rates than non-stunted children, perhaps indicating impaired absorption in chronically malnourished children.

BACKGROUND

In the last decade, antimalarial treatment strategies have changed radically with the introduction of artemisinin-based combination therapies (ACTs). The WHO now recommends ACTs as first line treatment for malaria in adults and children worldwide [1]. ACTs are three-day treatments which combine a short-acting, highly effective and rapidly eliminated artemisinin derivative (e.g. artemether or dihydroartemisinin) that quickly reduces parasite burden by 10,000-fold per reproductive cycle with a longer-acting partner drug (e.g., lumefantrine or piperaquine) which removes residual parasites [1]. The benefits of ACT regimens are two-fold: 1) the use of two efficacious antimalarial treatments confers mutual protection against parasite resistance and 2) ACTs reduce gametocyte carriage translating to a reduction of transmissibility and overall burden of malaria, particularly in areas of low to moderate transmission [1, 2]. Artemether-lumefantrine (AL) and dihydroartemisinin-piperaquine (DP) are two of the most important ACTs for the treatment of uncomplicated falciparum malaria with AL currently recommended as first line treatment in 48 countries, including 28 in Africa [3]. DP is a newer ACT, proven to be equivalent to or more effective than other ACT regimens in clinical trials [4-7] and is also recommended by the WHO as a first line-treatment [1].

Although the WHO recognizes that the pharmacokinetics (PK) of ACTs [1] in very young children and in the malnourished may be different from that of adults, dosing guidelines are deduced from adult-based regimens and adjusted for body weight only without consideration for age or nutritional status. In Africa, malnutrition is highly prevalent; 39%, 8%, and 28% of children are stunted (height for age z-score<-2), wasted (weight-for-height z-score<-2), or underweight (weight-for-age z-score<-2) respectively [8] and malnutrition and malaria frequently coexist [1]. Despite reported differences in drug absorption, metabolism, and elimination of other antimalarials such as quinine and chloroquine in malnourished children [9-12], to our knowledge, there are no PK studies of ACTs previously conducted in very young malnourished children. The few PK studies conducted in children treated with AL or DP have indicated children may be receiving suboptimal doses of antimalarials, presumably due to differences in drug disposition [13-16].

The purpose of this study is to assess the impact of two indicators of malnutrition, stunting and underweight, on the pharmacokinetic exposure of lumefantrine and piperaquine. This study was carried out in a large cohort of children in the Tororo District of Uganda, a region with the second highest malaria transmission intensity in the world [17].

METHODS

Study Area and Population

This study was conducted in rural eastern Uganda in the district of Tororo. Malaria transmission in this area is holoendemic, occurring perennially and with an entomological inoculation rate (EIR) estimated to be 562 infective bites per person-year [17]. Study participants were part of a

clinical trial designed to compare the efficacy of two ACT regimens, AL and DP, for the treatment of uncomplicated malaria in very young children. The clinical trial was part of a larger cohort study. The study protocol was approved by the Uganda National Council of Science and Technology and the institutional review boards of Makerere University, the University of California San Francisco, the US Centers for Disease Control and Prevention, and the University of Washington.

A full description of the study design has been presented elsewhere [7]. Briefly, convenience sampling was used to enroll 100 HIV-unexposed children (born to HIV-uninfected mothers), 48 HIV-infected children, and 203 HIV-exposed children (HIV-uninfected born to HIV-infected mothers) age 6 weeks to 12 months between August 2007 and April 2008. Parental/guardian informed consent was provided for all patients enrolled. All HIV-infected children received antiretroviral therapy (ART) consisting of nevirapine plus lamivudine plus zidovudine or stavudine, if eligible according to WHO criteria. All HIV-exposed children and HIV-infected children received daily trimethoprim-sulfamethoxazole (TS) prophylaxis. Following cessation of breastfeeding, HIV-exposed children who remained HIV-uninfected were randomized to continue or discontinue TS through 24 months of age. Children who were HIV-exposed and subsequently seroconverted continued TS prophylaxis.

Malaria diagnosis and treatment

Subjects were followed for all medical problems at a dedicated study clinic open 7 days a week. Subjects who presented to the clinic with a fever (tympanic temperature $\geq$ 38.0˚C) or reported history of fever in the past 24 hours provided blood obtained by finger prick for a thick blood smear. If the thick blood smear was positive, the patient was diagnosed with malaria regardless of parasite density. All episodes of malaria were classified as uncomplicated if the following criteria were met: fever ($\geq$ 38.0ºC tympanic) or history of fever in the previous 24 hours; positive thick blood smear; and absence of complicated malaria.

At the first diagnosis of uncomplicated malaria, study participants 4 months of age or older and at least 5 kg in weight were randomized to open-label treatment with AL or DP and received the same antimalarial treatment regimen for all subsequent episodes of uncomplicated malaria. A nurse administered study drugs according to weight-based guidelines as follows: AL (tablets of 20 mg of artemether and 120 mg of lumefantrine; Coartem; Novartis), administered as 1 (5-14 kg) or 2 (15-24 kg) tablets given twice daily for 3 days; and DP (tablets of 40 mg of dihydroartemisinin and 320 mg of piperaquine; Duocotecxin: Holley Pharm) targeting a total dose of 6.4 and 51.2 mg/kg of dihydroartemisinin and piperaquine, respectively, given as 3 equally divided doses to the nearest one-quarter tablet. Each dose was given once (for DP) or twice (for AL) a day over 3 days (days 0, 1 and 2). All patients were given a glass of milk or asked to breast-feed after each dose of study medication to optimize drug absorption. The first daily dose of study medication was administered in clinic and directly observed by a study nurse. Any patient who vomited the medication within 30 minutes of administration was retreated with a second dose.

Malaria Follow-up

Study participants diagnosed with malaria were asked to return to the clinic on days 1, 2, 3, 7, 14, 21, 28 or on any other day the parents thought the child was ill. At these visits and on any unscheduled day when a fever was documented or reported in the previous 24 hours, blood was obtained by finger prick for thick blood smears and filter paper collection. Study participants were actively followed through day 28 and treatment outcomes were classified according to the 2006 WHO treatment guidelines [18]. After 28 days of active follow-up, study participants were followed passively until their next episode of malaria.

Pharmacokinetic Sub-study Design

Starting in June 2008, any child who presented to the study clinic, was enrolled in the parent cohort study, and who was diagnosed with malaria was asked to participate in the PK sub-study. There were no refusals to participate. The study team aimed to collect 200 malaria episodes for each study treatment which was considered sufficient to ensure optimal precision in estimating the PK profile based on previous modeling exercises [19]. PK samples were collected on day 0, 2, 3, 7, 14, and 21, for DP and on days 0, 2, 3, 7, and 14 for AL. Subsequent to the start of the PK sampling, additional samples were collected for day 28 for patients randomized to DP. For the purpose of this analysis, only samples for day 3, 7, and 14 for DP and days 3 and 7 for AL were used. PK sampling for DP was completed in October 2008 and sampling for AL was completed in December 2008.

Anthropometric Measurements

Anthropomorphic measurements were collected in accordance with internationally accepted practices on the day malaria was diagnosed. Weight was taken using a spring scale for younger children (up to approximately 1 year of age) or with a standing scale for older children (Seca, Hamburg, Germany), both precise to the nearest 100 grams. Recumbent length measurements were taken using a steidiometer for children up to approximately 1 year of age. After that age, standing height measurements were taken. All length and height measurements were precise to the nearest 1 centimeter. Age was calculated using the date of birth of the child.

Laboratory Methods

Malaria Diagnosis: Thick and thin blood smears were stained with 2% Giemsa for 30 minutes and read by experienced laboratory technologists who were not involved in direct patient care. Parasite densities were calculated by counting the number of asexual parasites per 200 leukocytes (or per 500 leukocytes, if the count is <10 asexual parasites/200 leukocytes), assuming a leukocyte count of 8,000/μl. A blood smear was considered negative when the examination of 100 high power fields did not reveal asexual parasites. Thin smears were used for parasite species identification. For quality control, all slides were read by a second microscopist and a third reviewer settled any discrepant readings. Microscopists were blinded to the study participants' treatment assignments.

Piperaquine Sampling and Assay: Piperaquine PK samples were collected on days 0 (pretreatment/baseline), 3, 7, and 14. 125 to 200 μl of whole blood was obtained by finger prick and collected in a heparinized microtube, placed into an eppindorf, centrifuged, and the resulting plasma stored in liquid nitrogen. Samples were subsequently transferred to Mahidol Oxford Clinical Research Unit (MORU) for analysis. Plasma samples (50 μl were analyzed for piperaquine by a high throughput method using liquid chromatography/tandem mass spectrometry (LC tandem MS) and stable isotope labeled piperaquine was used as the internal standard as described previously [20]. The system used was the Agilent 1200 consisting of a binary LC pump, a vacuum degasser, a temperature-controlled micro-well plate autosampler set at 20° C, and a thermostatted column compartment set at 20° C (Agilent Technologies, Santa Clara, USA). Data acquisition and quantification were performed using Analyst 1.4 Applied Biosystems/MDS SCIEX, Foster City, USA). This method provided a lower limit of quantification (LOQ) set to 1.50 ng/ml.

Lumefantrine Sampling and Assay: Lumefantrine PK samples were collected on days 0 (pretreatment/baseline), 3, and 7. 100 μl of whole blood was obtained by fingerprick and collected on Whatman 31 ET Chr sampling paper (Whatman International, Maidstone, UK) pre-treated with 0.75 M tartaric acid (Fluka) and stored at room temperature. Samples were subsequently transferred to Uppsala University, Uppsala, Sweden for analysis. Lumefantrine was extracted from the sampling paper, then further purified using solid phase extraction and quantified with high performance liquid chromatography (HPLC) as described previously [21]. This method provided a lower LOQ of 0.25 μM and is validated according to the current FDA guideline for bioanalytical method validation (2001 U.S. Department of Health and Human Services).

Statistical Analysis

All analyses included patients with uncomplicated falciparum malaria and were stratified according to the treatment arm (AL or DP). The primary exposure variables of interest were measures of malnutrition classified according to height-for-age, and weight-for-age z-scores using the 2006 World Health Organization child growth standards. Stunting was defined as a height-for-age z-score (HAZ score) of <-2 and underweight as a weight-for-age z-score (WAZ score) of <-2. Wasting (weight-for-height z-score of <-2) was not evaluated in this analysis as less than 2% of the population was classified as wasted. Pairwise comparisons of categorical variables for each nutritional indicator were made using generalized estimating equations with adjustment for repeated measures in the same patient by using exchangeable correlation, binomial distribution, and robust standard errors [22] or in cases of non-convergence, with ordered logistic regression with individuals representing the clusters to simulate adjustment for repeated measures. Means of continuous variables were compared using generalized estimated equations as described above with Gaussian distribution and medians were compared using simultaneous quantile regression with inference derived using clustered nonparametric bootstrap with individuals representing the clusters to simulate adjustment for repeated measures. Univariate comparisons were made between selected baseline characteristics (including stunting

and underweight) and median piperaquine and lumefantrine levels using simultaneous quantile regression as described above with inference derived using clustered nonparametric bootstrap with individuals representing the clusters to simulate adjustment for repeated measures

Associations were evaluated between measures of malnutrition and 1) differences in piperaquine levels at day 3, 7, and 14; 2) differences in lumefantrine levels at day 3 and 7; and 3) differences in apparent clearance of piperaquine (CL/f_{pip}) (since DP was orally administered). *CL/f* of lumefantrine was unavailable for this analysis. Piperaquine and lumefantrine levels were evaluated as continuous variables and were log transformed to normalize distributions.

CL/f_{pip} was modeled with a Nonlinear Mixed Effect Model using NONMEM, version VI software (Icon Development Solutions, Maryland USA). The model was fit using the following equation: $CL_{typical}=12.5(AGE/466)^{0.684}$ with interindividual and interocassion variability modeled using a multiplicative exponential random effects model: CLi=CLtypical*(EXP(ETA(3)i+OCC1*ETA(5)i+OCC2*ETA(6)i+OCC3*ETA(7)i+OCC4*ETA(8)i) where ETA(3)i is the between-subject random effect accounting for the *i*th individual's deviation from the typical value having a mean of zero and a variance of w^2 and ETA(5)i to ETA(8)i is the inter-occasion random effect accounting for the *i*th individual's deviation from the typical value having a mean of zero and a variance of w^2. Residual variability was modeled using the log-transformed error model: $\ln(Y_{ij}) = \ln(F_{ij}) + EPS_{ij}$ where Y_{ij} is the observed concentration for the *i*th individual at time t_j, F_{ij} is the corresponding model-predicted concentration, and e_{ij} is the within-subject (residual) random effect, assumed to have mean of zero and a variance of s^2.

All comparisons were made using generalized estimating equations with adjustment for potential confounders and for repeated measures in the same patient by using exchangeable correlation, Gaussian distribution, and robust standard errors [22]. Potential confounding variables considered for each model included age, breastfeeding status, gender, TS use, ART use, place of residence (rural vs. urban), cumulative dose of piperaquine or lumefantrine, and piperaquine or lumefantrine concentration at baseline (day 0). Age was categorized as ≤ 12 months, > 12 to 18 months, and > 18 months. Cumulative doses of piperaquine and lumefantrine (provided over 3 days of dosing and based on mg/kg of body weight) were categorized based on cut-points corresponding to one SD interval away from the respective population mean of each drug. Models were fit separately for each comparison. All potential explanatory variables were included in each initial model and backwards selection procedures were used to remove variables that were not significant. Age, cumulative piperaquine or lumefantrine dose, and piperaquine or lumefantrine concentrations at baseline were forced into each model evaluating piperaquine and lumefantrine levels, respectively, and age and cumulative piperaquine dose were forced into the models for CL/f_{pip}.

Data was double entered in ACCESS (Microsoft Corporation, Redmond, WA). Statistical analysis was performed using STATA, version 9.0 (Stata Corporation, College Station, TX). For all analyses, a *P* value (two-sided) of less than 0.05 was considered statistically significant.

RESULTS

A total of 145 children were randomized to receive DP and 147 children were randomized to receive AL for treatment of uncomplicated malaria, leading to 981 and 1032 treatments, respectively. Of these, PK samples were collected from a subset of 106 patients treated with DP and 101 patients treated with AL. This provided a total of 214 treatments for DP (median=2, range=1-4 episodes per child) and 243 treatments for AL (median=2, range=1-6 episodes per child) for uncomplicated falciparum malaria which were included in this analysis (Figure 1). Children included in this dataset and treated for uncomplicated falciparum malaria ranged from 6 months to 24 months of age.

Demographic and anthropomorphic baseline characteristics of all episodes of uncomplicated falciparum malaria stratified by treatment are presented in Table 1. At the time of treatment, 55% were between 12 and 18 months of age, 47% were anemic (hemoglobin <10 g/dL), 37 % were taking TS prophylaxis, 9.4% were HIV-infected and 93% of these were taking ARTs. Forty-seven percent of the study participants had an HAZ z-score <-2 and 16% had a WAZ z-score of <-2, consistent with rates reported across Uganda [23].

Baseline demographics for all individual episodes of uncomplicated malaria treated with DP or AL stratified by measures of malnutrition are summarized in Table 2. At the time of each treatment with DP, 54% of the participants were stunted and 20% were underweight. Age differed significantly between stunted and non-stunted children and age, parasite density, place of residence, and cumulative dose of piperaquine differed significantly between underweight and non-underweight children. At the time of each treatment with AL, 40% of the participants were stunted and 13% underweight. Gender, age, breastfeeding status, and cumulative dose of lumefantrine differed significantly between stunted and non-stunted children and gender, presence of anemia at baseline, use of TS prophylaxis, and cumulative dose of lumefantrine differed significantly between underweight and non-underweight children.

Baseline (pretreatment) PK samples were collected for 65% of DP episodes and 59% of AL episodes. Of the baseline samples collected, blood levels were detectable for 83% (116/139) of treatments with DP and 6% (9/144) of treatments with AL. PK levels were available for 99% of DP treatments and 74% of AL treatments on day 3, 96% of DP and 88% of AL treatments on day 7 and 100% of DP treatments on day 14. On day 7, PK levels were below the LOQ for 13% (25/188) of the malaria episodes treated with AL. One day 14 piperaquine sample, and 1 day 3 and 1 day 7 lumefantrine samples were excluded from analyses as extreme outliers. However, including these samples in the analyses did not alter the results (results not shown).

Univariate associations of selected baseline characteristics with median day 3, 7, and 14 piperaquine levels and median day 3 and 7 lumefantrine levels are presented in Table 3. There was a trend toward lower median day 3 piperaquine levels in stunted children but this difference was not statistically significant (109 vs 129 ng/ml, p=0.054; Figure 2). The median day 7 or 14 piperaquine levels did not differ significantly between stunted vs non-stunted or between underweight vs. non-underweight children. Older children had significantly higher median day 3

piperaquine levels than younger children (p=0.037) yet the piperaquine cumulative dose received decreased with age (p<0.001).

Median day 3 or 7 lumefantrine concentrations were not significantly different when comparing children classified as stunted or underweight to children classified as not stunted or not underweight. Children taking TS prophylaxis received a significantly higher overall dose of lumefantrine (p=0.025) and had significantly higher median day 7 lumefantrine levels (p=0.014) than children not taking TS prophylaxis (Table 3).

Effect of nutritional status on piperaquine and lumefantrine concentrations. For each measure of malnutrition, piperaquine concentrations at day 3, 7, and 14 and lumefantrine concentrations at day 3 and 7 were compared in multivariate analyses. Each model was adjusted for age, cumulative dose of piperaquine or lumefantrine, piperaquine or lumefantrine levels at baseline, and repeated measures in the same patient. There was a significant association between stunting status and piperaquine levels at day 3 with stunted children more likely to have lower median piperaquine levels at day 3 than their non-stunted counterparts (OR=0.78, p=0.007). There were no significant associations between median day 7 or day 14 levels between stunted and non-stunted children nor between children classified as underweight and those classified as not underweight and any of the sampling days (Table 4).

For lumefantrine, there was no association between day 3 lumefantrine levels and stunting or underweight. Likewise, there was no association of day 7 levels and stunting. There was a trend of association between day 7 lumefantrine levels and underweight with underweight children more likely to have higher lumefantrine levels at day 7, though this did not reach statistical significance (OR=1.52, p=0.057) (Table 4).

Effect of nutritional status on apparent clearance of piperaquine. The mean CL/f_{pip} in participants treated with DP was 1.50 l/hr/kg (range 0.47-4.43). In stunted children, the mean CL/f_{pip} rate was 1.63 l/hr/kg vs 1.34 liters/hr in non-stunted children (p<0.001), which represents a 22% increase. In underweight children, the mean CL/f_{pip} was 1.63 l/hr/kg vs 1.46 l/hr/kg in non underweight children (p=0.133) (Figure 3). After adjustment for age, cumulative piperaquine dose, and repeated measures, stunting was associated with CL/f_{pip} with stunted children having higher CL/f_{pip} than non-stunted children (OR=1.32, p=0.001). There were no significant associations between underweight and CL/f_{pip}. One malaria episode was excluded from this analysis because it appeared to be an extreme outlier. However, including this data point in the analysis did not change the results.

DISCUSSION

To our knowledge, this is the first study assessing the effect of malnutrition on the PK of ACTs and one of the few studies evaluating PK in very young children with malaria. Moreover, this study was completed in the context of a longitudinal clinical trial which allowed for repeated PK evaluations in each child experiencing multiple episodes of clinical malaria during the course of

the study, thus providing one of the largest PK datasets in children available for DP and AL. Specifically our results represent 106 children treated with DP and 101 children treated with AL, all less than or equal to 2 years of age, and encompass 214 and 243 episodes of uncomplicated falciparum malaria for children randomized to treatment with DP or AL, respectively. In addition, this study utilized the new 2006 WHO growth standards which provide a more accurate tool for monitoring growth differences as they evaluate growth patterns for healthy children from around the world, rather than from one country. Our results indicate that in a high transmission setting, stunting, an indicator of chronic malnutrition does not have a significant effect on piperaquine or lumefantrine concentrations at day 7 in children age 2 years or younger. Chronic malnutrition appeared to be associated with day 3 piperaquine concentrations in adjusted analyses with stunted children having lower concentrations than non-stunted children. However the difference diminished when comparing results for day 7 and 14. Apparent clearance of piperaquine (CL/f_{pip}) was higher in children that were chronically malnourished which may be the consequence of a lower overall exposure to drug and is consistent with the lower piperaquine concentrations measured on day 3. Being underweight, which is non-specific and can arise when a child is stunted or wasted, did not have an effect on piperaquine drug concentrations, CL/f_{pip}, or on lumefantrine drug concentrations.

There are several biological mechanisms as to how malnutrition could impact antimalarial pharmacokinetics. First, malnutrition is associated with intestinal malabsorption, villous atrophy of the jejunal mucosa, reduced gastric acidity, and prolonged emptying time [24-26], all of which can impair drug absorption [26]. Decreased gastric acidity associated with malnutrition can result in decreased absorption of drugs requiring lower gastric pH for stability [27]. Piperaquine is better dissolved in an acidic (lower pH) environment which can explain why bioavailability is increased when co-administered with food [28, 29]. Our finding that chronic malnutrition was associated with day 3 piperaquine PK levels may be indicative of impaired absorption due to higher gastric pH. Similarly, in a study conducted in Nigerian children 24 to 42 months of age, peak plasma concentrations and the mean area-under the curve (AUC) of chloroquine, which has disposition pharmacokinetics similar to piperaquine, were found to be lower at all time points in children with kwashiorkor, indicating decreased bioavailability and impaired absorption [12]. However, contrary to the Nigerian study, the day 7 levels in our study were comparable between stunted and non-stunted children. As day 7 levels of the partner drug have been shown to be a strong determinant of therapeutic response for ACTs [15, 30, 31], the differences in day 3 levels may not have a significant impact on overall treatment efficacy, particularly since the initial therapeutic response with ACT treatment is entirely dependent on DHA through 4 days post start of treatment.

Lumefantrine is even less soluble in water than piperaquine and like piperaquine, is best dissolved in an acidic environment. The co-administration with food increases the bioavailability of lumefantrine by 16-fold [32, 33] and therefore it is recommended that AL be given with a small amount of fat such as in the form of milk or a biscuit [1]. Interestingly, contrary to the results seen with piperaquine, day 3 lumefantrine levels were similar between stunted and non-stunted children. Despite the fact that stunted children received higher

cumulative doses of lumefantrine, after adjustment for cumulative dose, stunting was still not associated with lumefantrine levels. However, given the biological plausibility that malnutrition can impact lumefantrine absorption, further exploration is warranted.

Malnutrition can also impact drug distribution, metabolism and elimination. Malnutrition can result in an increase in total body water leading to a greater volume of distribution and decreased protein binding due to reduced albumin levels, which in turn would result in lower blood concentrations and faster clearance [25]. Although lumefantrine is highly protein-bound (>99%) [33], we did not see differences in lumefantrine concentration between groups and unfortunately, were unable to evaluate clearance in the lumefantrine arm. The significant differences in clearance seen between groups may be a reflection of reduced distribution of this drug in stunted children. In addition, malnourished children, may exhibit altered metabolism due to altered hepatic oxidative drug biotransformation via the cytochrome P450 (CYP) enzymatic system [34, 35]. Rats on a protein-deficient diet administered chloroquine which is metabolized by several CYP enzymes including CYP3A4, exhibited a longer elimination half-life ($t_{½}$) than control rats, presumably due to a decrease in CYP2C8, CYP2D6, and CYP3A4 [36]. In a study conducted in Gabon in children under 5 years receiving quinine, also metabolized by the CYP3A4 enzyme, clearance was higher, blood concentrations lower, and elimination faster in malnourished children compared to normal children [11]. Moreover, full function of the CYP450 enzymatic system is thought to be dependent on age. Indeed, full maturation of the cytochrome P450 enzymes CYP1A2 and CYP2B6 are not achieved until peri- or post-puberty [37]. Both piperaquine and lumefantrine undergo CYP metabolism via the isoenzyme CYP3A4 [33] [Parikh S., unpublished data] thus, malnourished children treated with DP or AL may exhibit reduced distribution and diminished metabolism. In our study, chronically malnourished children had higher piperaquine clearance, perhaps indicative of inadequate CYP activity. As a previously published report of treatment efficacy in this patient population reported few recrudescences (<3%) [7], this difference in clearance rate does not translate to an increase in treatment failure. Whether it translates to a shorter post-treatment prophylactic effect should be elucidated.

Limitations of this study should be considered. First, we only evaluated two levels of malnutrition; stunting, which included children with a height-for-age z-score of <-2 and underweight, which included children with a weight-for-age z-score of <-2. We were unable to evaluate whether severe chronic malnutrition or whether measures of acute malnutrition (i.e., wasting) impacted piperaquine and lumefantrine drug levels as has been reported with other drugs known to have similar pharmacokinetic properties [11, 12, 39]. Second, we were unable to evaluate the effect of malnutrition on other pharmacokinetic parameters of piperaquine and lumefantrine including AUC and elimination half-life. Future studies evaluating the full pharmacokinetic profile of these treatment regimes is warranted. Finally, we were unable to extrapolate a day 7 piperaquine or lumefantrine level that could be predictive of treatment failure or recurrent infection in children in this age range. This may provide useful and definitive information as to whether dosing strategies in children should be readdressed.

Conclusion: Though stunted children age 2 years and younger were more likely to have lower piperaquine levels at day 3 and higher piperaquine clearance, it is unclear how these factors affect subsequent risk of recurrent infection. Malnutrition did not impact the pharmacokinetics of lumefantrine in Ugandan children age 2 years and younger in this research setting. However, this is the first study to evaluate the effects of malnutrition on lumefantrine pharmacokinetics and this finding should be confirmed. Future studies are warranted to evaluate the impact of piperaquine and lumefantrine pharmacokinetics on the subsequent risk of recurrent infection in chronically malnourished children.

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TABLES

Table 1. Demographic and anthropomorphic characteristics of all episodes of uncomplicated falciparum malaria treated with DP and AL.

	DP N=214	AL N=243
Male, n (%)	133 (62%)	119 (49%)
Age in months, mean (SD)	15.3 (3.6)	15.1 (3.5)
Age categories		
< 12 months, n (%)	42 (20%)	51 (21%)
12-18 months, n (%)	116 (54%)	137 (56%)
> 18 months, n (%)	56 (26%)	55 (23%)
Rural (v. urban) residence, n (%)	186 (87%)	227 (93%)
Breastfeeding, n (%)	94 (44%)	105 (43%)
HIV-infected, n (%)	21 (9.8%)	22 (9.1%)
Anemia at baseline, n (%)	96 (45%)	120 (49%)
Taking TS prophylaxis, n (%)	74 (35%)	93 (38%)
Taking ARTs, n (%)	19 (8.9%)	21 (8.6%)
Cumulative piperaquine dose (mg), mean (SD)	58.2 (8.5)	
Cumulative lumfantrine dose (mg), mean (SD)		79.6 (11.9)
Height-for-age (HAZ) z-score, mean (SD)	-2.18 (1.18)	-1.86 (1.03)
Stunted, n (%)	116 (54%)	97 (40%)
Weight-for-age (WAZ) z-score categories, n (%)	-1.07 (1.21)	-0.72 (1.09)
Underweight, n (%)	43 (20%)	32 (13%)

Table 2. Baseline characteristics of all episodes of uncomplicated falciparum malaria treated with DP and AL stratified by measures of malnutrition.

	Treatment with DP			
	Stunted (n=116)	**Not Stunted (n=98)**	**Underweight (n=43)**	**Not Underweight (n=171)**
Male, n (%)	78 (67%)	55(56%)	24 (56%)	109 (64%)
Age, mean (SD)	16.0 (3.3)*	14.5 (3.8)*	15.3 (3.6)*	15.4 (3.6)*
Anemia, n (%)	56 (48%)	40 (41%)	20 (47%)	76 (44%)
Parasite Density, geometric mean μL^{-1} (95% CI)	19201 (13720-26872)	16791 (11360-24818)	11557(5887-22691)*	20201.42 (15432-26444)*
Rural (v. urban) residence, n (%)	106 (91%)	80 (82%)	42 (98%)*	144 (84%)*
Breastfeeding, n (%)	54 (47%)	40 (41%)	20 (47%)	74 (43%)
HIV-infected, n (%)	16 (14%)	5 (5.1%)	9 (21%)	12 (7.0%)
Taking ARTs, n (%)	15 (13%)	3 (4.1%)	8 (19%)	11 (6.4%)
Taking TS prophylaxis, n (%)	37 (32%)	37 (38%)	14 (33%)	60 (35%)
Cumulative piperaquine dose (mg), mean (SD)	59.2 (9.2)	57.0 (7.6)	67.2 (9.8)*	55.9 (6.4)*
	Treatment with AL			
	Stunted (n=97)	**Not Stunted (n=147)**	**Underweight (n=32)**	**Not Underweight (n=211)**
Male, n (%)	60 (62%)*	59 (40%)*	24 (75%)*	95 (45%)*
Age, mean (SD)	15.6 (3.2)*	14.8 (3.7)*	15.1 (2.7)	15.2 (3.7)
Anemia, n (%)	51 (53%)	69 (47%)	24 (75%)*	96 (46%)*
Parasite Density, geometric mean μL^{-1} (95% CI)	12417 (8177-18854)	20349 (15303-27061)	22085 (12004-40634)	16015 (12339-20786)
Rural (v. urban) residence, n (%)	90 (93%)	137 (94%)	28 (88%)	199 (94%)
Breastfeeding, n (%)	32 (33%)*	73 (50%)*	8 (25%)	97 (46%)
HIV-infected, n (%)	6 (6.2%)	16 (11%)	2 (6.3%)	20 (9.5%)
Taking ARTs, n (%)	6 (6.2%)	15 (10%)	2 (6.3%)	19 (9.0%)
Taking TS prophylaxis, n (%)	43 (44%)	50 (34%)	21 (66%)*	72 (34%)*
Cumulative piperaquine dose (mg), mean (SD)	85.0 (11.5)*	76.1 (11.8)*	94.5 (9.7)*	77.4 (10.5)*

* $P<0.05$

Table 3. Association of selected baseline characteristics with median piperaquine and median lumefantrine levels.

	Piperaquine, ng/ml (median, IQR)			Lumefantrine, ng/ml (median, IQR)	
	PK Day 3	**PK Day 7**	**PK Day 14**	**PK Day 3**	**PK Day 7**
Stunted	109 (75-148)	38.5 (29.4-54.5)	23.5 (16.8-36.1)	2971.1 (1743.0-4675.9)	235.6 (144-355.2)
Not Stunted	129 (95.8-183)	42.7 (31.2-58.3)	26 (18.9-36)	2717.3 (1820.2-4930.5)	210.6 (129-321.7)
Underweight	128 (76.8-163)	48.1 (34.3-71.1)	28.3 (20.1-38.1)	3585.4 (968.7-6604.5)	261.8 (154.7-505.5)
Not Underweight	116 (87.7-163)	40 (29.1-51.7)	24.0 (17.3-33.1)	2770.6 (1787.0-4647.6)	215.8 (130-337.5)
Age					
< 12 months	143 (96.4-189)*	42.8 (29.2-62.3)	26.3 (16.6-41.1)	2634.6 (1916.9-4433.3)	238.3 (186.0-326.6)
12-18 months	124 (88.0-172)*	43 (31.7-58.4)	25.6 (18.4-36)	2971.1 (1677.6-5048.2)	215.5 (129-354.7)
> 18 months	106 (82.4-140)*	35.0 (26.2-47.2)	23.2 (16.5-30.8)	2685.3 (1712.5-4675.9)	226.3 (116-321.7)
HIV-infected	102 (74.7-117)	40.8 (29.9-55.9)	24.8 (18.4-29.8)	2597.9 (1753.8-4517.1)	283.6 (180.7-513.9)/
HIV-uninfected	124 (87.8-168)	41.4 (30.2-56)	24.4 (17.3-36.2)	2915.7 (1743.0-4923.7)	218.4 (129-338.1)
Taking ARTs	102 (74.6-127)	43.7 (33.9-56.4)	24.8 (18.4-29.9)	2597.9 (1753.8-4517.1)	283.6 (180.7-513.9)
Not Taking ARTs	123.5 (87.8-167)	40.9 (29.8-56)	24.4 (17.3-36.1)	2915.7 (1743.0-4923.7)	218.4 (129-338.1)
Taking TS	110 (76.8-155)	38.5 (29.6-56.4)	25.8 (18.4-35.9)	2904.9 (1753.8-4930.5)	270.0 (170-423.8)*
Not Taking TS	123.5 (88.1-166)	42 (30.4-56)	24.1 (17.3-36.1)	2793.2 (1743.0-4814.5)	207.5 (127-274.6)*

* $P<0.05$

Table 4. Association between median piperaquine levels at day 3, 7, and 14 and median lumefantrine levels at day 3 and 7 and measures of malnutrition[1].

	Piperaquine					
	PK Day 3		PK Day 7		PK Day 14	
	Odds Ratio (95% CI)	P-Value	Odds Ratio (95% CI)	P-Value	Odds Ratio (95% CI)	P-Value
Stunted	0.78(0.64-0.93)	0.007	0.91 (0.77-1.07)	0.247	0.90 (0.74-1.09)	0.280
Underweight	1.05 (0.82-1.33)	0.719	1.04 (0.88-1.23)	0.647	1.11 (0.91-1.37)	0.304

	Lumefantrine			
	PK Day 3		PK Day 7	
	Odds Ratio (95% CI)	P-Value	Odds Ratio (95% CI)	P-Value
Stunted	0.83 (0.62-1.11)	0.207	0.91 (0.66-1.24)	0.540
Underweight	0.90 (0.49-1.65)	0.735	1.52 (0.99-2.36)	0.057

1 - Adjusted for age, cumulative dose of piperaquine/lumefantrine, and piperaquine/lumefantrine level at baseline

FIGURES

Figure 1. Trial Profile

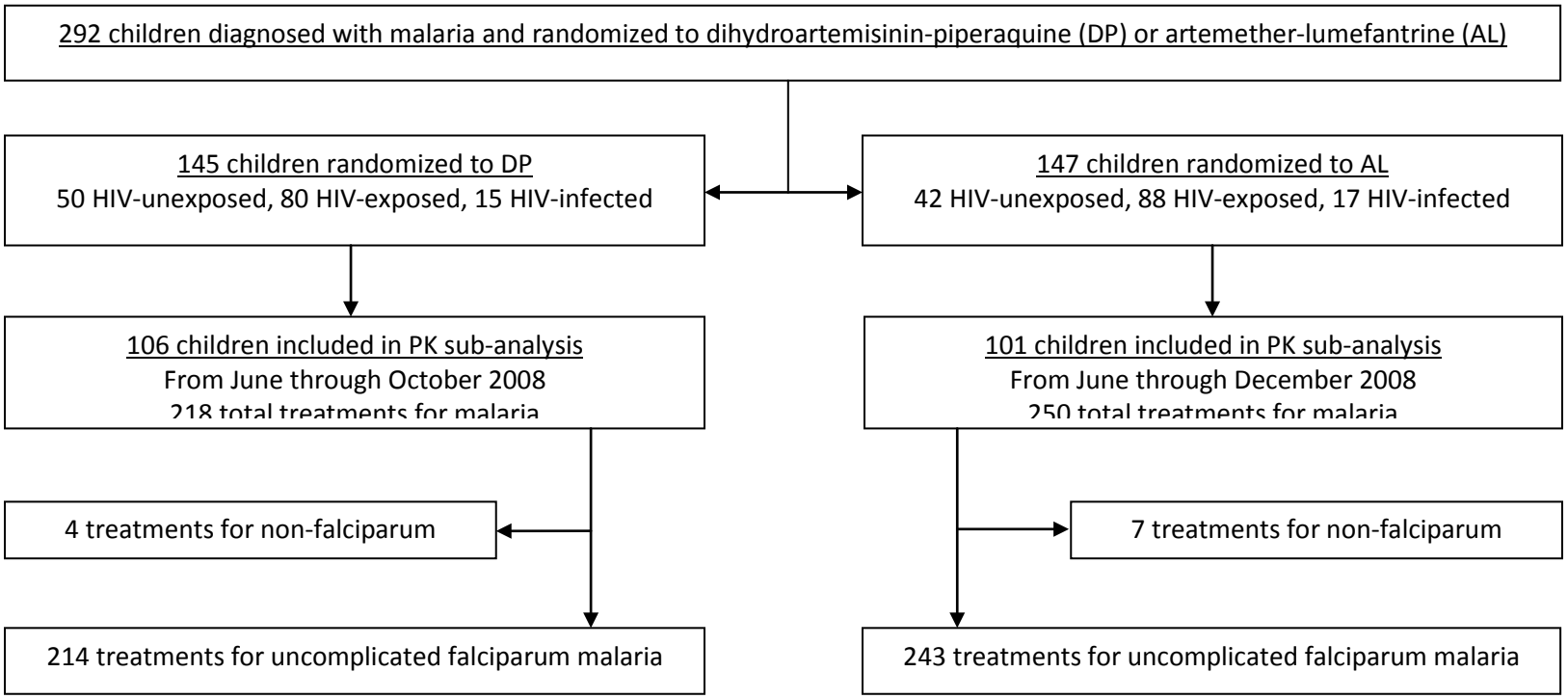


Figure 2. Mean plasma concentration vs time profile for piperaquine stratified by measures of malnutrition (stunted/not-stunted).

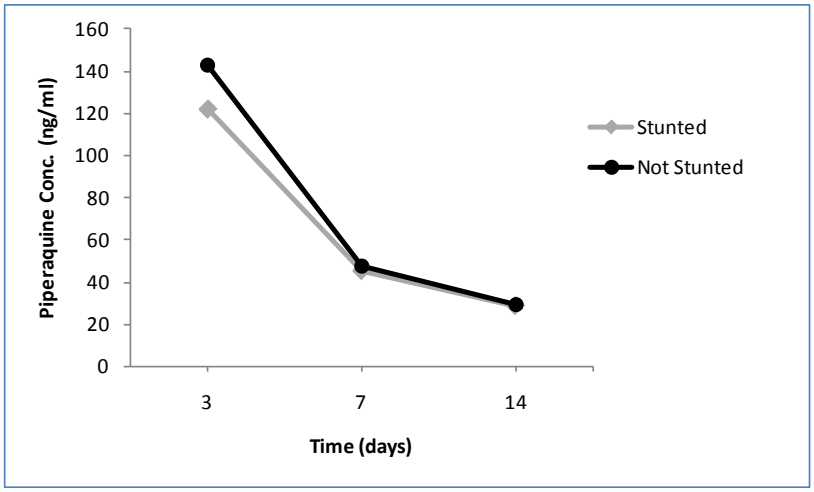
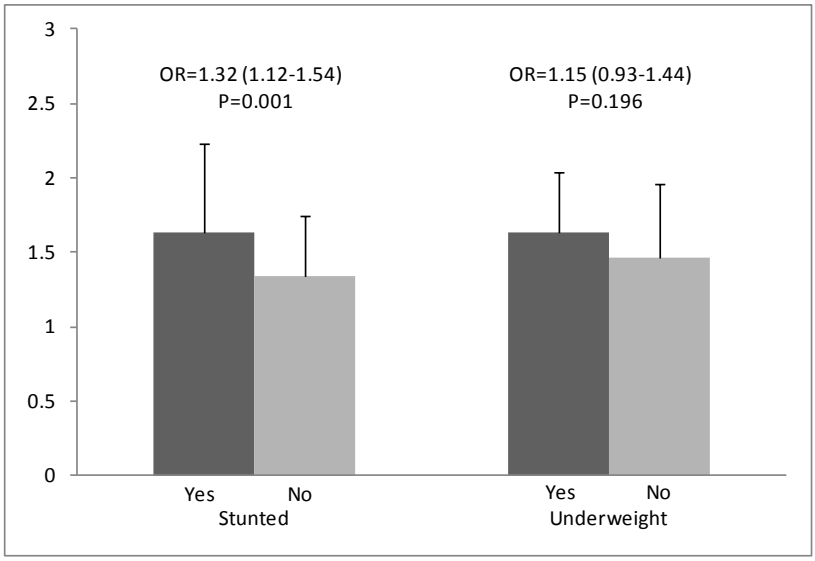


Figure 3. Piperaquine clearance (liters/hr/kg) stratified by measures of malnutrition. Odds ratios adjusted for age, total dose of piperaquine, and repeated measures in each patient.



CHAPTER 5

Summary and Future Directions

SUMMARY

This dissertation evaluated the effect of nutritional status on the response to treatment with artemisinin-combination therapy (ACT) in young Ugandan children with malaria using the preferred method of analysis for monitoring in vivo efficacy of antimalarial treatments established in Chapter 2. This dissertation went on to examine the effect of nutritional status on the pharmacokinetics (PK) of two ACT treatment regimens. The data from 14 comparative clinical trials conducted in Thailand between 1993 and 2005 and 15 comparative clinical trials conducted in Uganda and Burkina Faso between 2003 and 2007 was used for the comparison of statistical methods in Chapter 2 and data from the Tororo Child Cohort (TCC) Study conducted in Tororo, Uganda was used for the treatment response and PK analyses conducted in Chapters 3 and 4.

This dissertation began with a thorough review of malaria, including the epidemiology, parasite life cycle, and current treatment regimens for the disease, and a review of malnutrition including a summary of definitions and growth standards used, as well as the epidemiology of malnutrition (Chapter 1). This chapter continued with a discussion of the impacts of malnutrition on immunity and subsequently infectious disease and summarized the results of multiple studies assessing the association of malnutrition and malaria. Finally, a review of PK terminology was provided as well the potential impact of young age and malnutrition on the PK of ACTs. A summary of the common analytical approaches used for the interpretation of antimalarial clinical trials (intention to treat (ITT), modified intention to treat (mITT) and per protocol (PP)) was provided and analysis conducted to quantify the magnitude of the difference between efficacy estimates derived from these approaches (Chapter 2). This dissertation then examined the impact of indicators for malnutrition on the risk of recurrent parasitemia of two widely used ACTs, dihydroartemisinin-piperaquine (DP) and artemether-lumefantrine (AL), in young Ugandan children treated for repeated episodes of uncomplicated falciparum malaria (Chapter 3). This chapter also examined the association between these indicators of malnutrition and parasite clearance following therapy with DP or AL. Finally, this dissertation assessed the impact of two indicators of malnutrition, stunting, and underweight, on the pharmacokinetics of AL and DP in a subset of children enrolled in the TCC study (Chapter 4). The specific PK parameters evaluated included lumefantrine levels at day 3 and 7, piperaquine levels at day 3, 7, and 14, and apparent clearance of piperaquine (CL/f_{pip}). A summary of the findings in Chapters 2 through 4 are outlined below:

Chapter 2:

The risks of treatment failure unadjusted and adjusted by genotyping for each treatment arm of the individual studies were derived and compared using PP, ITT, and mITT analysis. The methods vary by who is considered in the evaluable population as well as how risks of failure are calculated. Risk of failure in the PP and ITT approaches are calculated via simple proportions and the risk of failure in the mITT approach is derived using the Kaplan-Meier product limit

formula with data censored for patients not classified as failures and with interrupted follow-up. Twenty-nine clinical trials with a total of 65 treatment arms were included in the analysis: 38 from Africa and 27 from Thailand. Of the 15,409 patients enrolled, 2,637 (17.1%) had incomplete follow up for the unadjusted analysis and 4,489 (33.4%) for the adjusted analysis. Estimates of treatment failure were consistently higher when derived from the ITT or PP analyses compared to the mITT approach in both unadjusted and adjusted analyses. In the unadjusted analyses the median difference between the ITT and mITT estimates was greater in Thai studies compared to African Studies (11.4% vs. 1.8%, $p<0.001$). In the adjusted analyses the median difference between PP and mITT estimates was 1.7%, but ranged from 0 to 30.9%. The discrepancy between estimates was correlated significantly with the proportion of patients with incomplete follow-up; $r_s = 0.740$, $p<0.0001$. The proportion of studies with a major difference (>5%) between adjusted PP and mITT, was 28% (16/57), with the risk difference greater in African (37% 14/38) compared to Thai studies (11% 2/19). In the African studies a major difference in the adjusted estimates was significantly more likely in high transmission sites (62% 8/13) compared to moderate transmission sites (24% 6/25); $p=0.035$.

Our findings highlight that caution is needed when generating temporal and geographical trends using different analytical methods, and that this is particularly apparent for studies with poorer patient adherence to follow-up, higher incidence of P. vivax relapse, and high incidence of *P. falciparum* new infections. Survival analysis should be the preferred approach for monitoring in vivo efficacy as it allows for all available data to contribute to the analysis, thus increasing the precision, avoids systematic biases introduced by dropping patients from the analysis that do not complete follow-up or classifying patients as failures who do not complete follow-up, and allows for data from patients with different follow-up periods to be combined to generate efficacy estimates thus enabling direct comparison between studies with different lengths of follow-up.

Chapter 3:

145 children with at least one diagnosis of uncomplicated malaria were randomized to DP and 147 were randomized to AL resulting in 981 and 1032 treatments for uncomplicated falciparum malaria, respectively. The primary exposure variables of interest were height-for-age (HAZ) and weight-for-age (WAZ) z-scores divided into four categories with the following cut-offs; ≥ 0, <0 and ≥ -1, <-1 and ≥ -2, and <-2. Less than 1% of patients had a positive blood smear at day 3 and 95% of patients treated with DP and 90% of patients treated with AL cleared all parasites by day 2 ($p<0.001$). There was no significant association between HAZ and WAZ scores and a positive blood smear two days following treatment with DP or AL. The risk of recurrent parasitemia was estimated using the Kaplan-Meier product limit formula with censoring for patients with incomplete follow-up. Measures of association between categories of malnutrition and risk of recurrent parasitemia were made using Cox proportional hazards models with adjustment for potential confounders and inference adjusted for repeated measures in the same patient. All models were stratified by cotrimoxazole (TS) use. The overall risk of recurrent parasitemia after 42 days of follow-up was 29% (95% CI 27 - 32%) and 54% (95% CI 51-57%)

in study participants treated with DP and AL, respectively. In children treated with DP not on TS, a decreasing HAZ score was independently associated with a higher risk of recurrent parasitemia. However, statistical significance was reached only when comparing HAZ scores <-1 with those $\geq$ 0 (HAZ $\geq$ -2 - <-1: HR=2.89, p=0.039; HAZ <-2: HR=3.18, p=0.022). In study participants treated with AL taking TS prophylaxis, a decreasing HAZ score was independently associated with a higher risk of recurrent parasitemia, although statistical significance was not achieved.

Overall, our results indicate that in a high transmission setting, both AL and DP are efficacious antimalarial treatments for children under 3 years of age, regardless of nutritional status and parasite clearance overall was excellence with over 99% of study participants clearing all primary parasites by day three. Children with signs of mild to moderate chronic malnutrition not taking TS prophylaxis were at higher risk of recurrent parasitemia but this was only significant in the group randomized to DP.

Chapter 4:

PK samples were collected from 106 patients treated with DP and 101 patients treated with AL providing a total of 214 treatments for DP and 243 treatments for AL for analysis. Stunting was defined as a height-for-age z-score (HAZ score) of <-2 and underweight as a weight-for-age z-score (WAZ score) of <-2. Using generalized estimating equations with adjustment for potential confounders and for repeated measures in the same patient, stunting was significantly associated with day 3 piperaquine levels with stunted children more likely to have lower median day 3 levels than non-stunted children (OR=0.78, p=0.007). Stunting was not associated with day 7 or 14 piperaquine levels or with day 3 or 7 lumefantrine levels. Underweight was not associated with piperaquine or lumefantrine levels on any sampling day. In stunted children, the mean CL/f_{pip} rate was 1.63 l/hr/kg vs 1.34 liters/hr in non-stunted children (p<0.001), which represents a 22% increase. Stunting was associated with CL/f_{pip} (OR=1.32, p=0.001) with stunted children having higher CL/f_{pip} than non-stunted children (1.63 l/hr/kg vs 1.34 liters/hr, p<0.001).

In summary, our results indicate that in a high transmission setting in children age two years or younger, chronic malnutrition does not have an effect of piperaquine or lumefantrine concentrations at day 7 – the PK parameter that has been found to be a useful determinant for treatment response. Chronic malnutrition appeared to be associated with day 3 piperaquine concentrations in adjusted analyses with stunted children having lower concentrations than non-stunted children which may be indicative of impaired absorption. CL/f_{pip} was higher in children that were chronically malnourished which may be the consequence of a lower overall exposure to drug and is consistent with the lower piperaquine concentrations measured on day 3.

FUTURE DIRECTIONS

The findings reported in this dissertation provide several pathways for future research examining the relationship between childhood malnutrition and its impact on how patients with malaria are treated:

1.) In chapter 2, we concluded that survival analysis is the optimal approach for monitoring in vivo efficacy and highlighted its benefits including providing increased precision, limiting systemic bias, and allowing for data from patients with different follow-up periods to be combined enabling comparisons between studies. We also highlighted the newly formed WorldWide Antimalarial Resistance Network (WARN - http://www.wwarn.org), which will serve as a global open-access resource for antimalarial efficacy data. Valuable data points that could be added to the WARN framework include patient age, height, and weight from which through programming, z-scores could be derived. Providing z-score information would provide researchers, particularly those without the computing power to calculate z-scores in the field, additional insight into their patient populations. In addition, it would provide the information needed to allow for researchers evaluate the impact of malnutrition on efficacy estimates in their patient population as well as to evaluate the impact of malnutrition on efficacy estimates across time and place. Understanding the impact of malnutrition on recrudescence as well as risk of new infection in areas of varying transmission settings and between different age ranges may give us a more conclusive answer as to if clinician's need to re-evaluate dosing strategies in patients with this co-morbidity.

2.) In Chapter 3, we found that children with signs of mild to moderate chronic malnutrition are at increased risk of recurrent parasitemia. It is important to understand the causal mechanisms behind this finding. To my knowledge, there have only been two other studies that have investigated the relationship between malnutrition, malaria, and the immunological factors that impact this relationship [1, 2]. One study found that prevalence of anti-IgG were significantly lower in chronically malnourished children (HAZ $\leq$ -2.5) than in controls [1]. The other found that the prevalence of cytokines were higher in stunted children than in well-nourished children while the humoral response (prevalence of specific antibodies) were greater in well-nourished than in wasted children [2]. More studies evaluating immune response should be conducted in very young children (under two years of age) and older children with malnutrition. Additional blood sampling is required which needs to be balanced with the ethical and logistical concerns of additional sampling in this vulnerable patient population.

3.) The other important causal factor behind the results reported in Chapter 3 is that the pharmacokinetics of ACTs may be very different in young malnourished children compared to well-nourished young children. Overall, our results indicate that in a high transmission setting, both AL and DP are efficacious antimalarial treatments for

treatment in children less than three years of age, regardless of nutritional status based on the high parasite clearance rates and low rate of recrudescence. Therefore indicating that the PK of artemisinin and the concentrations of piperaquine and lumefantrine reached by day 7 are adequate and do not negatively impact treatment response (i.e., treatment failure). However, more information needs to be elucidated as to whether malnutrition has an effect on the elimination half-life ($t_{½}$) of these partner drugs, thereby affecting the risk of recurrent parasitemia. In chapter 4, we were able to derive valuable information regarding the spot blood concentrations of piperaquine and lumefantrine and the apparent clearance of piperaquine. Additional PK testing and analysis should be conducted to derive the area-under the curve (AUC) and $t_{½}$ of these drugs to evaluate if malnutrition adversely impacts either parameter. A lower AUC or a shortening of the $t_{½}$ could indicate that the dosing strategies in this patient population should be readdressed so as to optimize the post-treatment prophylactic effect of these drug regimens while minimizing the potential for toxicity. Further pharmacokinetic analysis of piperaquine and lumefantrine in this patient population is currently ongoing and this research team plans to use this information to evaluate the impacts of malnutrition on the pharmacodynamics (PD) of these two partner drugs.

4.) As mentioned in Chapter 3, because this study was observational in nature and children could not be randomized to their "exposure", the causal effect of malnutrition on risk of recurrent parasitemia could not be determined. For instance, for these studies, the exposed group would be considered stunted children and the "unexposed" group would be considered non-stunted children. Through randomization, the initial conditions of the exposed and unexposed groups are distributed equally such that a causal association between malnutrition and risk of recurrent parasitemia can be established or refuted. In addition, in this patient population, nutritional status could be acting as a time-dependent confounder. A time-dependent confounder is a time –dependent covariate that is 1) a risk factor for or predictor of the outcome (i.e., risk of recurrent parasitemia) and also predicts subsequent exposure and 2) past exposure history predicts the subsequent level of exposure. Where a randomized clinical trial (RCT) is not possible and time dependent confounding may indeed exist, causal methods such as marginal structural models (MSMs) or history adjusted MSMs can be used to overcome the lack of exchangeability inherent in observational studies and to minimize bias introduced by time-dependent confounding [3, 4]. We plan to use causal methods in a future analysis to explore whether this provides a more precise and unbiased estimate of the effect of malnutrition on recurrent parasitemia risk.

Young children are at the highest risk of malaria morbidity and mortality, particularly in areas of high transmission and children with malnutrition may be at even greater risk. It is important to systematically include this vulnerable population in clinical trials in sufficient numbers to obtain a definitive understanding of the risk imposed by age and nutritional status. Ethical considerations must be balanced with optimizing the number of measurements and blood

samples required to derive a definitive causal mechanism that underlies the relationship between malnutrition and malaria risk.

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