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Challenges in the Management of Uncomplicated and Severe Malaria in Sub-Saharan Africa and New Interactions between Malaria and HIV treatments

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DECLARATION

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DEDICATION

I dedicate this thesis to my mother Mrs. Tabitha Ayerango Ojwang (RIP) and my father Mr. James Jago Ojwang for the special gifts that they have been in my life. For your selfless dedication to your children and for all the sacrifices you've made over the years. For setting an example of hard work, you have been my source of inspiration in many ways. You taught us that success does indeed come when you work hard. To you both, am deeply indebted and I share this success with you. To mum, I will forever cherish your memory and wish you were here to share this with me. To dad, I thank God for the gift that you have been to us and pray that the almighty adds more years to your life.

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ABBREVIATION

ABC	Abacavir
3TC	Lamivudine
ACT	Artemisinin- based combination therapy
AIDS	Acquired immunodeficiency syndrome
AL	Artemether-lumefantrine
AQ/AS	Amodiaquine/artesunate
ART	Antiretroviral therapy
AS	Artesunate
AZT	Zidovudine
CDC	Centers for Disease Control
CI	Confidence Interval
CTRP	Circumsporozoite thrombospondin-related protein
CYP	Cytochrome
D4T	Stavudine
DP	Dihydroartemsinin-piperaquine
EFV	Efavirenz
HIV	Human immunodeficiency virus
ITNs	Insecticide treated Nets
IV	Intravenous
LPV/r	Lopinavir-ritonavir
MSP	Merozoite surface protein
NNRTI	Non-nucleoside reverse transcriptase inhibitors
NRTI	Nucleoside reverse transcriptase inhibitors
NVP	Nevirapine
OPD	Out patient department
PCR	Polymerase chain reaction
RDTs	Rapid diagnostic tests
RLS	Resource limited settings
SD	Standard deviation
SP	Sulphadoxine-pyrimethamine

SSA	Sub-Saharan Africa
TB	Tuberculosis
TS	Trimethoprim-sulphamethoxazole
WHO	World Health Organization

SAMENVATTING

Achtergrond en motivering

In meer dan 100 landen, die samen 40% van de wereldbevolking herbergen, is malaria een volksgezondheidsprobleem. Het is één van de meest voorkomende tropische aandoeningen en wereldwijd lopen ongeveer 3,3 miljard mensen het risico op een malaria infectie. In 2010, waren er wereldwijd ongeveer 219 miljoen malariagevallen, en doodde de ziekte ongeveer 660 000 mensen, meestal kinderen minder dan vijf jaar oud. Meer dan 90% van deze gevallen zijn in Afrikaanse landen ten zuiden van de Sahara (SSA). Oeganda als land is een typisch voorbeeld voor het immense malaria probleem in SSA daar het grootste deel van zijn grondgebied malaria endemisch is. De rapporten van het Ministerie van Volksgezondheid duiden malaria aan als de belangrijke oorzaak van morbiditeit en mortaliteit met ongeveer 8-13 miljoen ziekte episodes per jaar, 30-50% van de poliklinische patiëntbezoeken, 35% van de ziekenhuisopnames, 9-14% van de ziekenhuissterfte, en dit voornamelijk in kinderen van minder dan 5 jaar oud. SSA draagt ook het grootste deel van de wereldwijde HIV epidemie met meer dan tweederde van de HIV-geïnfecteerden en 76% van alle AIDS doden in 2007.

Deze twee ziekten nemen samen jaarlijks meer dan 4 miljoen sterfgevallen voor hun rekening. De epidemiologische en geografische overlapping van HIV en malaria is zorgt voor een significant volksgezondheidsprobleem wat betreft behandeling en mogelijke interacties tussen de beide ziekten.

Malaria is nochtans een te voorkomen en te behandelen ziekte en efficiënte preventieve en curatieve middelen zijn beschikbaar. Deze omvatten (i) vectorcontrole, zoals geïmpregneerde muggennetten, binnenhuisverstuiving en in sommige specifieke omstandigheden controle van de muggelarven; (ii) chemoprofylaxis voor risicogroepen zoals zwangere vrouwen en zuigelingen; (iii) bevestiging van de diagnose door microscopie of sneltesten voor elk vermoedelijk malariageval; (iv) tijdige behandeling met de aanbevolen malariabehandelingen. Van deze strategieën is de behandeling het meest fundamentele en cruciale element. Er bestaan echter een aantal uitdagingen om de juiste behandelings strategien te instaleren. Ten eerste, is er de nood om sommige aanbevolen behandelingen voor ongecompliceerde malariabehandeling te herzien, bijvoorbeeld het gebruik van kinine per os met zijn complex doseringsschema. Ten tweede, de

actuele uitdagingen voor behandeling van gecompliceerde malaria in regio's met beperkte middelen leidt tot een significant 'mismeestering' en verhoogde mortaliteit. Ten derde, met de stijgende resistentie tegen antimalariamiddelen, is er een behoefte aan alternatieve behandelingen. Tot slot zijn er de specifieke uitdagingen van malaria behandeling in risicogroepen zoals HIV geïnfecteerde individuen waar de mogelijkheid voor druginteractie een invloed heeft op de doeltreffendheid en/of toxiciteit.

Doelstellingen

In het kader van het huidig malariacontrolebeleid, voerden wij een aantal studies uit om de uitdagingen van de behandeling van zowel ongecompliceerde als gecompliceerde malaria.. Onze Oegandese situatie is ook representatief voor verscheidene settings in SSA. De specifieke doelstellingen zijn i) de doeltreffendheid van kinine *per os* voor de behandeling van ongecompliceerde malaria evalueren, ii) de huidige behandeling van gecompliceerde malaria in Oegandese gezondheidscentra te beschrijven iii) de historische rol van kinine kritisch te evalueren, zijn huidig gebruik te documenteren, en inzicht te verstrekken in zijn mogelijk toekomstig gebruik in de malaria behandeling en iv) in HIV besmetten, het potentiële effect van simultane behandeling van HIV en malaria op de resultaten van de malariabehandeling te onderzoeken.

Methodes

Tussen 2008 en 2011, voerden wij verscheidene studies uit om verschillende aspecten van malariabehandeling te evalueren. De eerste studie was een gerandomiseerde klinische studie die de operationele doeltreffendheid van kinine *per os versus* artemether-lumefantrine voor de behandeling van ongecompliceerde malaria in Ugandese kinderen evalueerde. Het primaire eindpunt was het behandelingsresultaat, parasitologisch en klinisch, na 28 dagen. De tweede studie was een cross-sectionele evaluatie van de gecompliceerde malaria behandeling in geselecteerde gezondheidscentra in Oeganda via een gelaagde selectie methode. De evaluatie gebruikte een aan de lokale situatie aangepast onderzoeksinstrument van de

Wereldgezondheidsorganisatie: Hospital assessment tool (WGO, 2002). De belangrijkste evaluatie was de kwaliteit van de gecompliceerde malaria behandeling. De derde studie was ook een gerandomiseerde open klinische studie in HIV besmette kinderen van 2 maanden tot 5 jaar die aan de criteria voor ARV behandeling voldeden of reeds op een nNRTI gebaseerd regime van ARV behandeling waren. De deelnemers werden willekeurig op LPV/r- d gebaseerde of op een NNRTI- gebaseerde ARV behandeling geplaatst en werden 2 jaar opgevolgd. Ze werden behandeld met artemether-lumefantrine indien zij een ongecompliceerde malaria ontwikkelden. Het primaire eindpunt was de malaria incidentie. De secundaire resultaten omvatten de incidentie van ongecompliceerde malaria, doeltreffendheid en veiligheid van de antimalaria behandeling, en de farmacokinetiek van lumefantrine. Daarenboven hebben wij in een overzichtsartikel, de historische rol van kinine gedocumenteerd, zijn huidig gebruik geëvalueerd en zijn mogelijke toekomstige rol in de behandeling van malaria gesitueerd.

Resultaten

Kinine *versus* artemether-lumefantrine voor het behandelen van ongecompliceerde *falciparum* malaria

Om de operationele doeltreffendheid van kinine per os tegenover artemether-lumefantrine voor de behandeling van ongecompliceerde malaria te evalueren, werd 175 kinderen, van 6 tot 59 maanden, met niet gecompliceerde malaria willekeurig thuis behandeld met kinine per os of artemether-lumefantrine. Na 28 dagen was 96% in de artemether-lumefantrinegroep en 64% in de kininegroep genezen (HR:10,7; 95%CI 3,3-35,5; P=0,001). In de kininegroep waren 69% (18/26) van de terugkerende parasitemies toe te schrijven aan recrudescentie en in de artemether-lumefantrine groep geen enkele. De therapietrouw was 94,5% in de artemether-lumefantrine groep vergeleken met 85,4% in de kininegroep (P=0,0008). Therapie trouw van 80% of meer was geassocieerd met een verminderd risico van behandelingsfalen (HR:0,44; 0,19-1,02; P=0,06). De ongemakken, eventueel bijwerkingen, verschilden niet tussen beide groepen.

Behandelingsbeleid van gecompliceerde malaria in gezondheidscentra

Om de behandelingspraktijken voor gecompliceerde malaria in Oegandese gezondheidscentra te evalueren, deden wij een enquête in de gezondheidscentra in 11 districten in de oostelijke en mid-westelijke regio's van Oeganda. Honderd en vijf gezondheidscentra werden ondervraagd en 181 gezondheidswerkers en 868 patiënten/verzorgers geïnterviewd. Geen enkele van de centra met opname capaciteiten had alle zeven componenten van het standaardpakket voor het behandelen van gecompliceerde malaria constant beschikbaar in de 3 maanden voorafgaand aan het onderzoek. Indicaties voor verwijzing waren adequaat voor <10% (18/196) van de patiënten. Snelle en adequate zorg werd gerapporteerd door 29% (247/868) van de patiënten. Gecompliceerde malaria werd correct gediagnosticeerd in 233 (27%) van de patiënten. Hoewel de kininedosis en regime in het merendeel (611/868; 70,4%) van de patiënten correct was, werd het in slechts 18% (147/815) van de patiënten in correcte volumes van 5% dextrose toegediend. De meeste patiënten (80,1%) kregen verscheidene dosissen kinine toegediend in één enkele fles van 500ml 5% dextrose. De medicijnen en toebehoren werden door respectievelijk 385 (44%) en 478 patiënten (70,6%) zelf gekocht.

Malaria behandeling in HIV besmette kinderen onder antiretrovirale (ARV) behandeling

Wij recruteerden 176 HIV besmette kinderen van wie 170 een ARV behandeling ontvingen: 86 ontvingen een op NNRTI-gebaseerde en 84 ontvingen een op lopinavir-ritonavir-gebaseerde ARV behandeling. De malaria incidentie was lager in kinderen die het lopinavir-ritonavir-gebaseerde regime ontvingen dan die op een NNRTI-gebaseerde behandeling (1,32 *versus* 2,25 episodes per persoonjaar; IRR:0,59; 95%CI:0,36-0,97; p=0,04). Zo was ook het risico van een malaria herval na behandeling met artemether-lumefantrine (28,1% *versus* 54,2%; HR:0,41; 95%CI:0,22-0,76; p=0,004). Het mediaan pharmakokinetisch niveau van lumefantrine 7 dagen na de malaria behandeling was beduidend hoger in de lopinavir-ritonavirgroep dan in de groep NNRTI. In de lopinavir-ritonavirgroep was een lumefantrine niveau van meer dan 300ng per milliliter op dag 7 geassocieerd met een 85% lager risico van malariaherval tijdesns de 63 dagen na behandeling. Een groter aantal ernstige bijwerkingen kwam voor in de lopinavir-ritonavirgroep dan in de NNRTI groep (5,6% *versus* 2,3%; P=0,16). Jeuk, pruritus, kwam beduidend vaker voor in de lopinavir-ritonavirgroep, en alanine aminotransferase niveaus waren beduidend vaker hoger in de groep NNRTI groep.

Conclusies en de implicaties voor beleid

Deze studies leveren het bewijs dat beter gefundeerde malaria behandelingsstrategieën in verschillende doelgroepen een verbetering van de behandelingsresultaten tot gevolg hebben. Voor ongecompliceerde malaria betekent dit het consistent en adequaat gebruiken van artemisinin houdende malariabehandelingen (ACT) zowel voor de eerstelijns- als tweedelijnsbehandelings. Voor gecompliceerde malaria behandeling, zal een pakket van maatregelen nodig zijn om een substantiële verbetering te bekomen. Dit zijn onder andere maatregelen die de gezondheidssystemen versterken, de vaardigheden van de gezondheidswerkers verbeteren en beschikbaarheid van geneesmiddelen en toebehoren verbeteren. Voor HIV geinfecteerden, zal het strategische gebruik van op protease-inhibitoren gebaseerde antiretrovirale behandelingen beduidend bijdragen tot verminderde malaria-endemiciteit.

SUMMARY

Background and Rationale

Malaria is a public health problem in over 100 countries worldwide that are inhabited by 40% of the world's population. It is considered one of the world's most important tropical parasitic infections and about 3.3 billion people are considered at risk for malaria infection worldwide. In 2010 alone, an estimated 219 million malaria cases occurred globally, and the disease killed about 660 000 people, mostly children under five years of age. Countries in sub-Saharan Africa (SSA) account for more than 90% of these cases. Uganda as a country is emblematic of the immense malaria burden in SSA as a large majority of its territory is endemic for malaria. Reports by the Ministry of Health indicate that malaria is the leading cause of morbidity and mortality, accounting for approximately 8-13 million episodes per year, 30-50% of outpatient visits at health facilities, 35% of hospital admissions, 9-14% of hospital deaths, with nearly half of these occurring in children less than 5 years of age.

SSA also bears the greatest brunt of the worldwide HIV epidemic with more than two-thirds of the worldwide HIV-infected individuals living in SSA, where 76% of all AIDS-related deaths occurred in 2007. Together, these two diseases, account for a combined 4 million deaths annually. The epidemiological and geographical overlap of HIV and malaria therefore presents a significant public health problem in terms of the management of both infections and the potential interactions between the two diseases.

Malaria is however a preventable and treatable disease and effective preventive and curative tools are available. These include (i) vector control measures, namely ITNs, IRS and, in some limited settings, larval control; (ii) chemoprevention for the most vulnerable populations, particularly pregnant women and infants; (iii) confirmed diagnosis by microscopy or rapid diagnostic tests (RDTs) for every suspected case, and (iv) timely treatment with appropriate antimalarial medicines. Of these measures, disease management stands out as a fundamental and indispensable element of malaria control. However, a number of potential challenges exist that may impact on malaria case management of including the need to review treatment options available for uncomplicated malaria management, especially in light of difficulties in the utilization of oral quinine given its complex dosing regimen, challenges in the management of

severe malaria in resource limited settings that may lead to significant mismanagement and increased mortality, the need to identify alternative therapeutic agents in light of declining efficacy of previously utilized drugs and finally the challenges of management of malaria in special risk groups like HIV infected individuals where the potential for drug interactions could have an impact on of drug efficacy and occurrence of toxicity.

Objectives

In light of the current malaria controvl interventions, we designed a series of studies to better characterize and describe the challenges inherent to the treatment of both uncomplicated and severe malaria in Uganda, as a representative of similar settings in SSA. The specific objectives were i) to assess the effectiveness of oral quinine in the management of uncomplicated malaria, ii) to describe the current management practices for severe malaria at Ugandan health facilities iii) to critically review the historical role of quinine, document its current usage, and provide some insights into its appropriate future use in the treatment of malaria and iv) to explore the potential impact of co-treatment of HIV and malaria on malaria treatment outcomes in HIV infected populations.

Methods

Between 2009 and 2011, we conducted several studies to evaluate different aspects of malaria treatment. Sub-study 1 was an open label randomized trial evaluating the effectiveness of oral quinine and artemether-lumefantrine in the treatment of uncomplicated malaria in Ugandan children. The primary effectiveness outcomes were day-28 parasitological and clinical cure rates. Sub-study 2 was a cross sectional evaluation of severe malaria management practices in selected health facilities in Uganda using multi-stage sampling methods. The assessment employed survey instruments adapted from the World Health Organisation: Hospital assessment tool (WHO, 2002). The main outcome measure was quality of severe malaria case management. Sub-study 3 was a randomized open label trial of HIV-infected children aged 2 months to 5 years eligible for ART or currently receiving an NNRTI-based ART regimen. Participants were

randomized to receive either LPV/r-based or NNRTI-based ART and followed for 2 years, and were treated with artemether-lumefantrine when they developed uncomplicated malaria. The primary endpoint was incidence of malaria. Secondary outcomes included incidence of complicated malaria, efficacy and safety of antimalarial therapy, and lumefantrine pharmacokinetics. In addition, in a review article, we detail the historical role of quinine, considered its current usage and provided some insight into its appropriate future role in the treatment of malaria.

Major Findings

Quinine versus artemether-lumefantrine for treating uncomplicated falciparum malaria

To evaluate the effectiveness of oral quinine versus artemether-lumefantrine for the treatment of uncomplicated, 175 children aged 6 to 59 months with uncomplicated malaria were randomized to receive oral quinine or artemether-lumefantrine administered by care givers at home. Day 28 cure rates unadjusted by genotyping were 96% for the artemether-lumefantrine group compared with 64% for the quinine group (hazard ratio 10.7, 95% confidence interval 3.3 to 35.5, P=0.001). In the quinine group 69% (18/26) of parasitological failures were due to recrudescence compared with none in the artemether-lumefantrine group. The mean adherence to artemether-lumefantrine was 94.5% compared with 85.4% to quinine (P=0.0008). Having adherence levels of 80% or more was associated with a decreased risk of treatment failure (0.44, 0.19 to 1.02, P=0.06). Adverse events did not differ between the two groups.

Case management of severe malaria in health facilities

To evaluate the management practices for severe malaria in Ugandan health facilities, we did a cross sectional survey of health facilities in 11 districts in the eastern and mid-western parts of Uganda. One hundred and five health facilities were surveyed and 181 health workers and 868 patients/caretakers interviewed. None of the inpatient facilities had all seven components of a basic care package for the management of severe malaria consistently available during the 3 months prior to the survey. Referral practices were appropriate for < 10% (18/196) of the patients. Prompt care at any health facility was reported by 29% (247/868) of patients. Severe malaria was correctly diagnosed in 27% of patients (233).Though the quinine dose and regimen was correct in the majority (611/868, 70.4%) of patients, it was administered in the correct

volumes of 5% dextrose in only 18% (147/815). Most patients (80.1%) had several doses of quinine administered in one single 500ml bottle of 5% dextrose. Medications were purchased by 385 (44%) patients and medical supplies by 478 patients (70.6%).

Malaria treatment in HIV infected children receiving antiretroviral treatment (ART)

We enrolled 176 children HIV infected children of whom 170 received the study ART regimen: 86 received NNRTI-based ART and 84 received lopinavir-ritonavir-based ART. The incidence of malaria was lower among children receiving the lopinavir-ritonavir-based regimen than among those receiving the NNRTI-based regimen (1.32 vs. 2.25 episodes per person-year, IRR 0.59, 95% confidence interval [CI], 0.36-0.97, p=0.04), as was the risk of a recurrence of malaria after treatment with artemether-lumefantrine (28.1% vs. 54.2%, HR 0.41 95% CI 0.22-0.76, p = 0.004). The median lumefantrine level on day 7 after treatment for malaria was significantly higher in the lopinavir-ritonavir group than in the NNRTI group. In the lopinavir-ritonavir group, lumefantrine levels exceeding 300ng per milliliter on day 7 were associated with a reduction of more than 85% in the 63-day risk of recurrent malaria. A greater number of serious adverse events occurred in lopinavir-ritonavir group than in the NNRTI group (5.6% vs. 2.3%, P = 0.16). Pruritus occurred significantly more frequently in the lopinavir-ritonavir group, and elevated alanine aminotransferase levels significantly more frequently in the NNRTI group.

Conclusions and implications for policy

These studies provide evidence that more informed treatment approaches can be designed and utilized in the management of malaria in different populations with improvement in treatment outcomes. For uncomplicated malaria this will be achieved through the consistent and timely utilization of ACTs both for first-line and second-line treatment options. For severe malaria, a package of interventions addressing health systems weaknesses, health worker skills and availability of medicines and supplies will provide the necessary positive impact. For HIV infected populations, strategic utilization of protease-inhibitor based antiretroviral regimens will contribute significantly to reduced malaria burden.

CHAPTER 1

GENERAL INTRODUCTION AND BACKGROUND

1.1 Overview of malaria

Malaria is probably one of the oldest diseases known to mankind and has had a profound impact on our history. It is a public health problem in over 100 countries worldwide, that are inhabited by 40% of the world's population and is considered one of the world's most important tropical parasitic infection.[1] The scientific understanding of malaria begun after the discovery of the parasites by Charles Louis Alphonse Laveran in 1880 and the identification of mosquitoes as the vectors, first for avian malaria by Ronald Ross in 1897 and then for human malaria by Italian scientists Grassi, Bignami and Bastianelli between 1898 and 1900.[2] Malaria is caused by infection with protozoan parasites belonging to the genus *Plasmodium* and is transmitted by the bite of female *Anopheles* mosquitoes. Various species of the anopheles mosquito are responsible for transmission in different parts of the world. In Africa, the *A. gambiae* complex is the main vector. Both *A. gambiae* and another species common in Africa, *A. funestus,* are strongly anthropophilic (feed predominantly on humans).[3] Through this preference for biting humans rather than animals, the risk of transmitting malaria from person to person is greatly increased, making these two species the most efficient malaria vectors in the world. Five species of the *Plasmodium* parasite infect humans, namely *P. falciparum*, *vivax*, *ovale*, *malariae* and *knowlesi*, the latter a simian species that may infect humans.[4] Of these species, *P. falciparum* is the most virulent and is responsible for most severe illness and the majority of malaria mortality.

1.1.1 Life cycle of the malaria parasite

Malaria parasites require the presence of two hosts to complete their lifecycle; the definitive host is the anopheles mosquito and the mammal is the intermediate host. The female anopheles mosquito must take blood meals on a regular basis to support the development of successive batches of eggs. The malaria transmission cycle begins when a female anopheles mosquito bites an infected host and ingests gametocytes during its feed. [5] These gametocytes are the first sexual stages of parasite development. Within the gut of the mosquito, the gametocytes are immediately triggered by the fall in temperature usually > 5^0 C, and the presence of a mosquito factor known as xanthurenic acid to begin the formation of gametes. Both female and male gametocytes then break out of the infected red blood cells within the blood meal and quickly fuse to form a zygote. This zygote is adapted to the environment of the blood feed, being resistant to complement within the blood, but susceptible to attacks by phagocytes in the blood and to antibodies that recognize the parasite surface.[5] The zygote then undergoes a two-step meiosis and becomes transformed into a motile ookinete which penetrates the mid-gut wall. Invasion of the mid-gut wall requires the expression of a secretory protein - circumsporozoite, thrombospondin - related protein (CTRP). A fraction of the ookinete emerges through the basal plasma membrane of the mid-gut cell and bumps into the collagenous basal lamina where it comes to rest and initiates its differentiation into an oocyst. This process of transformation into an oocyst differs in detail between the different parasites and involves the rapid expansion of the cytoplasm as the parasite begins vegetative growth. Over the next week or so the ookinete develops and grows, undergoing several divisions to produce large numbers of sporozoites. Eventually, the oocyst wall becomes weakened and bursts, releasing the sporozoites which emerge through the cyst wall and basal lamina and enter into the open circulatory system of the mosquito where they migrate towards and invade the salivary glands.[2]

Sporozoites initially released from the oocyst are morphologically and physiologically different in a number of ways from those later found in the salivary glands. Oocyst sporozoites are poorly infectious to the vertebrate host but will infect mosquito salivary glands. They also express the circumsporozoite protein but poorly express a second surface protein TRAP. Salivary gland sporozoites on the other hand express TRAP at high levels and infect the invertebrate host and not salivary glands.[5] When the mosquito takes its next blood meal, the infective sporozoites are injected into the host along with the mosquito's anticoagulant-containing saliva.[5]

Infection in man begins when these sporozoites are injected into the blood stream during a blood meal by an infectious mosquito. Although it is assumed that one single sporozoite is capable of initiating infection in man, the number of sporozoites injected by a mosquito bite ranges from dozens to thousands. They remain in circulation for a short period of time before they invade liver hepatocytes where they undergo a phase of asexual multiplication (exoerythrocytic schizogony), resulting in the production of many uninucleate merozoites contained within a schizont.[6] The liver stage ends when the mature schizont ruptures to release merozoites into the hepatic sinusoids. Here, much of the released material is ingested by Kupffer cells, but some merozoites escape into the blood stream and invade red blood cells where they initiate a second phase of asexual multiplication (erythrocytic schizogony); each merozoite produces about 8-16 merozoites which invade other red blood cells. This process is repeated almost indefinitely and the time required for erythrocytic schizogony - which determines the interval between the releases of successive generations of merozoites - varies with the species of *Plasmodium* and is the basis of the classic periodicity of fever in malaria patients. [7] As the infection progresses, some young merozoites develop into male and female gametocytes that circulate in the peripheral blood until they are taken up by a female anopheles mosquito at the

time of the blood meal and undergo the process mentioned above also known as sporogony. In summary, malaria parasites undergo three distinct asexual replicative stages (exoerythrocytic schizogony, blood stage schizogony, and sporogony), resulting in the production of invasive forms (merozoites and sporozoites). Sexual reproduction occurs with the switch from vertebrate to invertebrate host and leads to the formation of the invasive ookinete.[7]

1.1.2 Pathogenesis of malaria

The pathological changes in malaria are related to the development of asexual parasites in the blood. Clinical illness is caused by the erythrocytic stages while no disease is associated with the sporozoites, the liver stages and corresponding merozoites and the gametocytes. [8] In the case of *P.falciparum*, the infected red blood cell undergoes a number of changes including altered membrane transport mechanisms, decreased deformability and other mechanical and rheological changes and development of knobs beneath the surface membrane. Other changes include the expression of variant surface antigens, development of cytoadherent and rosetting properties which result into the sequestration of red blood cells containing later trophozoites and schizonts in deep vascular beds and digestion of hemoglobin into pigment. The secondary effects of these changes are related to the host' immunological response to parasite antigens and altered RBC surface membranes. These include stimulation of the reticuloendothelial system, changes in regional blood flow and vascular endothelium, systemic complications of altered biochemistry, anemia, tissue and organ hypoxia and a systemic inflammatory response characterized by release of cytokines like interleukins and tumor necrosis factor- α. [9]

The first symptoms and signs of *P. falciparum* infections are associated with the rupture of erythrocytes due to the release in the blood stream of merozoites. The cytokines, reactive

oxygen intermediates, and other cellular products released during the immune response play a prominent role in pathogenesis, and are probably responsible for the fever, chills, sweats, weakness, and other systemic symptoms associated with malaria. The host's immune response plays a crucial role in modifying the symptoms of disease.[10] However, there are also parasite virulence factors that may account for the wide variation in clinical symptoms including the multiple facets of cytoadherence and initiation of host cytokine release. Thus, the outcome of a malaria infection is not a single homogenous disease, but reflects a number of possible pathophysiological processes arising from the exposure of genetically diverse populations to parasite strains of variable virulence.

1.1.2 Clinical features

The clinical outcome of a malarial infection depends on numerous parasite, host, geographic and social factors that converge in the individual and can result in a range of outcomes. Infection with *P.falciparum* infection can cause disease patterns of various intensities ranging from asymptomatic parasitemia, an acute but self-limiting febrile illness with mild constitutional symptoms and severe life- threatening illness.[11] Following inoculation of sporozoites into the bloodstream, symptoms usually appear after an average period of 12 days. Clinical malaria can be either uncomplicated malaria or severe malaria, differing in the presence of signs of severity or signs of vital organ dysfunction.[11] Uncomplicated malaria usually presents with fever and nonspecific symptoms, such as vomiting and/or diarrhea, a clinical picture that closely resembles that of many other common childhood infectious diseases. Uncomplicated malaria is therefore a relatively mild disease and is seldom fatal though, if not diagnosed early and treated promptly, may evolve towards the severe form.[12] Severe malaria is a complex multi-system disorder

presenting with a range of clinical features. Operationally it is defined as any malaria case associated with a high mortality (>5%), even after appropriate treatment in hospital.[11] The WHO criteria for severe falciparum malaria include at least one of the following criteria: coma, severe anemia, respiratory distress, hypoglycemia, circulatory collapse, spontaneous bleeding, haemoglobinaemia, acidosis and repeated convulsions. The supporting criteria include: impaired consciousness, jaundice, prostration, hyperpyrexia and hyperparasitaemia.[13] At the individual patient level, there is usually significant overlap of symptoms with multiple symptoms associated with an increased risk of mortality. [14]

1.2 Burden of Malaria

1.2.1 Global burden of malaria

Worldwide, 3.3 billion people are at risk for malaria infection. In 2010, an estimated 219 million malaria cases occurred globally, while the disease killed about 660 000 people, mostly children under five years of age, with countries in sub-Saharan Africa (SSA) accounting for more than 90% of these cases[1]. There are several reasons why sub-Saharan Africa (SSA) bears a large proportion of the world's malaria burden. First, most malaria infections in SSA are due to *Plasmodium falciparum*, the most virulent of the human Plasmodium species. Second, this region is also home to the most efficient malaria mosquito vectors which guarantee an intense and stable transmission.[15] Third, most African countries are very poor, lacking the basic infrastructure and resources necessary to mount sustainable malaria control efforts. [15] Malaria transmission in SSA is heterogeneous, both across and within countries. It is generally characterized by high rates of infection with transmission intensity in most endemic areas ranging from <10 to several hundred infective bites per person year.[16]

The risk of developing symptomatic disease is inversely proportional to the level of acquired immunity. Partial immunity develops through repeated exposure, leading first to protection against severe forms of disease, followed by protection against symptomatic illness.[17] The result of this phenomenon is that the burden of malaria in SSA is heavily borne by young children, with an estimated 75% of the worldwide malaria deaths occurring in African children under the age of 5 years. Other risk groups include pregnant women, patients with HIV/AIDS and non-immune travelers. These different groups therefore warrant particular attention and should be targeted by preventive interventions.

Another important aspect of the malaria burden is that it exerts a heavy economic burden on endemic countries, contributing to the cycle of poverty and limiting economic development. Representing 10% of the overall disease burden, malaria places a substantial strain on health services and each year costs SSA about US$ 12 billion in lost production. [18]

1.2.2 Malaria in Uganda

Uganda as a country is emblematic of the immense malaria burden in SSA as the large majority of its territory is endemic for malaria. The climate in Uganda also allows for stable, year round transmission, with very little seasonal variability in most areas. Indeed, some of the highest reported entomologic inoculation rates (EIR, number of infective mosquito bites per person per year) can be found in Uganda and range from 562 in Tororo district to 1586 in Apac district.[16] The most common malaria vectors in the country are *Anopheles gambiae s.l.* and *A. funestus,* with the former being often the dominant species in most locations.[16] *A. funestus* is seen more frequently in high altitude areas and during the short dry seasons, when permanent water bodies are the most common breeding sites. Within the *A. gambiae* complex, the predominantly

anthropophilic *A.gambiae s.s.* is by far the most common. *Anopheles gambiae s.s.* and *A. funestus* are both highly endophagic (feeding indoors) and endophilic (resting indoors). Because of these attributes, the preferable vector control strategies in these settings would include Insecticide Treated Nets and Indoor Residual Spraying.[19] Four species of malaria parasites exist in Uganda, though *P. falciparum* is responsible for the vast majority of cases. [20] Other species appear to account each for <5% of cases, with some mixed infections also reported. [21]

Reports by the Ministry of Health (MOH) indicate that malaria is the leading cause of morbidity and mortality, accounting for approximately 8-13 million episodes per year, 30-50% of outpatient visits at health facilities, 35% of hospital admissions, 9-14% of hospital deaths, nearly half of these occurring in children less than 5 years of age. [20] The situation in Uganda is also unique in that, despite reports of a decline in malaria burden in some African countries,[22] available data from multiple sources show that the malaria burden in Uganda has not decreased notably in recent years, and it may even have increased. [23, 24, 25] These reported high mortality rates and morbidity trends therefore call for renewed efforts in the fight against malaria in these settings.

1.3 Impact of HIV infection on malaria

Malaria and HIV are two of the most important infectious diseases worldwide, accounting for a combined 4 million deaths annually.[26] The two epidemics disproportionately affect SSA, which is home to only 11% of the world's population, but suffers the brunt of both diseases. The continent carries the bulk of the HIV/AIDS epidemic, with more than two-thirds (68%) of the worldwide HIV-infected individuals living in SSA, where 76% of all AIDS-related deaths occurred in 2007.[27, 28] Although HIV-infection and malaria

are driven by very different routes of transmission, their high prevalence and geographic overlap suggest that even small interactions in co-infected individuals could lead to substantial population effects. [29]

Concurrent HIV infection impacts on malaria in several ways. First, HIV infection could disrupt the acquired immune response to malaria and thereby may increase incidence and severity of malaria in HIV infected populations. [30] Several studies in a number of African countries provide evidence to support this observation. [28, 31-35] Using modeling methods, a study in Kisumu, Eastern Kenya, showed that HIV infection may have been responsible for about 10 % of adult malaria episodes translating into almost 980,000 excess malaria episodes in the town over the last 32 years. [36]Studies have also reported particularly higher risks of malaria with increasing immunosuppression.[28, 31, 35] Secondly, malaria treatment outcomes are a concern in HIV infected individuals as impaired cell-mediated immunity caused by HIV may impact on the response to standard antimalarial treatment. It has indeed been reported that HIV infection may be associated with reduced efficacy of antimalarial treatment with earlier studies reporting delayed parasite clearance. [37] Other aspects of these interactions include the fact that routine interventions for HIV may impact upon the incidence of malaria in HIV infected populations [38, 39] and the possibility that therapies for each infection may impact upon the other, leading to unanticipated effects on drug efficacy or toxicity. [40] However, with recent developments in the management of these two diseases and rapid scale-up of these interventions, there is emerging evidence suggesting that some of these previously documented interactions between HIV and malaria may be modified. It is important therefore that the effect of HIV infection on malaria morbidity and mortality is best described in the context of the current standard of care for malaria and HIV infection.

1.3.1: Malaria control issues in HIV infected populations

For the prevention and treatment of malaria in HIV infected populations current standard of care would include wide availability of insecticide treated nets (ITNs), use of trimethoprim-sulfamethoxazole (TS) prophylaxis, intermittent preventive treatment with sulfadoxine–pyrimethamine (SP) or TS in pregnant women and wide availability of different artemisinin-based combination therapies (ACTs) to treat malaria. For the treatment of HIV infection, increasing availability and rapid scale up of antiretroviral therapy has ensured wider coverage of the population that requires this treatment. With these multiple interventions, the effect of HIV infection on malaria is expected to have therefore changed over the past few years. For example, available data show that the wider implementation of ITN, TS prophylaxis, and antiretroviral therapy might substantially reduce the morbidity of malaria in HIV-infected patients.[38, 39] With these observations, it may therefore be expected that from a public health stand point, HIV-infection may no longer be considered a risk factor for malaria among those accessing care for HIV infection as such individuals are now, paradoxically, protected from malaria by TS prophylaxis and ITNs. These findings therefore highlight the need for confirmatory diagnosis of malaria in HIV infected individuals receiving these interventions and provision of malaria therapy only when the diagnosis is confirmed.

Regarding treatment for malaria, ACTs are now widely recommended as first line drugs for the treatment of uncomplicated malaria in several African countries. The most commonly used ACTs in these settings include artemether-lumefantrine (AL), and amodiaquine-artesunate (AQ/AS), with dihydroartemisinin-piperaquine (DP) also utilized in some settings. However, data on their safety and efficacy in HIV-infected populations is still limited and only a few previous studies have described these observations. In a randomized controlled trial of

artemether-lumefantrine versus sulfadoxine-pyrimethamine for the treatment of uncomplicated malaria in Zambian adults, the frequency of malaria treatment failure, with either therapy increased significantly with advancing immunosuppression.41 A study in Uganda using molecular genotyping showed that the increased risk of clinical treatment failure in HIV infected individuals was a result of new infections rather than recrudescence.42 In this latter study, the risk of clinical treatment failure due to new infections was >3-fold higher for HIV-1–infected adults than for HIV-uninfected patients (hazard ratio 3.28, $p = 0.02$). More recently however, a study in Uganda showed that both HIV infected and uninfected children responded well to AQ/ AS treatment for uncomplicated malaria.43 In yet another study, two cohorts of HIV-infected Ugandan children living in areas of medium and high transmission were evaluated for response to antimalarial therapy. The children in these cohorts were given ITNs, TS prophylaxis and antiretroviral therapy when indicated and uncomplicated malaria was treated with AS/AQ, AL or DP. All three ACTs were found to be 100% efficacious after adjustment by genotyping; however, AS/AQ was associated with a higher risk of neutropenia compared to AL and DP had the added benefit of lowering the risk of recurrent parasitemia compared to AL (7.1% vs. 34%, $p < 0.001$). In summary therefore, these findings show that AL and DP are highly efficacious and safe for the treatment of uncomplicated malaria in HIV-infected children. 44 AS/AQ, though highly efficacious, was poorly tolerated and associated with a high risk of neutropenia.43, 44 In addition, interactions between ARTs and antimalarials also deserve attention. Interactions may enhance antimalarial activity, but also exacerbate toxicity. Adjustment of antimalarial dosing may be necessary to avoid toxicity, but adequate data to guide dosing adjustments are not yet available. In this thesis we only address malaria treatment concerns in HIV infected individuals and describe the potential interactions between ARTs and antimalarial medicines when used for

treating uncomplicated malaria in HIV infected individuals receiving ART. This thesis does not address other malaria preventive and control strategies in this special risk population.

1.4 Malaria Control and Treatment approaches

1.4.1 Overview of control strategies

Malaria is a preventable and treatable disease, and effective preventive and curative tools are available. The desirable impact however will only be achieved when these currently recommended interventions are effectively implemented ensuring universal and consistent coverage. Several control and preventive strategies can be implemented and include (i) vector control measures, namely ITNs, IRS and, in some limited settings, larval control; (ii) chemoprevention for the most vulnerable populations, particularly pregnant women and infants; (iii) confirmed diagnosis by microscopy or rapid diagnostic tests (RDTs) for every suspected case, and (iv) timely treatment with appropriate antimalarial medicines.[15] Of these measures, disease management stands out as a fundamental and indispensable element of malaria control. Its aims are to avoid the progression of uncomplicated malaria to severe or complicated disease and to prevent death or sequelae.

Of the 200 million clinical episodes of malaria occurring annually among young African children, 4 to 6 million are severe and life threatening forms, resulting in 1-2 million deaths. [13] This implies that severe malaria represents only a small proportion of all malaria cases, which are mostly uncomplicated and can receive oral therapy. In the treatment of uncomplicated malaria, the main objective of treatment is to cure the infection. [11] The primary objective of antimalarial treatment in severe malaria is to prevent death. In cerebral malaria cases, prevention

of neurological deficit is also an important objective and for severe malaria in pregnancy, saving the life of the mother is the primary objective.

Efforts to reduce the burden of malaria have intensified over the recent few years.[45] These efforts have been made possible by the increased resources for malaria control provided by individual governments and international organizations. Whereas some settings have achieved some positive progress,[15] these success stories are not universal. In settings with no appreciable reduction in malaria, one major limitation or draw back has been the inability of these countries to scale up control interventions to ensure sufficient coverage to be able to obtain any appreciable impact. Of the various malaria control interventions, one that is of particular interest to clinicians is effective case management. Therapeutic approaches to malaria case management have greatly evolved over the last decade. For example in Uganda, treatment recommendations for uncomplicated malaria have changed from chloroquine monotherapy to combinations of chloroquine with sulphadoxine-pyrimethamine, and then to the currently recommended artemether-lumefantrine, an ACTs. For severe malaria, the mainstay of treatment has been intravenous quinine, though more recently intravenous artesunate has become the recommended first-line therapy.[11] However, despite such recommendations, in resource limited settings like Uganda there are still significant challenges in the management of malaria that require some consideration.

1.4.2 Potential challenges in the management of malaria

Currently, about 41 African countries have adopted ACTs as the first-line treatment for uncomplicated malaria. In most of these settings (29 countries), the recommended second line treatment for uncomplicated malaria is oral quinine.[11] However, important challenges with the

utilisation of oral quinine exist including complex dosing regimens, the prolonged treatment course and its unfavourable adverse event profile. However, data on the impact of these attributes on the effectiveness of oral quinine for the treatment of uncomplicated malaria are limited. Such evidence would be important as a justification for the need to recommend alternatives to oral quinine. In the event that poor effectiveness is demonstrated, identification of alternatives to oral quinine for second-line therapy for uncomplicated malaria would then become an important priority. ACTs are a potential option for second line therapy as they are very efficacious and generally have simple dosing regimens which enhance adherence to therapy and greatly improve treatment outcomes.

Severe malaria management presents yet another priority given that it is a medical emergency associated with an immediate threat to life and so requires prompt treatment. The priority requirement for successful treatment therefore is the early recognition of the signs and symptoms that should lead to emergency care in an in-patient setting. For patients with severe malaria, oral treatment is no longer possible and injectable or rectal drug administration is required; in such situations, any delays in referral to appropriate levels of care may be fatal. According to current WHO treatment guidelines, intravenous artesunate is the currently recommended drug of choice for treating severe malaria though intravenous quinine remains the most widely used treatment in most parts of Africa. Whereas the effective translation of such treatment policy guidelines into clinical practice is key for preventing deaths, many African countries face challenges with accessibility to good quality care as well as with the administration of intravenous drugs. In many cases, infusion is either impossible due to lack of equipment, lack of trained staff or potentially hazardous because of risk of infections such as HIV. When intravenous

administration is not possible, intramuscular treatment is usually recommended; however this too is associated with various complications including paralysis due to sciatic nerve damage and infections.[46-48] Management of severe malaria therefore presents several challenges in terms of adequacy of diagnosis and appropriateness of treatment practices. Unfortunately, both diagnostic approaches and treatment practices of severe malaria in resource limited settings (RLS) are not well documented and yet for any effective recommendations to be made for improvement in practice, these have to be based on available evidence.

Whereas malaria treatment with quinine marked the first successful use of a chemical compound to treat an infectious disease, [49] the continued role that quinine will play in the management of malaria needs scrutiny given the numerous developments in the malaria treatment arsenal to date. The management of severe malaria has seen new developments especially in light of recent findings from the AQUAMAT and SEAQUAMAT studies [50, 51] that showed that the use of intravenous artesunate was associated with a significantly lower mortality compared to intravenous quinine. Given these observations and the modifications in the WHO recommendations for the treatment of malaria, a comprehensive and objective evaluation of the continued role of quinine in the management of malaria is vital.

Therapeutic options in high risk populations also need further study as they may present important challenges given the potential for drug interactions. This is particularly important given the high prevalence of other co-existent co-morbidities in SSA. Of particular interest among these co-morbidities is HIV which also causes significant morbidity and mortality and together with malaria account each year for a combined 4 million deaths. [18] In Africa, HIV infected populations therefore present peculiar challenges in the co-management of both

diseases. This is of increasing importance because antiretroviral therapy is being rapidly scaled up, raising the possibility that in regions with high prevalence of both HIV and malaria, co-infected individuals will be frequently treated for both malaria and HIV. Co-administration of ART and antimalarial drugs creates potential for pharmacokinetic drug interactions due to the induction or inhibition of cytochrome (CYP) enzymes or drug transporters. [52] However, there are currently scanty clinical data on malaria treatment outcomes in individuals receiving antiretroviral drugs and the potential impact of any drug-drug interactions is not well documented. In addition, the majority of previous studies have been done in adult populations with limited data in children. Given that children bear the greatest brunt of malaria, it is important to understand the potential impact of concurrent treatment with antiretroviral drugs on malaria treatment outcomes in HIV infected children. The focus of this PhD work was therefore to fully characterize and describe these potential challenges in the management of malaria. The specific components addressed included the following

1. Evaluation of the current treatment policy for the management of uncomplicated malaria to provide evidence in support of these policy recommendations,
2. Assessment of the management practices for severe malaria in these resource limited settings
3. Evaluation of malaria management in HIV infected individuals as an important risk group in Africa.

1.5 Objectives of the thesis

Our general objective was to describe the challenges inherent to the treatment of both uncomplicated and severe malaria in Uganda as a representative of similar settings in SSA.

The specific objectives were;

1. To assess the effectiveness of oral quinine in the management of uncomplicated malaria.
2. To describe the current management practices for severe malaria at Ugandan health facilities.
3. To critically review the historical role of quinine, document its current usage, and provide some insights into its appropriate future use in the treatment of malaria.
4. To explore the potential impact of co-treatment of HIV and malaria on malaria treatment outcomes in HIV infected populations.

1.5.1 Organization and outline of the thesis

This thesis is organized into six chapters.

Chapter 1 is the general introduction and rationale of the studies herein. This chapter also details the general and specific objectives of the thesis and details the general organization and outline of the thesis.

Chapter 2 presents the results of a randomized controlled trial comparing the effectiveness of oral quinine and artemether-lumefantrine in the management of uncomplicated malaria in children.[53] This study was also an assessment of the 'real 'life effectiveness of the current malaria treatment policy in Uganda and other African countries that recommend oral quinine as the second-line treatment for uncomplicated malaria.

Chapter 3 presents findings of a cross-sectional study evaluating severe malaria treatment practices in Ugandan health facilities and provides some insight into severe malaria treatment practices that may be similar to practices seen in other SSA settings.[54]

Chapter 4 is a review article that presents a historical perspective of the role of quinine in the management of malaria. It details information on its current usage and provides a critical evaluation of what the potential future role of quinine would be in the management of malaria, especially in the light of recent developments and advances in malaria case management.[55]

Chapter 5 presents results of a randomized controlled trial in a population of HIV infected children receiving two different antiretroviral treatment (ART) regimens.[56] In this study we compared the differences in incidence of malaria and malaria treatment outcomes between two ART treatment arms.

Chapter 6 is a general discussion of our findings and their implications for malaria case management and policy as well as their potential public health impact. We also discuss the main strengths of these studies as well as their potential limitations and offer some conclusions and recommendations from this thesis.

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CHAPTER 2

EFFECTIVENESS OF QUININE VERSUS ARTEMETHER-LUMEFANTRINE FOR TREATING UNCOMPLICATED FALCIPARUM MALARIA IN UGANDAN CHILDREN: A RANDOMISED TRIAL

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ABSTRACT

Objective: To compare the effectiveness of oral quinine with that of artemether-lumefantrine in treating uncomplicated malaria in children.

Design: Randomized, open-label effectiveness study

Setting: Outpatient clinic of Mulago Hospital, Uganda's national referral hospital

Participants: 175 children aged 6 to 59 months with uncomplicated malaria.

Interventions: Participants were randomised to receive either oral quinine or artemether-lumefantrine administered by parents or guardians at home.

Main outcome measures: Primary outcomes were parasitological cure rates after 28 days of follow-up unadjusted and adjusted by genotyping to distinguish recrudescence from new infections; secondary outcomes included adherence to study medication, presence of gametocytes, haemoglobin recovery and safety profiles.

Results: Using survival analysis, the cure rate unadjusted by genotyping was 96.0% for the artemether-lumefantrine group compared to 64.4% for the quinine group (HR 10.7, 95% CI 3.3 to 35.5, p=0.001). In the quinine group 18 of 26 (69%) parasitological failures were due to recrudescence compared to none in the artemether-lumefantrine group. The mean adherence to artemether-lumefantrine was 94.5% compared to 85.4% for quinine (p=0.0008). Having adherence levels of ≥ 80% was associated with a decreased risk of treatment failure (HR 0.44, 95% CI 0.19-1.02, p = 0.06). Adverse events did not differ between the two treatment groups.

Conclusions: The effectiveness of a 7-day course of quinine for the treatment of uncomplicated malaria in Ugandan children was significantly lower than that of artemether-lumefantrine. These findings call into question the advisability of the recommendation for quinine therapy for uncomplicated malaria in Africa.

Trial registration: NCT00540202

INTRODUCTION

Most episodes of falciparum malaria are uncomplicated and treated with short courses of oral antimalarial medicines. Chloroquine was the mainstay of therapy for uncomplicated falciparum malaria until the late 1990s. With increasing resistance to chloroquine, sulfadoxine-pyrimethamine or amodiaquine were adopted as first-line therapy in a number of countries. However, increasing resistance to both of these drugs, has led to recommendations for combination therapy, preferably artemisinin-based combination therapies (ACTs) [1]. Consequently, artemether-lumefantrine or artesunate-amodiaquine have been adopted as first-line regimens for uncomplicated falciparum malaria in nearly all countries in Africa.

Quinine was the first established antimalarial drug, and it has been used to treat malaria for centuries [2]. Intravenous quinine is the standard therapy for severe falciparum malaria in all African countries. Many malaria endemic countries that recently adopted ACTs as first-line therapy recommend quinine as the second line regimen for uncomplicated malaria despite guidelines from the World Health Organisation (WHO) that antimalarial medicines should be used in combination [3]. Probably such a choice was influenced by the few alternatives to quinine at the time the policy change to ACT was done. Currently, of the 41 African countries that have adopted ACTs as the first-line treatment for uncomplicated malaria, 29 recommend quinine as second line therapy[4]. In addition, due to decreased efficacy of older agents and limited availability of ACTs, quinine is increasingly used as a first-line drug in the treatment of uncomplicated malaria in Africa.

In Uganda, according to the national malaria treatment policy, quinine in its tablet formulation is used as the second line treatment for uncomplicated malaria. A survey carried out in Uganda in 2007 reported that in rural health facilities quinine was prescribed for only 4% of all patients with uncomplicated malaria [5] while at the outpatient facility of Mulago Hospital, Kampala, this proportion was as high as 26% (unpublished data). Thus, although it is not listed as a first-line drug for uncomplicated malaria in any country, quinine is still widely used for this purpose.

Despite increasing use of quinine, few studies of its efficacy in the management of uncomplicated malaria have been published recently. In particular, few studies have used modern methods for outcome determination (at least 28 days of follow-up and molecular genotyping to distinguish recrudescence from new infections after treatment). Treatment of uncomplicated malaria with a shorter course (less than 7 days) of quinine was generally inferior to a 7-day treatment course [6]. Considering 7-day courses, available recent studies have shown 28-day quinine failure rates over 10% for uncomplicated malaria in Sudan[7] and Thailand [8]. Quinine efficacy may be particularly poor in pregnant women; both genotype-adjusted efficacy after supervised 7-day therapy in Thailand [9] and unadjusted effectiveness after 7-day therapy in Gabon [10] were only about 60%. However, other studies have shown excellent efficacy for quinine, including over 95% success after 28 days for the treatment of falciparum malaria in Equatorial Guinea [11], in Venezuela [12], and in returned travelers in France [13].

As study designs have varied, it is difficult to ascertain if limitations in quinine effectiveness in some studies were due principally to true drug resistance, varied pharmacokinetics, poor compliance with a 7-day regimen, or a combination of these factors. However, available evidence suggests that African strains of *P. falciparum* generally remain sensitive to quinine [14-

19] and that variability in quinine pharmacokinetics does not explain varied treatment responses [20]. Thus, poor compliance with a 7-day quinine regimen might contribute importantly to limitations in quinine efficacy. Poor compliance with quinine, compared to shorter regimens, has been documented previously [21]. Inadequate compliance is likely due both to the need for frequent dosing over one week and known poor tolerance of quinine, which frequently causes the syndrome of cinchonism, including nausea, headache, tinnitus, and blurred vision [22] at treatment doses. Considering the uncertain effectiveness of quinine and its increasing role in the treatment of uncomplicated malaria in Africa, it was of interest to compare its effectiveness with that of artemether-lumefantrine, the new first-line therapy for uncomplicated malaria in Uganda.

METHODS

Study design and study site

This was an open-label randomised effectiveness study to compare parasitological and clinical cure rates and adherence between quinine and artemether-lumefantrine in children with uncomplicated malaria. Participants were recruited from the main outpatient clinic at Mulago, Uganda's national referral hospital.

Participants

Children aged 6 to 59 months were included if they satisfied the following inclusion criteria: having axillary temperature $\geq 37.5\ ^{0}C$ or history of fever in the past 24 hours, microscopically confirmed *Plasmodium falciparum* mono infection with any asexual parasite density, ability to

tolerate oral therapy, and no history of antimalarial drug intake in the preceding two weeks. Patients were not recruited if they met at least one of the following exclusion criteria: a history of allergy to quinine or artemether-lumefantrine, evidence of severe malaria or other concomitant febrile illness or residence more than 20 km from the health clinic. Severe malaria was defined according to WHO criteria [23]

Enrolment procedures

Participants suspected to have malaria during an outpatient visit (Day 0) were assessed for eligibility and referred to the laboratory for thick (to assess parasite density) and thin (to assess parasite species) blood smears. Participants fulfilling all selection criteria had a standard baseline history and physical examination done by a study physician. Study data were recorded on case record forms, and participants were identified by their initials and study identification numbers. Hemoglobin was measured from finger-prick blood samples using a portable spectrophotometer (HemoCue, Angelhom, Sweden). Blood samples were also stored on Whatmann filter paper for subsequent molecular studies.

Randomisation and Treatment

Participants were randomly assigned to receive either oral quinine or artemether-lumefantrine. Block randomization was used with blocks of 20. Computer-generated randomization codes were prepared by an independent individual and enclosed in sequentially numbered opaque sealed envelopes, each of which contained a given treatment allocation. The envelopes were assigned in

sequential order to participants after inclusion. Participants in the quinine arm received a 7-day treatment course of quinine sulphate given as 10 mg/kg of body weight per dose thrice daily. Quinine sulphate was provided as tablets of 300 mg (Rene Pharmaceutical Industries, Kampala, Uganda); the quality of the drug was certified by the Uganda National Drug Authority. The dosage was weight-adjusted by dividing the tablets when necessary. Parents or caregivers were instructed by the study nurse that quinine tablets should be taken with clean water every 8 hours. Patients in the artemether-lumefantrine treatment arm received the WHO recommended weight-specific artemether-lumefantrine (Coartem®) blister packs (10.0 –14.9 kg: 1 tablet per dose; 15.0–24.9 kg: 2 tablets; 25.0–34.9 kg: 3 tablets; ≥ 35 kg: 4 tablets; Novartis Pharma AG, Basel, Switzerland). Parents or caregivers were instructed by the study nurse to administer a dose of artemether-lumefantrine eight hours after the first dose, and then each morning and evening for the following two days, with all doses given 30 minutes to 1 hour after food, preferably containing fat or oil. The first dose of the study drugs were administered under the supervision of the study nurse, who then provided the remaining doses to be taken at home. All patients were observed for 30 minutes; those who vomited within that time received a repeat dose. Parents or caregivers were informed that any subsequent dose vomited within 30 minutes of administration should be re-administered, with the option of coming to the clinic to collect a replacement dose. Parents or caregivers were counseled about the necessity to comply with the full treatment course and potential drug side effects. They were also encouraged to present to the study clinic in case of persistence or reappearance of symptoms. Concomitant medications were prescribed as needed and documented on case record forms; antibiotics with antimalarial activity like tetracyclines, macrolides and antifolates were not used in this study. At the end of the enrolment visit (Day 0) the patients were taken home by study personnel. Parents or caregivers were not

aware on leaving the clinic that they would be visited at home or that they would be assessed for adherence.

Follow-up

A field worker and a nurse visited participant's homes on Day 3 and day 7 for the artemether-lumefantrine and quinine group respectively, in both cases the day after treatment was scheduled to be completed. At this home visit a questionnaire was used to assess adherence. Information collected included basic sociodemographic data and information about the timing and method of administration of each dose. The blister pack of artemether-lumefantrine and the drug envelope for the quinine tablets were examined except when missing, for any remaining tablets. At the end of this home visit, patients in the artemether-lumefantrine arm were given appointments for subsequent follow-up visits and patients in the quinine group were brought back to the clinic. Participants were followed up for 28 days; follow-up visits to the clinic were scheduled on Days 7, 14 and 28 for both treatment groups. Patients were also seen at the clinic on any unscheduled day if they felt unwell. On each of these clinic visits a standardized history and physical examination were done, and blood was obtained by finger-prick for blood smears (for asexual parasite density and gametocytes) and hemoglobin assessment. Blood was also collected on filter paper for subsequent molecular studies. In the event of treatment failure, thin smears were examined for malaria parasite species. Participants who failed treatment were treated with dihydroartemisinin/piperaquine (Duocotexcin®) once daily for 3 days. Participants who developed severe malaria were referred for treatment with intravenous quinine. Participants who for any reason could not continue with prescribed medications, those who developed a serious concomitant illness that interfered with outcome classification, those who could not be located

within 48 hours of a scheduled visit or who withdrew consent were included in the analysis and censored on their last day of follow-up.

Adverse events defined as any unfavorable or undesirable medical occurrence were assessed at each visit and recorded on case record forms. Association of adverse events with the study drugs was classified as definite, probable, possible, unlikely or none; according to WHO and the International Conference on Harmonisation guidelines [24] . Adverse events were graded as serious if they were fatal or life-threatening, resulted in prolonged hospitalization, caused persistent disability or incapacity, or required medical/surgical interventions to prevent serious outcomes.

Definition of adherence

Adherence to treatment was assessed by patient report and pill counts; overall adherence was reported as the percentage of prescribed pills taken. In addition, for the artemether-lumefantrine arm, additional components of adherence assessment included i) presence of an empty blister pack, ii) count of the number of tablets administered based on those remaining in the blister pack, iii) caregiver reported dosing schedule and iv) caregiver report on whether tablets were given after food. Based on these components, adherence to artemether-lumefantrine was further qualified as optimal adherence (all 4 components present), good adherence (any 3 components present) or non-adherence (≤ 2 components present).

Laboratory procedures

Asexual malaria parasite density was determined using thick blood smears stained with 2% Giemsa for 30 minutes and counting parasites per 200 white blood cells. Results were expressed

as parasites per µl assuming a total white cell count of 8000 per µl. A thick blood smear was recorded as negative when no asexual forms were found on examination of 100 high power fields. All slides were read by a second microscopist and a third reviewer settled any discrepant readings. Microscopists were blinded to treatment assignments.

For participants with recurrent parasitemia after Day 3, DNA was extracted from filter paper blood samples collected at enrollment and on the day of failure using chelex [25]. To distinguish between recrudescence and new infection, samples from enrolment and the day of treatment failure were compared in a stepwise manner on the basis of polymorphisms in *msp-1, msp-2* and 4 microsatellites.[26]

Outcome classification

Treatment outcomes were assessed according to WHO guidelines as adequate clinical and parasitological response, early treatment failure, late clinical failure or late parasitological failure [27]. The primary effectiveness endpoint was the PCR-adjusted clinical and parasitological cure rate at day 28; secondary effectiveness outcomes included PCR-unadjusted cure rate at day 28, adherence with study treatment regimens, presence of gametocytes, haemoglobin recovery from baseline at day 28 and incidence of adverse events.

Sample size estimation

This study was designed to test the hypothesis that there would be no difference between the effectiveness of quinine and artemether-lumefantrine in the treatment of uncomplicated malaria. We calculated that 151 patients would be needed in each treatment arm using a 5% level of

significance and power of 90%, and assuming a difference in adherence between the two groups of 19%[21, 28] and a 10% loss to follow-up.

Statistical Analysis

Data were double entered into EPI-info software version 6.04 and analyzed using Stata version 10.0 (StataCorp, College Station, Texas). Effectiveness analysis was done using a modified intention to treat with survival analysis.Categorical data were compared with χ^2 or Fisher's exact tests. Differences between group means were analyzed using Student's *t* test, applying log-transformation when appropriate. Risks of treatment failure were estimated using Kaplan Meier survival analysis. Two tailed *P* values were used and all analyses were done at a 5% significance level.

RESULTS

Trial profile and baseline characteristics

A total of 325 participants were screened for eligibility between September 2007 and April 2008; of these 147 (46%) were excluded, most commonly for antimalarial drug use in the 2 weeks prior to enrollment or unwillingness to provide informed consent (Figure 1). A planned interim analysis was undertaken by the data and safety monitoring board after178 participants had been recruited. The oral quinine group met the pre-defined stopping rule of a significant difference in cure rate using the O'Brien Fleming method with a p value of < 0.001 and the study was halted.

89 participants were randomized to receive artemether-lumefantrine and 86 to receive quinine. During follow-up, 28/175 participants (16%) were withdrawn, 13 in the quinine arm (12 lost to follow-up and 1 took other antimalarials) and 15 in the artemether-lumefantrine arm (14 lost to follow up and 1 took other antimalarials). These participants were included in the analysis and censored on their last day of follow-up. Thus, primary outcomes (unadjusted and adjusted by genotyping) were available for 73/86 participants (85%) in the quinine treatment arm and 74/89 participants (83%) in the artemether-lumefantrine treatment arm. Baseline characteristics were comparable in the two groups (Table 1).

Figure 1. Trial Profile

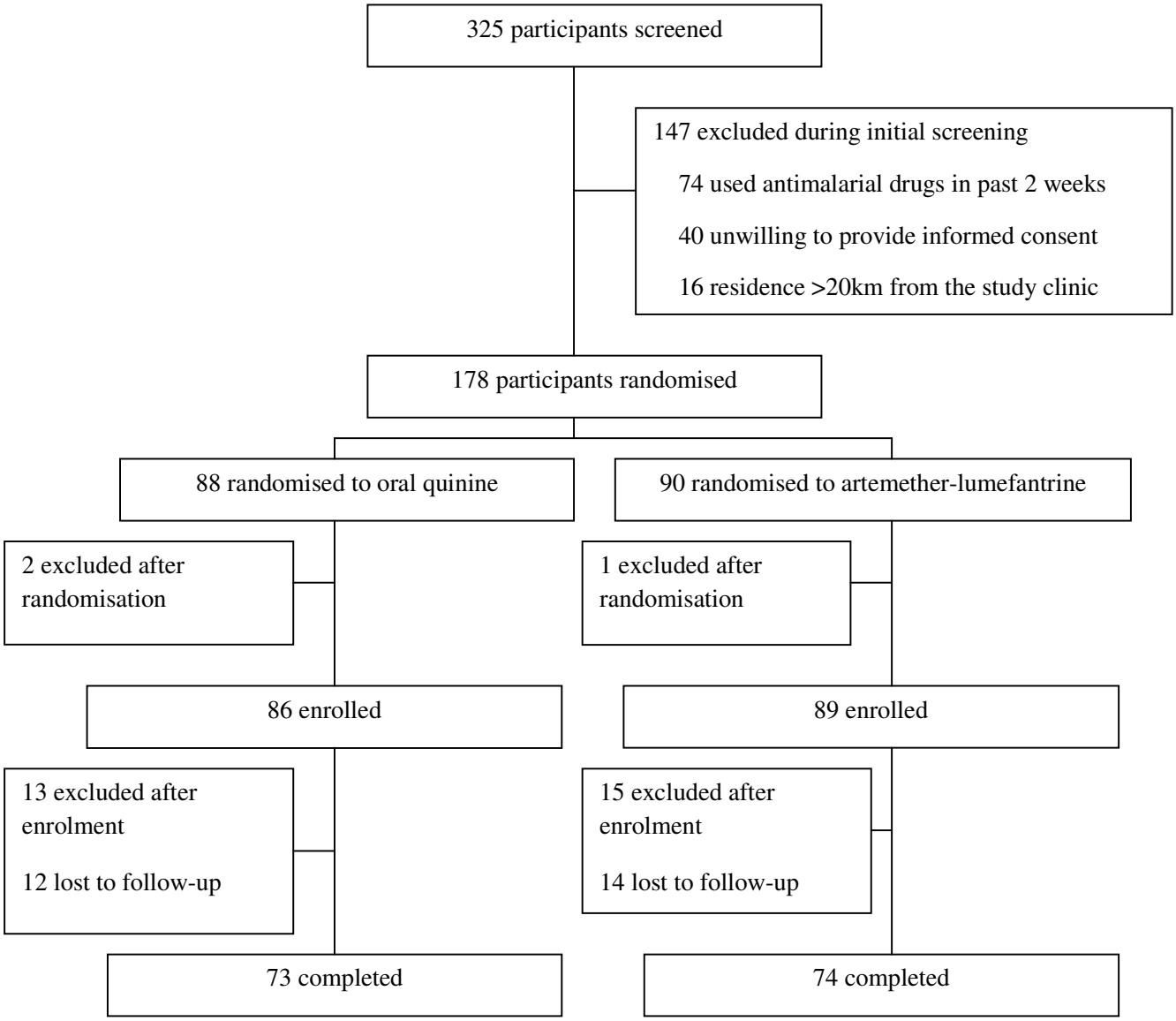


Table 1 Baseline characteristics of children with uncomplicated malaria randomised to receive quinine or artemether-lumefantrine. Values are means (standard deviations) unless stated otherwise

Characteristic	Quinine group (n=86)	Artemether-lumefantrine group (n=89)
Age (years)	2.0 (1.5)	2.1 (1.4)
No (%) girls	45 (52)	49 (55)
Weight (kg)	11.3 (3.3)	11.3 (3.0)
Axillary temperature (°C)	37.8 (1.4)	37.7 (1.3)
Haemoglobin concentration (g/l)	97 (20)	96 (21)
No (%) with gametocytes present	7 (8)	10 (11)
Parasite density (geometric mean/µl)	14 107	16 124
No (%) with parasitaemia <5000/µl	24 (28)	22 (25)

Primary treatment outcomes

Cure rates were significantly higher in the artemether-lumefantrine group compared to the quinine group. Using survival analysis, cure rates unadjusted by genotyping were 96% for the artemether-lumefantrine group compared to 64.4% for the quinine group ($p< 0.001$; Table 2). Early treatment failure was uncommon; only 2 cases were seen, both in the quinine arm: one child with repeated convulsions (> 2 in 24 hours) and another with profuse vomiting and prostration. They were both hospitalised and treated with intravenous quinine. Overall, participants were 10 times more likely to fail treatment with oral quinine compared to artemether-lumefantrine (HR 10.7, 95% CI 3.3 to 35.5, p=0.001). The risk of treatment failure unadjusted by genotyping was significantly higher in the quinine group (35.3%; 95% CI 25.6 to 47.4) than in the artemether-lumefantrine group (4.1%; 95% CI 1.3 to 12.0); risk difference

31.3%; 95% CI 19.4 to 31.1; p<0.0001 (Table 3, Figure 2). Genotyping showed that 18 of 26 (69 %) treatment failures in the quinine group were due to recrudescence and that all treatment failures in the artemether-lumefantrine group were due to new infections.

Table 2 Treatment outcomes of children aged 6 to 59 months with uncomplicated malaria after 28 days of follow-up. Values are percentages (numbers)

Treatment outcomes	**Quinine group (n=86)**	**Artemether-lumefantrine group (n=89)**
Cure rate*, †	64 (47)	97 (71)
Early treatment failure †	3 (2)	0
Late clinical failure: †		
Due to recrudescence	12 (10)	0
Due to new infection	2 (5)	2 (2)
Genotyping unsuccessful	0	0
Total	21 (15)	3 (2)
Late parasitological failure: †		
Due to recrudescence	2 (6)	0
Due to new infection	2 (2)	1 (1)
Genotyping unsuccessful	1 (1)	0
Total	7 (9)	1 (1)
No treatment outcome:		
Lost to follow-up	14 (12)	16 (14)
Use of other antimalarials	1 (1)	1 (1)
Total	15 (13)	17 (15)

*Adequate clinical and parasitological response.
† Concerns 73 children in quinine arm and 74 in artemether-lumefantrine arm in whom treatment outcome was assessed.

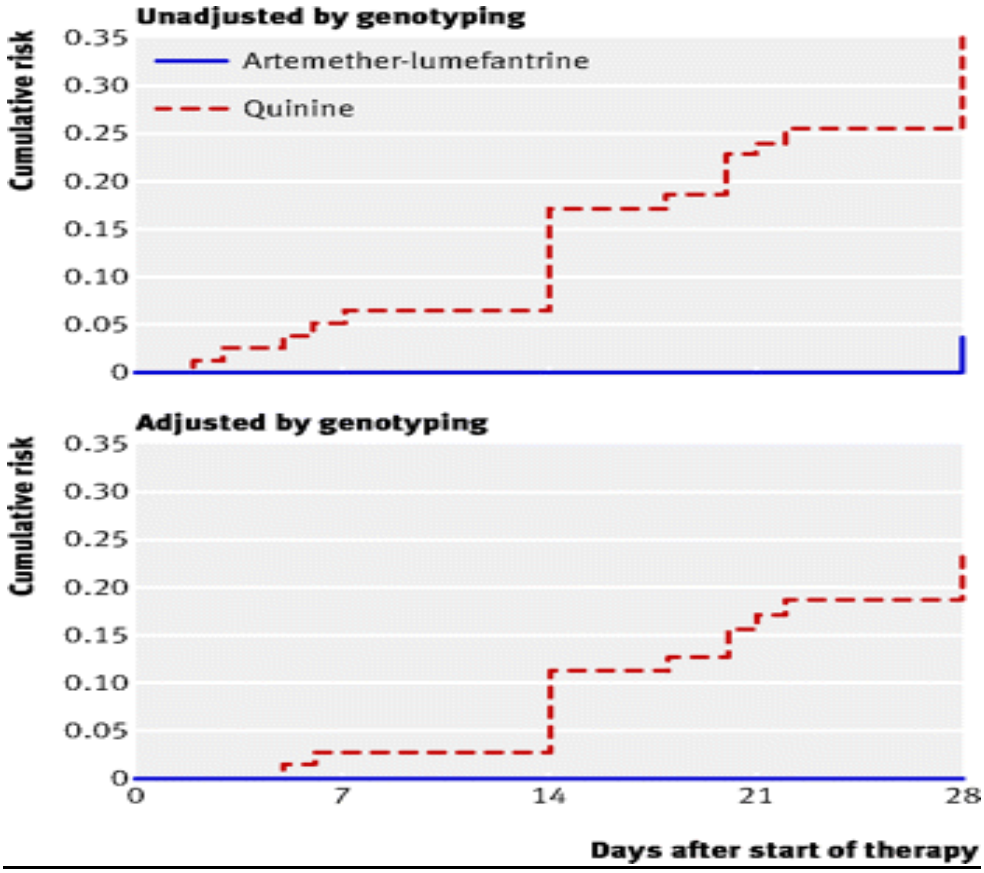
When only treatment failures caused by recrudescent parasites were considered, the risks of failure were 23.1% (95% CI 14.9 to 35.0) with quinine compared to 0 with artemether-lumefantrine (risk difference 23.1%; 95% CI 13.2 to 33.1, p <0.0001; Table 3). Using Cox regression analysis the predictors of treatment failure included treatment with oral quinine (HR 11.06, 95% CI 3.34 -36.57, P<0.001) and day 0 temperature > 37.5^0 C (HR 2.57, 95% CI 1.17-5.66, P = 0.02).

Table 3 Comparative effectiveness of oral quinine and artemether-lumefantrine treatment at day 28 in Ugandan children with uncomplicated malaria

	Risk of failure (95% CI)			
Outcome	**Quinine group (n=86)**	**Artemether-lumefantrine group (n=89)**	**Risk difference (95% CI)**	**P value**
Treatment failure (%)*	35.3 (25.6 to 47.4)†	4.1 (1.3 to 12.0)†	31.3 (19.4 to 31.1)	<0.001
Treatment failure (%) ‡	23.1 (14.9 to 35.0)§	0§	23.1 (13.2 to 33.1)	<0.001

*Any early treatment failure, late clinical failure, or late parasitological failure.
†Unadjusted by genotyping.
‡Any early treatment failure, late clinical failure, or late parasitological failure caused by recrudescence.
§Adjusted by genotyping.

Figure 2: Kaplan Meier curves for risk of treatment failure



Secondary outcomes

Certain early treatment responses, including fever clearance and parasite clearance, could not be assessed in this study, because patients were only seen at the study clinic on days 0 and 7. Hemoglobin levels improved equally in both groups during 28 days of follow-up. Gametocytaemia was more common in the quinine group at Day 7 compared to the artemether-lumefantrine group [10/73 (13.7%) vs. 1/74 (1.4%); p=0.001]. By Day 28 there was no

difference in gametocytaemia in the two groups (Table 4). Total gametocyte person time was 20 weeks for quinine compared to 5 weeks for artemether-lumefantrine ($p < 0.01$).

Table 4 Secondary treatment outcomes at 28 days of follow-up by treatment group. Values are numbers (percentages) unless stated otherwise

Outcome	Quinine group (n=86)	Artemether-lumefantrine group (n=89)	P value
Adherence*:			
None	41 (55)	14 (17)	<0.001
Difficulty taking drug	23 (31)	14 (17)	0.03
Appearance of gametocytes:			
Day 7	10 (17)	1 (1)	0.001
Day 14	5 (8)	1 (1)	0.07
Day 28	1 (2)	1 (1)	0.86
Mean (SD) change in haemoglobin concentration (g/l), day 0 *v* day 28	12 (22)	13(20)	0.78
Adverse events, days 0-28:			
Adverse event of any severity	21 (24)	16 (18)	0.30
Serious adverse event	2 (2)	1 (1)	0.54
Fever	15 (72)	7 (44)	0.49
Anorexia	0 (0)	1 (6)	0.47
Cough	1 (5)	2 (13)	0.74
Diarrhoea	0 (0)	3 (19)	0.47
Recurrent seizures	1 (5)	0 (0)	0.41
Repeated vomiting	1 (5)	2 (6)	0.45
Rash	1 (5)	0 (0)	0.41

*Concerns 75 children in quinine arm and 85 in artemether-lumefantrine arm.

Reported adverse events did not differ between the two treatment groups. Common side effects of quinine like nausea, headache, tinnitus and blurred vision were not observed. Severe adverse events were seen in only 3 patients. Two of these were in the quinine group, one patient with repeated seizures and the other with recurrent vomiting and prostration; they were both classified as early treatment failures. The third severe adverse event was in a patient in the artemether-lumefantrine group with repeated vomiting on day 7 and a negative malaria smear at that time; the patient was found to have a urinary tract infection and improved on antibiotic therapy.

Adherence

Adherence was assessed in 160 of 175 study participants (91%); 75 in the quinine group and 85 in the artemether-lumefantrine group. This was done successfully at the first home visit in 86% of the participants (138/160) and on a second home visit in 12% (19/160). Respondents were predominantly mothers 94% (150/160) who were unemployed and stayed at home 60% (96/160); 56% (89/160) were taking care of at least 2 children and 35% (56/160) were taking care of 3-4 children. About 90% (144/160) of these primary caregivers had at least a primary school education.

Mean adherence for artemether-lumefantrine was 94.5% (range 33-100%) compared to 85.4% (range: 14.3-100%) for quinine (p=0.0008). Overall, non adherence to treatment was higher in the quinine group 54.7% (41/75) compared to the artemether-lumefantrine group 16.5% (14/85); p = 0.001. On the 3rd day of treatment, the proportion of patients not adhering to treatment was similar in both treatment groups; 12% in the artemether-lumefantrine arm compared to 13% in

the quinine arm (p= 0.84). However the proportion of patients not adhering to quinine therapy increased to 18.6%, 30.6% and 44% on the 5th, 6th and 7th day of treatment respectively. For the artemether-lumefantrine group, only 29/85 (34.1%) of the participants had optimal adherence while 43/85 (50.6%) reported good adherence. For both groups, several reasons were given for non-adherence, these included; the parents or caregivers forgetting to administer drugs (23/46; 50%), the child vomiting the drugs (10/46; 22%), the child feeling better (3/46; 6.5%), the child unable to take the medication due to illness (2/46; 4.4%) and the parent or caregivers not understanding dosage instructions (2/46; 4.4%). Difficulty in taking the prescribed medication was reported by 23/86 (31.5%) of participants given quinine compared to 14/89 (18.9%) of those given artemether-lumefantrine; p = 0.03. In the quinine group participants reporting difficulty in taking medication were less likely to adhere to study medication compared to those that did not report any difficulty; 9/23 (39.1%) participants reporting difficulty in taking medication were non-adherent compared to only 13/63 (21.0%) who did not report any difficulty; p = 0.08. This trend was not observed in the artemether-lumefantrine group; 1/14 (7.1 %) participants reporting difficulty in taking medication were non-adherent compared to 7/75 (9.3 %) who did not report any difficulty; p = 0.79. In a multivariate analysis, predictors of non-adherence included treatment with oral quinine (p <0.001), being male (p<0.05) and presence of vomiting (p = 0.02). Having mean adherence of ≥ 80% was associated with a decreased risk of treatment failure, although this was not statistically significant (HR 0.44, 95% CI 0.19-1.02, p = 0.06).

DISCUSSION

We compared the effectiveness of oral quinine versus artemether-lumefantrine in the treatment of uncomplicated malaria in Ugandan children. To our knowledge this was the first

randomised comparison of the effectiveness of these two antimalarial drugs; artemether-lumefantrine was highly effective, with, after 28 days of follow-up, only 3 late failures (all new infections) among 74 evaluable patients (96.0% efficacy). In contrast, quinine was remarkably ineffective for treating uncomplicated malaria, with failure in 26 of 73 evaluable children (64.4% efficacy), including 2 early treatment failures and 16 late recrudescences. Patients treated with quinine were 10 times more likely to fail therapy compared to those treated with artemether-lumefantrine. These results have striking implications. Quinine is the most common second-line therapy, and also an increasingly used first-line therapy for uncomplicated malaria in Africa. Our results suggest that quinine is a poor choice for the treatment of uncomplicated malaria and its use as first- or second-line therapy for uncomplicated malaria needs to be reviewed.

Previous results for the efficacy and effectiveness of oral quinine for treating uncomplicated malaria have been mixed. Many studies have shown good efficacy against uncomplicated malaria for 7 day treatment courses of quinine [11-13], but some studies have shown efficacy [8] or effectiveness [7] below 90%, with particularly poor outcomes in studies in pregnant women [9, 10]. Several factors may have contributed to the poor effectiveness of quinine in our study. First, though the quinine used in this study had been certified by National Drug Authority as being of good quality, we did not conduct specific tests on its quality, such that poor drug quality cannot be ruled out with absolute certainty. Second, quinine resistance may have led to treatment failures. Diminished sensitivity of cultured *P. falciparum* has been demonstrated in Asia [29, 30] and South America [31], but it appears to be uncommon in parasites from Africa [14-19] Third, varied pharmacokinetics may have led to drug levels in some subjects that were inadequate to clear parasites after 7 days of treatment. However, there is little evidence for large variations in quinine pharmacokinetics [20]. The fourth and probably

most likely explanation for the poor effectiveness of quinine in our study was poor compliance with a thrice-daily 7-day treatment course. Quinine is well known to suffer from poor tolerability due to nausea, headache, tinnitus, blurred vision, and other symptoms that increase over a few days of therapy, and poor compliance with the drug has been documented previously [21]. In addition, even without tolerability problems subjects may discontinue therapy before completion if symptoms of malaria have resolved. Shortening the course of quinine has been proposed as a strategy to improve adherence; however shorter courses have generally shown decreased efficacy when compared to a 7-day regimen[6, 32]. In our study, multiple reasons for non-adherence were documented, and poor adherence with dosing guidelines was associated with treatment failures. However, associations between adherence and outcomes were modest and not statistically significant, and did not fully explain the remarkably poor effectiveness of quinine. Serum levels of quinine were not measured, and so adherence measures were dependent on patient reports and pill counts, which may have been inaccurate. It remains unclear if our inability to fully explain the poor effectiveness of quinine was due to limitations in our assessment of compliance or to the contribution of other factors.

In contrast to results with quinine, the effectiveness of artemether-lumefantrine, the new first-line drug for uncomplicated malaria in Uganda, was excellent. This result is consistent with those from other effectiveness studies, showing that unsupervised artemether-lumefantrine had the same efficacy (98%) as supervised therapy for the treatment of uncomplicated malaria in Uganda [33] and that artemether-lumefantrine had excellent effectiveness in a region of Tanzania with high rates of treatment failure with other antimalarial drugs [34]. Artemether-lumefantrine has a shorter treatment course than quinine and has excellent tolerability. However, it must be administered twice a day, ideally with a fatty meal, two factors that have led to concern

regarding its effectiveness. Our results and those of other recent studies reassure us that, even with sub-optimal adherence (18.9% of subjects had adherence <100%), artemether-lumefantrine will likely perform well as a routine first-line antimalarial therapy in Africa. However, other studies have shown levels of adherence to ACTs lower than those seen in our study [35], or observed that the unsupervised efficacy of ACTs was lower than that of supervised therapy [36, 37], emphasizing the need for increased attention to education of patients and caregivers regarding the appropriate dosing of new antimalarial therapies.

In summary, we found that the effectiveness of a 7-day course of quinine for the treatment of uncomplicated malaria in Ugandan children was significantly lower than that of the new ACT artemether-lumefantrine. These results call into question the advisability of the recommendation for quinine as therapy for uncomplicated malaria in Africa. Rather, it appears that another ACT will be more appropriate to treat uncomplicated malaria even after initial treatment failure with an ACT. This strategy seems reasonable in Africa, as nearly all clinical failures after use of highly effective ACTs (artemether-lumefantrine, artesunate-amodiaquine, and dihydroartemisinin-piperaquine) are new infections, not recrudescence. However, the use of ACTs as both first- and second-line therapy for uncomplicated malaria has not yet been tested in a therapeutic trial, and such evaluation should be an urgent priority. Further, the increasing use of quinine as first-line therapy for uncomplicated malaria should be discouraged. It is hoped that increasing access to new drugs will allow the vast majority of Africans with uncomplicated malaria to be treated promptly with ACTs, the most effective available therapies for this condition.

WHAT IS ALREADY KNOWN ON THIS TOPIC
Many malaria endemic countries recommend quinine as the second-line regimen for uncomplicated malaria, to be used after failure of first-line therapy. Due to decreased efficacy of older antimalarials and limited availability of new artemisinin-based combination therapies, quinine is increasingly used as the first-line treatment of uncomplicated malaria in Africa. No published studies have compared the effectiveness of quinine and an ACT for uncomplicated malaria.
WHAT THIS STUDY ADDS
The effectiveness of a 7-day course of quinine for the treatment of uncomplicated malaria in Ugandan children was lower than that of artemether-lumefantrine. These findings question the advisability of the recommendation for quinine as second-line therapy for uncomplicated malaria in most countries in Africa.

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Competing interest statement: All authors declare that the answer to the questions on the competing interest form are all no and therefore have nothing to declare.

Contributors: AJ devised the study, was involved in protocol development, implementation of the trial, data analysis and drafting the manuscript. JKT devised the study, obtained funding and was involved in protocol development and manuscript production. DK was involved in protocol development, implementation of the trial and reviewing the manuscript. AT devised the study, was involved in protocol development, manuscript production and is the guarantor. PJR was involved in implementation of the trial, interpretation of data, drafting and reviewing the manuscript. GD was involved in data analysis and interpretation and reviewing the manuscript. UD was involved in protocol development and reviewing the manuscript. MRK and FWM were involved in reviewing the manuscript. AJ and PJR wrote the first draft of the manuscript and all authors contributed to the final version of the paper.

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CHAPTER 3

CASE MANAGEMENT OF SEVERE MALARIA - A FORGOTTEN PRACTICE: EXPERIENCES FROM HEALTH FACILITIES IN UGANDA.

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ABSTRACT

Background: Severe malaria is a life threatening medical emergency and requires prompt and effective treatment to prevent death. There is paucity of published information on current practices of severe malaria case management in sub-Saharan Africa; we evaluated the management practices for severe malaria in Ugandan health facilities

Methods and Findings: We did a cross sectional survey, using multi-stage sampling methods, of health facilities in 11 districts in the eastern and mid-western parts of Uganda. The study instruments were adapted from the WHO hospital care assessment tools. Between June and August 2009, 105 health facilities were surveyed and 181 health workers and 868 patients/caretakers interviewed. None of the inpatient facilities had all seven components of a basic care package for the management of severe malaria consistently available during the 3 months prior to the survey. Referral practices were appropriate for < 10% (18/196) of the patients. Prompt care at any health facility was reported by 29% (247/868) of patients. Severe malaria was correctly diagnosed in 27% of patients (233).Though the quinine dose and regimen was correct in the majority (611/868, 70.4%) of patients, it was administered in the correct volumes of 5% dextrose in only 18% (147/815). Most patients (80.1%) had several doses of quinine administered in one single 500ml bottle of 5% dextrose. Medications were purchased by 385 (44%) patients and medical supplies by 478 patients (70.6%).

Conclusions: Management of severe malaria in Ugandan health facilities was sub-optimal. These findings highlight the challenges of correctly managing severe malaria in resource limited settings. Priority areas for improvement include triage and emergency care, referral practises, quality of diagnosis and treatment, availability of medicines and supplies, training and support supervision.

BACKGROUND

Severe malaria is a life threatening medical emergency that requires prompt and effective treatment to prevent death.[1, 2] However, effective management of severe malaria is relatively expensive and relies heavily on well equipped hospitals, with adequately trained health workers, both often lacking in sub-Saharan Africa.[3,4] Severe malaria has been described as a neglected disease that poses a significant economic burden on most African countries which typically have weak health systems and are unable to finance basic services and infrastructure. [5]

In Uganda, efforts to improve the management of severe malaria at formal health facilities started in 1998, [6] mainly through training workshops using adapted WHO training materials. Despite these efforts, severe malaria management remains challenging, as it depends on the availability of treatments, blood transfusion services, functional referral systems, good infrastructure and adequate organization of hospital services. There is limited information on management practices for severe malaria in resource constrained settings in Africa, with few studies reporting on this as part of integrated pediatric care evaluations.[7, 8] We evaluated these practices at different levels of health care in Uganda.

METHODS

Ethics statement

The study was approved by the Uganda National Council for Science and Technology and verbal consent was obtained from all participants. Verbal consent was considered more appropriate than

written consent for this survey as this was considered a routine audit/evaluation of health services. Verbal consent was documented as a tick on each case record form. .

Study design and setting

Between June and August 2009, a cross sectional assessment of severe malaria management practices was conducted in selected health facilities in 11 districts in Uganda. For patients with severe malaria, hospitals and health centre IVs run by specialists, medical officers and clinical officers provide inpatient services while health centre IIs and IIIs run by nurses typically provide outpatient and referral services.

Sampling methodology

Multi-stage sampling methods were used to select study sites. The eastern and mid-western regions of Uganda were selected to represent areas of high and low - medium malaria transmission settings, respectively. Out of 15 districts in these regions, 11 were randomly selected; 6 in eastern Uganda (Kumi, Soroti, Katakwi, Bukedea, Amuria and Kaberamaido) and 5 in mid-western Uganda (Bulisa, Hoima, Kibaale, Kiboga and Masindi). Within the districts, in order to obtain a representative sample of health facilities for each region, all hospitals and health centre IVs (in-patient facilities) were selected while among the 250 health centres II and III (lower level facilities) 30% were randomly selected. In all the selected health facilities, the director and the health workers involved in any aspect of care of malaria patients and available during the survey days were interviewed. In addition, after having obtained their or caregivers' verbal consent, randomly selected patients having malaria according to the admission register and hospitalized during the survey days were interviewed.

Data collection

The survey was conducted by 5 teams of 4 to 8 health workers working in parallel. The teams were trained for 1 week prior to the survey to ensure that interview questions were appropriately asked and responses consistently recorded. Training and concordance testing was done until the agreement of practice results of interviewers and trainers was >90%. District officials and health unit directors were informed about the survey only on the morning of the survey. A triangulation approach was used to collect data with the following methods: health facility assessments and health worker interviews at inpatient and lower level facilities as well as in-patient/caregiver interviews and reviews of patient's charts at inpatient facilities. Most survey instruments were adapted from the WHO hospital care assessment tools. Survey instruments can be found at www.plosone.org. Study coordinators reviewed all survey tools daily for completeness and accuracy. Health facility assessments collected information on staffing, triage systems, emergency care, presence of malaria treatment guidelines, laboratory practices and availability of medicines and supplies. Health worker assessments collected information on knowledge of severe malaria and its management, prescribing practices, training and support supervision. Knowledge on severe malaria management was further assessed using a clinical case scenario of a patient presenting with fever, convulsions and loss of consciousness. In-patient/caregiver interviews and chart reviews collected information on presenting complaints, time taken to receive care, diagnosis, patients' weight, laboratory investigations and treatment prescribed. On average, 17 patients were recruited in each health centre IV and 66 patients in each hospital. Patients/caregivers were asked to report their satisfaction with services provided on an ordinal scale (good, improvement needed or poor) and to suggest improvements. Any information not obtained through these two approaches was considered not documented. For missing weights we

used a weight equivalent to the 50% percentile for age according to the 2000 CDC growth charts. [9]

Definitions

Severe malaria case management was assessed according to the following definitions: correct diagnosis: documented fever or history of fever with a positive malaria test and at least one sign/symptom of severe disease according to WHO criteria[10]; prompt management: patient with severe malaria receiving care within 30 minutes of presentation at the health facility; correct initial parenteral antimalarial medicine prescribed : administration of parenteral quinine, artemether or artesunate; correct antimalarial drug dose and dosing regimen: IV quinine10 mg/kg every 8 hrs (margin of error +/- 20mg on total daily dose) or IM artemether 3.2 mg/kg on day 1, followed by 1.6 mg/kg daily or IV artesunate 2.4 mg/kg on admission at 12 hrs and then every 24 hrs (margin of error +/-5mg on total daily dose); all given until the patient was able to tolerate oral therapy. [10,11] Correct mode of administration: IV quinine in 10-20ml/kg of 5% dextrose, intramuscular administration of artemether or IV artesunate mixed with 5 mL of 5% dextrose and injected as a bolus; appropriate oral continuation therapy after initial parenteral treatment: either oral quinine at 10mg/kg every 8hrs until completion of a 7-day course or a full treatment course of an oral artemisinin based combination therapy according to appropriate weight-based dosing guidelines; [10] adequate referral practice: referral of a patient with severe malaria after administration of injectable quinine or rectal artesunate, provision of a referral note and transport[10,11]. Patients were considered appropriately treated if they received the correct antimalarial medicine, at the right dose and dosing regimen and with the correct mode of administration.

Sample size estimation, data management and analysis

For the inpatient interviews, a sample size of 869 inpatients was estimated assuming 50% of malaria inpatients are appropriately treated, at 95% level of confidence, with a tolerable error of 0.05, a design effect of 2 and allowing for 10% non-responsiveness.

Data were double entered in EPI-info software program version 6 and analysed using STATA version 10.0 (StataCorp LP, College Station, TX, USA). Results from all districts were combined and descriptive analysis was done at health facility, health worker and patient levels. Data are presented as proportions and frequencies adjusted for clustering by health facility. Fisher's exact tests were used to analyze differences in proportions. Two tailed p values and a 5% significance level were used.

RESULTS

In the 11 districts, 105 health facilities were included (83 lower level facilities and 22 inpatient facilities) and 181 health workers interviewed (151 at lower level and 50 inpatient facilities respectively). In addition, 868 inpatient interviews and chart reviews were conducted. No health worker or caregiver declined to participate.

Health facility characteristics

The majority of health facilities (83 %, 87/105) were government-run institutions. Despite health workers' reports of a defined triage system in most health facilities, triage was practised in less than half (44%, 46/105) of them (Table 1). Only 11.4% (12/105) of health units had separate

outpatient (OPD) queues for adults and children. Functional microscopes for malaria diagnosis were available in most inpatient units (77.3%, 17/22) and in about half (51.4%, 18/35) of the health centre IIIs (Table 1). Malaria rapid diagnostic tests were available in 14.4% (12/83) of health centre IIs and IIIs. Haemoglobin measurement was available at 39% (41/105) of the facilities. During the 3 months prior to the survey, 54.3% (57/105) of health facilities had consistent availability of parenteral quinine, while fewer facilities had quinine tablets (16.2%, 17/105) and artemether-lumefantrine tablets (33.3%, 35/105). None of the inpatient facilities had consistent availability of all seven components of a basic care package for severe malaria management (parenteral quinine, intravenous fluids, 50% dextrose, blood for transfusion, transfusion sets, IV giving sets, syringes). The most common stock outs were blood for transfusion (available in 4.5% of units), 50% dextrose (in 32%), 5% dextrose and transfusion sets (in 36.4%) (Table 1).

Table 1. Health facility characteristics

Characteristics	N= 105	
	No.	%
Health facility level		
Health centre II	48	(45.7)
Health centre III	35	(33.3)
Health centre IV	12	(11.5)
District hospital	8	(7.6)
Regional Referral hospital	2	(1.9)
Type of Health facility		
Government	87	(82.9)
Faith based	14	(13.3)
Private for profit	4	(3.8)
Treatment aide memoirs in outpatient units available	83	(79.0)
Health facilities with defined triage system	82	(78.1)
Triage practised	46	(43.8)
Presence of separate lines for adults and children in OPD	12	(11.4)
Functional weighing scale available	79	(75.2)
Thermometers available	83	(79.0)
Antimalarial medicines available on the day of survey		
Quinine injection	79	(75.2)
IV artesunate	2	(1.9)
Rectal artemisinin	5	(4.8)
Artemether Injection	10	(9.5)
Quinine tablets	41	(39.0)
Artemether-lumefantrine tablets	52	(49.5)
Sulphadoxine-pyrimethamine tablets	64	(60.9)
[‡]**Antimalarial medicines available in the 3 months prior to survey**		
Quinine injection	57	(54.3)
Quinine tablets	17	(16.2)
Artemether-lumefantrine tablets	35	(33.3)
[‡,†]**Supplies for severe malaria management available in the 3 months prior to survey at the inpatient units (N=22)**		
5% dextrose	8	(36.4)
50% dextrose	7	(31.8)
Blood for transfusion	1	(4.5)
Blood transfusion sets	8	(36.4)
IV giving sets	10	(45.5)
Availability of seven basic medicines and supplies for severe malaria management in the 3 months prior to survey at inpatient units[*]	0	(0)
[†]**Malaria testing facilities available**		
Functional microscope at inpatient units (N=22)	17	(77.3)
Functional microscope at health centre IIIs (N=35)	18	(51.4)
RDTs at health centre IIs and IIIs (N=83)	12	(14.4)

Health worker characteristics

At the inpatient units, nurses/midwives represented the majority of the staff (40%). Considering all health facilities visited, only 2 doctors were on duty on survey days. The percentage of health workers who could mention > 2 severe forms of malaria was 24% at the inpatient units and only 2.3% at the lower levels of care. In response to the clinical case scenario, 52% (26) of health workers at the inpatient level and 49.6% (65) at the lower levels of care were able to write an accurate prescription for a 4 year old patient (Table 2). Regarding on-site training, 22.2% (28/131) of health workers at the lower levels of care and 22.0% (11/50) at the inpatient units reported having received in-service training on severe malaria management within the year prior to the survey. Fewer health workers at the inpatient units (24.0%, 12/50) than those at the lower levels of care (41.9%, 55/131) (p= 0.025) reported having received at least one support supervision visit in the previous 6 months. (Table 2)

Table 2. Health worker Characteristics

Characteristics	Lower level units: Health centre II and III (N = 131) No. (%)	Inpatient units: Hospitals and Health centre IV (N=50) No. (%)	P value
Pre-service training			
Medical officer	0	2 (4.0%)	0.02
Clinical officer	9 (6.9%)	14 (28.0%)	0.00
Nurse/midwife	36 (27.5%)	20 (40.0%)	0.12
Nursing aide/assistant	86 (65.7%)	14 (28.0%)	0.00
In service at current post for > 12 months	97 (74%)	39 (78.0%)	0.58
Diagnosis of malaria based on clinical features and diagnostic tests (confirmatory)	11 (8.9%)	26 (52.0%)	0.00
Health worker ever undergone IMCI training	62 (49.6%)	28 (56.0%)	0.47
Received in-service training on severe malaria case management in last 12 months	28 (22.2%)	11 (22.0%)	-
Health worker has malaria treatment guidelines accessible	108 (82.4%)	43 (86.0%)	0.52
Knowledge on severe malaria			
Common forms of severe malaria listed			
Severe anaemia	6 (4.6%)	38 (76%)	0.00
Repeated convulsions	8 (6.1%)	35 (70%)	0.00
Cerebral malaria	93 (71.0%)	27 (54%)	0.03
Hypoglycaemia	14 (10.7%)	17 (34%)	0.00
Shock	2 (1.5%)	15 (30%)	0.00
Spontaneous bleeding	65 (49.6%)	5 (10%)	0.00
Pulmonary oedema	18 (13.7%)	1 (2)	0.02
Response to hypothetical clinical case			
Correct antimalarial medicine choice	113 (89.7%)	48 (98%)	0.03
Correct quinine prescription for child	65 (49.6%)	26 (52%)	0.81
Correct quinine prescription for adult	93 (71.1%)	40 (80%)	0.22

Patient assessment and emergency care

The majority of patients (76.3%, 663/868) were aged < 5 years; the median age being 2 years. Fever or history of fever was the commonest reason for attendance (96.6%). Mean duration of hospitalisation at the time of interview was 2.5 days (SD 1.5), with 546 patients (62.9%)

hospitalised for ≤ 2 days, 221 (25.5%) for ≤ 1 day and 142 (16.4%) for ≥ 4days. Malaria or severe malaria was the diagnosis documented in 93.8% of patients (814/868).Among these, 103 (11.9%) were recorded as malaria with severe anaemia (45% confirmed by microscopy) and 21 (2.4%) as cerebral malaria (57% confirmed by microscopy).

The median waiting time before receiving care at the facility was 3.0 hours (range 0-24 hours) with 28.5 % (247/868) of patients reporting having received care within the first 30 minutes and 52.3% (454/868) within 1 hour of attendance. At least 33 patients (3.8%) waited ≥8 hours before receiving any care. Though most patients were asked about their age (96.3%), history of fever (89 %), prior use of antimalarial therapy (58%) and history of repeated vomiting (55 %), patients/caretaker reports and chart reviews revealed that presence of common danger signs were not often elicited (history of convulsions in 303 (35%) and drowsiness in 248 (29%) patients). Body temperature and level of consciousness were assessed in 20.5% (178/868) and 23.6% (205/868) of patients, respectively. The proportion of patients with at least one sign or symptom of severe malaria documented was 27.9% (242/868). Malaria infection was confirmed by microscopy in 64.7% (432/668) of patients in health facilities where functional microscopy was available.

Case management practises

One hundred ninety six patients (23 %) had been referred from a lower level of health care. The main reasons for referral were: poor response to treatment (38%) or unavailability of either blood for transfusion (33%), intravenous fluids (18%) or beds (9%). Pre-referral medications were given to 145 (79%) patients, quinine in 39.3 % (57/145), often administered alone (65%, 37/57),

or with an antibiotic (13%), an antipyretic (15%) or diazepam (5%). No patient received pre-referral rectal artesunate, referral notes were provided for 58.7% (115/196) and transport for only 6.1% (12/196). Overall, 9.2% (18/196) patients referred had adequate referral practises; 1.3 % (1/76) in the low-medium transmission setting and 14.2% (17/120) in the high transmission setting (p=0.002).

Two hundred thirty three (27%) patients had a correct diagnosis of severe malaria. The proportion of patients with a correct diagnosis was higher in the high transmission setting, 29.7% compared to 17.9% in the low-medium transmission setting (p=0.001). Most patients were evaluated at least once a day during their hospitalization, though 102 (13%) were never assessed (Table 3). The majority of patients (95%, 823/868) received the correct initial parenteral antimalarial medicine, often at the recommended dose and dosing regimen (70.4%, 611/868). However, the dose was inappropriate in all 8 patients treated with artemether. For patients treated with quinine, 75% (611/815) were correctly dosed, 12.7% (104/815) were under dosed and 12.3% (12.3%) over dosed. Among patients treated with quinine, 18% (147/815) received the correct dosing regimen and mode of administration. Significantly, in most cases (75%) multiple doses of quinine were administered in a single 500ml bottle of 5% dextrose to run over 24 to 48 hours. The proportion of in-patients with a negative blood smear but receiving antimalarial treatment was 94.9% (129/136). Overall, only 16.9% of the patients were appropriately treated for severe malaria. (Table 3) Medications needed for treatment were purchased by 385 (44%) and medical supplies by 478 patients (70.6%) at a mean cost of $2.8 (SD 2.9) and $3.4 (SD 3.7), respectively.

Almost half of the patients (43.3%) considered that they had waited too long before seeing any health worker at presentation and 45 % thought that services offered needed further improvements. Quality of care at the health facilities was reported as good by 46.8 % of patients/caretakers, 45 % thought that services offered needed to be improved while 8.2 % thought services were poor. Suggestions for improvement included having sufficient medicines at health units (21.3%), improving the availability of supplies and sundries (11.6%), increasing the number of staff (8.3%), providing more beds and beddings (7.6%) and health workers having better attitudes towards patients and attendants (7.1%).

Table 3. Case management practises for patients hospitalised with a diagnosis of malaria

N= 868	**No.**	**%**	**95% CI (Cluster adjusted)**
Patients with a negative blood smear receiving antimalarial treatment (N=136)	129	94.9%	87.8- 100
Reported frequency of evaluation by health workers during hospitalization			
Once every day	410	50.1	
Twice or thrice daily	265	32.4	
Never seen	102	12.5	
Patients purchasing medications	385	44	34.8-53.9
Purchased medications	214	44.5	
Quinine	76	15.8	
Antibiotics	38	7.9	
Haematinics			
Patients purchasing medical supplies	478	70.6	61.7-79.5
Purchased medical supplies	223	33.4	
Intravenous cannula	162	24.3	
Intravenous fluids	109	16.3	
Syringes	101	15.0	
Giving sets	27	4.0	
Gloves			
Correct antimalarial treatment	823	94.8	91.7-98.7
Initial parenteral antimalarial medicine prescribed			
Quinine	815	93.9	
Artemether	8	0.9	51.9-87.3
Initial parenteral antimalarial medicine dose and dosing regimen	611	70.4	12.0-21.9
Initial parenteral antimalarial medicine , dosing regimen and mode of administration (appropriately treated)	147	16.9	
Oral continuation therapy (n = 486)	429	88.3	
Oral quinine	274	63.9	28.8-85.3
Artemether-lumefantrine	149	34.7	15.1-47.0
Dihydroartemisinin-piperaquine	6	1.4	0-3.4

DISCUSSION

In our survey, management of severe malaria in Ugandan health facilities was sub-optimal, with most facilities not fully complying with the national and international treatment guidelines. We found significant problems with case management at both the health system/health centre and provider levels. Indeed, this survey identified several problems at different levels of the health care system, from the referral practices at the lower level health centres to the availability of supplies and actual management of malaria cases in referral facilities. Despite the existence of some differences between the two regions, the problems identified in the management of severe malaria cases were similar, indicating that both regions needs similar attention and efforts to improve this unacceptable situation. Though the quality of documentation may have impacted on our assessment, we believe these findings accurately represent the management practices in these settings.

Practices related to severe malaria case management were deficient, from patient evaluation, for which the presence of danger signs were not systematically checked, to diagnosis, correctly done in <30% of patients, and treatment, which was usually correct in terms of dose and dosing regimen but for which drug administration was often not done as recommended. Deficiencies in correctly diagnosing severe malaria suggest that a significant proportion of these patients may have had uncomplicated malaria and did not require parenteral therapy or hospitalisation. This calls for measures to improve patient evaluation and promotion of the rational use of antimalarial medicines. Furthermore, none of the inpatient health facilities had all components of a basic care package for severe malaria management available, with blood for transfusion, 5% dextrose, and transfusion sets least available.

Though the survey was not designed to evaluate the impact of management practices on clinical outcome, it would be expected that such shortcomings would influence patient survival. The large majority of patients included in this survey had already gone through the first 24-48 hours of hospitalization, a known critical period,[12] and may not fully represent treatment practices in those with a fatal outcome. Therefore, the quality of case management might be worse than documented here.

When analysing these observations in more detail, patient triage, evaluation and diagnosis were extremely inadequate. More than half of health facilities did not practise triage and few had separate OPD queues for adults and children, an important element as the large majority of the patients were children <5 years of age. Such inefficient systems may explain the long waiting times prior to receiving care at the health facilities. Good quality emergency care and triage is a critical first step in improving hospital care; unfortunately, triage is often deficient in resource limited settings. [7,13]This worrying finding can be addressed by training health workers on emergency triage, assessment, and treatment [14] and by providing practical support through supervision and clinical audits. This strategy would not only improve the management of severe malaria cases but also that of other severely ill patients. Though health worker training has been shown to be critical for improving case management, [15] [16]the cadre of health workers to be targeted needs to be critically reconsidered. In our setting, nurses and nursing aides, though not primarily responsible for clinical management decision taking, should have the priority as they were the only cadre of staff consistently available at the units whereas medical and clinical officers, who theoretically have the primary responsibility, were consistently absent.

The proportion of in-patients with a negative blood smear but receiving antimalarial treatment was substantial. This finding has previously been reported in similar settings, with an increased risk of death in these patients when treated for malaria, possibly due to inappropriate treatment of other illnesses [17, 18, 19]. In our setting, there was also significant concurrent administration of antibiotics that could be attributed to diagnostic uncertainty. Routine treatment with parenteral antibiotics may be warranted, particularly when microscopy is not available or of insufficient quality, because of the increased risk of bacterial sepsis and associated mortality in malaria patients [20]. The recent decision by the Ugandan Ministry of Health to have all suspected malaria cases confirmed by microscopy or rapid diagnostic test may improve diagnosis. The challenge though remains to ensure consistent availability of these tools at all facility-based service delivery points.

The adequacy of treatment dose, dosing schedule and oral continuation therapy in our survey is reassuring; the latter was probably due to recent in-service training conducted on the management of uncomplicated malaria at the time of treatment policy change in Uganda in 2006. However, the method of quinine administration is a cause of concern. Most patients had multiple doses of quinine (for 24 to 48 hours) combined in a single 500ml bottle of 5% dextrose. The rationale for this practise is unclear; it may be due to the desire to minimize costs. Nevertheless, this practice is concerning and should be discouraged as it increases the risk of both quinine toxicity and fluid overload, particularly in children. The provision of smaller volume bottles for infusion, more suitable for paediatric patients, may overcome this problem. In addition, the use of artesunate injections may further improve treatment delivery as this regimen does not require rate-controlled infusion. The SEAQUAMAT[21] and recently published AQUAMAT study [22]

provide sufficient evidence of the superiority of artesunate over quinine in both children and adults and this should lead to severe malaria treatment policy change to intravenous artesunate in several Sub-Saharan countries, including Uganda. In our study, the alternative to quinine in a few patients was artemether, which was always administered at an incorrect dosage, possibly because the heath providers had little experience with this product.

Importantly, stock-outs of several items included in the basic care package for severe malaria management were common and could explain the high proportion of patients obliged to purchase medications and supplies needed for their management. This is certainly a major challenge, as improving clinical skills through training without ensuring availability of medicines and supplies will have limited impact on the quality of care. These shortages impact negatively on efforts to deliver effective treatment and undermine malaria control efforts. [23] Such stock-outs are caused by different factors and often reflect weaknesses in medicine and supplies procurement, management and distribution practices. Indeed, in this study inadequate and delayed funding, delayed drug deliveries and poor storage were identified as the main causes of stock-outs (data not presented). This problem must be addressed by the Ugandan Ministry of Health as a matter of priority to improve the quality of care and minimize out-of-pocket costs incurred by patients/caregivers, which were unacceptably high for a country like Uganda where 52% of the population lives below the international poverty line of US$1.25 per day[24]. Shortages may also be curtailed by measures to improve severe malaria diagnosis and by the use of diagnostic tests to improve targeting of treatment. It is also critical for resource limited countries like Uganda to look for more efficient ways of financing health care as the current system does not seem to mobilize sufficient resources to provide the desired levels for the entire population [25].

At the national and international level, much more attention appears to be focused on community-based health care interventions. There is need to shift some of this attention back to facility-based health care services, especially since community service delivery is linked to that at health units.

In conclusion, this study highlights the serious challenges faced in the management of severe malaria in a resource limited setting like Uganda. There is paucity of published information on current severe malaria management practices in sub-Saharan Africa, but the situation in many areas may not be very different from what we have observed in Uganda. Considering the problems identified, several priority areas at different points of care needing improvement would include: patient assessment, referral practices, quality of diagnosis, triage and emergency care, treatment practices, availability of medicines and supplies, health worker training and support supervision. Considering its huge toll on African children, improved management of severe malaria should be a priority.

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Contributors

All investigators contributed to the study design and planning as well as the development of study tools. Achan J, Kyabayinze D, Mawejje H and Mugizi R coordinated data collection, trained the survey staff and supervised the field work. Achan J and D'Alessandro U were responsible for data analysis and drafting of the manuscript and are the guarantors. All investigators reviewed the results, contributed to and approved the manuscript. No professional medical writer was involved in preparation of this manuscript.

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CHAPTER 4

QUININE, AN OLD ANTI-MALARIAL DRUG IN A MODERN WORLD: ROLE IN THE TREATMENT OF MALARIA

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Abstract

Quinine remains an important anti-malarial drug almost 400 years after its effectiveness was first documented. However, its continued use is challenged by its poor tolerability, poor compliance with complex dosing regimens, and the availability of more efficacious anti-malarial drugs. This article reviews the historical role of quinine, considers its current usage and provides insight into its appropriate future use in the treatment of malaria. In light of recent research findings intravenous artesunate should be the first-line drug for severe malaria, with quinine as an alternative. The role of rectal quinine as pre-referral treatment for severe malaria has not been fully explored, but it remains a promising intervention. In pregnancy, quinine continues to play a critical role in the management of malaria, especially in the first trimester, and it will remain a mainstay of treatment until safer alternatives become available. For uncomplicated malaria, artemisinin-based combination therapy (ACT) offers a better option than quinine though the difficulty of maintaining a steady supply of ACT in resource-limited settings renders the rapid withdrawal of quinine for uncomplicated malaria cases risky. The best approach would be to identify solutions to ACT stock-outs, maintain quinine in case of ACT stock-outs, and evaluate strategies for improving quinine treatment outcomes by combining it to antibiotics. In HIV and TB infected populations, concerns about potential interactions between quinine and antiretroviral and anti-tuberculosis drugs exist, and these will need further research and pharmacovigilance.

Background and historical perspective

The discovery of quinine is considered the most serendipitous medical discovery of the 17th century [1] and malaria treatment with quinine marked the first successful use of a chemical compound to treat an infectious disease[2]. Quinine, as a component of the bark of the cinchona (quina-quina) tree, was used to treat malaria from as early as the 1600s, when it was referred to as the "Jesuits' bark," "cardinal's bark," or "sacred bark." These names stem from its use in 1630 by Jesuit missionaries in South America, though a legend suggests earlier use by the native population[2]. According to this legend, an Indian with a high fever was lost in an Andean jungle. Thirsty, he drank from a pool of stagnant water and found that it tasted bitter. Realizing that the water had been contaminated by the surrounding quina-quina trees he thought he was poisoned. Surprisingly, his fever soon abated, and he shared this accidental discovery with fellow villagers, who thereafter used extracts from the quina-quina bark to treat fever [3] . The legend of quinine's discovery accepted in Europe differs though, and involves the Spanish Countess of Chinchon who, while in Peru, contracted a fever that was cured by the bark of a tree. Returning to Spain with the bark, she introduced quinine to Europe in 1638 and, in 1742, botanist Carl Linnaeus called the tree "Cinchona" in her honour [4].

Before 1820, the bark of the cinchona tree was first dried, ground to a fine powder, and then mixed into a liquid (commonly wine) before being drunk. In 1820, quinine was extracted from the bark, isolated and named by Pierre Joseph Pelletier and Joseph Caventou. Purified quinine then replaced the bark as the standard treatment for malaria [5]. Quinine and other cinchona alkaloids including quinidine, cinchonine and cinchonidine are all effective against malaria. The efficacies of these four alkaloids were evaluated in one of the earliest clinical trials, conducted from 1866 to 1868 in 3600 patients using prepared sulfates of the alkaloids. With the main

outcome measure of “cessation of febrile paroxysms”, all four alkaloids were found to be comparable, with cure rates of >98%[6]. However, after 1890 quinine became the predominantly used alkaloid, mainly due to a change in supply from South American to Javan cinchona bark, which contained a higher proportion of quinine [7]. Quinine remained the mainstay of malaria treatment until the 1920s, when more effective synthetic anti-malarials became available. The most important of these drugs was chloroquine, which was extensively used, especially beginning in the 1940s [6]. With heavy use, chloroquine resistance developed slowly. Resistance of *Plasmodium falciparum* to chloroquine was seen in parts of Southeast Asia and South America by the late 1950s, and was widespread in almost all areas with falciparum malaria by the 1980s. With increasing resistance to chloroquine, quinine again played a key role, particularly in the treatment of severe malaria [6]. To-date quinine continues to play a significant role in the management of malaria. This review, discusses the historical role of quinine, considers its current usage, and provides insight into the appropriate future use of quinine for the treatment of malaria. Information was obtained by searching published literature in the National Library of Medicine via Pub Med and MEDLINE search engines for research articles, reviews, books, and other reports. Identification of published reports was done using key word searches such as quinine and malaria treatment, quinine and drug resistance, quinine in pregnancy, quinine and antibiotic combinations, and quinine and HIV/TB infected populations.

Quinine properties

Quinine is a cinchona alkaloid that belongs to the aryl amino alcohol group of drugs. It is an extremely basic compound and is, therefore, always presented as a salt[6]. Various preparations exist, including the hydrochloride, dihydrochloride, sulphate, bisulphate, and gluconate salts; of

these the dihydrochloride is the most widely used. Quinine has rapid schizonticidal action against intra-erythrocytic malaria parasites. It is also gametocytocidal for *Plasmodium vivax* and *Plasmodium malariae,* but not for *Plasmodium falciparum*. Quinine also has analgesic, but not antipyretic properties. The anti-malarial mechanism of action of quinine is unknown.

Quinine is rapidly absorbed both orally and parenterally, reaching peak concentrations within 1-3 hours[8]. It is distributed throughout the body fluids and is highly protein bound, mainly to alpha-1 acid glycoprotein. The binding capacity in plasma is concentration dependent, but also depends on the levels of alpha-1 acid glycoprotein, which therefore makes comparisons between different studies difficult[9]. Quinine readily crosses the placental barrier and is also found in cerebral spinal fluid. Excretion is rapid - 80% of the administered drug is eliminated by hepatic biotransformation and the remaining 20% is excreted unchanged by the kidney [10-12]. The half-life of quinine ranges between 11-18 hours [13, 14]. Several pharmacokinetic characteristics of quinine differ according to the age of the subject and are also affected by malaria. The volume of distribution is less in young children than in adults, and the rate of elimination is slower in the elderly than in young adults. In patients with acute malaria the volume of distribution is reduced and systemic clearance is slower than in healthy subjects; these changes are proportional to the severity of the disease. As a result, plasma quinine levels are higher in patients with malaria. Protein binding of quinine is increased in patients with malaria as a result of an increased circulating concentration of alpha-1 acid glycoprotein [15].

Quinine has a low therapeutic index, and adverse effects with its use are substantial [16]. The side effects commonly seen at therapeutic concentrations are referred to as cinchonism, with

mild forms including tinnitus, slight impairment of hearing, headache and nausea. Impairment of hearing is usually concentration dependent and reversible [17]. More severe manifestations include vertigo, vomiting, abdominal pain, diarrhea, marked auditory loss, and visual symptoms, including loss of vision. Hypotension may occur if the drug is given too rapidly, and venous thrombosis may occur following intravenous injections [10]. Intramuscular administration is painful and may cause sterile abscesses. Hypoglycaemia is yet another common side effect of quinine therapy [15, 18] and is a particular problem in pregnant women[19]. Hypoglycaemia has been reported to occur in up to 32% of patients receiving quinine therapy[18]. However in more recent studies, hypoglycaemia occurred in only 3% of adults and 2.8% of African children receiving quinine [20, 21]. Less frequent but more serious side effects of quinine therapy include skin eruptions, asthma, thrombocytopaenia, hepatic injury and psychosis [22].

Overview of quinine use in the management of malaria

Quinine remains an important anti-malarial drug, almost 400 years after Jesuit priests first documented its effectiveness. The 2010 World Health Organisation (WHO) guidelines recommend a combination of quinine plus doxycycline, tetracycline or clindamycin as second-line treatment for uncomplicated malaria (to be used when the first-line drug fails or is not available) and quinine plus clindamycin for treatment of malaria in the first trimester of pregnancy [23]. Based on recent trials, intravenous artesunate should be used for the treatment of severe falciparum malaria in adults [20] and children[21], in preference to quinine.

By 2009, 31 African countries recommended quinine as second-line treatment for uncomplicated malaria, 38 as first-line treatment of severe malaria and 32 for treatment of malaria in the first trimester of pregnancy [24]. In most of Africa, quinine is still used as monotherapy, contrary to the WHO recommendations[23, 24]; the reason for this practice may be the higher costs of quinine-antibiotic combinations. Quinine continues to play a significant role in the management of malaria in sub-Saharan Africa and other malaria endemic areas, and its use in routine practice may not be restricted to the stated WHO recommendations. In Cameroon, even one year after the introduction of ACT, quinine continued to be used as first-line therapy, with 45% of adults receiving oral quinine for uncomplicated malaria [25]. Recent surveillance data from sentinel sites in Uganda showed that quinine was prescribed for up to 90% of children < 5 years with uncomplicated malaria [26].

The use of quinine for uncomplicated malaria cases should have decreased due to toxicities, poor compliance and the implementation of newer and better tolerated therapies such as ACT. However, the limited availability of ACT and the increasing resistance to chloroquine and antifolates have actually increased its use in recent times [27]. Therefore, studies evaluating the role of quinine in the management of malaria have been reviewed.

Quinine for uncomplicated malaria

In several settings, oral quinine continues to be used as treatment for uncomplicated malaria, a practice mainly resulting from frequent stock-outs of the recommended ACT [26, 28]. Previous

studies of the effectiveness and efficacy of quinine for uncomplicated malaria showed mixed results (Table 1). The majority of these studies were conducted in settings with reported declining efficacy of quinine in Southeast Asia and South America. Earlier studies in these regions, using varying dosing regimens, showed cure rates ranging from 76 % to 98 %. The lower cure rates were mainly observed with shorter regimens (3 days) and higher cure rates when the drug was combined with sulphadoxine-pyrimethamine, tetracycline or clindamycin [29-34]. Similar findings were reported in Vietnam, where a three-day course of quinine plus artesunate had a cure rate of only 50%, compared to a five-day course, which had a cure rate of 76%[35]. Studies in Southeast Asia using quinine monotherapy for 7 days showed cure rates of 85-87 % [29, 33] , which is similar to what was observed over 15 years earlier [36], (Table 1).

Table 1: Summary of studies of quinine for the treatment of uncomplicated malaria

Study site	Year	Sample size and study population	Drug Regimens	Duration of follow-up	Treatment outcome	Comment	Ref
Thailand, region with multidrug resistant malaria	1984-1985	66 children 2–12 years	Quinine Quinidine	28 days DOT	Cure rates: Quinine- 85% Quinidine – 88%	Treatment failures only RI responses	[29]
Cambodia, region with multidrug resistant malaria	1983	119 adults, >15 years	Mefloquine +SP (MSP) 3 days quinine+tetracycline (Q3T7)	28 days DOT	Cure rates: MSP: 98% Q3T7: 76%	Q7T7 still gives good cure rate	[30]

			7 days of quinine+ tetracycline (Q7T7)		Q7T7: 92%		
Brazil, setting with quinine resistance	1985	100 patients 18–55 years	Mefloquine 1000mg single dose (MQ) 3 days quinine+SP (Q3+SP)	42 days DOT	Cure rates: MQ: 96% Q3+SP: 98%	Four RI responses in Q3+SP group	[31]
Thailand, region with multidrug resistant malaria	1994	102 patients 16–60 years	Mefloquine+tetracycline (MQT) 7 days of Quinine+ tetracycline (Q7T7)	28 days DOT	Cure rates: MQT: 94% Q7T7: 98%	MQ+Tetra as effective as Q7T7	[34]
Thailand, region with multidrug resistant malaria	1995-1997	204 male patients 15–64 years	7 days quinine (Q7) Quinine + tetracycline (Q7T7) Quinine + clindamycin (Q7C7)	28 days Directly observed therapy	Cure rates: Q7: 87% Q7T7: 98% Q7C7: 100%	Tetracycline or clindamycin improves quinine cure rates	[33]
Equatorial Guinea, setting with no quinine resistance	1999	114 children 6–59 months	7days quinine (Q7) Chloroquine (CQ) Sulfadoxine/pyrimethamine (SP)	14 day follow-up	Cure rates: Q7: 94.5% CQ: 60% SP: 90%	Quinine is effective against P.falciparum malaria	[43]
Cameroon, High transmission setting	2005	30 children 0.5–6 years	5 days quinine (Q5)	14 day follow-up	Cure rates: 100%		[41]
Burundi Perennial transmission	1992-1995	472 children 0–14 years	Chloroquine (CQ) 5 days quinine	7 day follow-up	Failure rates Q5: 1992-1993:		[40]

setting			(Q5)		4.2% 1994-1995: 7.1%		
Guinea-Bissau Perennial transmission setting	1994-1995	203 children 0.7–13 years	3 days quinine (Q3) 5 days quinine (Q5) 7 days quinine (Q7)	28-35 day follow-up	Day 28 recurrent parasitemia: Q3: 79% Q5: 90% Q7: 11%	3 day quinine regimens should not be used.	[37]
Gabon High transmission setting	1993-1994	120 adults ≥ 15years	3 days quinine (Q3) 3 days quinine+clindamycin (Q3C3) 3 days quinine+doxycycline (Q3D3)	28 day follow-up	Day 28 cure rates: Q3: 38% Q3C3: 92% Q3D3: 91%	The two short course combinations of quinine had excellent cure rates	[109]
Uganda Meso-endemic transmission setting	2007-2008	175 children 6months - 5 years	7 days quinine (Q7) 3 days artemether-lumefantrine (AL)	28 day follow-up	Cure rates: Q7: 64% AL: 97%	Results question the advisability of quinine use for uncomplicated malaria	[45]

Moreover, the addition of either tetracycline or clindamycin to quinine in the Thai study improved cure rates to 98% and 100% respectively and also delayed the appearance of *Plasmodium vivax* infection, suggesting additional activity against this species [33].

In Africa, studies evaluating three-day quinine treatment regimens have usually found unacceptably high failure rates [37], with recurrent infections at day 28 post-treatment experienced in 30% - 50% of patients[37-39]. However most of these studies did not perform

PCR analyses to distinguish between recrudescence and re-infection, leading to possible underestimation of efficacy. In interpreting these results, the malaria transmission intensity at the study sites needs to be taken into consideration, as high treatment failure rates in high transmission settings may be due to a high risk of new infections. Additional PCR unadjusted studies that have evaluated five-day regimens of quinine have found recurrent infection rates on day 7 between 4% and 7% [40] and day 14 treatment failure rates of 0 to 5% (Table 1) [41, 42]. In Equatorial Guinea, five-day courses of quinine were associated with day 14 PCR unadjusted failure rates as high as 22%. These latter results prompted a change in the quinine treatment regimen for this region to a 7 day course, with subsequent significant decrease in treatment failure rates to 3% -5.5% [43]. This study also reported that treatment failure rates with quinine remained stable over the five-year period of surveillance.

Even with seven-day treatment durations, evaluations of different quinine dosage regimens have revealed interesting trends. Doses of 10mg/kg/day given twice daily for 7 days were associated with day 28 treatment failure rates as high as 30 %[37]. Increasing the quinine dosage to 15mg/kg/ day or 20mg/kg/day improved treatment outcomes, with failure rates ranging from 8% to 14%[37], although potential increases in toxicity with higher dosages are a concern. The treatment regimen currently recommended in sub-Saharan Africa is 10mg/kg of the base given 8 hourly for 7 days. This regimen was associated with a lower rate of recurrent infections on day 28 (6.3%) compared to the 10mg/kg twice daily regimen (16.1%)[44].

The advent of ACT has provided important new therapeutic options for the management of uncomplicated malaria in regions with high prevalence of multi-drug resistant malaria. A few

available trials have shown superiority of ACT over quinine in the management of uncomplicated malaria [32, 45, 46]. In Brazil, patients treated with artemether-lumefantrine (AL) had significantly faster parasite clearance times when compared to those treated with quinine+doxycycline [46]. Considering the extensive available data, quinine should not be used to treat uncomplicated malaria when ACT is available [27, 45]. ACT has the advantages of simplicity of dosing, which promotes adherence to therapy when compared with the seven-day treatment courses of quinine [32, 45], better tolerance and decreased risks of serious toxicity.

Nevertheless, despite their scale up in Africa, the cost and availability of ACT in the public sector remains a major challenge. In 2008, ACT coverage in the public sector in high-burden African countries was only 42%[47]. Similarly, a survey carried out during the same year in seven African countries showed that the percentage of fever cases in children < 5 years treated with ACT was only 16% [47]. The sustainability of ACT supplies in resource limited settings therefore presents a huge problem, with stock-outs consistently occurring in health facilities [48]. Quinine, on the other hand, is a relatively cheap drug and often the only available option, rendering its rapid withdrawal for uncomplicated malaria cases risky. The best approach in these settings would be to proactively identify solutions to ACT stock-outs and maintain quinine as a fall-back drug only in case of ACT stock-outs. Additionally, improving quinine treatment outcomes by combining it with antibiotics, such as tetracycline or clindamycin [49-51], could be investigated and promoted. More recently, combinations of quinine and newer antibiotics with shorter treatment regimens that would improve adherence to therapy as well as minimize related adverse events have been evaluated. One such combination is that with azithromycin which is of particular interest, as the drugs act synergistically [52]. This combination offers promise for use

especially in pregnant women and children < 8 years, since, unlike tetracyclines, both drugs are safe in these groups. A study in Thailand showed comparable efficacy in the treatment of multidrug resistant malaria, with cure rates of 100%, for a seven-day course of quinine+doxycycline and a three-day course of quinine+azithromycin [49]. These drug combinations will need further evaluation to confirm these findings and may offer a solution to the compliance problems associated with seven-day courses of quinine.

Quinine for malaria in pregnancy

Malaria in pregnancy causes several adverse outcomes that include maternal anaemia, intrauterine growth retardation, low birth weight, preterm deliveries and abortion. Prevention and treatment of malaria in pregnancy is, therefore, critical to avoid these adverse outcomes. Currently the WHO recommends the use of quinine plus clindamycin for treating malaria in the first trimester of pregnancy, as the safety of artemisinin compounds during this period is not yet established [23]. As most clinical trials exclude women in their first trimester of pregnancy, information on the efficacy and safety of anti-malarial drugs during this period is extremely limited. Evidence for the safety of quinine in pregnancy is mostly historical and there are few clinical trials published [50, 53]. Clindamycin on the other hand has a good safety record in pregnancy [54] and its pharmacokinetic properties are usually unchanged by pregnancy[55]. The combination of quinine and clindamycin has proven highly efficacious against multidrug-resistant strains of *P. falciparum*, with 42 day cure rates of 100% in one study [50]. The only concern with this combination is that it is usually not affordable for most resource limited settings. For the second and third trimester of pregnancy, quinine monotherapy seems to have

unacceptably low efficacy in areas with multidrug resistant malaria when compared to ACT. Studies in these regions have shown that ACT performs better than oral quinine in terms of parasite clearance and fever clearance. Two studies in Thailand [56, 57] reported fewer treatment failures at day 63 with artesunate plus atovaquone-proguanil and artesunate plus mefloquine, when compared with quinine. The occurrence of adverse events experienced by the pregnant women was similar in all groups, although tinnitus was more frequent in the quinine group. In these studies, the considerably inferior efficacy of quinine was attributed to both drug resistance and to the varying pharmacokinetic properties of quinine during pregnancy. In Africa however, available evidence suggests that *Plasmodium. falciparum* generally remains sensitive to quinine [58] and low cure rates with quinine monotherapy in pregnant women has been mainly attributed to poor compliance to treatment [59]. Thus in Africa, quinine monotherapy remains the most widely used treatment for malaria in the first trimester of pregnancy and is also considered safe during all trimesters of pregnancy. A recent study from Uganda provides important reassurance of continued efficacy of quinine monotherapy in these regions of Africa. In this study, quinine and artemether-lumefantrine had similar efficacy for the treatment of uncomplicated malaria in the second and third trimesters of pregnancy [60]. The evidence for safety of ACT use during the first trimester of pregnancy is currently limited [61]. Therefore, until more data become available, the recommendation to use quinine in the first trimester of pregnancy will remain and ACT should only be used in the second and third trimesters of pregnancy. Patient education and counseling will however be critical to promote compliance with therapy.

Quinine in HIV or tuberculosis infected populations

Interactions between HIV and malaria remain a major public health concern in areas affected by both diseases. Very few studies have evaluated the role of quinine in the management of malaria in HIV infected populations. The earliest study was done in the Congo in 1986 and it showed malaria cure rates of 92% in HIV infected patients treated with oral quinine with comparable results in HIV-negative patients [62]. In a subsequent study in the same region, no significant differences in treatment response were observed between children with progressive HIV infection and HIV-uninfected controls treated with oral quinine [63]. Such findings and other available data suggest that malaria treatment policy in HIV infected populations can generally follow the standard practices. Concerns however remain about potential interactions between anti-malarial and anti-retroviral drugs. Currently, there is little published information on the co-administration of antiretroviral therapy (ART) and anti-malarial drugs, yet this will become increasingly important with the rapid scale-up of ART in Africa. In Nigeria, concurrent administration of nevirapine and quinine led to significant reductions in the plasma levels of quinine and elevated plasma levels of 3-hydroxyquinine, the major metabolite of quinine [64]. This could potentially reduce the efficacy of quinine while increasing toxicity, since 3-hydroxyquinine has higher toxicity and lower anti-malarial activity than quinine. Interactions with ritonavir have also been described, with concurrent administration of these drugs leading to marked elevations in plasma levels of quinine and decreases in levels of 3-hydroxyquinine [65]. These results suggest the need for downward dosage adjustments of quinine with concurrent administration of ritonavir, including ritonavir-boosted protease inhibitor regimens.

The co-existence of tuberculosis (TB), malaria and HIV in sub-Saharan Africa and other settings causes additional concerns about their treatment. Interactions between rifampicin (a major

component of first-line anti-TB treatment regimens) and quinine would be expected as rifampicin is a potent inducer of hepatic enzymes and quinine is metabolised mainly by the human CYP 3A isoenzyme. In vivo studies in healthy volunteers showed that when quinine was administered with rifampicin its mean clearance was significantly greater and mean elimination half-life shorter [66]. Interesting observations of the effect of combined quinine and rifampicin therapy were additionally reported in Thai patients with uncomplicated malaria [67]. In this study, parasite clearance times were shorter in the quinine-rifampicin group than in the group given quinine monotherapy, suggesting that the anti-malarial activity of rifampicin augmented that of quinine initially. However, recrudescence rates were five times higher in the quinine-rifampicin group than in the quinine-alone group[67]. These observations were explained by marked differences in the plasma quinine concentrations when rifampicin was combined with quinine. These results suggest that the quinine dosage might need to be increased in patients receiving rifampicin as an anti-TB drug.

Concerns also exist about potential interactions with the concurrent use of antiretroviral drugs and artemisinin-based combination therapy [68-70]. Further research and pharmacovigilance will be critical to facilitate the development of targeted treatment recommendations. Presently, it is not possible to elucidate advantages associated with the use of any particular anti-malarial drug for HIV or TB infected populations.

Quinine in the management of severe malaria

The treatment of severe malaria requires prompt, safe, and effective intravenous anti-malarial drugs. Over the years, quinine has been the mainstay in the treatment of severe malaria and still remains the first line drug in most African countries [24]. Though quinine dosing regimens have varied, the WHO recommends a dose of 20 mg salt/kg by intravenous infusion, then 10mg/kg every eight hours [23]. The rationale for the loading dose is the urgent need to achieve therapeutic plasma concentrations. One systematic review showed that a loading dose of quinine reduced fever and parasite clearance times, but there was insufficient data to demonstrate its impact on risk of death [71].

More recently, intravenous artesunate is the recommended treatment of choice for severe falciparum malaria in adults [23]. This recommendation was made on the basis of the dramatic results of the SEAQUAMAT trial conducted in Southeast Asia that showed a 35% reduction in the case-fatality rate in adults with severe malaria treated with intravenous artesunate compared to intravenous quinine[20]. Subsequent systematic reviews have also provided additional evidence for this recommendation [72]. However, about 80% of malaria deaths occur in sub-Saharan Africa among children aged < 5 years. The therapeutic options previously recommended by WHO for the paediatric group included intravenous artesunate, intramuscular artemether or intravenous quinine[23]. Several trials and meta-analyses comparing intramuscular artemether with intravenous quinine have consistently shown no benefit of treatment with artemether over quinine in children with severe malaria in sub-Saharan Africa [73-75] (Table 2). The recently concluded AQUAMAT study now provides conclusive evidence of the superiority of intravenous artesunate over quinine in children <15 years, with a relative reduction of 23% in mortality associated with the use of artesunate.[21] These observations recently led to a change

in WHO recommendations, with intravenous artesunate now advocated in preference to quinine for the management of severe malaria in children. The most critical issues that will need to be addressed, however, are the availability of intravenous artesunate for the patients who need it, especially in resource-limited settings, and its effectiveness in real-life settings. Until recently, the available formulations of injectable artesunate that have been used in several clinical trials were not produced according to Good Manufacturing Practices (GMP) and this could be a problem for African countries relying on donors who do not permit purchase of non-GMP artesunate. WHO recently pre-qualified intravenous artesunate manufactured by Guilin Pharmaceuticals in China and this may resolve problems of procurement of GMP artesunate. However, it is unclear whether supplies will be sufficient for the thousands of patients in need. Until these procurement and supplies issues are resolved, intravenous quinine may remain the only readily available drug for treating severe malaria in sub-Saharan Africa and other resource-limited settings. Furthermore, there are several health systems challenges related to the management of severe malaria in resource limited settings that impact on treatment outcomes, independent of the parenteral anti-malarial drugs used. Consequently, changes in treatment policies, in this case from quinine to artesunate, may not offer improvements without considering drug availability as well as additional measures to strengthen health systems.

Another important aspect of severe malaria case management is pre-referral treatment, which is treatment given to a patient with severe malaria before they are referred to a health facility. This is critical, as most malaria deaths, especially in Africa, occur outside hospitals, either in the communities or at lower levels of care. Studies evaluating the role of rectal artesunate and artemether as pre-referral treatment have found these options to be highly efficacious [76, 77].

However, the biggest challenge faced in resource limited settings has been the non-availability of these preparations in health facilities. A recent survey in Uganda found that rectal artemisinins were available in only 5% of the health facilities despite the fact that this is the recommended pre-referral drug [78]. A feasible alternative is rectal quinine, which has been found to have comparable efficacy with intravenous quinine in the management of severe malaria in children [79-84] (Table 2) and could play a more significant role than currently acknowledged as pre-referral treatment for severe malaria. More recent studies in Senegal and Mali provide additional support for the efficacy and feasibility of this route and also show that a pre-referral kit of rectal quinine was acceptable to both caretakers and health workers [85, 86].

Following successful administration of parenteral treatment for severe malaria, it is recommended to continue with an oral anti-malarial drug once a patient is able to tolerate oral therapy. The current practice is to continue the same medicine orally as given parenterally to complete a full treatment course [23]. The options for oral continuation therapy that are available in many African settings would therefore include oral quinine or an ACT. In non-pregnant adults, doxycyline would also be added to either of these drugs and given twice daily for 7 days. Where available, clindamycin may be substituted in children, since doxycyline is contraindicated in this age group [23]. The choice of oral continuation therapy following initial parenteral treatment of severe malaria may also have an impact on clinical outcomes, particularly on parasite clearance, fever clearance and potentially the risk of recurrent parasitaemia. In this regard completing intravenous quinine treatment with an ACT instead of oral quinine may improve the overall treatment outcome of parenteral quinine therapy. Studies evaluating this approach to therapy are limited. A study in Kenya during 2000–2002, showed that completing

the intravenous quinine dose with oral malarone (atovaquone + proguanil) was associated with improved clinical outcomes compared to intravenous quinine followed by oral quinine [87] (Table 2). Additional studies should explore other options, in particular ACT, for improving therapeutic outcomes with intravenous quinine treatment.

Table 2: Summary of studies of quinine for the treatment of severe malaria

Study site	Year	Sample size and Study population	Drug Regimens	Treatment outcome	Comment	Reference
Gambia	1992-1994	576 children 1-9 years Cerebral malaria	Intramuscular artemether (IMA) Intravenous quinine (IVQ)	Mortality: IMA:20.5% IVQ: 21.5% Neurological sequelae: IMA: 3.3% IVQ: 5.3%	Artemether is as effective as quinine in treatment of cerebral malaria in children	[186]
Malawi	1992-1994	183 children Cerebral malaria	Intramuscular artemether (IMA) Intravenous quinine (IVQ)	Mortality: IMA: 11% IVQ: 16% Survival with neurological sequelae: IMA: 19% IVQ: 12%	Results do not suggest artemether would confer a survival advantage over quinine	[185]
Kenya	2000-2002	360 patients 1-60 years Severe malaria	IV Quinine + oral malarone (QM) IV Quinine +oral quinine (QQ)	Day 28 cure rates: QM: 98.7% QQ: 90%	Using malarone after IV quinine is safer and as effective as IV quinine +oral quinine	[193]
Burkina Faso	2001-2002	898 children 1-15 years Moderately severe malaria	Rectal quinine (RQ) Intramuscular quinine (IMQ)	Early treatment failure (day 3): RQ: 6%	Rectal quinine had acceptable safety profile and could be used as	[194]

				IMQ: 3% Fever recurrence on day 7: RQ: 5% IMQ: 10%	early treatment for severe malaria	
Uganda	2002-2003	103 children 0.5-5 years Cerebral malaria	Rectal artemether (RA) Intravenous quinine (IVQ)	Mortality: IVQ: 11.7% RA: 19.2%	Rectal artemether was effective and well tolerated	[188]
S.E Asia (Four countries)	2003-2005	1461 patients >2 years Severe malaria	Intravenous artesunate (IVA) Intravenous quinine (IVQ)	Mortality: IVA: 15% IVQ: 22% Absolute reduction in mortality: 34.7%	Intravenous artesunate should be treatment of choice for severe malaria in adults	[50]
Uganda	2003-2004	110 children 0.5-5 years Cerebral malaria	Rectal quinine (RQ) Intravenous quinine (IVQ)	Mortality: RQ: 7% IVQ: 9% Comparable clinical and parasitological outcomes	Rectal quinine was efficacious and could be used as a treatment alternative	[191]
Africa (Nine countries)	2005-2010	5425 children < 15years Severe malaria	Intravenous artesunate (IVA) Intravenous quinine (IVQ)	Mortality: IVA: 8.5% IVQ: 10.9% Relative reduction in mortality: 22.5%	Parenteral artesunate should replace quinine as the treatment of choice for severe malaria	[51]

Potential explanations for quinine treatment failure

Quinine resistance. Parasite drug resistance is probably the greatest problem faced by malaria control programs worldwide and is an important public health concern. Over the years, malaria parasites have developed resistance to a number of commonly used anti-malarial drugs. However the development of resistance to quinine has been slow. Although its use started in the 17th century, resistance to quinine was first reported in 1910 [88]. In comparison, resistance to chloroquine and proguanil emerged within only 12 [89] and 1 year [88, 90] of their introduction, respectively. Resistance to quinine is usually low grade, with the drug retaining some activity but having its action delayed or diminished. Diminished sensitivity of *P. falciparum* to quinine has been widely documented in Asia [91] and South America [92] but it seems relatively uncommon in Africa where conflicting results of no resistance [93, 94] or varying degrees of resistance [95], [96] have been reported. A recent study from Thailand showed significant reductions in efficacy of quinine, artemisinin and mefloquine when compared to previous reports from the same area, suggesting further increase in drug resistance in this region [97]. No convincing evidence of high grade quinine resistance in the treatment of severe malaria has been reported. Findings from a recent systematic review of about 435 clinical trials published between 1966 and 2002 showed that the recrudescence rates for quinine reported over these past 30 years remained roughly constant [98]. These findings are encouraging and may suggest that efficacy of quinine has been preserved.

Variations in quinine pharmacokinetics. Treatment failures with quinine could also be explained by varying pharmacokinetic profiles of the drug. It is known that quinine

pharmacokinetic properties and therapeutic responses vary with age, pregnancy, immunity and disease severity [99]. Also, as patients recover from malaria, there is usually an expansion of the volume of distribution and an increase in systemic clearance of quinine resulting in a decline in the average concentration of quinine in plasma [100]. These variations may lead to drug levels that may be inadequate to completely clear infection. The possibility that pharmacokinetic factors may explain quinine treatment failure was initially raised about 20 years ago when a Thai patient who had fatal severe malaria and apparent RIII resistance was found to have abnormally low levels of quinine despite adequate dosing [101]. Additional evidence for the impact of unusual quinine pharmacokinetics on treatment outcomes was provided by a more recent study describing early treatment failure in a patient with severe malaria with an abnormally high volume of distribution and increased quinine clearance, resulting in abnormally low quinine concentrations [102]. A few studies have proposed that an increase in the quinine dosage after the third day could compensate for declines in plasma drug levels during recovery, especially in areas with resistant *P. falciparum* [99]. However, this is not routinely practiced. Despite these anecdotal observations, there is little evidence for large variations in quinine pharmacokinetics [103] and the exact role that variations in drug levels play in quinine treatment responses is unclear.

Quinine drug quality and treatment compliance. The quality of quinine used in routine care could play a key role in clinical outcomes. Poor quality drugs remain a problem worldwide and are a serious public health threat. A study in Nigeria evaluating the quality of different anti-malarial drugs found that 37 % of 225 anti-malarial drugs did not meet the tolerance limits set by United Sates Pharmacopeia (USP) for the amount of active ingredient, and 46% of these were

formulations of quinine [104]. In Congo, Burundi and Angola only 89% of the declared active substance was found in quinine tablets, with high quantities of impurities reported [105]. Another worrying situation was unveiled in a survey in Cameroon, where nearly 74% of 70 quinine samples had no active ingredient [106]. Several other studies have also described varying problems with quinine drug quality in different settings [107, 108]. Ideally, branded anti-malarial drugs should be used, but unfortunately, branded quinine products are not universally available in Africa and other malaria endemic settings. In addition, national drug regulators need to strengthen their roles in the monitoring of anti-malarial drug quality.

Another potential explanation for quinine treatment failures may be poor compliance. Quinine's prolonged treatment course and significant tolerability problems may lead to poor compliance, and hence poor therapeutic outcomes [32, 45, 59]. In this aspect, ACT has an advantage over quinine since it is administered once or twice daily over three days. A recent study in Uganda showed comparable compliance on day 3 of treatment in patients taking either quinine or artemether-lumefantrine. However, non-compliance to quinine greatly increased with increasing days on therapy to about 44% by day 7[45]. Promotion of shorter courses of quinine, especially in combination with antibiotics, should improve compliance as well as treatment outcomes [39, 109].

Conclusion

In the near future, quinine will continue to play a significant role in the management of malaria, particularly in resource limited settings. Following the results of the SEAQUAMAT and AQUAMAT trials, artesunate is now recommended as the treatment of choice for severe malaria

patients, with quinine only acting as an alternative when artesunate is not available. The role of rectal quinine as pre-referral treatment for severe malaria has not been fully explored, but this remains a promising intervention given the limited availability of rectal artemisinin preparations in resource limited settings. Quinine continues to play a critical role in the management of malaria in the first trimester of pregnancy, and will remain so until safer alternatives become available. The continued use of quinine in the management of uncomplicated malaria is a concern. Clearly, the seven day duration of therapy and thrice daily administration of quinine present a major challenge to completion of therapy, leading to sub-optimal treatment outcomes. In these situations, ACT is a better option given the simplicity of dosing and shorter treatment duration. However, because of the frequent ACT stock outs, the rapid withdrawal of quinine as a treatment option for uncomplicated malaria cases is risky. The best approach would be, besides improving the supply system, to maintain quinine as a fall-back drug in case of ACT stock-outs.

Competing interests

The authors declare that they have no competing interests.

Author's contribution

AJ, PJR and UD conceived the idea and wrote the first draft of the manuscript. All authors read and approved the final version

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CHAPTER 5

ANTIRETROVIRAL AGENTS AND PREVENTION OF MALARIA IN HIV-INFECTED UGANDAN CHILDREN

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ABSTRACT

Background

Human immunodeficiency virus (HIV) protease inhibitors show activity against *Plasmodium falciparum* in vitro. We hypothesized that the incidence of malaria in HIV-infected children would be lower among children receiving lopinavir–ritonavir–based antiretroviral therapy (ART) than among those receiving nonnucleoside reverse-transcriptase inhibitor (NNRTI)–based ART.

Methods

We conducted an open-label trial in which HIV-infected children 2 months to 5 years of age who were eligible for ART or were currently receiving NNRTI-based ART were randomly assigned to either lopinavir–ritonavir–based ART or NNRTI-based ART and were followed for 6 months to 2 years. Cases of uncomplicated malaria were treated with artemether–lumefantrine. The primary end point was the incidence of malaria.

Results

We enrolled 176 children, of whom 170 were started on the study regimen: 86 received NNRTI-based ART, and 84 lopinavir–ritonavir–based ART. The incidence of malaria was lower among children receiving the lopinavir–ritonavir–based regimen than among those receiving the NNRTI-based regimen (1.32 vs. 2.25 episodes per person-year; incidence-rate ratio, 0.59; 95% confidence interval [CI], 0.36 to 0.97; P = 0.04), as was the risk of a recurrence of malaria after treatment with artemether–lumefantrine (28.1% vs. 54.2%; hazard ratio, 0.41; 95% CI, 0.22 to 0.76; P = 0.004). The median lumefantrine level on day 7 after treatment for malaria was significantly higher in the lopinavir–ritonavir group than in the NNRTI group. In the lopinavir–ritonavir group, lumefantrine levels exceeding 300 ng per milliliter on day 7 were associated with a reduction of more than 85% in the 63-day risk of recur- rent malaria. A greater number of serious adverse events occurred in the lopinavir–ritonavir group than in the NNRTI group (5.6% vs. 2.3%, P = 0.16). Pruritus occurred significantly more frequently in the lopinavir–ritonavir group, and elevated alanine aminotransferase levels significantly more frequently in the NNRTI group.

Conclusions

Lopinavir–ritonavir–based ART as compared with NNRTI-based ART reduced the incidence of malaria by 41%, with the lower incidence attributable largely to a significant reduction in the recurrence of malaria after treatment with artemether–lumefantrine. (Funded by the Eunice Kennedy Shriver National Institute of Child Health and Human Development; ClinicalTrials.gov number NCT00978068.)

INTRODUCTION

Malaria and human immunodeficiency virus (HIV) infection impose immense and overlapping burdens in sub-Saharan Africa. Prophylaxis with trimethoprim–sulfamethoxazole and insecticide-treated bed nets reduce the incidence of malaria among HIV-infected children, but protection is incomplete, and even with the use of these protective strategies, the burden of malaria remains great in areas of high-intensity transmission.[1] Thus, new approaches to the prevention of malaria are an important public health priority.

Antiretroviral protease inhibitors show in vitro activity against *Plasmodium falciparum*[2-4] (the cause of most malaria cases in Africa), probably owing to inhibition of plasmodial aspartic proteases that are biochemically similar to the HIV-1 protease.[3,5] Lopinavir is the most potent of these inhibitors and is active at levels well below those achieved with standard doses of coformulated lopinavir–ritonavir.[2,3] Since lopinavir–ritonavir is increasingly available for the treatment of HIV infections in Africa, it may represent a valuable tool for the prevention of malaria. However, because ritonavir inhibits the metabolism of many drugs, it has the potential for interactions with antimalarial agents, including widely used artemisinin-based combination therapies, potentially affecting the efficacy and safety of the drugs.[6] We assessed the incidence of malaria in HIV-infected Ugandan children who were randomly assigned to receive a lopinavir–ritonavir–based antiretroviral regimen or a nonnucleoside reverse-transcriptase inhibitor (NNRTI)–based antiretroviral regimen.

METHODS

Study Participants

We conducted the study in Tororo, Uganda, an area of high-intensity malaria transmission.[7] Eligible children were 2 months to 5 years of age, with confirmed HIV infection. Participants had either never received antiretroviral therapy (ART) and were eligible for initiation of ART according to national guidelines or were currently receiving standard first-line ART, comprising one NNRTI plus two nucleoside reverse-transcriptase inhibitors (NRTIs), and had an HIV RNA level of less than 400 copies per milliliter, as assessed in the most recent measurement during the preceding 6 months. A complete description of the entry criteria is provided in the Supplementary Appendix and in the protocol, both of which are available with the full text of this article at NEJM.org. The parents or guardians provided written informed consent for the participation of their children in the trial.

Study Design

This was an open-label trial with randomization stratified at the time of enrollment according to prior or no prior receipt of ART. Randomization was performed in permuted blocks of 2 or 4. Participants received either lopinavir–ritonavir (Abbott Laboratories) plus two NRTIs or an NNRTI — nevirapine (for children <3 years of age) or efavirenz (for children ≥3 years of age) — plus two NRTIs. The NRTIs that were used in the regimens were lamivudine and zidovudine, with stavudine or abacavir replacing zidovudine in children who had anemia. Children who were already receiving ART were randomly assigned to continue their current regimen or to switch to lopinavir–ritonavir while continuing the same NRTIs. Parents and guardians received counseling on adherence before the children underwent randomization and

were given a 4-week supply of ART at each monthly visit. Insulated coolers were provided when liquid lopinavir–ritonavir was dispensed for young children. Adherence to ART was assessed monthly on the basis of caregiver reports and pill counts.

Study Procedures

At the time of enrollment, children received a long-lasting insecticide-treated bed net, a hygienic water-storage container, multivitamins, and trimethoprim–sulfamethoxazole to be taken daily. Participants received all their medical care at a study clinic that was open every day. Routine visits were scheduled every 4 weeks, and routine laboratory tests were performed every 12 weeks. Parents or guardians were encouraged to bring a child to the clinic any time the child was ill. In the case of children who presented with a documented fever (a tympanic temperature ≥38.0°C) or a history of fever in the previous 24 hours, blood was obtained by finger prick for examination of a thick blood smear. The diagnosis of malaria was made if the smear was positive for malaria parasites. Children with uncomplicated malaria were treated with artemether–lumefantrine, which is the recommended first-line treatment in Uganda, and the parents or guardians were instructed to bring the children back for follow-up evaluation on days 1, 2, 3, 7, 14, 21, and 28. The administration of each first daily dose of artemether–lumefantrine was directly observed in the study clinic, and each second daily dose was administered at home. A complete blood count and measurement of the alanine aminotransferase level were performed on days 0 and 28. From July 13, 2010, through June 8, 2011, blood samples were obtained by means of finger prick 7 days after initiation of artemether–lumefantrine therapy, for measurement of lumefantrine levels. After November 15, 2010, electrocardiograms were obtained and were read by trained study physicians on days 0 and

3 to assess corrected QT (QTc) intervals. Outcomes of malaria treatment were classified according to World Health Organization (WHO) guidelines.[8] Adverse events were assessed at every visit and were graded according to standardized criteria.[9]

Study Oversight

The study was approved by the Makerere University School of Medicine Research and Ethics Committee, the Uganda National Council for Science and Technology, and the University of California, San Francisco, Committee for Human Research. All the authors vouch for the completeness and accuracy of the data and analyses and for the fidelity of the study to the protocol.

Laboratory Procedures

Parasite density was assessed from blood smears as described previously.[10] If a participant had a recurrence of malaria 4 to 63 days after the initiation of malaria therapy, genotyping was performed, as described previously, to distinguish recrudescence from new infection.[10] Lumefantrine levels were measured in 25-μl samples as described previously.[11] The interassay and intra- assay precision (percentage coefficient of variation) was 5.3 to 6.1% and 2.2 to 10%, respectively. The interassay and intra-assay accuracy was 103.5 to 107.1% and 99.5 to 109.7%, respectively. The lower limit of quantification was 50 ng per milliliter. At the time of the diagnosis of malaria, in vitro culturing of selected *P. falciparum* isolates was performed as described previously.[12] After 2 to 4 weeks, aliquots were frozen in glycerol and stored in liquid nitrogen; they were subsequently thawed and cloned by means of a limiting-dilution technique.[13] Drug-interaction studies were performed on clones from two different patients with the use of

the checkerboard technique, as described previously.[2]

Study End Points

The primary end point was the incidence of malaria, which was defined as the number of incident episodes of malaria per time at risk. Malaria that was diagnosed within 14 days after a prior episode was not considered to be an incident event. The time at risk was calculated as the time from the day after initiation of study treatment to the last day of observation, minus 14 days after each incident episode of malaria. In the calculation of the time at risk, discounting the 14 days after each incident episode of malaria is a standard approach. Secondary outcomes included the incidence of complicated malaria, the efficacy and safety of antimalarial therapy, and pharmacokinetic characteristics of lumefantrine.

Statistical Analysis

To test the hypothesis that lopinavir–ritonavir–based ART would reduce the incidence of malaria, we assumed that the incidence of malaria in the NNRTI group would be 0.70 episodes per person-year and estimated that we would need a sample of 300 participants for the study to have 80% power to show a 35% reduction in the incidence of malaria in the lopinavir–ritonavir group, at a two-sided significance level of 0.05. We subsequently observed an incidence of malaria in the NNRTI group that was higher than anticipated (2.19 episodes per person-year) and revised the sample size to 150 participants, who would be followed for at least 6 months.

The statistical analysis was performed according to the intention-to-treat principle, with the use of Stata software, version 11. For the between group comparisons of the incidence of

malaria, we used a negative binomial regression model. Time to events was estimated with the use of the Kaplan–Meier product-limit formula, and comparisons were made with the use of a Cox proportional-hazards model with adjustment for repeated measures in the same patient. Pairwise comparisons of categorical and continuous variables at the level of each episode of malaria were made with the use of generalized estimating equations with adjustment for repeated measures in the same patient and with exchangeable correlation and robust standard errors. P values of less than 0.05 were considered to indicate statistical significance.

RESULTS

Study Participants and Follow-up

From September 2009 through July 2011, a total of 404 children were screened for eligibility; 228 were found not to be eligible, including 136 who did not meet the criteria for initiation of ART (Fig. 1). A total of 176 children underwent randomization: 89 were assigned to NNRTI-based ART and 87 to lopinavir–ritonavir–based ART. Study drugs were initiated in 86 children in the NNRTI group and 84 in the lopinavir–ritonavir group, and these children were followed for a median of 366 days. The baseline characteristics were similar between the two groups (Table 1). During monthly routine assessments, 97% of caregivers reported 100% adherence to ART.

Figure 1: Trial Profile - Screening, Enrollment, Randomization, and Follow-up.

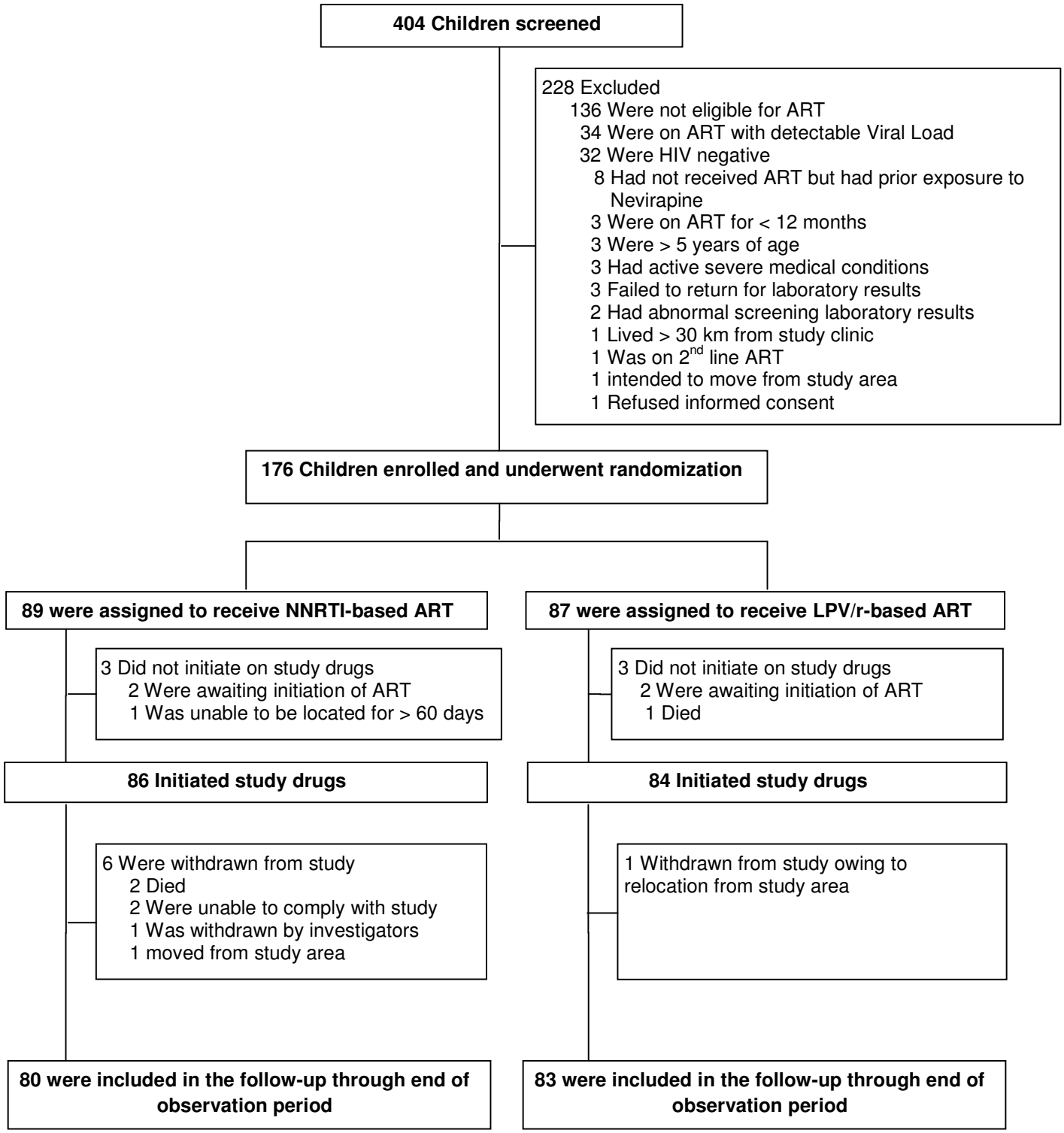


Table 1. Baseline characteristics of participants who received study drugs

Characteristic	NNRTI-based ART (n=86)	LPV/r-based ART (n=84)
Age in years		
Median	3.1	2.9
Range)	0.5-5.9	0.7-6.0
Female sex - no. (%)	41 (48)	41 (49)
No previous ART - no. (%)	58 (67)	57 (68)
WHO clinical HIV stage, no. (%)		
I	66 (77)	60 (71)
II	15 (17)	16 (19)
III	1 (1.2)	2 (2.4)
IV	4 (4.7)	6 (7.1)
CD4 percentage†		
No previous ART		
Median	16	14
Range	2-43	2-44
Previous ART		
Median	30	31
Range	10-45	8-51
Viral load - copies/ml		
No previous ART		
Median	5.5	5.4
Range	BLD-6.4	BLD-6.4
Previous ART		
Median	BLD	BLD
Range	BLD	BLD
Mean hemoglobin g/dL	10.6±1.5	10.4±1.3
Blood smear positive for asexual parasites, no. (%)	11 (13)	10 (12)

* Plus–minus values are means ±SD. There were no significant differences between the groups in baseline characteristics.

BLD denotes below the level of detection (<400 copies per microliter), and WHO World Health Organization.

† CD4 percentage is the preferred measurement for children younger than 5 years of age, s recommended by the WHO. The percentage represents the percent- age of white cells that are CD4 cells.

Incidence of Malaria

Among the study participants, there were 285 new episodes of malaria during 162 person-years of follow-up; all the episodes of malaria were due to *P. falciparum* infection. The incidence of malaria was significantly lower in the lopinavir–ritonavir group than in the NNRTI group (1.32 vs. 2.25 episodes per person-year), resulting in protective efficacy of 41% with lopinavir–ritonavir (incidence rate ratio, 0.59; 95% confidence interval [CI], 0.36 to 0.97; P = 0.04) (Table 2). Complicated malaria occurred infrequently, with a similar incidence in the two groups. To assess the effect of ART independently of potential interactions with antimalarial therapy after treatment for malaria, we compared the two groups with respect to the time to the first episode of malaria (Fig. 2A). The 6-month risk of a first episode of malaria was 40.7% in the lopinavir–ritonavir group, as compared with 52.5% in the NNRTI group (hazard ratio with lopinavir–ritonavir, 0.71; 95% CI, 0.45 to 1.12; P = 0.14) (Table 2).

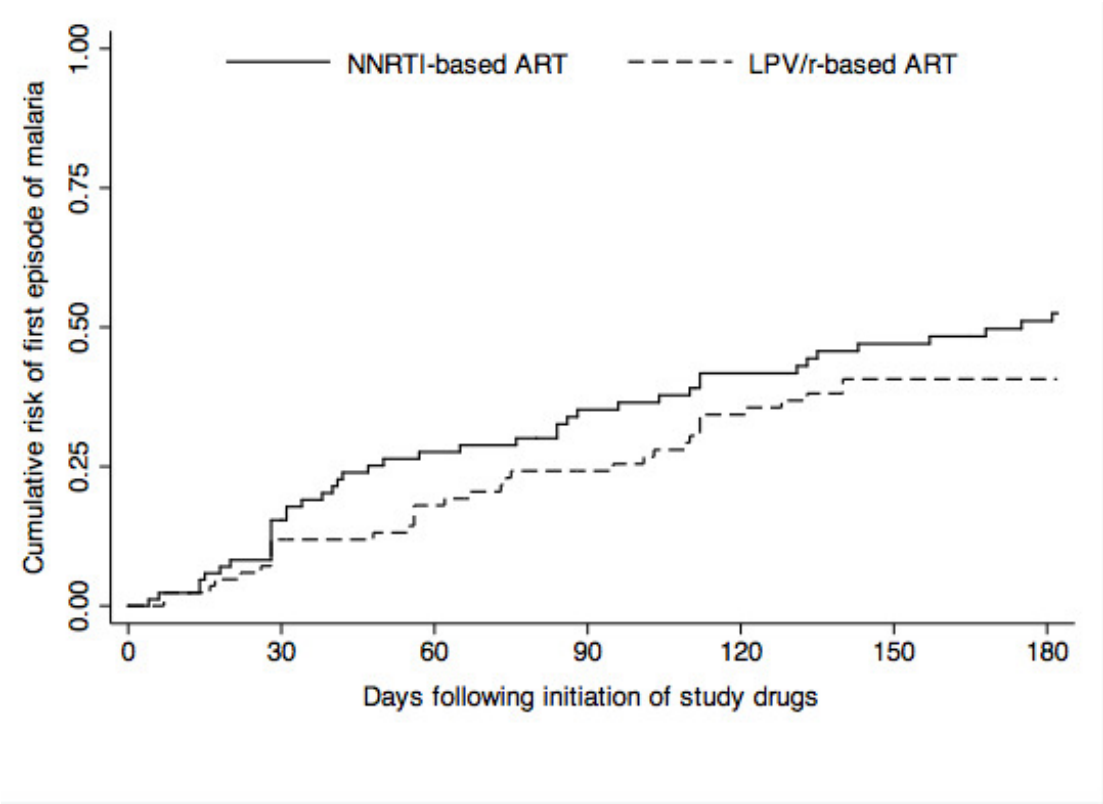
Table 2. Main Study Outcomes

Outcome	NNRTI-based ART			LPV/r-based ART			IRR** (95% CI)	P-value
	No. of Events	Person-Yr at risk	Episodes/ Person-Yr	No. of Events	Person-Yr at risk	Episodes/ Person-Yr		
Episodes of malaria								
All episodes	176	78.2	2.25	109	82.3	1.32	0.59 (0.36-0.97)	0.04
Complicated malaria	2	78.2	0.026	2	82.3	0.024	0.80 (0.06-11.16)	0.87

	NNRTI-based ART		LPV/r-based ART			
	No. of Events	Cumulative risk (95% CI)	No. of Events	Cumulative risk (95% CI)	HR*** with LPV/r (95% CI)	P-value
Risk of malaria						
6-month risk of first episode of malaria	86	52.5% (42.0-63.9%)	84	40.7% (30.9-52.2%)	0.71 (0.45-1.12)	0.14
28-day risk of recurrent parasitemia[§]	174	40.8% (33.9-48.6%)	107	14.0% (8.7-22.2%)	0.31 (0.14-0.68)	0.004
63-day risk of recurrent malaria[§]	174	54.2% (46.4-62.2%)	107	28.1% (20.2-38.3%)	0.41 (0.22-0.76)	0.004

[§] The risk of recurrence was assessed among patients who had had uncomplicated malaria that had been treated with artemether–lumefantrine. IRR denotes Incidence Rate Ratio, HR denotes Hazard Ration

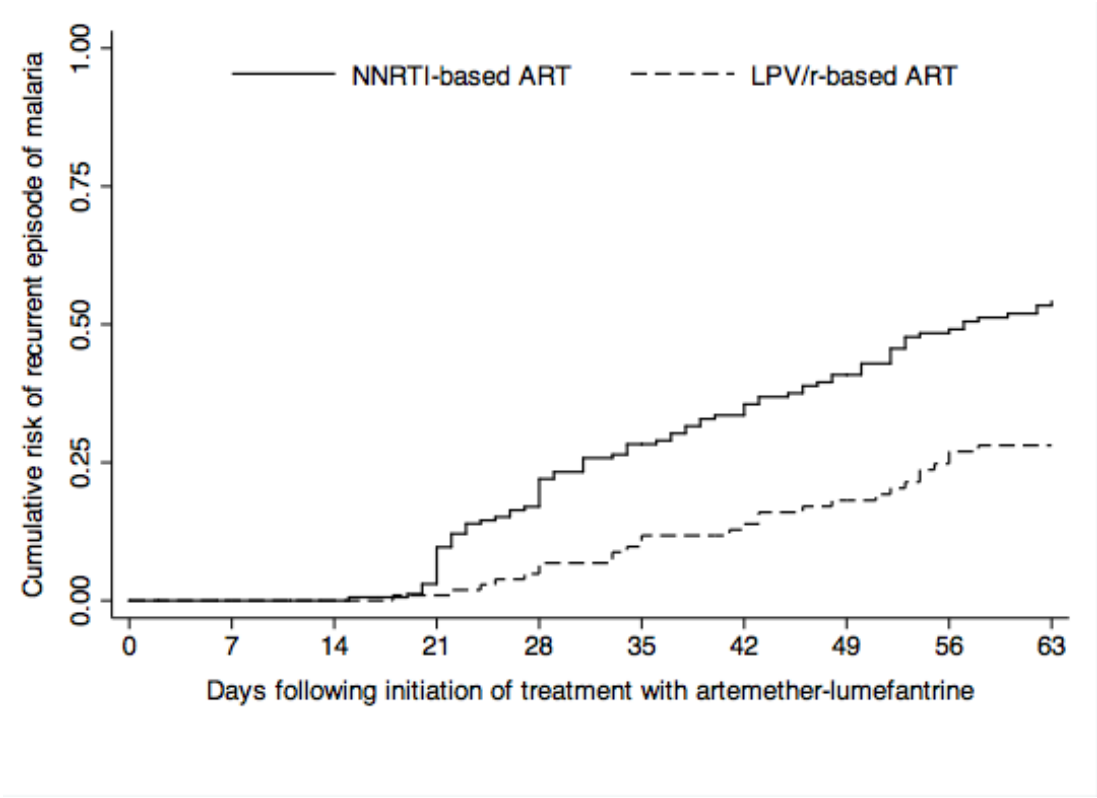
Figure 2B. Kaplan Meier curve for Time to First Episode of Malaria stratified by ART group



Efficacy and Safety Outcomes after Antimalarial Therapy

Of the 285 new episodes of malaria, 281 (98.6%) were uncomplicated and were treated with artemether–lumefantrine. To assess the effect of potential interactions between ART and artemether–lumefantrine, the risks of recurrent parasitemia and recurrent malaria were compared between the two groups. The 28-day risk of re- current parasitemia was significantly lower in the lopinavir–ritonavir group than in the NNRTI group (14.0% vs. 40.8%, P = 0.004), as was the 63-day risk of recurrent malaria (28.1% vs. 54.2%, P = 0.004) (Table 2 and Fig. 2B).

Figure 2B. Kaplan Meier curves for 63-day risk of recurrent malaria following treatment with artemether-lumefantrine stratified by ART group



Among patients in whom parasites were cleared by 7 days, there were 111 episodes of recurrent malaria within 63 days; 107 cases were successfully genotyped, and all were classified as new infections. Resolution of fever, parasite clearance, and recovery of mean hemoglobin levels after antimalarial treatment were similar in the two groups (Table S1 in the Supplementary Appendix). There were no significant differences in the rates of adverse events over the course of the 28-day period after anti- malarial therapy, except that pruritus was more common in the lopinavir–ritonavir group (5.6% vs. 1.2%, P = 0.04), and elevation of alanine

aminotransferase levels occurred more frequently in the NNRTI group (13.5% vs. 3.3%, P = 0.003) (Table S2 in the Supplementary Appendix). A total of 10 serious adverse events occurred during the standard WHO 28-day period for malaria follow- up, with a trend toward a higher frequency in the lopinavir–ritonavir group (5.6% vs. 2.3%, P=0.16).

All the serious adverse events were considered to be unrelated to the study drugs with the exception of one episode of the Stevens–Johnson syndrome in the NNRTI group, which led to discontinuation of the study drug, and two episodes of neutropenia in the lopinavir–ritonavir group, which resolved without discontinuation of therapy. Electrocardiograms obtained on day 3 after initiation of treatment for malaria in a subgroup of 120 patients showed no episodes of prolongation of the QTc interval (Table S2 in the Supplementary Appendix).

Table S1. Secondary efficacy outcomes 28 days following treatment with AL

Treatment Outcome	**ART Group**		**P-value**
	NNRTI-based ART	**LPV/r-based ART**	
Fever Clearance			
Fever present on day 1	77/173 (44.5%)	50/107 (46.7%)	0.69
Fever present on day 2	18/171 (10.5%)	13/107 (12.2%)	0.70
Fever present on day 3	9/171 (5.3%)	2/105 (1.9%)	0.18
Parasite clearance			
Positive blood smear on day 2	9/171 (5.3%)	9/107 (8.4%)	0.35
Positive blood smear on day 3	2/171 (1.2%)	2/105 (1.9%)	0.61
Appearance of gametocytes on days 2-28*	12/145 (8.3%)	6/99 (6.1%)	0.51
Hemoglobin recovery, mean gm/dL (SD)	0.61 (1.14)	0.56 (1.07)	0.58

***** does not include patients with gametocytes on day 0

Table S2. Adverse events 28 days following treatment with AL

Treatment Outcome	ART Group		P-value
	NNRTI-based ART	LPV/r-based ART	
Cough	77/174 (44.3%)	43/107 (40.2%)	0.42
Elevated temperature	39/174 (22.4%)	22/107 (20.6%)	0.84
Diarrhea	14/174 (8.1%)	11/107 (10.3%)	0.46
Vomiting	17/174 (9.3%)	8/107 (7.5%)	0.53
Anorexia	11/174 (6.3%)	8/107 (7.5%)	0.72
Weakness	8/174 (4.6%)	3/107 (2.8%)	0.51
Pruritus	2/174 (1.2%)	6/107 (5.6%)	0.04
Rash	3/174 (1.7%)	1/107 (0.9%)	0.58
Chills	2/174 (1.2%)	3/107 (2.8%)	0.31
Dysphagia	1/174 (0.6%)	1/107 (0.9%)	0.78
Respiratory distress	1/174 (0.6%)	0/107 (0%)	N/A[‡]
Jaundice	0/174 (0%)	3/107 (2.8%)	N/A[‡]
Nausea[*]	1/125 (0.8%)	1/69 (1.5%)	0.67
Abdominal pain[*]	7/124 (5.7%)	1/68 (1.5%)	0.24
Headache[*]	4/125 (3.2%)	1/69 (1.5%)	0.56
Anemia	6/163 (3.7%)	5/104 (4.8)	0.70
Neutropenia	44/152 (29.0%)	36/102 (35.3%)	0.38
Thrombocytopenia	11/158 (7.0%)	3/102 (2.9%)	0.19
Elevated ALT	20/148 (13.5%)	3/91 (3.3%)	0.003
Prolonged QTc interval[†]	0/66 (0%)	0/54 (0%)	N/A[‡]
Any adverse event	138/174 (79.3%)	76/107 (71.0%)	0.13
Any serious adverse event	4[a]/174 (2.3%)	6[b]/107 (5.6%)	0.16

[*] Only assessed in children over 3 years of age
[†] Only assessed in episodes occurring after November 15th 2010
[‡] Unable to generate due to lack of convergence
[a] 1 neutropenia, 1 elevated ALT, 1 vomiting, 1 respiratory distress
[b] 3 neutropenia, 2 anemia, 1 elevated AL

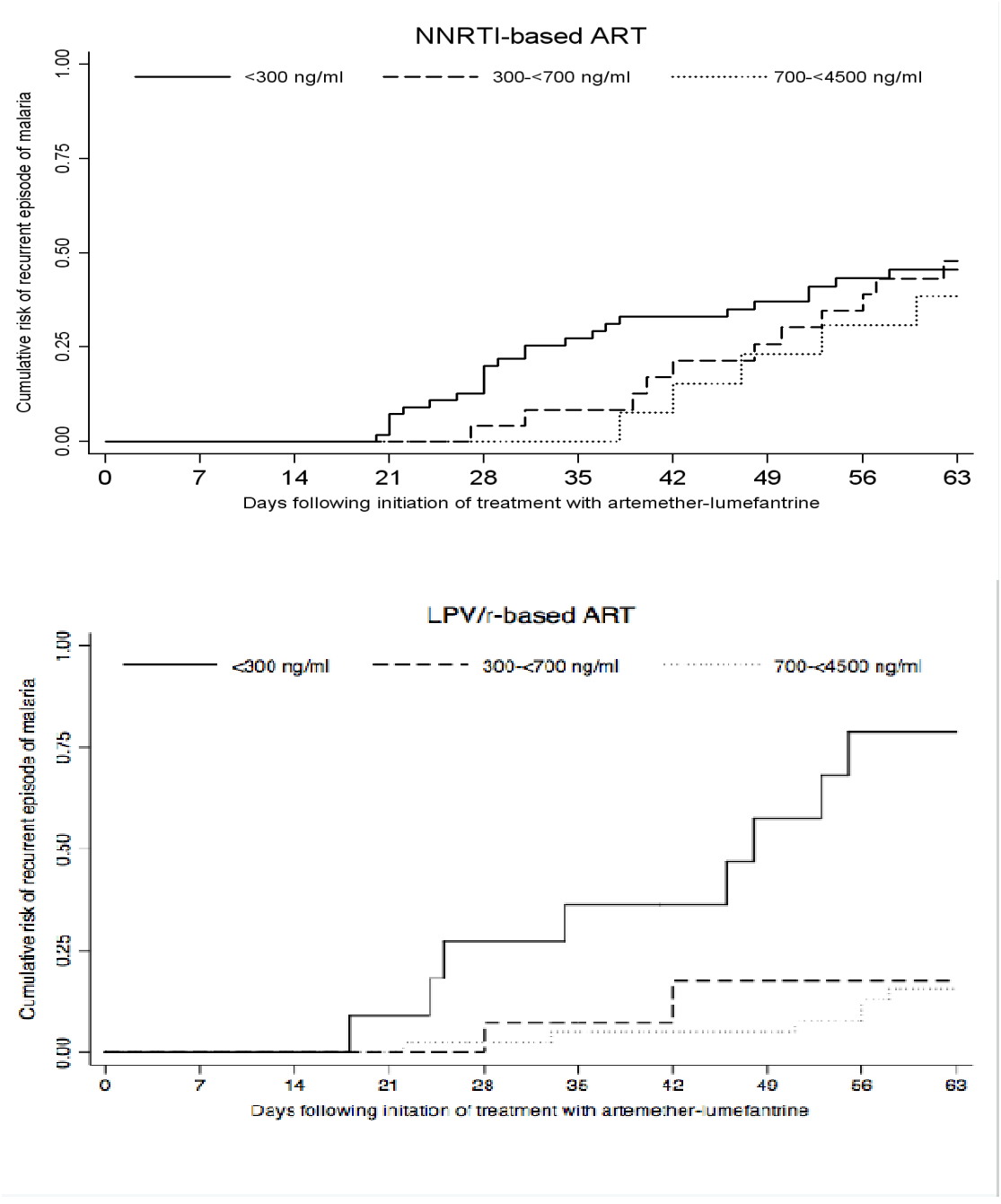
Pharmacokinetic Characteristics of Malaria Therapy and Drug Interactions

During the period in which blood samples were obtained to assess drug levels, there were 103 episodes of malaria in the NNRTI group and 73 in the lopinavir–ritonavir group; lumefantrine levels were successfully measured on day 7 after initiation of malaria treatment in the case of 92 episodes (89%) and 65 episodes (89%), respectively. The median lumefantrine level was significantly higher in the lopinavir–ritonavir group than in the NNRTI group (926 ng per milliliter [inter- quartile range, 473 to 1910] vs. 200 ng per milliliter [interquartile range, 108 to 510], P<0.001). In addition, in the NNRTI group, the median lumefantrine level on day 7 was significantly higher among the 67 patients who were taking nevirapine than among the 25 patients who were taking efavirenz (388 ng per milliliter [interquartile range, 164 to 563] vs. 97 ng per milliliter [interquartile range, 61 to 124], P<0.001). There was no significant association between the lumefantrine level on day 7 and the 63-day risk of recurrent malaria in the NNRTI group. In contrast, in the lopinavir–ritonavir group, children with lumefantrine levels of 300 ng per milliliter or higher on day 7, as compared with children with lower drug levels, had a significantly reduced risk of recurrent malaria within 63 days (Fig. 3, and Table S3 in the Supplementary Appendix).

Table S3. Association between day 7 lumefantrine levels and 63-day risk of recurrent malaria

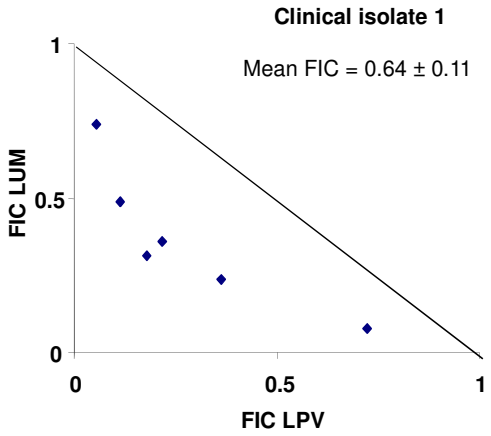
Day 7 lumefantrine level ng/ml	NNRTI-based ART				LPV/r-based ART			
	No.	Risk of recurrent malaria after 63 days	HR* (95% CI)	P-value	No.	Risk of recurrent malaria after 63 days	HR* (95% CI)	P-value
< 300	55	45.7% (33.2-60.2%)	1.0 (reference)	-	11	78.8% (50.7-96.7%)	1.0 (reference)	-
300-<700	24	48.0% (30.0-69.8%)	0.87 (0.42-1.80)	0.71	14	17.5% (4.5-54.9%)	0.15 (0.04-0.56)	0.005
700-<4500	13	38.5% (18.2-69.2%)	0.66 (0.23-1.88)	0.44	40	15.6% (7.3-31.4%)	0.11 (0.03-0.35)	<0.001

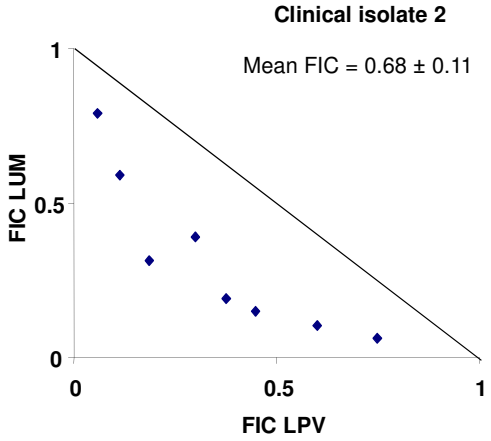
Figure 3. Kaplan Meier curves for 63-day risk of recurrent malaria stratified by ART group and day 7 lumefantrine levels



To determine whether there were direct anti- malarial interactions between lopinavir and lumefantrine, two field isolates were cloned for in vitro drug-sensitivity testing. Modest synergy between lopinavir and lumefantrine was observed, as evidenced by concave isobologram curves and mean fractional inhibitory concentrations near 0.5 (Fig. S1 in the Supplementary Appendix).

Figure S1. Isobolograms describing the interaction between lumefantrine (LUM) and lopinavir (LPV) for two cloned clinical isolates. The mean fractional inhibitory concentration (FIC) index and standard deviation are shown for each analysis.





DISCUSSION

Among HIV-infected children in Uganda, a lopinavir–ritonavir–based antiretroviral regimen as compared with an NNRTI-based regimen reduced the incidence of malaria by 41%. The primary benefit in the lopinavir–ritonavir group was conferred by a dramatic reduction in the risk of recurrent malaria after treatment with artemether–lumefantrine.

The protective effect of lopinavir–ritonavir against malaria could have resulted from direct antimalarial activity of lopinavir–ritonavir; inhibition of lumefantrine metabolism after treatment with artemether–lumefantrine, which would have extended the post-treatment

prophylactic effect; or antimalarial synergy between lumefantrine and lopinavir. The possibility of direct antimalarial effects of HIV protease inhibitors is supported by in vitro studies showing activity of lopinavir at levels achievable in humans,[3,5] in vivo studies showing activity against the rodent parasite *P. chabaudi*,[5] activity against clinical isolates of *P. falciparum* and *P. vivax*,[14] in vitro activity against *P. falciparum* in serum samples from patients receiving protease inhibitors,[15] and action against *P. falciparum* cytoadherence and phagocytosis.[16] In vitro antimalarial activity has not been reported with levels of NNRTIs that are attained with standard doses.[2]

The antimalarial activity of HIV protease inhibitors could theoretically be due to inhibition of plasmodial aspartic proteases that are bio- chemically similar to the HIV protease.[3,5] However, we found only a non significant trend toward a reduced risk of a first episode of malaria in the lopinavir–ritonavir group. Rather, protection against recurrent malaria accounted for most of the benefit of lopinavir–ritonavir, suggesting that the principal role of lopinavir–ritonavir with respect to protection against malaria is an effect on lumefantrine exposure. Children in the lopinavir–ritonavir group had significantly higher lumefantrine levels 7 days after the initiation of artemether–lumefantrine therapy than did children in the NNRTI group and this effect was associated with a 59% reduction in the 63-day risk of recurrent malaria in the lopinavir–ritonavir group as compared with the NNRTI group. Increased lumefantrine exposure with concomitant lopinavir–ritonavir therapy, which was thought to be the result of inhibition of cytochrome P450 3A4 metabolism by ritonavir, was previously observed in healthy adults.[6] In contrast, nevirapine and efavirenz both induce cytochrome P450 3A4 pathways, and the concurrent administration of either of these drugs with artemether–

lumefantrine might diminish lumefantrine exposure. However, day 7 lumefantrine levels in the nevirapine group were similar to those reported previously in HIV-uninfected children receiving artemether–lumefantrine, [17] suggesting that the greater protection in the lopinavir–ritonavir group than in the NNRTI group was due to inhibition of lumefantrine metabolism by lopinavir–ritonavir rather than to enhancement of cytochrome P450 3A4 metabolism by the NNRTIs.

The higher lumefantrine levels in the lopinavir–ritonavir group were not associated with a significantly increased risk of adverse events, with the exception of pruritus. Halofantrine, a related drug, is associated with prolongation of the QT interval and cardiac rhythm disturbances. In this study, no episodes of prolongation of the QTc interval were observed 3 days after the initiation of artemether–lumefantrine.[18] We noted a trend toward a higher risk of serious adverse events after antimalarial therapy in the lopinavir–ritonavir group, with two episodes of neutropenia possibly related to the study drug. Given our limited statistical power for the comparison of uncommon events in this study and our limited evaluation of potential cardiotoxic effects, future studies of the safety of co administration of lopinavir–ritonavir and lumefantrine are warranted. Increased lumefantrine exposure might also increase the selection of lumefantrine-resistant parasites. Genetic polymorphisms associated with decreased activity are selected by arte- mether–lumefantrine,[19] suggesting that continued surveillance of the efficacy of artemether–lumefantrine therapy is warranted.

In vitro synergy in antimalarial activity between lopinavir and lumefantrine has been observed in laboratory strains of *P. falciparum*[2] and in two isolates obtained from children in this trial. Perhaps the synergistic antimalarial activity between lopinavir and lumefantrine enhanced the protective effective of prolonged lumefantrine exposure. Our results suggest

that a strategy of pharmacologic enhancement of exposure to antimalarial agents may be useful in reducing the burden of malaria particularly in areas where transmission intensity is high and recurrent malaria after treatment is commonplace. Although artemether–lumefantrine was very effective in clearing infections, the high risk of recurrent malaria after therapy, even with the use of insecticide-treated bed nets and trimethoprim–sulfamethoxazole prophylaxis, highlights the importance of post-treatment prophylaxis in high transmission areas such as Uganda. In HIV- infected children requiring ART, it may be possible to use pharmacologic enhancement with lopinavir–ritonavir to provide protection from malaria. It is unlikely that an increase in the antimalarial dose without lopinavir–ritonavir therapy will achieve the same effect that we observed with lopinavir–ritonavir therapy in our cohort. The effects that are seen with increases in exposure of a drug may be several magnitudes greater than the effects that are possible with dose escalation — a finding that has been shown with respect to the enhancement of HIV protease inhibitors with ritonavir.[20] In addition, lumefantrine exhibits saturable absorption, with the result that only modest increases in exposure are seen after dose escalation.[21]

In a randomized trial comparing lopinavir–ritonavir therapy with NNRTI therapy in children, virologic suppression rates were higher with lopinavir–ritonavir than with NNRTI — a finding that also supports the use of lopinavir–ritonavir.[22,23] However, before changes in policy are implemented, the relative antiretroviral efficacies of various regimens should be studied further, the applicability of our findings to areas with a lower intensity of malaria transmission should be considered, and logistical challenges must be addressed. We are continuing to follow our cohort in order to evaluate long-term HIV efficacy outcomes.

Previously, the high cost of the drugs and complex storage requirements represented considerable challenges in administering lopinavir–ritonavir in low-income countries. However, the declining cost of the drugs and the availability of a heat stable lopinavir-ritonavir formulation suggest that these challenges are now surmountable. In conclusion, pharmacologic enhancement with the use of agents that alter drug metabolism may offer a means of improving the control of malaria.

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CHAPTER 6

General Discussion

The management of malaria in SSA clearly presents many challenges, some of them highlighted in this thesis. However, with well designed approaches, supported by the evidence generated herein, these challenges are not insurmountable and should lead to optimal treatment approaches for all malaria patients.

6.2 Role of oral quinine for the management of uncomplicated malaria

For uncomplicated malaria, the main objective of treatment is to cure the infection or clear parasitemia as rapidly as possible. This requires the utilization of effective and efficacious drugs that ideally should be easy to administer and readily available. In this thesis, we have been able to demonstrate for the first time the sub-optimal effectiveness of oral quinine, largely due to poor adherence, when used for treating uncomplicated malaria[1]. Therefore, patients in need of second line treatment, when the first line is not as efficacious as expected, would be better treated with another ACT rather than with quinine. ACTs, compared to quinine, would offer simpler dosing regimens and a shorter treatment course. The only challenge this approach presents is ensuring sustainable supplies of ACTs for both first-line and second-line treatment options. Uganda has acknowledged this evidence and has revised its treatment policy for the management of uncomplicated malaria, replacing oral quinine with dihydroartemsisin-piperaquine as the preferred second-line therapy [2]. The uptake of this new policy and its impact on health worker practice and patient treatment outcomes will need further evaluation. A recent study that

evaluated the efficacy of 3 different antimalarial drugs for rescue treatment following failure of first-line antimalarial therapy provides evidence in support of this recommendation [3]. This study showed that in areas of intense transmission, the risk of recurrent malaria following rescue therapy was unacceptably high with oral quinine (70%) compared to dihydroartemisin-piperaquine (25%). In the event that ensuring availability of ACTs as both first-line and second-line treatment options are not feasible, other strategies for improving treatment outcomes with oral quinine are needed. These may include better patient education and closer patient follow-up to ensure adherence to therapy or possibly using shorter quinine treatment courses in combination with antibiotics like azithromycin [4, 5], though this would need some evaluation. Such studies would provide evidence for the optimal quinine-antibiotic combinations for use as well as best dosing regimens for any such formulations. These challenges also show the urgent need for newer antimalarial compounds in order to increase the available therapeutic options once first-line therapy fails.

6.3 Severe malaria treatment practices

Chapter 3 describes severe malaria treatment practices in real-life settings and provides an objective assessment of what the major limitations and challenges in severe malaria case management are[6]. These observations provide a useful baseline for the development of possible interventions to improve practice. The key areas identified as priority areas for improvement including triage and emergency care, referral practises, quality of diagnosis and treatment, availability of medicines and supplies, training and support supervision are very critical in ensuring favourable treatment outcomes for patients with severe malaria. Having made these

critical recommendations, there is need for evidence to support the fact that improvements in these basic approaches can indeed lead to better treatment outcomes for patients with severe malaria. There are several studies that have demonstrated a positive impact of some aspects of these recommendations on treatment outcomes in critically ill patients. Training on emergency treatment and triage (ETAT) decreased mortality in the emergency care settings of several African countries[7, 8]. In addition, health worker training on malaria management has also been shown to improve patient evaluation and treatment outcomes [9]. These training programs indeed provide evidence that such approaches are effective and beneficial in these resource limited settings. Despite the feasibility of training as an intervention in these settings, stock-outs of medicines and supplies remain a major challenge in several settings in SSA[10] and more sustainable solutions to this need to be developed. This could be achieved through promotion of better procurement practices that ensure better quantification and forecasting of required supplies and medicines. In addition, improved stock taking practises are important to ensure that limited stocks are easily noticed and flagged for re-stocking. Ultimately, better financing for health care will be the most important approach to ensure availability of basic resources and requirements for health facilities.

Stock outs may also be curtailed through the promotion of more targeted treatment prescriptions and the avoidance of presumptive diagnosis and treatment. Antimalarial drugs should only be prescribed for a parasitologically confirmed case of malaria – this can be achieved by improving the availability of parasite based diagnostics either through the use of RDTs or microscopy; both currently readily available in many African countries. Treatment of only confirmed cases of malaria prevents unnecessary wastage of antimalarial drugs; especially in settings where over diagnosis of malaria is a common occurrence [11]. Further evidence for the utility of this practise

is derived from results of studies in Uganda and Tanzania that showed increased risk of mortality in patients with negative blood smears treated with antimalarials[12]. These observations suggest that presumptive treatment may lead to mismanagement of other possible causes of illness hence a higher risk of mortality. Our study did not collect information on mortality in these health facilities and we are therefore unable to comment on the factors associated with death in these settings or evaluate the impact of these practices on patient outcomes. However ongoing in-patient sentinel site surveillance reports in 6 district health facilities located in areas of varying malaria transmission settings in Uganda report mortality rates ranging from 1 to 6% (unpublished data). In a study that evaluated the validity of verbal autopsy procedures for determining malaria deaths in Uganda, malaria was reported as a cause of death in 49% of cases in a high transmission setting and 10% in a medium transmission setting[13]. Both reports however do not provide data on factors associated with death – this should be an area of further study to better inform interventions for this group of patients.

6.4 Optimal drug choices for treatment of severe malaria

Currently available evidence suggests that intravenous artesunate (IV AS) is better than quinine for the treatment of severe malaria and indeed the WHO and some African countries (Uganda included) have changed their treatment policy for severe malaria from quinine to IV AS. The benefits of IV AS included a reduced risk of death and decreased incidence of severe adverse events such as hypoglycemia and seizures [14, 15]. The evidence base for this change in treatment policy is clear and not under any contention. However, we need to proceed with caution, particularly when considering the findings described in chapter 3 and the knowledge that severe

malaria related mortality is usually the result of the spectrum of complications [16] that need adequate supportive care. Given the currently weak health systems in resource limited settings and the inadequate supportive treatment provided to patients with severe malaria, changing treatment policy from intravenous quinine to IV AS alone may not achieve the significant mortality benefits observed in the clinical trial setting. There is therefore the urgent need, beyond the introduction of this new treatment approach, to strengthen the health systems in these settings, improve the health worker skills for the diagnosis and management of these patients and ensure that medications and supplies needed for supportive care are available. Without this combined approach and these additional interventions in place, the benefit of IV AS may be lost.

6.5 Malaria management in patients with HIV infection

Our findings in the HIV infected population of children in Uganda demonstrates the need for continued pharmacovigilance to enhance our understanding of the potential drug interactions that may occur when different co-morbidities are concurrently managed. ACTs will remain the mainstay of treatment for uncomplicated malaria and with the scale up of antiretroviral therapy in SSA, all potential interactions between these agents should be well characterized, particularly their potential impact on drug efficacy and toxicity. Our observations show a dramatic benefit of the interactions between Lopinavir/ritonavir and artemether-lumefantrine in terms of improved malaria treatment outcomes, with no apparent increase in risk of toxicity[17]. These findings have huge public health implications on the potential benefit of this kind of interaction for co-infected individuals, particularly those living in areas of high malaria transmission intensity. Clearly in these settings the LPV/r based ART regimen had significant advantages over the NNRTI based

ART regimen as it was associated with a marked reduction of malaria risk. This regimen could therefore be strategically utilized in high transmission settings in high risk populations, like children < 5years, for the dual benefits of protection against malaria and also as a superior regimen for HIV treatment [18]. This approach should be feasible even in resource limited settings especially with increasing availability of protease inhibitors, particularly the heat stable formulations that would be most suitable for African settings. Price reductions of these drugs over the years would be particularly important for the success of these approaches. Translating these findings into policy would also be a cost –effective strategy considering the dual benefit of better therapeutic outcomes for both diseases as well as the marked impact on reduction in the number of malaria episodes/cases.

The use of LPV/r in this population was associated with a 41% reduction in the risk of recurrent episodes of malaria. This presents an additional tool for malaria prevention in HIV infected populations following the positive impact of the utilizations for cotrimoxazole prophylaxis and ITNs [19, 20 21]. This is particularly important given the increasing resistance to cotrimoxazole in many settings in Africa and the uncertainty about its long-term protective efficacy[22] . The impact of LPV/r for prevention of malaria in pregnancy has not been documented however studies are currently underway to answer this research question. In this population, the current standard of care in Uganda and other similar settings is the use of ITNs and cotrimoxazole prophylaxis. Our findings and available literature do not support the need to have different malaria treatment guidelines for HIV-infected populations hence the current guidelines used for the non-HIV infected populations should apply. However these findings suggest close follow up and clinical observation during malaria treatment in this population. Indeed for some of the drug combinations like amodiaquine-artesunate, clear spectrums of

adverse events have been documented[23] [24] and this provides good guidance for clinicians in their evaluations. However the spectrum of potential adverse events with concurrent administration of other the antimalarial drugs and antiretroviral drugs are not well characterized. These will need standardized evaluations to ensure that any such events are consistently documented. This will become increasingly important with the development of newer antimalarial and antiretroviral drugs. Regarding utilization of quinine, the recommendations in HIV uninfected populations should still apply in HIV infected populations. There is limited literature on the interaction between quinine and antiretroviral drugs [25, 26] but the available literature does not suggest and obvious contraindications to its use. Quinine will therefore continue to play a role in the treatment of malaria in HIV infected populations - as an alternative for severe malaria, for treatment of malaria in the first trimester of pregnancy, and as an alternative second-line treatment of uncomplicated malaria.

It is however important to note that all these observations are not necessarily limited to antimalarial drugs and would also apply to other drugs used for the management of other co-morbidities in HIV infected individuals. It is critical therefore, that health workers are adequately equipped with the necessary skills and knowledge to evaluate for all these potential events/occurrences. These observations highlight the need for continued patient education in the recognition of these events in so doing promoting active pharmacovigilance.

6.6 Implications for further research

Our findings highlight several potential areas for further research to inform policy and practice.

For uncomplicated malaria, evaluations for better approaches to improve quinine treatment outcomes and additional therapeutic options for the different risk groups will be important. For severe malaria, research in the area of accurate documentation of mortality and causes of mortality will be needed. The role of clinical audits in resource limited settings as well as their impact on improved treatment practices also needs further study. There is also limited literature on the impact of several interventions like training, mentorship and support supervision on malaria treatment outcomes and mortality. With the current recommendation of IV artesunate for severe malaria, it will be critical to document its impact on mortality in real life clinical settings in Africa. In HIV- infected patients receiving treatment for malaria, continued evaluations and better understanding of the spectrum and clinical implications of any interactions remain crucial. In addition studies to improve our understanding of the pharmacokinetic and molecular basis of any such interactions would be useful to guide management

Conclusion

Whereas malaria continues to be a major cause of morbidity and mortality in SSA, our findings reported in this thesis provide sufficient evidence that effective interventions can curtail the burden of this disease. This thesis highlights management issues for uncomplicated malaria, severe malaria and malaria in HIV infected populations. We believe that results provide evidence that more informed treatment approaches can be designed and utilized in the management of malaria in these different populations with improvement in treatment outcomes. For uncomplicated malaria improvement in treatment outcomes will be achieved through the consistent and timely utilization of ACTs both for first-line and second-line treatment options. For severe malaria, a package of interventions addressing health systems weaknesses, health worker skills and availability of medicine and supplies will provide the necessary positive

impact. For HIV infected populations, strategic utilization of protease-inhibitor based antiretroviral regimens will contribute significantly to reduced malaria burden.

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APPENDICES

Appendix S: Survey Instruments for Sub-study 2

Appendix S1: Severe malaria survey tool for Inpatient Interview

Appendix S2: Severe malaria survey tool - For Outpatients' Health Centre II and III

Appendix S3: Severe malaria survey tool For Inpatient unit/ward

Appendix S4: Severe malaria survey tool - Checklist for each Health Facility

Appendix S5: Severe malaria survey tool for pharmacy

Appendix S1: Severe malaria survey tool for Inpatient Interview

Date__/___/___

Instructions

1. Complete the blank space with the answers given

2. Select the most appropriate option by clearly ticking the correct one/s with a pencil.

3. Do not prompt with the listed answers unless prompting is specified

4. If the Health centre III does not have admission facilities then use the form for outpatients

A. Geographic and Demographic information (GD)

1. Name of Health facility:_____2. Status (circle one): HCII, HCIII, Hospital

3. Facility code (circle one): H (High malaria transmission) L (Low malaria transmission)

4.Patient's names Initial_____ 5. IP number_____

6.Patient's age:_____7. Sex (circle one): M (Male), F (Female)

8.Caretaker characteristics (circle one): M (Male), F (Female)

9 Caretaker relationship to patient: (circle one)

1. Biological mother
2. Biological father
3. Spouse
4. Other relative (Specify)_____

B. Medical History (MH)

1. Why did you come to the health facility (complaints at admission)
   a. Fever or h/o fever (Y/N)
   b. Diarrhoea/vomiting (Y/N)
   c. Fast breathing/ difficulty in breathing (Y/N)
   d. Child difficult to wake (Y/N)
   e. Child convulsing (Y/N)
   f. Ear problem (Y/N)
   g. Other (Specify)_____

2 .....How long did the patient wait before receiving medical care?.....(.hours)?

3 Was your patient immediately assessed and given priority treatment over others?(triage)……(Y/N)

4 For how long have you/your patient been on the ward?_____-(days)

Ask the patient or caretaker if at the time of admission they were asked the following questions:{Y/N}

1. .Does the patient's present illnesses involve a fever? ..... [ ]
2. If yes, was fever present in last 24 hours? ..... [ ]
3. Did patient receive any antimalarial/antibiotic for this illness prior to this visit ..... [ ]
4. Was the patient asked which name and formulation of the medicine taken ..... [ ]
5. Did the patient have convulsions? ..... [ ]
6. Did the patient become drowsy, confused or lose consciousness? ..... [ ]
7. Was the patient extremely weak, unable to sit or stand..... [ ]
8. Was the patient unable to eat or drink or refused to breastfeed? ..... [ ]
9. Did the patient suffer from cough? ..... [ ]
10. Did the patient suffer from breathing problems? ..... [ ]
11. Did the patient vomit repeatedly? ..... .[ ]
12. Did the patient have diarrhoea? ..... [ ]
13. Did the patient have yellow eyes (Jaundice)? ..... [ ]
14. Did the patient suffer from stomach ache? ..... [ ]
15. Did the patient have any ear problems like discharge ..... [ ]
16. Does the patient suffer from any underlying illness(Y/N)..... .[ ]
17. Are you on any medication for that illness? (Y/N)..... [ ]

Does any of the patients contacts suffer from a similar condition?..... [ ]

C. Examination and Investigations (EI)

1) Did any HW **ask/record patient's age** during this visit? ..... [__]
2) Did any HW **measure weight**? (Y/N) ..... [__]
3) Did any HW **measure temperature**? (Y/N) ..... [__]
4) Did any HW take the patient's pulse (Y/N)..... [__].
5) Did any HW take the patients pressure? ..... [__]
6) Did the health worker physically examine the patient? (Y/N)..... [__]
7) If yes, which of these procedures did he/she perform?

Examine (look at or touch) the following

- Eyes.....
- Ears.....
- Mouth.....
- Tongue.....
- Skin for lesions,
- Chest with a stethoscope.....
- Take pulse (feel the wrists).....
- Touch the abdomen.....
- Level of consciousness (Try to arouse patient).....

8; Was the patient sent to the laboratory for investigations (Y/N) ..... [__]

What specimen (samples) were taken

a) blood
b) blood smear (on a glass slide)
c) urine
d) stool
e) CSF (water off the back)

D. Communication and counseling

Answer Yes or No for all the questions below

1. Did the HW offer you or your patient reassurances? (Y/N) [ ]
2. Did they explain the diagnosis [ ]
3. Did the explain to you the treatment given (Y/N) [ ]
4. Did the HW tell you how often to take the medicine [ ]
5. Where you told who to call when the patients condition worsened [ ]
6. Did they seek consent for the tests? (Y/N) [ ]
7. Did they interpret results to the patient and care givers? (Y/N) [ ]
8. Did the HW tell you to continue feeding or breastfeeding? (Y/N) [ ]
9. Did the health worker educate you on any health issues like Immunization [ ]
10. Did the HW talk to you about referral? (Y/N) [ ]
11. Did the HW ask you to return immediately if you or your child **becomes sicker**? [ ]
12. Were you or your patient given any injectable antimalarials?
    1. Yes
    2. No
    3. Doesn't know
13. If yes:

    a. What was the name of the injection?

    _____

    b. What was the injection for?

    ---

    c. How was the injection given? [ ]
        i. Intravenous (In a drip) (Y/N)
        ii. On the buttocks (Y/N)
        iii. On the thighs (Y/N)
        iv. Not sure (Y/N)

    d. How many injections were you given per day? [ ]
        i. One (Y/N)
        ii. Two (Y/N)
        iii. Three (Y/N)
        iv. Other, Specify_____

    e. For how many days were the injections given?
        i. One (Y/N)
        ii. Two (Y/N)
        iii. Three (Y/N)
        iv. Four (Y/N)
        v. Five (Y/N)
        vi. Other, Specify_____

Did you buy any medicines that were not available in the hospital? [ ]

What medicine did you buy _____

How much did you spend on these medicines?_____

Did you buy any medical supplies [ ]

List what you bought _____

How much did this cost you?_____

C. Satisfaction with care given

5. What do you think of the services provided at this facility? (read all options to the patient/caretaker)
    1. Good as they are
    2. Should be improved. If this option chosen, list what should be improved
        i. _____
        ii. _____-
        iii. _____
        iv. _____

    3. Doesn't know

6. What do you think about the time you had to wait to see the health worker on the day you/your patient was admitted? (read all options to the patient/caretakers)
    1. Definitely too long
    2. Long
    3. Acceptable
    4. Short
    5. Doesn't know
7. While on the ward, how often were you/your patient examined by the health workers? (read all options to the patient/caretakers)
    1. Once every day
    2. Twice daily
    3. Three times daily
    4. On alternate days
    5. Once in three days
    6. Never seen
    7. Others, Specify_____

8. What type of medication did the health worker give or prescribe for you or your patient?
    1. Injectables
    2. Oral medication
    3. Both

E. Patient triage (PT)

1. How long did the patient wait before receiving medical care?.....?

2Was your patient immediately assessed and given priority treatment over others?(triage)……(Y/N)

3. Were there health workers look out for very sick patients and getting them quick attention (Y/N)
[ ]

C. Referral History

1. Where you referred to this facility from a lower health centre? (Y/N) [2.What type of facility _____

3. Where you given any pre-referral medication [ ]

4. What medicines were you given_____

3. What were the reasons why you were referred?

i. Lack of blood for transfusion at the facility (Y / N)

ii. Poor response to treatment given (Y / N)

iii. Lack of I.V fluids (Y / N)

iv. Lack of Oxygen (Y / N)

v. No beds available to admit patient (Y / N)

vi. Others, specify_____

4. Were you given any support to get to this health facility? (Y/N)

5. What support were you given to help you get to this health facility

_____

F Record reviews for the patient: (R)

Medical record number: _____

Section 1: History

Is the clinical history documented in the medical record? *Please indicate if the symptom is commented on in the medical record, not whether the symptom was present or absent.*			
History / Symptom	**Recorded?**	**History / Symptom**	**Recorded?**
Age	Yes No	Loss of consciousness	Yes No
LC1 chairman name	Yes No	Refusal to feed / breastfeed	Yes No
Fever	Yes No	Vomiting	Yes No

Weakness	☐ Yes ☐ No	Diarrhea	☐ Yes ☐ No
Pallor	☐ Yes ☐ No	Cough	☐ Yes ☐ No
Jaundice	☐ Yes ☐ No	Fast breathing	☐ Yes ☐ No
Convulsions	☐ Yes ☐ No	Edema	☐ Yes ☐ No

Is the past history documented in the medical record?
Please indicate whether the following aspects of the history are commented on in the medical record.

Comment on past medical history: ☐ Yes ☐ No	Comment on gestational history: ☐ Yes ☐ No
Allergies to medications recorded: ☐ Yes ☐ No	Comment on feeding history: ☐ Yes ☐ No
Record of any prior treatment: ☐ Yes ☐ No	Record of immunization history: ☐ Yes ☐ No

If treatment recorded, tick all that apply:

☐ Chloroquine ☐ Coartem ☐ Amoxacillin ☐ Other:_____

☐ SP (Fansidar) ☐ AQ+AS ☐ Septrin ☐ Other:_____

☐ Homapak ☐ Quinine ☐ Chloramphenicol ☐ Other:_____

Section 2: Physical examination

Is the physical exam documented in the medical record?
Please indicate whether the physical exam is recorded, not whether the findings were normal or abnormal.

Exam finding	Recorded?	Exam finding	Recorded?
Temperature	☐ Yes ☐ No	Evidence of dehydration	☐ Yes ☐ No
If yes, record temperature	|_____|_____| •|_____| ° C	Evidence of weakness	☐ Yes ☐ No
Weight	☐ Yes ☐ No	Inability to sit or stand	☐ Yes ☐ No
If yes, record weight	|_____|_____| kg	Comment on mental status	☐ Yes ☐ No
Respiratory rate	☐ Yes ☐ No	Comment on neck stiffness	☐ Yes ☐ No
Pulse rate	☐ Yes ☐ No	Comment on Kernig's sign	☐ Yes ☐ No
Comment on nasal flaring	☐ Yes ☐ No	Chest exam	☐ Yes ☐ No

Evidence of pallor/anemia	☐ Yes ☐ No	Comment on chest indrawing	☐ Yes ☐ No
Evidence of jaundice	☐ Yes ☐ No	Abdominal exam	☐ Yes ☐ No

Section 3: Investigations

Are laboratory and radiology investigations documented in the medical record?
Please indicate whether the tests were ordered, and if results were recorded, not if the tests were abnormal.

Investigation	**Ordered?**	**Investigation**	**Recorded?**	**What was the result?**
Blood smear	☐ Yes ☐ No	If BS ordered, is result recorded?	☐ Yes ☐ No	
Hemoglobin (Hb)	☐ Yes ☐ No	If Hb ordered, is result recorded?	☐ Yes ☐ No	
Complete blood count	☐ Yes ☐ No	If CBC ordered, is result recorded?	☐ Yes ☐ No	
Glucose	☐ Yes ☐ No	If glucose ordered, is result recorded?	☐ Yes ☐ No	
Lumbar puncture	☐ Yes ☐ No	If LP ordered, is result recorded?	☐ Yes ☐ No	
Chest X-ray	☐ Yes ☐ No	If CXR ordered, is result recorded?	☐ Yes ☐ No	
Other X-ray	☐ Yes ☐ No	If Xray ordered, is result recorded?	☐ Yes ☐ No	

Section 4: Impression

Is the impression of the suspected diagnosis documented in the medical record?
What diagnoses were recorded? Tick all that apply.

Impression	**Recorded?**	**Impression**	**Recorded?**
Malaria	☐ Yes ☐ No	Pneumonia	☐ Yes ☐ No
Severe malaria	☐ Yes ☐ No	Diarrhea / Dysentery	☐ Yes ☐ No
Cerebral malaria	☐ Yes ☐ No	Malnutrition / PEM	☐ Yes ☐ No
Severe anemia	☐ Yes ☐ No	Measles	☐ Yes ☐ No
Meningitis	☐ Yes ☐ No	Other:	☐ Yes ☐ No
Final diagnosis on face sheet	☐ Yes ☐ No	If yes, please indicate Dx:	
Final diagnosis in notes	☐ Yes ☐ No	If yes, please indicate Dx:	

Outcome / disposition	☐ Yes ☐ No	If yes, please indicate:	☐ Discharged ☐ Died ☐ Ran away

Section 5: Treatment

Is the treatment plan documented in the medical record?
What medications were ordered? Tick all that apply.

Medication	**Ordered?**	**Medication**	**Ordered?**
Penicillin / PCN	☐ Yes ☐ No	Coartem	☐ Yes ☐ No
Chloramphenicol	☐ Yes ☐ No	IV fluids	☐ Yes ☐ No
Gentamicin	☐ Yes ☐ No	Dextrose	☐ Yes ☐ No
Ceftriaxone	☐ Yes ☐ No	Blood transfusion	☐ Yes ☐ No
Quinine IV or IM	☐ Yes ☐ No	Nasogastric tube	☐ Yes ☐ No
Quinine tablets	☐ Yes ☐ No	Other	☐ Yes ☐ No

If antimalarial treatment ordered, record complete dosing schedule prescribed:

Appendix S2: Severe malaria survey tool - For Outpatients' Health Centre II and III

Instructions

1. Complete the blank space with the answers given

2. Select the most appropriate option by clearly ticking the correct one/s with a pencil.

3. Do not prompt with the listed answers unless prompting is specified

4. If the Health centre has admission facilities then complete the form for Inpatients as well

A. Geographic, Historical and Demographic information (GD)

1. Name of health facility: _____

2. Cadre to be interviewed:

i. Nursing Aid / Asst

ii. Clinical Officer

iii. Nursing Officer

iv. Enrolled Nurse

v. Midwife only

vi. Comprehensive nurse

vii. MO

viii. SMO

ix. Consultant

x. Other_____

3. Duration you have been at current post:

i. < 6 mths

ii. 6 – 12 mths

iii. > 12 mths

B. Knowledge on severe malaria and its management (KW)

1. Ask to list the types of severe malaria *(tick those mentioned without prompting)*

i. Cerebral malaria

ii. Severe anaemia

iii. Renal failure

iv. Pulmonary oedema

v. Hypoglycaemia

vi. Shock

vii. Spontaneous bleeding

viii. Repeated convulsions

ix. Acidosis

x. Haemoglobinuria

2. Commonest type of severe malaria seen in the last week [ ]

3. Ask to list danger signs that indicate the need for urgent attention in a very sick patient *(tick those mentioned)*

i. Rapid breathing [ ]

ii. Deep breathing [ ]

iii. Chest indrawing [ ]

iv. Unable to localise painful stimuli [ ]

v. Extreme generalised body weakness / cannot feed [ ]

vi. Convulsions / fits [ ]

vii. Very pale mucous membranes / palms [ ]

viii. Yellowing of the white part of the eyes [ ]

ix. Body temperature above 39.5^{o}C [ ]

x. Has sunken eyes / fontanelle [ ]

xi. Has reduced skin turgor [ ]

xii. Repeated vomiting [ ]

xiii. Does not know any [ ]

4. Which of the following practices are important in saving the lives of patients with severe malaria

Rate from 1-5 as below	
Not important practice for saving life	1
Important practice for saving life	2
Very important practice for saving life	3
No idea / No response	4
Not applicable	5

i. Take a long and detailed history [ ]

ii. Carry out a short but thorough examination [ ]

iii. Measure and monitor respiratory rate in those under 5 years [ ]

iv. Measure and monitor body temperature [ ]

v. Tepid sponging if febrile [ ]

vi. Fanning if febrile [ ]

vii. Measure blood glucose if unconscious [ ]

viii. Identify patients with danger signs from other patients [ ]

ix. Start prompt treatment with IV quinine [ ]

x. Do blood slide to confirm malaria parasites in blood [ ]

xi. Nurse in the lateral positions if unconscious [ ]

xii. Blood transfusion for those with severe anaemia [ ]

xiii. Educate attendants on bednets before starting treatment [ ]

5. Are there other conditions that can present like severe malaria (Y / N)

6. If Y, which ones occur in your unit?

i. _____

ii. _____

iii. _____

iv. _____

C. Diagnosis and Treatment (DT)

1. Do you usually make a final diagnosis based on:

i. clinical features only (presumptive) [ ]

ii. clinical features and diagnostic tests (confirmatory) [ ]

iii. Both [ ]

2. What antimalarial drugs do you routinely give severe malaria cases (as treatment)i. Quinine

ii. Chloroquine

iii. Artemether

iv. Artemether-lumefantrine

v. Amodiaquine

vi. Artesunate

vii. Chloroquine+SP tablets

viii. Other _____

xi. Referred without treatment

3. What route do you routinely use to give the antimalarial?

i. IM injection

ii. IV infusion

iii. Oral tablets or syrup

iv. Rectal

v. Not applicable

4. Are children weighed before an antimalarial is prescribed? (Y / N)

5. A patient presents to you with history of fever for 4 days, associated with convulsions and now is unconscious. You think this patient has severe malaria. What antimalarial treatment will you give this patient?

i. Quinine (Y/N)

ii. Artemether (Y/N)

iii. Artesunate (Y/N)

iv. Others, specify_____

6. For how long will you administer the antimalarial for?_____

7. If this patient was a 4 year old child, write the exact prescription of the antimalarial you would prescribe

_____

_____

_____

7. If this patient was an adult, write the exact prescription of the antimalarial you would prescribe

_____

_____

_____

8. What additional supportive treatment would you give to these patients?

i._____

ii._____

iii._____

iv._____

v._____

9. What problems do you face in managing severe malaria cases in your unit:

a) _____

b) _____

c) _____

d) _____

10. Have you had the opportunity to improve your skills at severe malaria case management in the last 12 months? (Y / N)

11. If Y, how?

i. At workshops

ii. Teaching by a colleague/senior from within the health facility

iii. Teaching by someone from outside the health facility

vi. Reading printed material / self teaching

v. Other, specify _____

12. Have you ever undergone IMCI training? (Y / N)

D. Stock **(ST)**

Complete the checklist for supplies and equipment

1. How often did you get stock outs lasting more than one week of the items listed below in the previous three months in your unit?

Code	
Not available (but should be)	0
Available and never out-of-stock	1
1-2 stock-outs	2
3-4 stock-outs	3
More than 4 stock-outs	4
Not applicable	5

Items	**Frequency**	**Main reason for stock-out**

i. Quinine (parenteral)		
ii. Normal saline		
iii. 50% dextrose		
iv. Blood for transfusion		
v. IV giving sets		
vi. Blood transfusion set		
vi. Syringes		

2. Are there particular months of the year when you are more likely to get stock-outs of:

i. Quinine inj (Y / N)

ii. Blood for transfusion (Y / N)

3. If Y, when? i. Quinine inj _____

ii. Blood _____

E. Patient triage (PT)

1. Who is usually the first to meet the patient and attendants when they arrive at the health facility?

i. 9am - Midday: _____

ii. 10pm – 1am: _____

2. Is there a method of screening very sick patients from the queue?

(Y / N)

3. If Y, who identifies them? _____

_____

4. If Y, what are the most useful signs that are used to identify very sick patients in the queue?

i. _____

ii. _____

iii. _____

5. If Y, are very sick patients marked in any way? (Y / N)

6. If Y, how are they marked? _____

7. If N, how would you want them marked? _____

_____

8. What is done for those who are screened? _____

_____

F Timing (TI)

1. Complete the table below using information from the health worker

Code:	
Within 30 mins	1
>30 mins – 1 hour	2
>1 hour – 3 hours	3
>3 hours	4
Not applicable	5

Component of triage	**9am - midday**	**10pm – 1am**
i. Arrival to seeing the relevant health worker		
ii. Clinical assessment to getting results of blood smear		
iii. Clinical assessment to getting first treatment dose		
v. Clinical assessment to getting a blood transfusion		
vi. Getting referral note to departure from the health facility		

G. Referral system (R)

1. Number of patients with severe malaria that have been referred that day
[|]

2. What are the reasons why you decide to refer patients with severe malaria?

i. Lack of blood for transfusion at the facility (Y / N)

ii. Poor response to treatment given (Y / N)

iii. Lack of I.V fluids (Y / N)

iv. Lack of Oxygen (Y / N)

v. No beds available to admit patient (Y / N)

vi. Others, specify_____

3. Do you use the presence of some clinical signs to make referral decisions?

(Y / N)

4. If Y, what signs do you use?

i. Rapid breathing [ ]

ii. Deep breathing [ ]

iii. Chest indrawing [ ]

iv. Unable to localise painful stimuli [ ]

v. Extreme generalised body weakness / cannot feed [ ]

vi. Convulsions / fits [ ]

vii. Very pale mucous membranes / palms [ ]

viii. Yellowing of the white part of the eyes [ ]

ix. Body temperature above 39.5^{o}C [ ]

x. Has sunken eyes / fontanelle [ ]

xi. Repeated vomiting [ ]

xii. Others, specify_____

5. When you refer to another health facility do you give any pre-referral medications
(Y / N)

6. If Y, what do you give? _____

_____

7. If N, why not? _____

8. Do you give a referral note? (Y / N)

9. Where do you refer the patients to (name)?

i. _____ approx distance from unit _____ km

ii. _____ approx distance from unit _____ km

10. Do you give the attendants directions to get to the health facility? (Y / N)

11. Do you tell the attendants what form of transport to use? (Y / N)

12. Do you tell them where to report when they get to the health facility? (Y / N)

13. Do you give any other advice (Y / N)

11. If Y, what? _____

14. Do you have a method of finding out the outcome of the referral? (Y / N)

15. If Y, how _____

16. If N, would you like to know the outcome? (Y / N)

H. Supervision on Malaria Case Management (SU)

1. Have you undergone any form of supervision on the management of malaria in the last 6 months? (Y / N)

2. If Y, were you comfortable with the process? (Y / N)

3. Who has supervised you in the last 6 months?

Within the health facility

i. Colleague [ ]

ii. Immediate senior [ ]

iii. Head of unit [ ]

iv. Head of health facility [ ]

From outside the health facility

v. Malaria focal person [ ]

vi. Malaria zonal coordinator [ ]

vii. Staff from health subdistrict [ ]

viii. Consultant from the nearest referral hospital [ ]

ix. Ministry of Health technical staff [ ]

x. Health worker from abroad [ ]

4. How often have you been supervised in the last 6 months?

i. Once

ii. Twice

iii. Thrice

iv. Monthly

v. None

5. What methods have you been supervised with in the last 6 months?

i. Direct observation of care

ii. Interviews

iii. Inspection

iv. Feedback

v. Problem-solving

vi. Coaching

vii. Training

viii. Decision-making

ix. Clinical audit

x. Other, Specify_____

6. Do you feel support supervision for malaria is useful? (Y / N)

7. If yes, how is it useful?

i. Improved competence / skills

ii. Improved compliance with national guidelines

iii. Improved care given to patients

iv. Improved motivation

v. Other, specify _____

8. Can you list any international organisations or NGOs that are involved in malaria work where your facility is located?

i. _____
ii. _____
iii. _____

9. How have these international organisations or NGOs been useful to you or the community?

i._____

ii._____

iii._____

iv._____

I. Roles and Responsibilities (RR)

1. Were you given a job description when you started your current post?
(Y / N)

2. If Yes, was it i. written or ii. verbal

3. What would you consider as your role in the routine management of patients with severe malaria?

i. _____

ii. _____

iii. _____

J. Aides Memoir (AM)

1. Which of the following severe malaria case management aides are available at the unit?

i. Posters on the wall (Y / N)

ii. Wall charts (Y / N)

ii. Leaflets / Pamphlets (Y / N)

iii. Reference textbooks (Y / N)

iv. Desk aids (Y / N)

Others, specify _____

2. Which do you prefer as a reminder?

Rate from 1 to 5 as below	
Not useful	1
A good reminder	2
A very good reminder	3
No idea / No response	4
Not applicable	5

i. Posters on the wall [ ]

ii. Wall charts [ ]

ii. Leaflets / Pamphlets [ ]

iii. Reference textbooks [ ]

iv. Desk aids [ ]

Others, specify _____

K. Adverse reactions (AR)

1. Do you inform attendants of the adverse reactions of the antimalarial which the patient is getting?
(Y / N)

2. If Y, which ones do you mention for quinine

Drug	Adverse Reaction
Quinine	i. _____
	ii. _____
	iii._____

3. If N, why not_____

4. Do you record and report suspected adverse reactions of any of the drugs that you use in your facility?
(Y / N)

5. If Y, were do you record _____

6. Who do you report to _____

7. If N, why not? _____

L. Death due to severe Malaria

3. On what days of the week do most of these deaths occur?

i. Monday to Wednesday (Y / N)

ii. Thursday to Friday (Y / N)

iii. Weekends (Y / N)

4. At what times do these deaths commonly occur?

i. Mornings (Y/N)

ii. Afternoons (Y/N)

iii. Evenings (Y/N)

iv. Nights (Y/N)

M. Quality of care (QC)

1. How do you rate the quality of care that your unit gives to patients with severe malaria?
[ ]

Rate from 1 to 5 as below	
Poor quality	1
Good quality	2
Very good quality	3
No idea / No response	4
Not applicable	5

i. Quality of diagnosis [ ]

ii. Quality of treatment [ ]

iii. Quality of nursing care [ ]

iv. Quality of supportive care [ ]

v. Quality of follow-up [ ]

vi. Quality of management of the health facility [ ]

2. What specific aspects of care are weak in your health facility?

i. _____

ii. _____

iii. _____

3. What specific aspects of care are done very well in your health facility?

i. _____

ii. _____

iii. _____

4. What suggestions do you have to improve the quality of care given to patients with severe malaria in your health facility?

i. _____

ii. _____

iii. _____

iv. _____

Date: ___ / ____ / 2009 Time _____ am /pm

Completed by: _____ (name)

Appendix S3: Severe malaria survey tool For Inpatient unit/ward

Instructions

1. Complete the blank space with the answers given

2. Select the most appropriate option by clearly ticking the correct one/s with a pencil.

3. Do not prompt with the listed answers unless prompting is specified

4. If the Health centre III does not have admission facilities then use the form for outpatients

A. Geographic, Historical and Demographic information (GHD)

1. Name of health facility: _____

2. Cadre to be interviewed:

i. Nursing Aid / Asst
ii. Clinical Officer
iii. Nursing Officer
iv. Enrolled Nurse
v. Midwife only
vi. Comprehensive nurse
vii. MO
viii. SMO
ix. Consultant
x. Other _____

3. Duration you have been at current post:

i. < 6 mths ii. 6 – 12 mths iii. > 12 mths

B. Knowledge on severe malaria and its management (KW)

1. Ask to list the **types** of severe malaria *(tick those mentioned without prompting)*

i. Cerebral malaria
ii. Severe anaemia
iii. Renal failure
iv. Pulmonary oedema
v. Hypoglycaemia
vi. Shock

vii. Spontaneous bleeding

viii. Repeated convulsions

ix. Acidosis

x. Haemoglobinuria

2. Commonest manifestation admitted in the last week [ ]

3. Ask to list **danger signs** that indicate the need for urgent attention in a very sick patient *(tick those mentioned without prompting)*

i. Rapid breathing [ ]

ii. Deep breathing [ ]

iii. Chest indrawing [ ]

iv. Unable to localise painful stimuli [ ]

v. Extreme generalised body weakness / cannot feed [ ]

vi. Convulsions / fits [ ]

vii. Very pale mucous membranes / palms [ ]

viii. Yellowing of the white part of the eyes [ ]

ix. Body temperature above 39.5^{o}C [ ]

x. Has sunken eyes / fontanelle [ ]

xi. Has reduced skin turgor [ ]

xii. Repeated vomiting [ ]

xiii. Does not know any [ ]

4. Which of the following practices are important in **saving the lives** of patients with severe malaria

Rate from 1-5 as below	
Not important practice for saving life	1
Important practice for saving life	2
Very important practice for saving life	3
No idea / No response	4
Not applicable	5

i. Take a long and detailed history [ ]

ii. Carry out a short but thorough examination [ ]

iii. Measure and monitor respiratory rate in those under 5 years [ ]

iv. Measure and monitor body temperature [ ]

v. Tepid sponging if febrile [ ]

vi. Fanning if febrile [ ]

vii. Measure blood glucose if unconscious [ ]

viii. Identify patients with danger signs from other patients [ ]

ix. Start prompt treatment with IV quinine [ ]

x. Do blood slide to confirm malaria parasites in blood [ ]

xi. Nurse in the lateral positions if unconscious [ ]

xii. Blood transfusion for those with severe anaemia [ ]

xiii. Educate attendants on bednets before starting treatment [ ]

5. Are there other conditions that can present like severe malaria (Y / N)

6. If Y, which ones occur in your unit?

i. _____ iii. _____

ii. _____ iv. _____

C. Diagnosis and Treatment (DT)

1. Do you usual make a **<u>final</u>** diagnosis based on:

i. clinical features only (presumptive) [ ]

ii. clinical features and diagnostic tests (confirmatory) [ ]

2. What antimalarial drugs do you **<u>routinely</u>** give severe malaria cases (as treatment)

i. Quinine

ii. Chloroquine

iii. Artemether

iv. Artemether-lumefantrine

v. Amodiaquine

vi. Artesunate

vii. Chloroquine / SP tablets

viii. Other _____

xi. Referred without treatment

3. What route do you routinely use to give the antimalarial

i. IM injection

ii. IV infusion

iii. Oral tablets or syrup

iv. Rectal

v. Not applicable

4. Are children weighed before an antimalarial is prescribed? (Y / N)

5. A patient presents to you with history of fever for 4 days, associated with convulsions and now is unconscious. You think this patient has severe malaria. What antimalarial treatment will you give this patient?

i. Quinine (Y/N)

ii. Artemether (Y/N)

iii. Artesunate (Y/N)

iv. Others, specify_____

6. For how long will you administer the antimalarial for?_____

7. If this patient was a 4 year old child, write the exact prescription of the antimalarial you would prescribe

_____

_____

_____

7. If this patient was an adult, write the exact prescription of the antimalarial you would prescribe

_____

_____

_____

8. What additional supportive treatment would you give to these patients?

i._____

ii._____

iii._____

iv._____

v._____

9. What problems do you face in managing severe malaria cases?

a) _____

b) _____

c) _____

d) _____

10. Have you had the opportunity to improve your skills at malaria case management in the last 12 months? (Y / N)

11. If Y, how?

i. At workshops

ii. Teaching by a colleague/senior from within the health facility

iii. Teaching by someone from outside the health facility

iv. Reading printed material / self teaching

v. Other, specify _____

12. Have you ever undergone IMCI training? (Y / N)

D. Stock **(ST)**

Complete the checklist for supplies and equipment

1. How often did you get stock outs lasting for more than one week of the items listed below in the previous three months in your unit?

Code	
Not available (but should be)	0
Available and never out-of-stock	1
1-2 stock-outs	2
3-4 stock-outs	3
More than 4 stock-outs	4
Not applicable	5

Items	**Code**	**Main reason for stock-out**
i. Quinine (parenteral)		
ii. Normal saline		
iii. 50% dextrose		
iv. Blood for transfusion		
v. IV giving sets		
vi. Blood transfusion sets		
vii. Syringes		

2. Are there particular months of the year when you are more likely to get stock-outs of:

i. Quinine inj (Y / N)

ii. Blood for transfusion (Y / N)

3. If Y, when? i. Quinine inj _____

ii. Blood _____

E. Patient triage (PT)

1. Who is usually the first to meet the patient and attendants when they arrive at the health facility?

i. 9am - Midday: _____

ii. 10pm – 1am: _____

2. Is there a method of screening very sick patients from the queue?

(Y / N)

3. If Y, who identifies them? _____

_____

4. If Y, how are they identified? _____

_____

5. What is done for those who are screened? _____

_____

6. If N, how would you want them identified? _____

_____

7. If no one has the responsibility to screen very sick patients in the OPD, who do you think is the **best person** to do the screening?

_____

F Timing (TI)

1. Complete the table below using information from the health worker

Code:	
Within 30 mins	1
>30 mins – 1 hour	2
>1 hour – 3 hours	3
>3 hours	4
Not applicable	5

Component of triage	**9am - midday**	**10pm – 1am**
i. Arrival to seeing the relevant health worker		
ii. Clinical assessment to getting results of blood smear		
iii. Clinical assessment to getting first treatment dose		
v. Clinical assessment to getting a blood transfusion		
vi. Getting referral note to departure from the health facility		

G. Referral system (R)

(Do not complete this section if the health facility does not refer patients or if the interviewee is not involved in referring patients)

1. Number of patients with severe malaria that have been referred that day

[ ]

2. What are the reasons why you decide to refer patients with severe malaria?

i. Lack of blood for transfusion at the facility (Y / N)

ii. Poor response to treatment given (Y / N)

iii. Lack of I.V fluids (Y / N)

iv. Lack of Oxygen (Y / N)

v. No beds available to admit patient (Y / N)

vi. Others, specify_____

_____

3. Do you use the presence of some clinical signs to make referral decisions?

(Y / N)

4. If Y, what signs do you use?

i. Rapid breathing [ ]

ii. Deep breathing [ ]

iii. Chest indrawing [ ]

iv. Unable to localise painful stimuli [ ]

v. Extreme generalised body weakness / cannot feed [ ]

vi. Convulsions / fits [ ]

vii. Very pale mucous membranes / palms [ ]

viii. Yellowing of the white part of the eyes [ ]

ix. Body temperature above 39.5^{o}C [ ]

x. Has sunken eyes / fontanelle [ ]

xi. Repeated vomiting [ ]

xii. Others, specify_____

5. When you refer to another health facility do you give any pre-referral medications

(Y / N)

6. If Y, what do you give?

Generic name	Route of administration
i. _____	_____
ii._____	_____
iii. _____	_____

7. If N, why not? _____

8. Do you give a referral note? (Y / N)

9. Where do you refer the patients to *(name)*?

i. _____ approx distance from unit _____ km

ii. _____ approx distance from unit _____ km

10. Do you give the attendants directions to get to the health facility?
(Y / N)

11. Do you tell the attendants what form of transport to use? (Y / N)

12. Do you tell them where to report when they get to the health facility?
(Y / N)

13. Do you give any other advice (Y / N)

11. If Y, what? _____

14. Do you have a method of finding out the outcome of the referral?
(Y / N)

15. If Y, how _____

16. If N, would you like to know the outcome? (Y / N)

H. Supervision on Malaria Case Management (SU)

1. Have you undergone any form of supervision on the management of malaria in the last six months? (Y / N)

2. If Y, were you comfortable with the process? (Y / N)

3. Who has supervised you in the last six months?

Within the health facility

i. Colleague [ ]

ii. Immediate senior [ ]

iii. Head of unit [ ]

iv. Head of health facility [ ]

From outside the health facility

v. Malaria focal person [ ]

vi. Malaria zonal coordinator [ ]

vii. Staff from health subdistrict [ ]

viii. Consultant from the nearest referral hospital [ ]

ix. Ministry of Health technical staff [ ]

x. Health worker from abroad [ ]

4. How often have you been supervised in the last 6 months? i. Once

ii. Twice

iii. Thrice

iv. Monthly

v. None

5. What methods have you been supervised with in the last 6 months?

i. Direct observation of care

ii. Interviews

iii. Inspection

iv. Feedback

v. Problem-solving

vi. Coaching

vii. Training

viii. Decision-making

ix. Clinical audit

x. Other, specify _____

6. Do you feel support supervision for malaria is useful? (Y / N)

7. If yes, how is it useful?

i. Improved competence / skills

ii. Improved compliance with national guidelines

iii. Improved care given to patients

iv. Improved motivation

v. Other, specify _____

8. Can you list any international organisations or NGOs that are involved in malaria work where your facility is located

i. _____
ii. _____
iii. _____

9. How have these international organisations or NGOs been useful to you or the community?

i._____

ii._____

iii._____

iv._____

I. Roles and Responsibilities (RR)

1. Were you given a job description when you started your current post?
(Y / N)

2. If Yes, was it i. written or ii. verbal

3. What would you consider as your role in the routine management of patients with severe malaria?

i. _____

ii. _____

iii. _____

J. Aides Memoir (AM)

1. Which of the following severe malaria case management aides are available at the unit?

i. Posters on the wall (Y / N)

ii. Wall charts (Y / N)

ii. Leaflets / Pamphlets (Y / N)

iii. Reference textbooks (Y / N)

iv. Desk aids (Y / N)

Others, specify _____

2. Which do you prefer as a reminder?

Rate from 1 to 5 as below	
Not useful	1
A good reminder	2
A very good reminder	3
No idea / No response	4
Not applicable	5

i. Posters on the wall [ ]

ii. Wall charts [ ]

ii. Leaflets / Pamphlets [ ]

iii. Reference textbooks [ ]

iv. Desk aids [ ]

Others, specify _____

K. Adverse reactions (AR)

1. Do you inform attendants of the adverse reactions of the antimalarial which the patient is getting? (Y / N)

2. If Y, which ones do you mention for quinine

Drug	Adverse Reaction
Quinine	i. _____
	ii. _____
	iii._____

3. If N, why not_____

4. Do you record and report suspected adverse reactions of any of the drugs that you use in your facility? (Y / N)

5. If Y, were do you record _____

6. Who do you report to _____

7. If N, why not? _____

L. Care and monitoring **(CM)**

1. What cadre of staff are always available on the ward *(fill table below with the options from the key)*

Cadre	Code
Nursing Aid / Asst	1
Registered Nurse	2
Enrolled Nurse	3
Clinical officer	4
Doctor	5

Period	Always available
i. 8am – midday	
ii. 1pm – 5pm	
iii. 6pm – 10pm	
iv. 11pm – 3am	
v. 4am – 8am	

2. Do you think that qualified nurses are competent enough to start patients with severe malaria on treatment with IV quinine without waiting for the clinical officer or doctor to prescribe?
(Y / N)

3. Are there occasions when you give a loading dose of quinine? (Y / N)

4. If Y, when do you do so _____

5. If N, why not _____

6. Do you routinely monitor unconscious patients with

i. Glasgow coma scale (Y / N)

ii. Blantyre coma scale (Y / N)

7. How often do you measure the following parameters in patients with severe malaria on the first day of admission?

Parameter	Frequency (hourly)
i. Temperature	
ii. Pulse	
iii. Blood pressure	
iv. Respiratory rate	
v. Unconsciousness	
vi. Blood glucose	
vii. Convulsions	
viii. Haemoglobin concentration	
ix. Parasitaemia	

M. Investigations **(IG)**

1. List the investigations that can routinely be carried out on patients with severe malaria in your health facility?

i. _____

ii. _____

iii. _____

iv. _____

v. _____

vi. _____

vii. _____

viii. _____

2. In severe malaria patients, do you routinely repeat the blood smear for malaria parasites to monitor parasite clearance?

3. If Y, how often? _____

4. If N, why not? _____

5. Who routinely takes the blood specimens from the patients? _____

6. Who routinely takes the specimens to the laboratory? _____

7. Are urgent laboratory requests marked in any special way? (Y / N)

8. If Y, in what way? _____

9. If Y, does the laboratory process them urgently? (Y / N)

10. If Y, what is the average time to get them back? ____ (hours)

11. If N, why not _____

13. Who routinely collects the lab results from the laboratory? _____

N. Follow-up **(FU)**

1. Are survivors of severe malaria followed-up? (Y / N)

2. If Y, what assessments are carried out during follow-up?

i. Hb measurement (Y / N)

ii. Blood smear for malaria parasites (Y / N)

iii. Growth monitoring (Y / N)

iv. Assessments for neurological sequelae (Y / N)

v. Other _____

2. How often are these patients followed-up?_____

O. Death due to severe Malaria

1. On what days of the week do most of these deaths occur?

i. Monday to Wednesday (Y / N)

ii. Thursday to Friday (Y / N)

iii. Weekends (Y / N)

2. At what times do these deaths commonly occur?

---

i. Mornings (Y / N)

ii. Afternoons (Y / N)

iii. Evenings (Y / N)

iii. Nights

P. Quality of care (QC)

1. How do you rate the quality of care that your unit gives to patients with severe malaria?
[ ]

Rate from 1 to 5 as below	
Poor quality	1
Good quality	2
Very good quality	3
No idea / No response	4
Not applicable	5

i. Quality of diagnosis [ ]

ii. Quality of treatment [ ]

iii. Quality of nursing care [ ]

iv. Quality of supportive care [ ]

v. Quality of follow-up [ ]

vi. Quality of management of the health facility [ ]

2. What specific aspects of care are weak in your health facility?

i. _____

ii. _____

iii. _____

3. What specific aspects of care are done very well in your health facility?

i. _____

ii. _____

iii. _____

4. What suggestions do you have to improve the quality of care given to patients with severe malaria in your health facility?

i. _____

ii. _____

iii. _____

iv. _____

Date: ___ / ____ / 2005 Time _____ am /pm

Completed by: _____ (name)

Appendix S4: Severe malaria survey tool - Checklist for each Health Facility

Instructions

1. Complete the blank spaces

2. Select the most appropriate option by clearly ticking the correct one/s with a pencil.

3. Complete this checklist by observing what goes on in the units of the health facility

A. Geographic, Historical and Demographic information (GDC)

1. Name of health facility: _____

2. Grade of health facility

i. HC II

ii. HC III

iii. HC IV

iv. District Hospital

v. Other _____

3. Type of health facility

i. Government facility

ii. Faith-based facility

iii. Private-for-profit based facility

iv. Other _____

4. Name of village: _____ 5. Parish: _____

6. Subcounty: _____ 7. District: _____

8. Approximate size of population in catchment area _____

B. HUMAN RESOURCES (HRS)

Record the number of personnel by cadre, carefully recording the following information:

- Number of staff employed in the facility
- Number of staff scheduled to be on duty on the day of survey
- Number of staff present during the survey

Cadre	**Number required according to MOH staffing norms**	**Number of staff employed in facility**	**Number of staff scheduled for duty today**	**Number of staff present on duty today**
Medical Doctor				
Health officer				
Clinical Nurse				
Public Health Nurse				
Midwife				
Comprehensive Nurse				
Community health worker				
Nursing Aids				
Laboratory technicians				
Nursing Aids				
Other (Specify)				

C. Records **(RCC)**

1. Is there a register for keeping record of patients seen in OPD (Y / N)

2. If Y, is it uptodate (by yesterday) (Y / N)

3. Is there a register for keeping record of patients admitted (Y / N / NA)

4. If Y, is it uptodate (by yesterday) (Y / N)

5. Do the records note:

i. Age of patient (Y / N)

ii. Type of severe malaria manifestation (Y / N)

iii. If microscopy was performed (Y / N)

iii. Records are not clear

6. In April 2009, what was the number of severe malaria cases?

i. Referred _____ or Not applicable

ii. Admitted _____ or Not applicable

iii. Died in health facility _____ or Not applicable

8. Of the patients admitted with severe malaria last year, how many died? (Also mention the total number admitted with severe malaria during this period)

i. Number of adults_____

ii. Number of children_____

9. Of the patients admitted with severe malaria last month, how many died? (Also mention the total number admitted with severe malaria during this period)

i. Number of adults_____

ii. Number of children_____

10. What are the common causes of death in patients presenting with severe malaria at this health facility

1. Cerebral malaria (Y/N)

2. Severe anaemia (Y/N)

3. Hypoglycaemia (Y/N)

4. Severe dehydration (Y/N)

5. Respiratory distress (Y/N)

6. Others, specify_____

11. How are records of death kept in this facility?

1. Inpatient register

2. Death register

3. Other, specify_____

12. Were source documents of death records verified by the interviewer? (Y/N)

13. If yes, comment on the quality of records

1. Good quality

2. Poor quality

3. Accurate

4. Inaccurate/incomplete

5. Other, specify_____

D. Supplies and Equipment (SAEC)

1. Which of the following diagnostic facilities are available and functional **within** the unit specified *(A=available, F=functional, AF=available and functional, N=None, use these letters to indicate the pertaining situation)*

Test	OPD	Children ward
i. No diagnostic facilities		
ii. Malaria Rapid test kit		
iii. Parasight F		
iv. ParaCheck		
v. Optimal		
vi. Hand-held Glucometer		
vii. Glucose dipstick		
viii. Urine dipstick		
ix. Hb colou r scale		
x. HemoCue™ haemoglobinometer		
xi. Microscopy		

2. Complete this table for the OPD noting the supplies that the staff in the OPD currently have **access to**.

	Item	Specification	√/×	NA
	Drugs			
1	Quinine	Injectable		
2		Oral		
3	Chloroquine	Injectable		
4	Sulphadoxine-pyrimethamine	Oral		
5	Artemether-lumefantrine	Oral		
6	Artemether	Injectable		
7	Artemisinin	Rectal		
8	Artesunate	Iv		
9		Rectal		
10	Arteether	Injectable		

11	Diazepam	Injectable		
12		Rectal		
13	Dextrose	50%		
14		30%		
15		25%		
16	Paracetamol	Oral		
17		suppositories		
18	Phenobarbitone	Injection		
19	Furosemide	Injection		
	Item	**Specification**	**√/×**	**NA**
	Fluids			
20	Dextrose	5%		
21		10%		
22		50%		
23	Saline	0.9%		
24	Darrow's solution	Half strength		
25		Full strength		
26	Ringer lactate	500ml		
27	Fluid bottles	100ml		
28		200ml		
29		500ml		
30	Water for injection			
	Medical			
31	NG tube	Paediatric sizes		
32		Adult sizes		
33	IV giving sets			
34	Blood transfusion sets			
35	IV cannulae	Paediatric sizes		
36		Adult sizes		

37	Scalp vein butterfly needles			
38	Needles disposable			
39	Syringes	2ml		
40		5ml		
41		10ml		
42		20ml		
43	Syringe feeding	50/60ml		
44	Gloves	Sterile		
45		Disposable		
46	Cotton wool			
47	Adhesive tape			
48	Lancets			
49	Oxygen in cylinders			
	Equipment			
50	Thermometer			
51	Weighing scale	Hanging/Salter		
52		Electronic		
53		Bathroom		
54		Other		
55	Examination table			
56	Stethoscope			
57	Clock/Watch			
58	BP machine			
59	Ophthalmoscope			
60	Otoscope			
61	Oral airways			
62	Ambubag			
	Item	**Specification**	**√/×**	**NA**
63	Torch			

64	Glucometer			
65	Glucose dipsticks			
66	Urine dipsticks			

3. Complete this Table for the children's ward noting the supplies that the staffs there currently have **access to**.

	Item	**Specification**	**√/×**	**NA**
	Drugs			
1	Quinine	Injectable		
2		Oral		
3	Chloroquine	Injectable		
4	Sulphadoxine-pyrimethamine	Oral		
5	Artemether-lumefantrine	Oral		
6	Artemether	Injectable		
7	Artemisinin	Rectal		
8	Artesunate	Iv		
9		Rectal		
10	Arteether	Injectable		
11	Diazepam	Injectable		
12		Rectal		
13	Dextrose	50%		
14		30%		
15		25%		
16	Paracetamol	Oral		
17		suppositories		
18	Phenobarbitone	Injection		
19	Furosemide	Injection		
	Fluids			

20	Dextrose	5%		
21		10%		
22	Saline	0.9%		
23	Fluid bottles	100ml		
24		200ml		
25		500ml		
26	Darrow's solution	Half strength		
27		Full strength		
28	Ringer lactate			
29	Water for injection			
30	Blood for transfusion	Packed cells		
31		Whole blood		
	Item	**Specification**	**√/×**	**NA**
	Medical			
32	NG tube	Paediatric sizes		
33		Adult sizes		
34	IV giving sets			
35	Blood transfusion sets			
36	IV cannulae	Paediatric sizes		
37		Adult sizes		
38	Scalp vein butterfly needles			
39	Needles disposable			
40	Syringes	2ml		
41		5ml		
42		10ml		
43		20ml		
44	Syringe feeding	50/60ml		
45	Lumbar puncture needles	Paediatric sizes		
46		Adult sizes		

47	Intraosseous needles	Paediatric sizes		
48		Adult sizes		
49	Gloves	Sterile		
50		Disposable		
51	Urinary catheters Foley	Paediatric sizes		
52		Adult sizes		
53	Urinary catheters condom	Small size		
54		Medium size		
54		Large size		
56	Suction catheters	Paediatric		
57		Adult		
58	Cotton wool			
59	Adhesive tape			
60	Lancets			
	Equipment			
61	Thermometer			
62	Weighing scale	Hanging/Salter		
63		Electronic		
64		Bathroom		
65	Examination table			
66	Stethoscope			
67	Clock/Watch			
68	BP machine			
69	Ophthalmoscope			
70	Otoscope			
71	Oral airways			
72	Ambubag			
73	Oxygen	Cylinders		

74		Dispenser		
	Item	**Specification**	**√/×**	**NA**
75	Suction machine			
76	Torch			
77	Glucometer			
78	Glucose dipsticks			
79	Urine dipsticks			
80	HB colour scale			
81	HemoCue/Haemoglobinometer			
82	Specimen bottles/vacutaniers	EDTA		
83		Plain		
84		Sodium citrate		
85		Clot activator		
86	Microscope slides			

E. Patient triage **(PTC)**

1. Is there a defined triage system in place *(observation)*? (Y / N)

2. If N, why not? _____

_____

3. What is the entry point to the facility _____

4. Visible directions tell people where to go (Y / N)

5. Screening of sick patients at OPD queue (Y / N / NA)

6. Separate lines for children and adults at OPD queue (Y / N / NA)

7. Screening of sick patients for urgent treatment on admission queue (Y / N / NA)

8. Urgent attention given to sick patients at OPD queue (Y / N / NA)

9. Urgent attention given to sick patients on admission queue (Y / N / NA)

10. Lab requests marked for urgent response (Y / N / NA)

11. Lab results that are urgent are given priority (Y / N / NA)

12. Lab results that are urgent are returned to requester as priority (Y / N / NA)

13. Describe any other features not captured above _____

_____

F. Aides Memoir (AMC)

1. Which of the following severe malaria case management aides are located in **visible** areas for the staff in the **OPD**?

i. Posters on the wall (Y / N)

ii. Wall charts (Y / N)

ii. Leaflets / Pamphlets (Y / N)

iii. Reference textbooks (Y / N)

iv. Desk aids (Y / N)

Others, specify _____

2. Which of the following severe malaria case management aides are located in **visible** areas for the staff at the **children's ward**?

i. Posters on the wall (Y / N)

ii. Wall charts (Y / N)

ii. Leaflets / Pamphlets (Y / N)

iii. Reference textbooks (Y / N)

iv. Desk aids (Y / N)

Others, specify _____

G. Communication (COC)

1. Is there an easy and quick means of communicating with the other departments within the health facility (Y / N)

2. If Y, which forms of communication exist

i. Direct communication in a small unit [ ]

ii. Telephone [ ]

iii. Other_____

3. Is there a means of communicating with the other health facilities in the district?

(Y / N)

4. If Y, which forms of communication exist

i. Radio [ ]

ii. Telephone [ ]

iii. Other_____

5. Are there regular meetings with other staff in the facility? (Y / N)

6. If Y, how often? _____

H. Quality of care (QCC)

1. How do you rate the quality of care that the unit gives to patients with severe malaria?
[ ]

Rate from 1 to 5 as below	
Poor quality	1
Good quality	2
Very good quality	3
No idea / No response	4
Not applicable	5

i. Quality of diagnosis [ ]

ii. Quality of treatment [ ]

iii. Quality of nursing care [ ]

iv. Quality of supportive care [ ]

v. Quality of follow-up [ ]

vi. Quality of management of the health facility [ ]

2. What specific aspects of care are weak in the health facility?

i. _____

ii. _____

iii. _____

3. What specific aspects of care are done very well in the health facility?

i. _____

ii. _____

iii. _____

Date: ___ / ____ / 2009 Time _____ am /pm

Completed by: _____ (name)

Appendix S5: Severe malaria survey tool for pharmacy

Instructions

1. Complete the blank space with the answers given

2. Select the most appropriate option by clearly ticking the correct one/s with a pencil.

3. Do not prompt with the listed answers unless prompting is specified

A. Geographic, Historical and Demographic information (GHD)

1. Name of health facility: _____

2. Cadre to be interviewed: _____

3. Duration you have been at current post:

i. < 6 mths ii. 6 – 12 mths iii. > 12 mths

4. Any previous history of training on supply chain management of drugs (Y / N)

B. Records (RC)

1. Register for keeping record of antimalarial usage (Y / N)

2. If Y, are they uptodate (yesterday) (Y / N)

C. Stock (SK)

Complete the checklist for supplies and equipment

1. How often did you get stock outs lasting more than one week of the items listed below in the previous three months in your unit?

Code	
Not available (but should be)	0
Available and never out-of-stock	1
1-2 stock-outs	2
3-4 stock-outs	3
More than 4 stock-outs	4
Not applicable	5

Items	**Frequency**	**Main reason for stock-out**
i. Quinine (parenteral)		
ii. Normal saline		
iii. 50% dextrose		
iv. 5% dextrose		
v. Blood for transfusion		
vi. IV giving sets		
vii. Blood transfusion set		
viii. Syringes		
ix. Quinine tablets		

2. Are there particular months of the year when you are more likely to get stock-outs of:

i. Quinine inj (Y / N)

ii. Blood for transfusion (Y / N)

iii. Quinine tablets (Y / N)

3. If Y, when? i. Quinine inj _____

ii. Blood _____

iii. Quinine tablets _____

D. Supplies and Supply management (SSM)

1. Which of the following are available in the pharmacy unit?

Item	Specification	Yes	No
Drugs			
Quinine	Injectable		
	Oral		
Chloroquine	Injectable		
Sulphadoxine-pyrimethamine	Oral		
Artemether-lumefantrine	Oral		
Artemether	Injectable		
Artemisinin	Rectal		
Artesunate	IV		
	Rectal		
Arteether	Injectable		
Diazepam	Injectable		
	Rectal		
Paracetamol	Oral		
	Suppositories		
Phenobarbitone	Injection		
Furosemide	Injection		
Fluids			
Dextrose	50%		
	30%		

	25%		
	10%		
	5%		
Saline	0.9%		
Fluid bottles	100ml		
	200ml		
	500ml		
Darrow's solution	Half strength		
Item	**Specification**	**Yes**	**No**
	Full strength		
Ringer lactate			
Water for injection			

2. Is there a method in place for preventing stocks-outs? (Y / N)

3. If Y, what is done? _____

_____

4. If N, why not? _____

_____

5. In the last year has quinine expired in the pharmacy/store? (Y / N)

6. If Y, why? _____

7. Is there a method in place for quantifying the antimalarial needs? (Y / N)

8. If Y, what is it? _____

_____

9. If N, why not? _____

10. Is your supply of antimalarials sufficient for the patients that are admitted?
(Y / N)

11. If N, which ones are not? _____

12. If Y, how do you ensure adequate supply? _____

_____

13. Do you supply IV fluids in smaller bottles (100ml/200ml) for children (Y / N)

14. If Y, what have been the benefits _____

_____

15. If N, why not? _____

_____

16. Do you keep oxygen for use on the inpatient medical and paediatric wards
(Y / N)

17. If N, why not? _____

17. What specific aspects of drug management and supply are weak in your health facility?

i. _____

ii. _____

iii. _____

18. What specific aspects of drug management and supply do you think are performed very well in your health facility?

i. _____

ii. _____

iii. _____

19. What suggestions do you have to improve the quality of treatment given to patients with severe malaria in your health facility?

i. _____

ii. _____

iii. _____

iv. _____

E. Supervision (SUp)

1. Have you undergone any form of supervision in the last six months?
(Y / N)

2. If Y, were you comfortable with the process? (Y / N)

3. Who has supervised you in the last six months?

Within the health facility

i. Colleague

ii. Immediate senior

iii. Head of unit

iv. Head of health facility

From outside the health facility

Specify _____

4. How often have you been supervised in the last six months?

i. Once

ii. Twice

iii. Thrice

iv. Monthly

v. None

F. Pharmacovigilance (PV)

1. Are you informed of any adverse reactions of the drugs that are used in your facility?
(Y / N)

2. If Y, which drugs do you routinely keep records for their reactions? _____

_____

3. If Y, who informs you and how? _____

7. If Y, who do you report them to? _____

_____

8. If N, why not? _____

Date: ___ / ____ / 2009 Time _____ am /pm

Completed by: _____ (name)

Appendix 6: Severe malaria survey tool for laboratory

Instructions

1. Complete the blank space with the answers given

2. Select the most appropriate option by clearly ticking the correct one/s with a pencil.

3. Do not prompt with the listed answers unless prompting is specified

A. Geographic, Historical and Demographic information (GHD)

1. Name of health facility: _____

2. Cadre to be interviewed:

i. Laboratory assistant
ii. Lab technician
iii. Lab technologist
iv. Senior lab technologist
v. Microscopist
vi. Other _____

3. Duration you have been at current post:

i. < 6 mths ii. 6 – 12 mths iii. > 12 mths

4. Any training on malaria laboratory diagnosis in the last 12 months (Y / N)

B. Records (RC)

1. Register for keeping record of patients investigated (Y / N)

2. If Y, are they uptodate (yesterday) (Y / N)

4. Do the records note the:

i. Age of patient (Y / N)

ii. Type of severe malaria manifestation (Y / N)

iii. Records are not clear

C. Laboratory diagnosis (LD)

1. Which of the following diagnostic investigations are done in your laboratory?

Investigation	**(√ ×)**
Blood smear thick film	
Blood smear thin film	
Blood smear parasite count	
Malaria Rapid test kit	
Hb estimation – Sahl's method	
Hb estimation – Hb colour scale	
HB estimation - HemoCue™ haemoglobinometer	
Glucose blood concentration – Portable glucometer	
Glucose blood concentration – Glucose-oxidase method	
Glucose blood concentration – Glucostick method	
White blood cell count – total	
White blood cell count – differential	
Red blood cell count	
Platelet count	
Haematocrit	
Blood film	
ESR	
CSF analysis – glucose concentration	
CSF analysis – protein concentration	
CSF analysis – white cell count	

CSF analysis – Gram stain	
CSF analysis – India ink	
CSF analysis – ZN stain	
CSF analysis – culture and sensitivity	
Urinalysis – dipstick	
Serum electrolytes	
Sickling test	
HIV serology	

2. Are there any other diagnostic investigations not listed above? (Y / N)

3. If Y, which ones? _____

4. In patients with malaria admitted to the wards, are you **routinely** asked to repeat the blood smear for malaria parasites to monitor parasite clearance?

(Y / N)

5. If Y, how often _____

6. If N, why not? _____

7. Who **routinely** brings the blood specimens to the laboratory *(how are the blood specimens collected)*?
_____

8. Are urgent laboratory requests marked in any **special** way? (Y / N)

9. If Y, in what way? _____

10. If Y, does the laboratory process them **urgently**? (Y / N)

11. If Y, what is the average time to get urgent results back to the ward? _____ hrs _____ mins

12. If N, why not _____

13. Do you have a designated area in the lab where urgent specimens are put? (Y / N)

14. Where are the results put once investigations have been done?

i. Non-urgent results _____

ii. Urgent results _____

15. How do the results get back to the wards? _____

16. Do you have a **working** microscopy? (Y / N)

17. If Y, is it i. mono-ocular or ii. bi-ocular

18. If Y, do you use i. electricity ii. sunlight or iii. both

19. If Y, what quality control measures do you have to make sure that the results of microscopy are accurate and up to standard on a **regular** basis? i. _____

ii. _____

iii. _____

20. Do you have the opportunity to discuss your laboratory results with the clinicians on the wards? (Y / N / NA)

D. Supervision on Malaria laboratory diagnosis (SUL)

1. Have you undergone any form of supervision on malaria laboratory diagnosis in the last six months? (Y / N)

2. If Y, were you comfortable with the process? (Y / N)

3. Who has supervised you in the last six months?

Within the health facility

i. Colleague

ii. Immediate senior

iii. Head of unit

iv. Head of health facility

From outside the health facility

v. Malaria focal person

vi. Laboratory focal person

vii. Staff from health sub-district

vii. Consultant from the nearest referral hospital

viii. Ministry of Health technical staff

4. How often have you been supervised in the last six months?

i. Once

ii. Twice

iii. Thrice

iv. Monthly

v. None

5. What methods have you been supervised with in the last six months?

i. Direct observation of slides (practical)

ii. Interviews

iii. Inspection

iv. Feedback

v. Problem-solving

vi. Coaching

vii. Training

viii. Decision-making

ix. Clinical audit

x. Other, specify _____

6. Do you feel support supervision for malaria is useful? (Y / N)

7. If yes, how is it useful?

i. Improved competence / skills

ii. Improved compliance with national guidelines

iii. Improved effectiveness of care

iv. Improved motivation

v. Other, specify _____

E. Aides Memoir (AM)

1. Which of the following malaria laboratory diagnosis aides are **<u>available</u>** at the unit?

i. Posters on the wall (Y / N)

ii. Wall charts (Y / N)

ii. Leaflets / Pamphlets (Y / N)

iii. Reference textbooks (Y / N)

iv. Desk aids (Y / N)

Others, specify _____

2. Which do you prefer as a reminder?

Rate from 1 to 5 as below	
Not useful	1
A good reminder	2
A very good reminder	3
No idea / No response	4
Not applicable	5

i. Posters on the wall [ ]

ii. Wall charts [ ]

ii. Leaflets / Pamphlets [ ]

iii. Reference textbooks [ ]

iv. Desk aids [ ]

Others, specify _____

Date: ___ / ____ / 2009 Time _____ am /pm

Completed by: _____ (name)

Appendix N: Supplementary Materials for sub-study 4 entitled "Antiretroviral Agents and Prevention of Malaria in HIV-Infected Ugandan Children"

Inclusion criteria

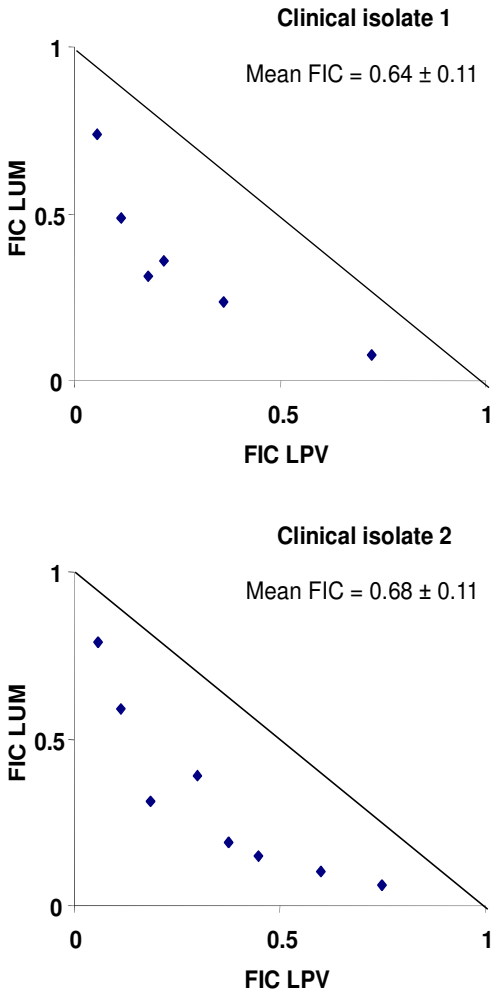
1) Age 2 months to < years
2) Confirmed HIV diagnosis: Children ≥ 18 months: Documentation of HIV status must come from two assays. Assays include DNA PCR, HIV RNA, Western blot, or rapid HIV antibody test. Children < 18 months: Documentation included DNA PCR confirmation only along with documentation of testing from the referral entity
3) ART-naïve patients eligible for ART initiation per WHO/Uganda guidelines or Patients receiving first line ART regimen with NNRTI +2 NRTI with at least one HIV RNA <400 copies/ml within the past 6 months
4) Agreement to come to the study clinic for any febrile episode or other illness
5) Agreement to avoid medications administered outside study protocol
6) Provision of informed consent by parent/guardian and agreement to have child's care at the clinical site
7) Lives within 50 km of study site

Exclusion criteria

1) ART-naïve children: children or their mothers that have received any dose of Nevirapine in the past 24 months
2) Active medical problem requiring in-patient evaluation at the time of screening or enrollment
3) History of cardiac conduction disorder or known significant cardiac structural defect
4) Moderate, Severe or Life-threatening (Grade 2, 3, or 4) AST or ALT found within 4 weeks prior to enrollment: AST: >113U/L (>2.5xULN) and ALT: >113U/L (>2.5xULN)
5) Life-threatening (Grade 4) screening laboratory value found within 4 weeks prior to enrollment for the following: Absolute neutrophil count: <500 mm^3 , Hemoglobin: <6.5 g/dL, Creatinine: ≥3.5xULN, Platelets: <25,000/mm^3

FIGURES

Figure S1. Isobolograms describing the interaction between lumefantrine (LUM) and lopinavir (LPV) for two cloned clinical isolates. The mean fractional inhibitory concentration (FIC) index and standard deviation are shown for each analysis.



TABLES

Table S1. Secondary efficacy outcomes 28 days following treatment with AL

Treatment Outcome	ART Group		P-value
	NNRTI-based ART	LPV/r-based ART	
Fever Clearance			
Fever present on day 1	77/173 (44.5%)	50/107 (46.7%)	0.69
Fever present on day 2	18/171 (10.5%)	13/107 (12.2%)	0.70
Fever present on day 3	9/171 (5.3%)	2/105 (1.9%)	0.18
Parasite clearance			
Positive blood smear on day 2	9/171 (5.3%)	9/107 (8.4%)	0.35
Positive blood smear on day 3	2/171 (1.2%)	2/105 (1.9%)	0.61
Appearance of gametocytes on days 2-28*	12/145 (8.3%)	6/99 (6.1%)	0.51
Hemoglobin recovery, mean gm/dL (SD)	0.61 (1.14)	0.56 (1.07)	0.58

***** does not include patients with gametocytes on day 0

Table S2. Adverse events 28 days following treatment with AL

Treatment Outcome	ART Group		P-value
	NNRTI-based ART	LPV/r-based ART	
Cough	77/174 (44.3%)	43/107 (40.2%)	0.42
Mild	73	42	
Moderate	4	1	
Elevated temperature	39/174 (22.4%)	22/107 (20.6%)	0.84
Mild	21	11	
Moderate	10	8	
Severe	8	3	
Diarrhea	14/174 (8.1%)	11/107 (10.3%)	0.46
Mild	13	11	
Moderate	1	0	
Vomiting	17/174 (9.3%)	8/107 (7.5%)	0.53
Mild	16	8	
Moderate	1	0	
Anorexia	11/174 (6.3%)	8/107 (7.5%)	0.72
Mild	9	7	
Moderate	2	1	
Weakness	8/174 (4.6%)	3/107 (2.8%)	0.51
Mild	5	1	
Moderate	3	2	
Pruritus	2/174 (1.2%)	6/107 (5.6%)	0.04
Mild	2	6	

Rash	3/174 (1.7%)	1/107 (0.9%)	0.58
Mild	3	1	
Chills	2/174 (1.2%)	3/107 (2.8%)	0.31
Mild	2	2	
Moderate	0	1	
Dysphagia	1/174 (0.6%)	1/107 (0.9%)	0.78
Mild	0	1	
Moderate	1	0	
Respiratory distress	1/174 (0.6%)	0/107 (0%)	N/A[‡]
Severe	1	0	
Jaundice	0/174 (0%)	3/107 (2.8%)	N/A[‡]
Mild	0	3	
Nausea[*]	1/125 (0.8%)	1/69 (1.5%)	0.67
Mild	1	1	
Abdominal pain[*]	7/124 (5.7%)	1/68 (1.5%)	0.24
Mild	7	1	
Headache[*]	4/125 (3.2%)	1/69 (1.5%)	0.56
Mild	2	1	
Moderate	2	0	
Anemia	6/163 (3.7%)	5/104 (4.8)	0.70
Mild	5	2	
Moderate	1	1	
Severe	0	0	
Life-threatening	0	2	
Neutropenia	44/152 (29.0%)	36/102 (35.3%)	0.38

Mild	20	22	
Moderate	17	8	
Severe	6	3	
Life-threatening	1	3	
Thrombocytopenia	11/158 (7.0%)	3/102 (2.9%)	0.19
Mild	4	1	
Moderate	7	2	
Elevated ALT	20/148 (13.5%)	3/91 (3.3%)	0.003
Mild	18	2	
Moderate	1	0	
Severe	0	0	
Life-threatening	1	1	
Prolonged QTc interval[†]	0/66 (0%)	0/54 (0%)	N/A[‡]
Any adverse event	138/174 (79.3%)	76/107 (71.0%)	0.13
Any serious adverse event	4[a]/174 (2.3%)	6[b]/107 (5.6%)	0.16

[*] Only assessed in children over 3 years of age

[†] Only assessed in episodes occurring after November 15th 2010

[‡] Unable to generate due to lack of convergence

[a] 1 neutropenia, 1 elevated ALT, 1 malnutrition, 1 respiratory distress

[b] 3 neutropenia, 2 anemia, 1 elevated ALT

Table S3. Association between day 7 lumefantrine levels and risk of recurrent parasitemia

Day 7 lumefantrine level ng/ml	NNRTI-based ART				LPV/r-based ART			
	N	Risk of recurrent malaria after 63 days	HR* (95% CI)	P-value	N	Risk of recurrent malaria after 63 days	HR* (95% CI)	P-value
< 300	55	45.7% (33.2-60.2%)	1.0 (reference)	-	11	78.8% (50.7-96.7%)	1.0 (reference)	-
300-<700	24	48.0% (30.0-69.8%)	0.87 (0.42-1.80)	0.71	14	17.5% (4.5-54.9%)	0.15 (0.04-0.56)	0.005
700-<4500	13	38.5% (18.2-69.2%)	0.66 (0.23-1.88)	0.44	40	15.6% (7.3-31.4%)	0.11 (0.03-0.35)	<0.001

* Hazard ratio