Longitudinal Outcomes in a Cohort of Ugandan Children Randomized to Artemether-Lumefantrine Versus Dihydroartemisinin-Piperaquine for the Treatment of Malaria

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Background. Artemisinin-based combination therapy (ACT) has become the standard of care for the treatment of uncomplicated Plasmodium falciparum malaria. Although several ACT regimens are approved, data guiding optimal choices of ACTs are limited. We compared short- and long-term outcomes in a cohort of young Ugandan children randomized to 2 leading ACTs.

Methods. Overall, 312 children were randomized to artemether-lumefantrine or dihydroartemisinin-piperaquine (DP) at the time of the first episode of uncomplicated malaria (median age, 10.5 months). The same treatment was given for all subsequent episodes of uncomplicated malaria and children were followed until they reached 5 years of age. The cohort included a subgroup that was human immunodeficiency virus (HIV) infected (n = 44) or HIV exposed (n = 175) and prescribed trimethoprim-sulfamethoxazole (TMP-SMX) prophylaxis. Outcomes included time to recurrent malaria following individual treatments and the overall incidences of treatments for malaria, complicated malaria, and hospitalizations.

Results. Among children not prescribed TMP-SMX prophylaxis, 4443 treatments for malaria were given over 790 person-years following randomization. Treatment with DP was associated with a lower hazard of recurrent malaria over the 84 days after treatment (hazard ratio, 0.66; 95% confidence interval [CI], .61–.70; P < .001). Children randomized to DP had a lower incidence of all treatments for malaria (incidence rate ratio [IRR], 0.85; 95% CI, .75–.96; P = .01), complicated malaria (IRR, 0.12; 95% CI, .04–.39; P < .001), and hospitalizations (IRR, 0.31; 95% CI, .13–.77; P = .01). Among children prescribed TMP-SMX prophylaxis, there were no significant differences in longitudinal outcomes.

Conclusions. Compared to artemether-lumefantrine, the use of DP to treat uncomplicated malaria delayed the time to recurrent malaria and reduced the incidences of treatments for malaria, complicated malaria, and hospitalizations.

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Keywords. malaria; artemether-lumefantrine; dihydroartemisinin-piperaquine; Uganda; cohort.
in a significantly lower risk of recurrent infections [3–6]. Comparative antimalarial efficacies are generally assessed in clinical trials evaluating individual episodes of malaria, with patient follow-up of 28–63 days [7]. However, important differences in drug impacts may only be appreciated after longer durations of follow-up, covering repeated episodes of malaria. This may be especially important in young children living in highly endemic areas, who commonly suffer multiple episodes of malaria each year. In 2009, we published the initial results of a longitudinal clinical trial following a cohort of young Ugandan children living in a highly endemic area and randomized to AL or DP for the treatment of all episodes of uncomplicated malaria [4]. Our initial report included a median follow-up period of 4 months after randomization and 671 treatments with study drugs. Compared with AL, DP was associated with a significantly lower risk of recurrent malaria after 28 days (11% vs 35%; P < .001), but we did not detect significant differences in risks of recurrent malaria or the incidence of malaria when follow-up was extended. This report from the same cohort includes a much longer period of observations, with median follow-up after randomization of almost 4 years and 5564 treatments with study drugs. With extended follow-up, we assessed the long-term impacts of study drugs, including the incidences of all treatments for malaria, complicated malaria, hospitalizations, and hospitalizations with malaria.

METHODS

Study Area and Enrollment of Cohort
The study was conducted in Tororo, an area with very high malaria transmission intensity [8]; details have been described elsewhere [4]. In brief, convenience sampling was used to enroll children from a postnatal clinic at Tororo District Hospital. Enrollment was based on targeted numbers of HIV-unexposed (uninfected, born to uninfected mothers), HIV-exposed (uninfected, born to infected mothers), and HIV-infected children. At enrollment, all study participants received a long-lasting insecticide-treated bednet (ITN). HIV-unexposed children were not prescribed trimethoprim-sulfamethoxazole (TMP-SMX) prophylaxis; HIV-infected children were prescribed TMP-SMX prophylaxis for the entire observation period; and HIV-exposed children were initially prescribed daily TMP-SMX prophylaxis, and if they remained HIV uninfected 6–8 weeks after breastfeeding cessation, were randomized to stop TMP-SMX at that time, 2 years of age, or 4 years of age. HIV-infected children were also provided antiretroviral therapy according to national guidelines.

Follow-up of Study Participants
Subjects were followed up for all medical problems at a study clinic open 7 days a week. Parents/guardians were encouraged to bring their children to the study clinic whenever they were ill, and after-hours care was available. Children who presented with new medical problems underwent a standardized medical evaluation. Medications with antimalarial activity were avoided for the treatment of nonmalarial illnesses. Monthly assessments were done in the study clinic.

Malaria Diagnosis and Management
Subjects who presented to the study clinic with a documented fever or history of fever in the previous 24 hours had blood obtained by finger-prick for a thick smear. If the thick smear was positive, the patient received a diagnosis of malaria. Study participants aged ≥4 months and weighing ≥5 kg were randomly assigned to receive open-label AL or DP at the time their first episode of uncomplicated malaria was diagnosed. Participants received the same regimen for all subsequent episodes of uncomplicated malaria. Episodes of uncomplicated malaria in children <4 months of age or weighing <5 kg, as well as episodes of complicated malaria and treatment failures within 14 days, were treated with a 7-day course of quinine (oral or parenteral). All children with malaria were followed up on days 1, 2, 3, 7, 14, 21, and 28 following the diagnosis.

Treatment Allocation and Study Drug Administration
Treatment allocation was based on a computerized randomization list. Study drugs were administered according to weight-based guidelines as previously described [4], including AL (Coartem, Novartis) administered twice daily for 3 days and DP (Duocotexcin, Hollelypharm) administered once a day for 3 days. The first daily dose of study drug was directly observed at the study clinic. After each dose, children were observed for 30 minutes, and the dose was readministered if vomiting occurred. For patients randomized to receive AL, parents/guardians were instructed to give each second daily dose at home.

Laboratory Procedures
Methods used for the preparation and reading of blood smears have been previously described [4]. Hemoglobin was measured at the time of each malaria diagnosis using a portable spectrophotometer (HemoCue, Ängelholm, Sweden). For a subset of episodes of recurrent malaria occurring 4–63 days after the initiation of treatment, molecular genotyping was used to distinguish new from recrudescent infections as previously described [9].

Ethics Statement
Informed consent was obtained from the parents or legal guardian of all study participants. The study protocol was approved by the Uganda National Council of Science and Technology and the institutional review boards of the University of California, San Francisco; Makerere University; the University of Washington; and the Centers for Disease Control and Prevention (CDC).
Statistical Methods
The study was originally powered to test the hypothesis that the risk of recurrent parasitemia after 28 days would be lower for DP than for AL, estimating a risk of recurrent parasitemia of 50% in the AL arm based on a previous study in Tororo [10]. These original estimates were based on following study participants to 21 months of age and an effect size of an 11% or greater risk difference between the 2 groups, as previously described [4]. The study protocol was amended and follow-up extended from 21 months to 5 years of age primarily to compare the protective efficacy of TMP-SMX prophylaxis given for different durations. No sample size calculations were made a priori for the longitudinal outcomes described below.

Data were entered into an Access database (Microsoft, Redmond, Washington) and analyzed using Stata software version 12 (StataCorp, College Station, Texas). Outcomes were assessed at the level of each treatment with study drugs and at the level of each participant randomized to study drugs. Treatment level outcomes included 28-day outcomes using the standardized WHO classification system [1] and the 84-day risk of recurrent malaria. The 28-day treatment outcomes were compared using generalized estimating equations with adjustment for repeated measures in the same patient. Cumulative risks of recurrent malaria were estimated using the Kaplan-Meier product limit formula with data censored for patients who did not complete follow-up. Pairwise comparisons of the hazard of recurrent malaria were made using a Cox proportional hazards model with adjustment for repeated measures in the same patient. Longitudinal outcomes for individual study participants included the incidence of all treatments for malaria, the incidence of all treatments for complicated malaria defined as severe malaria or danger signs [1], the incidence of all hospitalizations, and the incidence of all hospitalization in association with malaria. For longitudinal outcomes, the observation period began 1 day after study participants were diagnosed with their first episode of uncomplicated malaria and ended when they turned 5 years of age or were prematurely withdrawn from the study. Comparisons of longitudinal outcomes were made using a negative binomial regression model. All analyses were stratified by person-time according to whether the participant was prescribed TMP-SMX prophylaxis (ie, HIV-exposed children contributed person-time to both strata). A \( P \) value <.05 was considered statistically significant.

RESULTS

Trial Profile
Of 351 enrolled children, 312 were diagnosed with at least 1 episode of uncomplicated malaria, 34 were excluded prior to...
randomization, and 5 reached 5 years of age without being diagnosed with uncomplicated malaria (Figure 1). Of the 312 children randomized to therapy, 76 (24.4%) were excluded prior to reaching 5 years of age. Reasons for exclusion after randomization included movement out of the study area (n = 27), discretion of the study investigators (n = 25), death (n = 8), loss to follow-up (n = 8), withdrawal of informed consent (n = 6), and inability to tolerate study drugs (n = 2).

No significant differences were found in the characteristics of children between the treatment arms (Table 1). The median age at randomization was 10.5 months, and children were followed for a median of 3.9 years following randomization. At randomization, 29.8% of children were HIV unexposed, 56.1% HIV exposed, and 14.1% HIV infected. Following randomization, 49.0% were not prescribed TMP-SMX prophylaxis, 32.7% were prescribed TMP-SMX prophylaxis, which was stopped during follow-up, and 14.1% were prescribed TMP-SMX prophylaxis for the entire follow-up period. At routine assessments following randomization, 98.0% of children were reported to have slept under an ITN during the prior night.

### Treatment Outcomes for Individual Episodes of Malaria Treated With Study Drugs

Standardized WHO treatment outcomes after 28 days of follow-up and the cumulative risks of recurrent malaria after 84 days stratified by whether or not the child was prescribed TMP-SMX prophylaxis are presented in Table 2 and Figure 2, respectively. Considering children who were not prescribed TMP-SMX prophylaxis, early treatment failures requiring rescue therapy within 3 days were uncommon, but significantly less frequent following treatment with DP (2/2176) compared with AL (14/2371) (odds ratio [OR], 0.14; 95% confidence interval [CI], 0.03–0.64; \( P = .01\)). The risk of any WHO treatment failure by day 28 of follow-up was markedly lower following treatment with DP compared with AL (8.9% vs 51.0%, \( P < .001\)). When follow-up was extended to 84 days, the majority of patients experienced a recurrent episode of malaria requiring therapy and the cumulative risks were similar following treatment with DP (82.7%; 95% CI, 81.0%–84.2%) and AL (83.9%; 95% CI, 82.3%–85.3%). However, the hazard of recurrent malaria over 84 days was significantly lower following treatment with DP compared with AL (hazard ratio [HR], 0.66; 95% CI, 0.61–0.70; \( P < .001\)).

It was important to consider children receiving TMP-SMX prophylaxis separately, as this regimen offers significant protection against malaria [11]. As expected, in this group, the risks of treatment failure after 28 days and recurrent malaria after 84 days were lower compared with children not prescribed TMP-SMX prophylaxis. Furthermore, differences in treatment outcomes between the 2 malaria treatment arms were less pronounced in those not prescribed TMP-SMX, although the risk of treatment failure by day 28 of follow-up (8.1% vs 32.8%; \( P < .001\)) and the hazard of recurrent malaria over 84 days (HR, 0.80; 95% CI, .69–.93; \( P = .004\)) were both significantly lower following treatment with DP.

All episodes of recurrent malaria occurring 4–63 days after initiation of therapy with study drugs through February 2009 and then a random subset of 50 per treatment arm per year from 2009 to 2012 were genotyped to distinguish recrudescence from new infections. The proportions of episodes of recurrent malaria classified as recrudescences were equally low for both the DP (24/545 [4.4%]) and AL treatment arms (22/600 [3.7%]) (\( P = .68\)) and did not change significantly over time or have any significant association with the age of the child (data not shown).

### Longitudinal Outcomes at the Level of Individual Children Randomized to Therapy

To compare the longer-term impacts of the 2 ACT study drugs, the incidence of key long-term outcomes was compared...
between children randomized to receive AL and those randomized to receive DP (Table 3). The overall incidence of malaria following randomization was very high, with 5.62 treatments for malaria per year among children not prescribed TMP-SMX and 3.35 treatments per year among those prescribed TMP-SMX (incidence rate ratio [IRR], 0.60; 95% CI, .53–.68; \( P < .001 \)).

Considering observed time for children not prescribed TMP-SMX, those randomized to DP had a 15% lower incidence of treatments for malaria compared with those randomized to AL (IRR, 0.85; 95% CI, .75–.96; \( P = .01 \); Table 3), an effect that was consistent over age (Figure 3). Only 42 of 4443 (0.9%) malaria treatments (in 23 different children) were for presentations meeting criteria for complicated malaria (convulsions = 23, severe anemia = 17, cerebral malaria = 1, and respiratory distress = 1). Importantly, children randomized to DP had an 88% lower incidence of complicated malaria compared with children randomized to AL (IRR, 0.12; 95% CI, .04–.39; \( P < .001 \)). Of note, among 2099 treatments for malaria in children randomized to DP and not prescribed TMP-SMX, only 1 met the WHO criteria for severe malaria [1]. A total of 55 hospitalizations occurred in 31 different children (malaria = 46, malnutrition = 2, pneumonia = 2, febrile convulsions without malaria = 2, diarrheal illness = 1, meningitis = 1, and septicemia = 1). Compared with children randomized to AL, children randomized to DP had a 69% lower incidence of hospitalization (IRR, 0.31; 95% CI, .13–.77; \( P = .01 \)), which could be fully explained by the 78% lower incidence of hospitalization associated with malaria (IRR, 0.22; 95% CI, .08–.62; \( P = .004 \)).

For children prescribed TMP-SMX prophylaxis, children randomized to DP had a 7% lower incidence of treatments for malaria compared with children randomized to AL, but this difference was not statistically significant (IRR, 0.93; 95% CI, .70–1.24; \( P = .63 \)). In contrast to children not prescribed TMP-SMX, in those prescribed TMP-SMX there were no significant differences between children randomized to DP vs AL in the incidences of complicated malaria, hospitalizations, or hospitalizations with malaria (Table 3).

There were no differences in mean hemoglobin levels at the time malaria was diagnosed between the AL and DP treatment arms following randomization, both for those children not prescribed TMP-SMX (10.5 vs 10.5 g/dL; \( P = .42 \))

### Table 2. World Health Organization Treatment Outcome by Day 28 of Follow-up Unadjusted by Genotyping

<table>
<thead>
<tr>
<th>Treatment Outcomes</th>
<th>Children Not Prescribed TMP-SMX Prophylaxis</th>
<th>Children Prescribed TMP-SMX Prophylaxis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AL(^a) (n = 2371)</td>
<td>DP(^a) (n = 2176)</td>
</tr>
<tr>
<td>No treatment outcome, No. (%)</td>
<td>20 (0.8)</td>
<td>16 (0.7)</td>
</tr>
<tr>
<td>Lost to follow-up</td>
<td>17</td>
<td>15</td>
</tr>
<tr>
<td>Other antimalarial use</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Withdrew informed consent</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Early treatment failure, No. (%)</td>
<td>14 (0.6)</td>
<td>2 (0.1)</td>
</tr>
<tr>
<td>Late clinical failure, No. (%)</td>
<td>421 (17.8)</td>
<td>38 (1.7)</td>
</tr>
<tr>
<td>Late parasitological failure, No. (%)</td>
<td>774 (32.6)</td>
<td>153 (7.0)</td>
</tr>
<tr>
<td>Adequate clinical and parasitological response, No. (%)</td>
<td>1142 (48.2)</td>
<td>1967 (90.4)</td>
</tr>
</tbody>
</table>

Abbreviations: AL, artemether-lumefantrine; DP, dihydroartemisinin-piperaquine; TMP-SMX, trimethoprim-sulfamethoxazole.

\(^a\) Three patients randomized to DP received AL; 2 patients randomized to AL received DP.
and for those children prescribed TMP-SMX (10.2 vs 10.1 g/dL; $P = .43$).

**DISCUSSION**

In this longitudinal trial in a cohort of Ugandan children, AL and DP were both highly efficacious for the treatment of uncomplicated malaria. However, in this area of very high malaria endemicity, recurrent episodes of malaria were common and DP was associated with a significant delay in the time to the next episode of malaria. This extended posttreatment prophylactic effect of DP translated into 1 fewer treatment for malaria per child per year and a significant reduction in the risk of complicated malaria and hospitalizations. Interestingly, in a subset of HIV-exposed and HIV-infected children prescribed TMP-SMX prophylaxis, the long-term benefits of DP over AL were less pronounced and did not reach statistical significance.

ACTs have become the recommended first-line therapy for uncomplicated falciparum malaria throughout the world, and the WHO recommends 5 regimens: AL, DP, artesunate plus amodiaquine (AS + AQ), artesunate plus mefloquine (AS + MQ), and artesunate plus sulfadoxine-pyrimethamine (AS + SP) [1]. Several factors contribute to choosing the most appropriate ACT in a particular country or region, including the level of resistance to the partner drug, safety, tolerability, cost, and availability. For example, the combinations AS + MQ and AS + SP have not been widely deployed in Africa due to concerns about tolerability in young children and resistance to SP, respectively [1]. DP has an excellent efficacy and safety profile, but it has not been prequalified by the WHO and has yet to be adopted as a first-line regimen in any African country [1]. Indeed, currently all countries in Africa recommend either AL and/or AS + AQ as their first-line antimalarial regimen [12].

Table 3. Comparative Incidence of Outcomes of Interest Following Randomization

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Observation Period Among Children Not Prescribed TMP-SMX Prophylaxis</th>
<th></th>
<th>Observation Period Among Children Prescribed TMP-SMX Prophylaxis</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AL (382 Person-years)</td>
<td>DP (408 Person-years)</td>
<td>AL (134 Person-years)</td>
<td>DP (128 Person-years)</td>
</tr>
<tr>
<td></td>
<td>Events</td>
<td>Incidence$^a$</td>
<td>Events</td>
<td>Incidence$^a$</td>
</tr>
<tr>
<td>All treatments for malaria$^b$</td>
<td>2344</td>
<td>6.14</td>
<td>2099</td>
<td>5.14</td>
</tr>
<tr>
<td>Complicated malaria</td>
<td>37</td>
<td>0.097</td>
<td>5</td>
<td>0.012</td>
</tr>
<tr>
<td>All hospitalizations</td>
<td>42</td>
<td>0.110</td>
<td>13</td>
<td>0.032</td>
</tr>
<tr>
<td>Hospitalizations in association with malaria</td>
<td>38</td>
<td>0.099</td>
<td>8</td>
<td>0.020</td>
</tr>
<tr>
<td></td>
<td>457</td>
<td>3.42</td>
<td>418</td>
<td>3.27</td>
</tr>
<tr>
<td>Complicated malaria</td>
<td>7</td>
<td>0.052</td>
<td>4</td>
<td>0.031</td>
</tr>
<tr>
<td>All hospitalizations</td>
<td>16</td>
<td>0.120</td>
<td>19</td>
<td>0.149</td>
</tr>
<tr>
<td>Hospitalizations in association with malaria</td>
<td>11</td>
<td>0.082</td>
<td>8</td>
<td>0.063</td>
</tr>
</tbody>
</table>

Abbreviations: AL, artemether-lumefantrine; CI, confidence interval; DP, dihydroartemisinin-piperaquine; IRR, incidence rate ratio; TMP-SMX, trimethoprim-sulfamethoxazole.

$^a$ Per person-year.

$^b$ Does not include first treatment for uncomplicated malaria with study drugs.

**Figure 3.** Incidence of all treatments for malaria over age for children randomized to artemether-lumefantrine (AL) vs dihydroartemisinin-piperaquine (DP). Data limited to children not prescribed trimethoprim-sulfamethoxazole (TMP-SMX) prophylaxis.
AL is the first-line regimen in most East African countries, where resistance to amodiaquine is a concern and studies have reported superior efficacy of AL compared with AS + AQ [13, 14].

In Uganda, AL has been the first-line therapy for uncomplicated malaria since 2005, and multiple studies have demonstrated this regimen to have an excellent efficacy and safety profile [3, 5, 13, 15]. DP has also been extensively studied in Uganda and has also been shown to have excellent efficacy and safety [3–5, 15, 16]. One distinct advantage of DP over AL is its extended posttreatment prophylactic effect, resulting from a terminal elimination half-life for piperaquine of 3–4 weeks, compared to about 3 days for lumefantrine [17, 18]. Several comparative trials in Africa, including this one, have demonstrated a consistent and significant reduction in the risk of recurrent parasitemia or malaria within 28–63 days following treatment with DP, compared with AL, across a range of transmission intensities [3–5, 15, 16]. In this study we were able to estimate the impact of the posttreatment prophylactic effect of DP on longitudinal outcomes measured at the level of individual children followed over an extensive period, covering thousands of treatments for malaria. Children randomized to DP had a 15% reduction in the number of treatments for malaria compared with children randomized to AL, resulting in an average of 1 fewer treatment for malaria per year. Although episodes of complicated malaria were rare in this study (<1% of episodes), children randomized to DP also had a lower incidence of complicated malaria and hospitalizations compared with children randomized to AL. We hypothesize that the protection against complications of malaria afforded by DP was primarily due to the benefits of delaying the time to recurrent malaria and allowing children more time to recover from their previous episode; however, we did not find an association between hemoglobin levels and time since last episode of malaria (data not shown). One limitation of this study is that we did not evaluate differences in the prevalence of asymptomatic parasitemia between the 2 treatment arms, which could have important implications for malaria transmission dynamics. Interestingly, in the subset of HIV-exposed and HIV-infected children who were prescribed TMP-SMX prophylaxis, the long-term benefits of DP were less pronounced, presumably due to the independent protective effect of TMP-SMX against malaria, resulting in a lower overall risk of recurrent malaria following therapy, diluting the impact of DP. Indeed, despite widespread resistance to antifolates in Uganda, TMP-SMX prophylaxis offers some antimalarial preventive efficacy. For example, in a previously published analysis from this same cohort, HIV-exposed children randomized to continue TMP-SMX prophylaxis beyond the cessation of breastfeeding had a 39% reduction in the incidence of malaria and a significant reduction in the risk of recurrent parasitemia within 28 days of therapy [11].

In summary, in a cohort of Ugandan children living in a highly endemic area, the use of DP for the treatment of uncomplicated malaria offered significant long-term benefits compared with the use of AL. This study provides further evidence that DP should be considered as a first-line regimen for the treatment of malaria, especially in high-risk groups living in areas of high transmission intensity.

Notes

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Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References


