Protective efficacy of prolonged co-trimoxazole prophylaxis in HIV-exposed children up to age 4 years for the prevention of malaria in Uganda: a randomised controlled open-label trial

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Summary

Background WHO recommends daily co-trimoxazole for children born to HIV-infected mothers from 6 weeks of age until breastfeeding cessation and exclusion of HIV infection. We have previously reported on the effectiveness of continuation of co-trimoxazole prophylaxis up to age 2 years in these children. We assessed the protective efficacy and safety of prolonging co-trimoxazole prophylaxis until age 4 years in HIV-exposed children.

Methods We undertook an open-label randomised controlled trial alongside two observational cohorts in eastern Uganda, an area with high HIV prevalence, malaria transmission intensity, and antifolate resistance. We enrolled HIV-exposed infants between 6 weeks and 9 months of age and prescribed them daily co-trimoxazole until breastfeeding cessation and HIV-status confirmation. At the end of breastfeeding, children who remained HIV-uninfected were randomly assigned (1:1) to discontinue co-trimoxazole or to continue taking it up to age 2 years. At age 2 years, children who continued co-trimoxazole prophylaxis were randomly assigned (1:1) to discontinue or continue prophylaxis from age 2 years to age 4 years. The primary outcome was incidence of malaria (defined as the number of treatments for new episodes of malaria diagnosed with positive thick smear) at age 4 years. For additional comparisons, we observed 48 HIV-infected children who took continuous co-trimoxazole prophylaxis and 100 HIV-unexposed uninfected children who never received prophylaxis. We measured grade 3 and 4 serious adverse events and hospital admissions. All children were followed up to age 5 years and all analyses were by intention to treat. This study is registered with ClinicalTrials.gov, number NCT00527800.

Findings 203 HIV-exposed infants were enrolled between Aug 10, 2007, and March 28, 2008. After breastfeeding ended, 185 children were not infected with HIV and were randomly assigned to stop (n=87) or continue (n=98) co-trimoxazole up to age 2 years. At age 2 years, 91 HIV-exposed children who had remained on co-trimoxazole prophylaxis were randomly assigned to discontinue (n=46) or continue (n=45) co-trimoxazole from age 2 years to age 4 years. We recorded 243 malaria episodes (2·91 per person-years) in the 45 HIV-exposed children assigned to continue co-trimoxazole until age 4 years compared with 503 episodes (5·60 per person-years) in the 46 children assigned to stop co-trimoxazole at age 2 years (incidence rate ratio 0·53, 95% CI 0·39–0·71; p<0·0001). There was no evidence of malaria incidence rebound in the year after discontinuation of co-trimoxazole in the HIV-exposed children who stopped co-trimoxazole at age 2 years, but incidence increased significantly in HIV-exposed children who stopped co-trimoxazole at age 4 years (odds ratio 1·78, 95% CI 1·19–2·66; p=0·005). Incidence of grade 3 or 4 serious adverse events, hospital admissions, or deaths did not significantly differ between HIV-exposed, HIV-unexposed, and HIV-infected children.

Interpretation Continuation of co-trimoxazole prophylaxis up to 4 years of age seems safe and efficacious to protect HIV-exposed children living in malaria-endemic areas.

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Introduction

To reduce the risk of HIV-associated opportunistic infections, WHO recommends that infants born to HIV-infected mothers and exposed to HIV through breastfeeding (HIV-exposed children) receive co-trimoxazole prophylaxis from 6 weeks of age until breastfeeding ends and HIV infection can be excluded.[1,2] The rationale is that co-trimoxazole prophylaxis protects children who acquire HIV from their mothers against opportunistic infections including malaria and is inexpensive.[3–8] However, this recommendation can be difficult to implement because many HIV-exposed children are not retained in care beyond the last routine measles immunisation at 9–12 months of age, when breastfeeding might not have ended.[9,10] Moreover, exclusion of HIV infection in infants younger than 12 months has to be done via the detection of HIV DNA due to the persistence of maternal antibodies in HIV-exposed children's blood until 12–18 months of age,[11,12] and HIV DNA testing can be costly and difficult to access.[13,14]

Every year, HIV and malaria combined cause 2·2 million deaths worldwide.[15,16] In sub-Saharan Africa, more than 1 million deaths every year are attributed to dual infection in children.[16] In 2005, a randomised trial provided evidence that co-trimoxazole prophylaxis protected children aged 5–15 years against malaria in an area of Mali with low HIV prevalence.[6] In 2011, we reported the results of a randomised controlled trial showing that prolongation of co-trimoxazole prophylaxis in HIV-exposed children beyond breastfeeding cessation to age 2 years was safe and resulted in a 39% protective efficacy against malaria.[17] We now present the final results of the trial up to age 5 years. Although incidence and mortality of severe *Plasmodium falciparum* malaria tend to peak in young children, especially in areas of high transmission intensity, the incidence of uncomplicated clinical malaria is relatively evenly distributed across the first 10 years of life in children in sub-Saharan Africa, independent of malaria transmission and seasonality patterns.[18] Moreover, in areas of high transmission intensity such as our study setting of eastern Uganda, malaria incidence gradually increases until about 30 months of age and then gradually decreases, both in HIV-exposed and HIV-unexposed children.[19] Our present hypotheses were that extending co-trimoxazole prophylaxis from age 2 to age 4 years would be associated with a reduction in the incidence of malaria and that between age 4 and age 5 years, the incidence of malaria would be higher among children who stopped co-trimoxazole at 4 years of age compared with children who stopped co-trimoxazole after cessation of breastfeeding or at 2 years of age.

Methods

Study design and participants

We did a randomised controlled open-label trial with additional observational comparisons. Detailed setting characteristics and enrolment procedures have been described previously and are available online.[17] Our study was done in Tororo, Uganda, where malaria is holoendemic, the entomological inoculation rate is 591 infective bites per person-year,[20] and the prevalence of antifolate resistance (*dhfr*/*dhps* quintuple mutant, composed of *dhfr* 51I, 59R, and 108N, and *dhps* 437G and 540Q mutations) exceeds 90%.[17,21,22] We used convenience sampling to enrol an interventional cohort of HIV-exposed infants and two observational cohorts of HIV-unexposed and HIV-infected infants from Tororo District Hospital and the AIDS Support Organization in Tororo, eastern Uganda.

For the **protocol** see http://clinicaltrials.gov/ct2/show/NCT00527800?term=NCT00527800&rank=1

Eligibility criteria for HIV-exposed infants were: babies born to an HIV-infected mother, age 6 weeks to 9 months,[23] HIV-negative status, currently breastfeeding, residing within 30 km of Tororo District Hospital, parental agreement to bring the child to Tororo District Hospital for any illness, avoidance of drugs outside the study protocol, absence of medical problems at screening, and parental provision of informed consent. Apart from HIV status, inclusion criteria were the same for HIV-infected and HIV-unexposed children, except that age of inclusion was from 6 weeks to 12 months for HIV-infected children. All participants were given an insecticide-treated bednet with instructions for use. We provided daily co-trimoxazole prophylaxis drugs according to national guidelines.[23,24] All mothers were counselled to exclusively breastfeed their infants until at least 6 months of age and then to wean according to national guidelines.[23,25]

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, CA, USA; Makerere University, Kampala, Uganda; the University of Washington, Seattle, WA, USA; and the Centers for Disease Control and Prevention, Atlanta, GA, USA. We obtained written informed consent from parents or guardians.

Randomisation and masking

Simple randomisation lists were computer generated by GD at the University of California, San Francisco, CA, USA. Sequentially numbered sealed envelopes containing the treatment group assignments were prepared from these lists, and allocated sequentially by a study nurse. Intervention assignments were not masked to investigators, parents, and assessors.

Procedures

Before enrolment, we established mothers' HIV status by rapid HIV testing (Determine Rapid Test for HIV-1, Abbott Laboratories, Abbott Park, IL, USA) and tested infants for HIV DNA by PCR assay (Amplicor HIV-1 DNA PCR Test, version 1.5; Roche, Branchburg, NJ, USA) following Ugandan national HIV testing algorithms.[26] We retested children 6–8 weeks after cessation of breastfeeding by HIV DNA PCR assay, and determined their final HIV status at age 18 months with rapid HIV testing and HIV DNA PCR. Positive PCR results were confirmed by repeat bleeding and quantitative HIV RNA PCR testing (Cobas Amplicor HIV-1 RNA Monitor Test, version 1.5; Roche).

We randomly assigned (1:1) HIV-exposed children who remained uninfected with HIV 6–8 weeks after breastfeeding cessation to discontinue co-trimoxazole immediately or to continue prophylaxis to age 2 years.[17] At age 2 years, children previously assigned to continue prophylaxis were, for a second time, randomly assigned (1:1) to stop co-trimoxazole prophylaxis at age 2 years or to continue to age 4 years. At age 4 years, co-trimoxazole prophylaxis was stopped for all children. We followed up all children to age 5 years.

Participants attended a dedicated Tororo District Hospital clinic for all scheduled and unscheduled study visits. The clinic was open 7 days a week with after-hours care provided at the hospital. We monitored co-trimoxazole adherence, bednet use, and children's breastfeeding status and feeding practices through recall

by parent or guardians at routine monthly visits.[16] Infants assigned to continue prophylaxis were given a month-long supply of co-trimoxazole at 30-day scheduled routine visits according to weight-based guidelines.[16] We did a complete blood cell count every 90 days. Children who presented with tympanic temperature higher than 38°C or a history of fever in the previous 24 h were bled by finger prick for laboratory assessment of thick smear. Thick smears were stained with 2% Giemsa stain for 30 min and parasite density estimated.[4] Children with a

Figure 1: **Study profile**
CTX=co-trimoxazole. *Results of the first randomisation have been previously reported.[17] †Unrelated to CTX: this child had recurrent episodes of hemolytic anaemia after treatment with dihydroartemisinin-piperaquine that were later shown to be due to glucose-6-phosphate dehydrogenase deficiency.

	Study cohort, HIV-exposed children, second randomisation		Comparison cohorts	
	Daily CTX from age 2–4 years (n=45)	Stopped daily CTX at age 2 years (n=46)	HIV-unexposed children not on CTX (n=88)	HIV-infected children on daily CTX since HIV diagnosis (n=48)
Girls	24 (53%)	18 (39%)	36 (41%)	25 (52%)
Median age at study enrolment (months)	3·0 (2·3–5·2)	3·3 (2·2–5·6)	5·4 (3·4–7·5)	5·6 (2·8–8·5)
Median age at breastfeeding cessation (months)	7·5 (6·0–10·6)	7·2 (6·4–8·9)	21·1 (18·3–23·2)	17·0 (9·6–20·8)
Median age at first randomisation (months)	10·1 (8·2–13·1)	9·6 (8·5–11·2)	..	..
Time on co-trimoxazole before second randomisation or 2 years of age (cumulative person-years)	74·8	75·9	0	72·8
Proportion using an insecticide-treated bednet*	97%	98%	98%	99%
Residing in Tororo town	12 (27%)	7 (15%)	18 (20%)	13 (27%)
Malaria incidence per person-year before second randomisation or 2 years of age	1·93† (1·63–2·27)	2·87†‡ (2·50–3·27)	4·68§ (4·32–5·05)	2·01 (1·69–2·37)

Data are n (%), median (IQR), or incidence (95% CI), unless otherwise indicated. CTX=co-trimoxazole. *Proportion of participants who reported sleeping under an ITN the previous night at the time of monthly routine clinic visits. †These incidence values are lower than those reported in our previous study[17] or in table 2 because we used all observed person-time during follow-up to calculate time at risk to provide a better estimate of the number of treatments for malaria expected over time. In the previous study, we subtracted 14 days out of time at risk after each incident and treated episode of malaria. ‡p <0·06 for comparison with HIV-exposed children assigned to continue CTX until age 4 years. §p<0·0001 for comparison with either group of HIV-exposed children.

Table 1: **Baseline characteristics of HIV-exposed children (age 2 years) and comparison cohorts at the time of the second randomisation**

positive thick smear were diagnosed with malaria, irrespective of parasite density. Haemoglobin measurements were made by spectrophotometry (HemoCue, Ängelholm, Sweden) on days 0 and 28 of a malaria episode. We defined complicated malaria using standardised criteria.[27]

All children aged 4 months of age or older and weighing 5 kg or more diagnosed with uncomplicated malaria received either open-label artemether-lumefantrine or dihydroartemisinin-piperaquine.[28] Children with uncomplicated malaria younger than 4 months of age or weighing less than 5 kg and children diagnosed with complicated malaria were treated with standard doses of quinine. Children diagnosed with malaria returned on days 1, 2, 3, 7, 14, 21, 28, and on any day they were ill. Drugs with known antimalarial activity were avoided for the treatment of non-malarial infections.

At monthly visits, study clinicians assessed children for adverse events and serious adverse events using the International Conference on Harmonization Guideline criteria.[29] Children were withdrawn from the study if there was: a change of residence outside the study area, more than 60 days' loss to follow-up, withdrawal of informed consent, poor adherence to study procedures, or inability to tolerate study drugs.

Outcomes

The primary outcome of this extension study was the incidence of malaria in HIV-exposed children who were allocated to co-trimoxazole at first randomisation and were subsequently allocated at second randomisation to stop or continue treatment until age 4 years. We also assessed incidence of malaria to 5 years in the two observational cohorts of HIV-unexposed children who did not receive any prophylaxis and HIV-infected children who received co-trimoxazole therapy until age 5 years. Our secondary endpoints were incidence of complicated malaria, diarrhoeal illnesses and respiratory tract infections, and rebound malaria in the year after stopping of co-trimoxazole prophylaxis. Our safety endpoints were numbers of adverse events, severe adverse events, hospital admissions, and death.

Statistical analysis

On the basis of findings from our previous trial of prolonging of co-trimoxazole prophylaxis to age 2 years (first randomisation),[17] we assumed normally distributed data, 10% loss to follow-up, and two-sided α=0·05 with 80% power to detect a 38% difference in malaria incidence (3·04 *vs* 2·20 episodes per person-year) between two intervention groups of 100 HIV-exposed children each in our second randomisation. We defined time-at-risk from day 1 post-randomisation until day of next randomisation or withdrawal from study, whichever came first. Malaria incidence was defined as the number of new malaria episodes per person-time at risk and was reported in malaria episodes per person-year with associated 95% CIs. We compared malaria incidence and safety outcomes among all groups with negative binomial regression, adjusting for location of residence (urban *vs* rural, a characteristic previously associated with a lower incidence of malaria in urban areas[30]) and antimalarial treatment group. We defined the protective efficacy of a treatment with an incidence rate ratio (IRR) of the malaria incidence among children continuing co-trimoxazole divided by the incidence among those discontinuing co-trimoxazole. We used generalised estimating equations to measure associations between increasing age and daily risk of

malaria. A p<0·05 was considered statistically significant. We generated malaria incidence curves over age among children stratified by co-trimoxazole randomisation groups using Lowess smoothing with a bandwidth of 0·8. We double-entered data in Epi-Info and did statistical analysis with Stata version 10. All analyses were done on an intention-to-treat basis. This study is registered with ClinicalTrials.gov, number NCT00527800.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between Aug 10, 2007 and March 28, 2008, we enrolled 203 HIV-exposed infants, 100 HIV-unexposed–uninfected infants, and 48 HIV-infected infants (figure 1). 185 HIV-exposed uninfected infants remained in the study at the time of the first randomisation; 98 were assigned to continue co-trimoxazole after breastfeeding ended, of these, seven (7%) withdrew before age 2 years, thus at second randomisation, we assigned 46 HIV-exposed children to stop co-trimoxazole and 45 HIV-exposed children to continue co-trimoxazole prophylaxis up to age 4 years (figure 1). Overall, 146 (79%) of 185 children in the primary analysis completed follow-up to age 5 years. In the observational cohorts, 21 of 48 (44%) HIV-infected children completed follow-up to age 5 years and 73 (73%) of the 100 HIV-unexposed and uninfected children remained in follow-up to age 5 years.

The groups of HIV-exposed children did not differ in age at enrolment, breastfeeding cessation, or first randomisation, or in adherence to co-trimoxazole before first randomisation (table 1). Across the interventional groups and the two observational cohorts, bednet use, treatment of uncomplicated malaria before second randomisation, or urban residence also did not significantly differ (table 1). Compared with HIV-exposed children, HIV-unexposed and HIV-infected children were enrolled at a slightly older age and were breastfed for longer as expected (table 1). Malaria incidence before the second randomisation was lower in HIV-exposed children assigned to continue co-trimoxazole from age 2 to 4 years than in those assigned to stop co-trimoxazole at age 2 years, although this difference did not reach statistical significance (IRR 1·48, 95% CI 0·98–2·22; p=0·06).

The 91 HIV-exposed children assigned to discontinue or continue co-trimoxazole prophylaxis from age 2 years to 4 years had 746 malaria episodes. Of these, 243 (2·91 per person-years) episodes occurred among the 45 children randomly assigned to continue co-trimoxazole until age 4 years, whereas 503 episodes (5·60 per person-years) occurred in the 46 children randomly assigned to stop co-trimoxazole at age 2 years (table 2). Thus at age 4 years, the 45 HIV-exposed children who continued co-trimoxazole had a 47% lower risk of malaria than had the 46 children who stopped co-trimoxazole at age 2 years (IRR 0·53, 95% CI 0·39–0·71, p<0·0001). Similarly, the non-randomised comparison between the 45 HIV-exposed children who continued co-trimoxazole from age 2–4 years and the 80 children who stopped taking co-trimoxazole at breastfeeding cessation indicated an IRR of 0·53 (95% CI 0·43–0·67; p<0·0001; table 2).

By age 4 years, the 40 HIV-infected children on continuous daily co-trimoxazole prophylaxis had 166 malaria episodes (2·66 per person-years)—a 53% lower risk of malaria than the 80 HIV-exposed children who stopped taking co-trimoxazole at breastfeeding cessation (IRR 0·47, 95% CI 0·37–0·60; p<0·0001; table 2), and a 51% lower risk of malaria than had the 88 HIV-unexposed children who never received prophylaxis (0·49, 0·38–0·61; p<0·0001).

	n	CTX prophylaxis status	Episodes	Person-years	Incidence PPY	IRR (95% CI)*	p value
Enrolment (end of BF)							
HIV-exposed	203	On CTX since age 6 weeks	142	105·5	1·35	..	..
Before age 2 years†							
HIV-exposed	87	CTX stopped after end of BF	400	86·9	4·60	Ref	..
HIV-exposed	98	On CTX until age 2 years	305	104·1	2·93	0·66 (0·52–0·82)	0·0003
HIV-unexposed‡	100	Not on CTX	674	145·7	4·63	..	..
HIV-infected‡	48	On CTX since age 6 weeks	142	73·5	1·93	..	..
Between age 2 years and 4 years							
HIV-exposed	80	CTX stopped after end of BF	900	151·2	5·95	Ref	..
HIV-exposed	46	CTX stopped at age 2 years	503	89·9	5·60	0·96 (0·78–1·17)	0·69
HIV-exposed	45	On CTX until age 4 years	243	83·4	2·91	0·53 (0·43–0·67)	<0·0001
HIV-unexposed‡	88	Never on CTX	959	164·8	5·82	0·99 (0·84–1·17)	0·91
HIV-infected‡	40	On CTX since HIV diagnosis	166	62·4	2·66	0·47 (0·37–0·60)	<0·0001
Between age 4 years and 5 years							
HIV-exposed	71	CTX stopped after end of BF	371	69·7	5·32	Ref	..
HIV-exposed	36	CTX stopped at age 2 years	204	42·4	4·81	0·93 (0·73–1·18)	0·54
HIV-exposed	45	CTX stopped at age 4 years	167	36·2	4·61	0·92 (0·71–1·20)	0·55
HIV-unexposed‡	79	Never on CTX	383	76·8	4·99	0·97 (0·79–1·18)	0·74
HIV-infected‡	21	On CTX since HIV diagnosis	59	22·8	2·59	0·55 (0·42–0·69)	<0·0009

PPY=per person-year of follow-up. IRR= incidence rate ratio. CTX=co-trimoxazole. BF=breastfeeding. *Malaria IRR (95% CI), controlling for place of residence and antimalarial treatment group. †For randomised groups, incidence is from cessation of breastfeeding to 2 years of age. For observational cohorts is from enrolment (6 weeks to 12 months of age) to 2 years of age. ‡Observational cohorts, not randomised.

Table 2: **Incidence of any malaria among groups according to co-trimoxazole randomisations and ages**

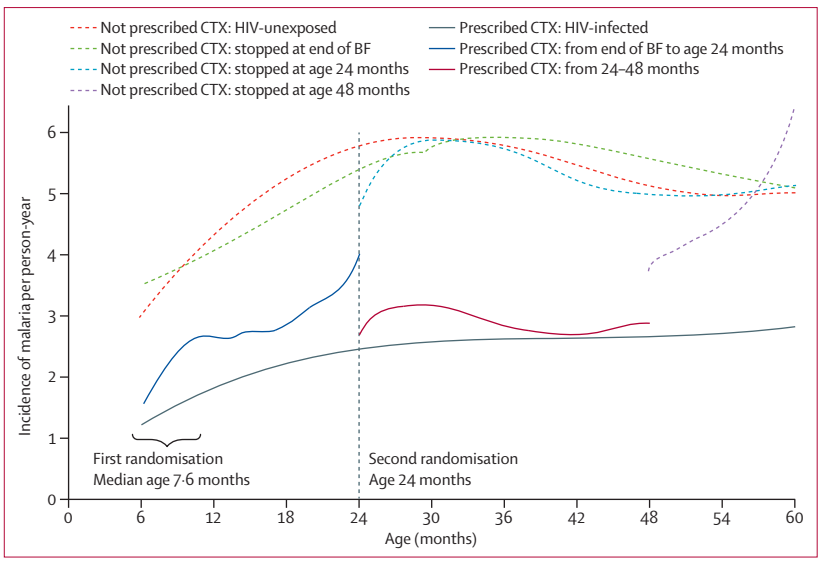

Figure 2: **Malaria incidence in relation to age for all groups**

CTX=co-trimoxazole. BF=breastfeeding. Full lines represent time when children were on CTX. Dotted lines represent time when children were not on CTX. The first randomisation of HIV-exposed children occurred at the end of breastfeeding, between 6 weeks and 9 months of age. The second randomisation of HIV-exposed children occurred at age 24 months.

	Age range	OR* (95% CI)	p value
HIV-unexposed: never on CTX	30–60 months	0·84 (0·78–0·90)	<0·0001
HIV-exposed: CTX stopped after breastfeeding	30–60 months	0·86 (0·79–0·93)	<0·0001
HIV-exposed: CTX stopped at age 24 months	30–60 months	0·86 (0·78–0·94)	<0·0001
HIV-infected: always on CTX	30–60 months	1·08 (0·83–1·39)	0·57
HIV-exposed: CTX stopped at age 48 months	48–60 months	1·78 (1·19–2·66)	0·005

OR=odds ratio. CTX=co-trimoxazole. *Daily odds of being diagnosed with malaria per 12 month increase in age within the indicated range using generalised estimating equations to measure the associations between increasing age and the daily risk of malaria for the different CTX exposure groups.

Table 3: **Daily risk of malaria with increasing age in relation to HIV status and exposure to co-trimoxazole prophylaxis**

Overall, from first randomisation after breastfeeding cessation to age 4 years, 133 children not prescribed co-trimoxazole had 5·50 (95% CI 5·25–5·76) malaria episodes per person-years, whereas 98 children prescribed co-trimoxazole had 2·92 (2·68–3·18) malaria episodes per person-years, representing a protective efficacy of 43% (IRR 0·57, 95% CI 0·47–0·69, p<0·0001). Thus, daily co-trimoxazole prophylaxis prevented an average of 2·63 episodes of malaria per year per HIV-exposed child between the ages of 2 years and 4 years.

To assess whether the protective efficacy of co-trimoxazole changed over time and whether discontinuation of co-trimoxazole prophylaxis was followed by a rebound incidence of malaria, we plotted malaria incidence after the first and second randomisations in relation to age (figure 2). Moreover, to complement table 2 and figure 2, we looked at associations between malaria incidence and age between ages 30 months and 60 months (table 3). There were no significant differences in malaria incidence in the year after randomisation between children who stopped co-trimoxazole prophylaxis after breastfeeding (odds ratio [OR] 0·84; 95% CI 0·78–0·90), at age 2 years (OR 0·86; 95% CI 0·79–0·93) and at age 4 years (OR 0·86; 95% CI 0·78–0·94; table 3). The difference in malaria incidence between HIV-exposed children randomised after breastfeeding cessation or at age 2 years was constant with increasing age, as was the difference in incidence between HIV-unexposed children and HIV-infected children, and between these groups and the randomised HIV-exposed children (figure 2).

See Online for appendix

Malaria incidence in HIV-unexposed children who never received prophylaxis was similar to that in HIV-exposed children who discontinued co-trimoxazole at breastfeeding cessation, whereas malaria incidence among HIV-infected children who remained on co-trimoxazole until age 5 years was similar to that of HIV-exposed children who continued co-trimoxazole prophylaxis to age 4 years (table 2, figure 2). Malaria incidence between 48 months and 60 months of age was stable or decreased for HIV-exposed children who discontinued co-trimoxazole at breastfeeding cessation or age 2 years, whereas it rose rapidly for children who discontinued co-trimoxazole at age 4 years (OR 1·78, 95% CI 1·19–2·66; p=0·005; figure 2). In keeping with these patterns, table 3 shows a significant and consistent association between increasing age and decreasing risk of malaria from 30–60 months of age for HIV-unexposed children and HIV-exposed children who stopped co-trimoxazole after breastfeeding cessation or age 2 years. By contrast, increasing age was associated with increased risk of uncomplicated malaria between 48–60 months of age in HIV-exposed children who stopped prophylaxis at age 48 months, whereas there was no significant association between age and risk of any malaria among HIV-infected children aged 30–60 months taking co-trimoxazole prophylaxis (table 3). None of the children who were on extended co-trimoxazole prophylaxis until 2 or 4 years of age had complicated malaria during follow-up from age 4 years to 5 years.

Few episodes of complicated malaria occurred. In the two intervention groups and two observational cohorts, only 59 of 5618 (1%) treated malaria episodes were complicated (appendix). Of these, 45 occurred in children not prescribed co-trimoxazole (incidence 5·2 per 100 person-years) compared with 14 episodes among children on co-trimoxazole (incidence 3·1 per 100 person-years) showing a 55% protective efficacy of co-trimoxazole against complicated malaria (IRR 0·45; p<0·0001).

We recorded 116 hospital admissions for all causes among all groups of children during the 5 years of follow-up, 83 (72%) of which occurred in HIV-exposed children.

The rate of admissions did not significantly differ between any group at any point during follow-up (appendix). Overall, 43 admissions occurred among HIV-exposed children on co-trimoxazole from enrolment to age 4 years, compared with 38 admissions in HIV-exposed children not on co-trimoxazole during the same period. We found no significant differences in the incidence of pneumonia or diarrhoea between the randomised prophylaxis groups at any age (appendix).

HIV-exposed children who remained on co-trimoxazole up to age 4 years had a 49% lower risk of grade 3 and 4 serious adverse events compared with HIV-exposed children who discontinued co-trimoxazole at age 2 years. This difference could be attributed to the significant difference in incidence of raised temperature at the time of malaria diagnosis (table 4). There was no significant difference between the groups of HIV-exposed children in either median haemoglobin levels at day 0 of each malaria episode, or mean changes in haemoglobin between day 0 and day 28 of malaria follow-up (data not shown). The incidence of neutropenia and thrombocytopenia from scheduled quarterly complete blood cell counts was not significantly different between groups (table 4). Lastly, 13 children died in total: two HIV-exposed children not on co-trimoxazole; five HIV-exposed children on co-trimoxazole; five HIV-infected children on co-trimoxazole; and one HIV-unexposed child never on co-trimoxazole (figure 1). No deaths were attributed to co-trimoxazole administration.

	Discontinued CTX prophylaxis at age 2 years	Continued CTX prophylaxis from age 2–4 years	IRR (95% CI)	p value
All grade 3–4 adverse events	215 (1·216)	111 (0·592)	0·51 (0·36–0·72)	<0·0001
Individual grade 3–4 adverse events*				
Raised temperature	133 (0·752)	48 (0·256)	0·35 (0·24–0·51)	<0·0001
Anaemia	67 (0·379)	51 (0·272)	0·69 (0·34–1·40)	0·31
Neutropenia	10 (0·057)	9 (0·048)	0·85 (0·34–2·15)	0·73
Thrombocytopenia	5 (0·028)	2 (0·011)	0·36 (0·06–2·33)	0·28
All serious adverse events	33 (0·187)	31 (0·165)	0·91 (0·42–1·98)	0·81

All data given as n (incidence per person-years at risk). There were 177 person-years at risk in the discontinued CTX group and 188 person-years at risk in the continued CTX group. CTX=co-trimoxazole. *Only includes children with at least five total events. †Raised temperature defined as tympanic temperature higher than 38°C.

Table 4: **Safety outcomes in HIV-exposed children randomly assigned to discontinue or continue CTX prophylaxis to age 4 years**

Discussion

This is the first randomised trial assessing the protective safety and efficacy of prolonged daily co-trimoxazole prophylaxis against malaria in HIV-exposed children to age 4 years (panel). Continuation of co-trimoxazole prophylaxis beyond the period of HIV exposure (end of breastfeeding) until age 4 years was safe and provided an overall protective efficacy against malaria of 43% in an area characterised by very high malaria transmission intensity and antifolate plasmodium resistance.[17] Moreover, compared with children not receiving prophylaxis, children receiving prophylaxis had a 55% lower risk of complicated malaria, a major cause of death in children younger than 5 years.

We noted no evidence of malaria incidence rebound in the year after co-trimoxazole prophylaxis when stopped after breastfeeding cessation or at age 2 years. Indeed, after children reached 30 months of age there was an inverse association between age and risk of malaria in HIV-unexposed children, and in HIV-exposed children who stopped co-trimoxazole after breastfeeding cessation or at age 2 years. These findings are probably due to the development of naturally acquired antimalarial immunity in these children,[35–37] and are consistent with findings from a 2009 pooled analysis of six randomised controlled trials, and two more recent trials on intermittent preventive therapy in infancy in Mozambique and

Panel: **Research in context**

Systematic review

We searched PubMed, Medline, the National Library of Medicine, and CAB Abstracts for papers or abstracts published between 2000 and 2014, in English and French, with the keywords: "co-trimoxazole", "malaria", "HIV", "children", "infants", "Africa", "prophylaxis", and "randomized". Although there have been no randomised controlled trials for the protective efficacy of co-trimoxazole prophylaxis against malaria in HIV-exposed uninfected infants apart from the first publication from this study,[17] co-trimoxazole prophylaxis has shown many benefits in HIV-infected and HIV-uninfected children in sub-Saharan Africa. In 2004, Chintu and colleagues[8] reported a 43% reduction in mortality in HIV-infected Zambian children aged 1–14 years randomly assigned to co-trimoxazole prophylaxis compared with placebo without increased adverse events and despite high levels of common bacterial resistance to co-trimoxazole. Shortly thereafter, Mermin and colleagues[31] showed that mortality for HIV-negative Ugandan family members living with an HIV-infected person decreased by 63% and these family members had a reduced incidence of malaria after the HIV-infected person started daily co-trimoxazole prophylaxis. In a setting of low antifolate resistance, Thera and colleagues[6] reported that co-trimoxazole prophylaxis among Senegalese children aged 5–15 years had a 99·5% protective efficacy against clinical malaria compared with no prophylaxis, and that co-trimoxazole did not induce antifolate resistance in plasmodia infecting these children. However, these children were followed up for 3 months and were not tested for HIV.[6] Kamya and colleagues[4] showed that co-trimoxazole prophylaxis in combination with bednets had a 97% protective efficacy against malaria in HIV-infected children aged 1–11 years in Kampala, Uganda. Moreover, several African observational studies have reported that HIV-exposed uninfected infants had significantly higher morbidity and mortality risks than did infants born to HIV-uninfected mothers, and that this risk increased with advanced HIV disease in the HIV infected mothers, prompting strong calls for child survival interventions, including co-trimoxazole prophylaxis for all HIV-exposed children.[32–34] None of these studies, nor a trial to assess the safety and efficacy of nevirapine prophylaxis in combination with co-trimoxazole prophylaxis until breastfeeding cessation in HIV-exposed children,[3] reported evidence of adverse events associated with co-trimoxazole prophylaxis.

Interpretation

Our randomised comparison of HIV-exposed children allocated to receive different durations of co-trimoxazole prophylaxis reliably reinforce existing evidence that continuing co-trimoxazole prophylaxis in HIV-exposed children beyond the period recommended by WHO up to age 4 years was safe and efficacious to prevent malaria.

Kenya.[37–39] In these studies, no substantial rebound effect occurred after intermittent preventive therapy in the second year of life of the children who received it. However, none of these trials followed up children beyond age 2 years or studied continuous daily prophylaxis. Our observation that malaria incidence increased significantly from age 4 years to 5 years in children who stopped co-trimoxazole at 4 years of age raises the possibility that extension of co-trimoxazole prophylaxis to age 4 years might have delayed the development of natural immunity. The curves in figure 2 all show an inflexion point around 36 months of age, suggesting that this might be the time of acquisition of natural immunity. However, the fact that neither the incidence of complicated malaria nor that of adverse events (data not shown) increased in the year after stopping of co-trimoxazole prophylaxis at any age suggests that the benefits of preventing of nearly half of all malaria infections earlier in life outweighs the risks associated with rebound. Why co-trimoxazole can have such a marked effect on malaria incidence in an area of high endemicity and high grade of multiple antifolate resistance to sulfadoxine–pyrimethamine is not entirely clear. Possible explanations include the fact that daily dosing of co-trimoxazole might achieve higher blood concentrations and longer periods of protection than does intermittent preventive therapy with sulfadoxine–pyrimethamine, and that resistant parasites do not have identical resistance profiles to co-trimoxazole and sulfadoxine–pyrimethamine.[40–42]

This trial had several limitations: although HIV-exposed treatment groups were randomly assigned, the HIV-infected and HIV-unexposed children were not randomised and thus were not directly comparable to HIV-exposed children. The self-reported nature of co-trimoxazole adherence, bednet use, and infant feeding practice might have been affected by respondent bias. At the time of the second randomisation, the baseline incidence of malaria was lower in HIV-exposed children randomised to continue co-trimoxazole from age 2 years to age 4 compared to those randomised to stop co-trimoxazole at age 2 years (table 1). Although this difference occurred by chance, it might have resulted in a slight overestimation of the protective efficacy of co-trimoxazole in HIV-exposed children between age 2 years and age 4 years. There were also insufficient complicated malaria episodes to allow for meaningful statistical comparisons of this outcome across the HIV-exposed intervention groups and the two observational cohorts. Safety outcomes were not systematically collected for the observational cohorts or for HIV-exposed children after discontinuation of co-trimoxazole prophylaxis at age 4 years. Thus, safety outcomes could not be compared across the HIV-exposed intervention groups and the two observational cohorts. Although the trial was not designed to assess the efficacy of co-trimoxazole against bacterial infections or the development of resistance to bacterial pathogens potentially attributable to co-trimoxazole, we noted no significant differences in the incidence of pneumonia or diarrhoea between the randomised prophylaxis groups at any age (appendix) and ample evidence exists to show that the efficacy of continuous co-trimoxazole prophylaxis in HIV-infected children is neither affected by, nor increases, pathogens' resistance to the drug.[6,8,43] Lastly, we did not follow up children beyond age 5 years, thereby limiting our ability to assess further the effect of stopping prophylaxis on malaria incidence and rebound in older children.

The 2013 Consolidated WHO Guidelines for Treating and Preventing HIV Infection recommend to start lifelong triple antiretroviral drug therapy (ART) in HIV-infected pregnant women, irrespective of WHO clinical stage or CD4 cell count.[2] When it achieves virological suppression, ART started in pregnancy can reduce the risk of antenatal and intrapartum HIV transmission from infected mother to infant by more than 98%,[44] and is expected to cause a similar reduction in the cumulative risk of mother-to-child HIV transmission through breastfeeding. As these guidelines are widely implemented, the number of children infected by their mother will greatly decrease, and the number of HIV-exposed uninfected children will increase accordingly. Given the lack of evidence regarding the protective efficacy of co-trimoxazole prophylaxis against diarrhoea and respiratory infections in HIV-exposed uninfected children, the benefits of the standing recommendation for these children have been questioned and the potential risks of toxicity and resistance raised.[45,46] Our previous and present findings, as well as the studies reviewed, clearly establish the substantial protection co-trimoxazole prophylaxis confers against malaria for all children, irrespective of HIV status and antifolate resistance levels.[47,48] As malaria continues to exert high mortality on all children younger than 5 years worldwide,[15,16,19,30,49] this combined evidence provides a rationale for safe and efficacious continued co-trimoxazole prophylaxis against malaria in HIV-exposed children up to at least 2 years of age and possibly to 4 years if the benefits of malaria prevention between ages of 2 years and 4 years outweigh the risk of malaria incidence rebound after 4 years of age. This could prove to be a simple and highly cost-effective measure to reduce drastically the public health burden and social impact of malaria in these children and their families in areas where malaria is endemic. An important implementation question would be whether to continue recommending daily co-trimoxazole or a better antimalarial for prophylaxis given the possibility of emergence of additional high-level antifolate resistance-mediating mutations that might follow the widespread use of co-trimoxazole prophylaxis.[21] In the high malaria transmission intensity setting of this study, intermittent preventive therapy with artemisinin-based combination therapy in infants has been shown to provide 58% protective efficacy as compared with no chemoprevention

in HIV-unexposed infants aged 6 months to 2 years.[48] It remains to be seen whether similar combinations are equally safe and effective for children up to 5 years old.

In any event, innovative means to ensure practical implementation and monitoring of malaria chemoprophylaxis, whether extended to age 2 years or beyond, and of other health, nutritional, and environmental interventions[50] that play an important role in malaria prevention, are critically needed to close the wide gap that currently exists between the millions of HIV-exposed and infected children who need co-trimoxazole prophylaxis worldwide and the less than 10% of those who are presently taking it.[1,51]

Contributors

JH, GD, and JWT co-conceived and designed the study, developed the study proposal and protocol, planned study implementation, and analysed and interpreted the data. GD also overviewed study implementation and data collection, and did all data analyses. EA coordinated study implementation and data collection. HW, AK, VB and MM implemented the study and collected all the data. MRK and TGS participated in planning and coordinating the study, developing the protocol, and analysing and interpreting the data. JH and JWT wrote the Article. All authors reviewed the manuscript.

Declaration of interests

We declare no competing interests.

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