

BMJ Open Randomised controlled trial testing the effect of cotrimoxazole prophylaxis on morbidity and mortality outcomes in breastfed HIV-exposed uninfected infants: study protocol

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To cite: Coutsooudis A, Daniels B, Moodley-Govender E, *et al*. Randomised controlled trial testing the effect of cotrimoxazole prophylaxis on morbidity and mortality outcomes in breastfed HIV-exposed uninfected infants: study protocol. *BMJ Open* 2016;**6**:e010656. doi:10.1136/bmjopen-2015-010656

► Prepublication history for this paper is available online. To view these files please visit the journal online (<http://dx.doi.org/10.1136/bmjopen-2015-010656>).

Received 24 November 2015
Revised 17 May 2016
Accepted 17 May 2016



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ABSTRACT

Introduction: No randomised controlled trial (RCT) has examined the efficacy of cotrimoxazole (CTX) prophylaxis in HIV-exposed uninfected (HEU) infants during the breastfeeding period, in this new era of effective prevention of mother-to-child transmission (PMTCT) prophylaxis. The efficacy of CTX prophylaxis has presently been demonstrated only in HIV-infected children. The absence of proven benefits in HEU breastfed infants associated with infectious diseases justifies an RCT as proposed. Herewith lies the rationale for conducting the proposed study.

Methods: A partially blinded RCT is proposed to evaluate the efficacy of CTX prophylaxis administered from 6 weeks of age to HEU infants receiving a PMTCT regimen. A non-inferiority design will be used, randomising 1298 infants to receive CTX or not to receive CTX. Participants will be reviewed at the following time points: 6 weeks (enrolment and randomisation), 10 weeks, 14 weeks, 4 months and monthly thereafter until 12 months of age. They will be evaluated for anthropometric growth, interval illness, CTX adherence, signs and symptoms of study drug toxicity, concomitant medication use, breastfeeding status and HIV infection status. The study will compare the incidence of grade 3 and grade 4 common childhood illnesses (focusing on pneumonia and diarrhoea) and all-cause mortality until 12 months of age. In a subset of participants, we will compare grade 3 and grade 4 haemoglobin and alanine aminotransferase results as well as investigate gut integrity.

Ethics and dissemination: The study has ethical approval from the University of KwaZulu-Natal Biomedical Research Ethics Committee (BFC212/13).

Trial registration numbers: PACTR201311000621110 and DOH-27-0614-4728; Pre-results.

INTRODUCTION

Currently, the WHO guidelines recommend cotrimoxazole (CTX) prophylaxis

Strengths and limitations of this study

- This is the first randomised controlled trial testing the effect of cotrimoxazole (CTX) prophylaxis in breastfeeding HIV-exposed uninfected infants.
- The study outcomes could result in the possible reduction in inappropriate antibiotic use.
- The study could reduce the burden of healthcare workers and the burden on infants' caregivers.
- No placebo will be used for this study.
- Changes in prevention of mother-to-child transmission programmes and breastfeeding promotion efforts could introduce temporal trends.

for HIV-infected infants, as well as HIV-exposed uninfected (HEU) infants. This recommendation is based on the efficacy data from a single trial studying untreated HIV-infected children¹ who were protected against infection and death from *Pneumocystis pneumonia* (PCP). The policy to advise CTX for HEU infants was developed in the context of limited access to HIV diagnostic testing to identify HIV-infected infants, and the use of prevention of mother-to-child transmission (PMTCT) drug interventions with lesser efficacy than the current regimens. In the current era of greatly expanded access to infant HIV testing, HIV-infected infants are identified early and placed on lifelong antiretroviral treatment (ART) and CTX prophylaxis. Additionally, PMTCT regimens are now vastly more effective and accessible such that only 1–2% of infants born to HIV-infected mothers are expected to be infected intrauterine, intrapartum and through breastfeeding. Having such a small number of infants expected to be infected

with HIV, and now being identified by healthcare systems, it is imperative to reconsider the impact, cost-effectiveness and appropriateness of a public health 'blanket' approach of providing CTX prophylaxis to all infants born to HIV-infected mothers. This proposal has been prompted by two policy briefs^{2 3} encouraging re-consideration of the guidelines for CTX prophylaxis in HEU infants, as well as the recent WHO World Health Day call⁴ for renewed attention to the appropriate use of antibiotics to contain antimicrobial resistance which focuses specifically on the effectiveness of CTX in breastfed HEU infants.

Benefit of breastfeeding for HEU infants that could negate the need for CTX prophylaxis

Human breastmilk has significant protective benefits against infections, due to its immune properties.⁵⁻⁷ CTX prophylaxis is, therefore, likely to confer little additional advantage in the HEU population who accessed adequate PMTCT services and are breastfed.

Breastmilk induces a gut microbiota rich in bifidobacteria, which contributes to the development of immune responses⁶ and a lower incidence of diarrhoea and allergy in breastfed infants compared to formula-fed infants.⁸ Epidemiological studies in resource-limited settings reveal that artificially fed infants were at threefold to 10-fold higher risk of infection, particularly enteric or intestinal infections, resulting from pathogens such as harmful bacteria, fungi, parasites and viruses.^{9 10} A systematic review and meta-analysis demonstrated that formula-fed infants had a 14-fold higher risk for all-cause mortality and an eightfold increased risk for infection-related mortality than exclusively breastfed infants.¹¹ Evidence exists that growth faltering in Gambian infants was associated with impaired gut intestinal barrier function.¹² There seems to exist a dynamic interplay between breastmilk and gut microbiota.¹³ Furthermore, it is suggested that the milk microbiome may modulate the human milk composition and therefore influence the immune components of breastmilk.¹⁴ The use of antibiotics may also influence the type of microorganisms that colonise the infant's gastrointestinal tract.^{15 16} Findings are increasing that indicate the short-term and long-term implications of perturbations of the microbiota of young infants, which may predispose them to the risk of lifelong disease related to immune modulation.¹⁶⁻²⁰

Benefit of CTX prophylaxis for HEU infants

There lies equipoise whether CTX prophylaxis is able to prevent common childhood infections (other than PCP) and improve health outcomes in HEU infants. It is understood that even if CTX is no longer needed for PCP prophylaxis in HEU infants, it would be prudent to retain CTX prophylaxis so that it can protect against infections, as HEU infants are thought to be at an increased risk compared to non-HIV-exposed infants in terms of morbidity and mortality. Studies have

consistently observed immune abnormalities in HEU infants²¹⁻²⁶ as well as increased risk of disease and death.²⁷⁻²⁹ However, there is debate about whether these vulnerabilities will persist with improvements in maternal health with lifelong ART and if they do, it is by no means clear that CTX prophylaxis would reduce them. A recent, as yet unpublished, report of a randomised controlled trial (RCT) testing the efficacy of CTX prophylaxis in reducing mortality in 2848 HEU infants in Botswana showed no significant difference in the cumulative mortality by 18 months of age.³⁰

Several studies have demonstrated variable effectiveness of CTX prophylaxis against bacterial infections and malaria in select populations.³¹⁻³⁵ Of note, is a randomised trial of CTX prophylaxis conducted in HEU infants from Uganda that showed a reduction in malaria incidence rates, but CTX prophylaxis did not show an effect on secondary outcomes such as diarrhoea and pneumonia. Infants in the CTX arm had an incidence of 1.96 diarrhoea episodes/person-year (95% CI 1.67 to 2.27) compared to 1.83 (95% CI 1.57 to 2.10) in the no CTX arm. Incidence of respiratory tract infections was 0.32 episodes/person-year (95% CI 0.22 to 0.45) for infants receiving CTX compared to 0.23 (95% CI 0.14 to 0.36) in the group not receiving CTX. Furthermore, the rates of mortality and hospitalisation were higher in the CTX group compared to the control arm (10.2% vs 5.7%—difference not significant as not powered for this secondary objective).³⁶ Importantly, this trial was initiated only after cessation of breastfeeding and thus did not test the efficacy of CTX prophylaxis on breastfed HEU infants. A study in Malawi³⁴ examining the association of breastfeeding cessation on morbidity and mortality as part of a secondary analysis examined the impact of standard-of-care CTX on any illness or hospitalisation. Although as part of the standard of care, not all infants received CTX and hence the study was able to examine only the association with CTX prophylaxis during the period 12–15 months of age. CTX prophylaxis was associated with a 23% reduction related to illness or hospitalisation (RR 0.77, 95% CI 0.63 to 0.92). As this was a secondary analysis of data and not an RCT, it is possible that CTX was a proxy for other aspects of care and that the infants who accessed CTX prophylaxis were in fact different from those who did not access prophylaxis—for example, access could have been an indicator of improved maternal care and/or increased access to general healthcare. Secondary analysis of data from the Malawian BAN study reported that CTX prophylaxis was associated with a short-lived positive effect on malaria; however, there was no protection against the combined outcome of severe illness or death.³⁷ However, a subsequent report from this study where Kourtis *et al*³⁸ examined outcomes separately (severe febrile illness, diarrhoea/growth faltering or death) with all such events (and not just the first) contributing through conditional gap time models showed that infant CTX significantly decreased morbidity

namely malaria (HR 0.33); diarrhoea (HR 0.64) and pneumonia (HR 0.8). Two preliminary observational studies in Durban, South Africa,^{31 32} conducted secondary analyses of data and reported a non-significant reduction in the frequency of lower respiratory tract infections and a non-significant increase in risk of diarrhoea. Finally, as already mentioned, the presentation of results of the Botswana study at CROI 2016 reported no benefit of CTX prophylaxis on mortality. Of note, this was despite 80% of these infants being formula fed and presumably at higher risk of diarrhoea and pneumonia.³⁰

The details of the studies mentioned above are summarised in [table 1](#).

Whatever the efficacy of CTX prophylaxis on HEU infants, policymakers need to consider the cost-effectiveness of providing an intervention such as this to a population located in settings where growing PMTCT programmes result in relatively few exposed infants becoming infected. If CTX prophylaxis in HEU infants is effective in protecting against malaria, as has been shown in HIV-infected children and adults, then the question remains whether or not all children in malaria endemic settings would benefit from CTX prophylaxis rather than restricting the intervention to HIV-exposed children only. Additionally, one needs to consider the cost and the obvious logistical difficulties of providing daily CTX compared with other perhaps more targeted interventions of proven efficacy, such as intermittent malaria chemoprophylaxis provided at the time of childhood vaccinations or seasonal malaria chemoprophylaxis. It may be more practical and cost-efficient to use drugs specifically targeted for malaria.

Nevertheless, this particular study will be undertaken in a non-malaria area (South Africa) to assess whether or not there are benefits of CTX prophylaxis for severe morbidity and mortality unrelated to malaria.

Hypotheses

This RCT will examine the following two main hypotheses:

1. The breastfed HEU infant who does not receive CTX will not have an inferior outcome in terms of incidence of grade 3 or grade 4 common childhood illnesses or mortality.
2. The breastfed HEU infant who is exposed to CTX will experience a disruption in the normal development of gut immunity and changes in the gut microbiota compared to the infant who is not exposed to CTX.

In summary, the efficacy of CTX prophylaxis has been strongly demonstrated in only HIV-infected children and for the prevention of malaria in HEU infants. The absence of proven benefit in HEU breastfeeding infants in relation to other (non-malaria) infectious diseases thus justifies an RCT, as proposed.

METHODS AND ANALYSIS

Study design

A randomised trial is proposed to evaluate the efficacy of CTX prophylaxis from 6 weeks of age given to HEU infants receiving a PMTCT regimen. A non-inferiority hypothesis will be tested. The study is registered with the Pan African Clinical Trials Registry (PACTR201311000621110) and the South African National Clinical Trials Registry (DOH-27-0614-4728).

Study participants will be randomised to receive CTX from 6 weeks of age until confirmed HIV-negative 6-week post cessation of breastfeeding or not to receive CTX. It is noted that a placebo-controlled study would provide stronger results, but the investigators are apprehensive that even the use of a placebo (which is usually a sugar solution) has the potential to interfere with the gut and disrupt the benefits of exclusive breastfeeding, thus a placebo will not be administered. Additionally, the time and cost involved with developing a colour-matched and taste-matched placebo that did not contain sugar was prohibitive. Furthermore, as we are investigating only grade 3 and grade 4 clinical events and mortality, doctor bias ascertainment of end points is unlikely. The laboratory staff will, however, be blinded to the study arm for the blood and stool analyses.

Study randomisation and blinding

Block randomisation was used with random block sizes, which were computer-generated. Study randomisations were divided into a large number of blocks of sizes 2, 4, 6 or 8 and randomisation was performed within each block by a statistician external to the study team. Randomisation allocations were then put into sequentially numbered, sealed opaque envelopes by the study Principle Investigator. Groups of 20 sequentially numbered envelopes are then placed into a randomisation box by study counsellors, from which mothers randomly choose an envelope. The randomisation process is explained to the mother by the study counsellor, who also issues the CTX to the mother at each visit. The study counsellor asks study adherence questions at every follow-up visit. In addition, mothers are to be thoroughly counselled and reminded not to discuss the infants' study arm with the clinical team at every follow-up visit. Only the mother and study counsellor are aware of the infant's randomisation arm and clinical and laboratory staff remain blinded at all times. In addition, case report forms filled in by the clinician do not have any questions pertaining to the receipt of CTX.

Main objectives and end points

Primary objective

The primary objective is to compare the incidence of grade 3 and grade 4 common childhood illnesses or all-cause mortality until 12 months of age in HEU infants receiving CTX or no CTX. For the proposed study, common childhood illnesses are defined as pneumonia and diarrhoea.

Table 1 Summary of studies in HEU infants investigating the effect of CTX prophylaxis on general morbidity

Country (years)	Sample size and study population	Type of study	CTX outcomes
Uganda (2007–2008) ³³	185 HIV-exposed, uninfected infants who have received CTX prophylaxis while breastfeeding	Non-blinded RCT to evaluate the protective efficacy of CTX prophylaxis against malaria in HIV-exposed children. All children who remained HIV uninfected (n=185) after breastfeeding cessation were then randomised to stop CTX prophylaxis immediately or continue CTX until 2 years old.	CTX prophylaxis yielded a 39% reduction in malaria incidence, after adjustment for age at randomisation (incidence rate ratio 0.61 (95% CI 0.46 to 0.81), p=0.001). There were no significant differences in the incidence of complicated malaria, diarrhoea, pneumonia, hospitalisations or deaths between the two treatment arms.
Malawi (2004–2010) ³⁷	1522 Infants born to HIV-infected mothers as part of the BAN study	RCT evaluating the effect of a maternal nutritional supplement in addition to a three-group antiretroviral intervention (triple-drug antiretroviral regimen for the mother (maternal-regimen group), daily dose of NVP for the infant (infant-regimen group) or neither (control antiretroviral group)).	Secondary analysis: a significant decrease in malaria with CTX; no significant difference in diarrhoea, pneumonia or severe illness/death.
Malawi (2004–2010) ³⁸	2250 infants born to HIV-infected mothers as part of the BAN study	A later analysis of data from the BAN study above included more infants. All 2250 infants who had information on the outcomes of interest. Additionally, all outcomes were examined separately with all such events contributing through conditional gap time models—provided a more accurate analysis.	Secondary analysis: infant CTX significantly decreased morbidity, namely malaria (HR 0.33); diarrhoea (HR 0.64) and pneumonia (HR 0.8).
Malawi (2004–2009) ³⁴	1543 infants born to HIV-positive mothers	PEPI-Malawi study was a randomised clinical trial to assess efficacy of extended infant antiretroviral prophylaxis to reduce postnatal HIV transmission. All received CTX prophylaxis.	Secondary analysis: lower risk of illness and/or hospital admission with CTX (OR 0.56 at 6–9 months; OR 0.65 at 9–12 months and OR 0.77 at 12–15 months).
South Africa (2003–2010) ³¹	480 breastfed infants who tested negative for HIV at 6 weeks of age.	Assessed the impact of CTX on diarrhoeal and respiratory morbidity in breastfed, HIV-exposed-negative infants in a community. CTX was received by 50.8% of infants for >60 days, whereas the remainder for 60 days or less, and the median duration of breastfeeding was 181 days.	Use of CTX for >60 days showed no consistent evidence of benefit for LRTI, although the incidence rate ration (IRR) was lower (0.71) and the CIs were wide in both directions (95% CI 0.39 to 1.26; p=0.241). Use of CTX for >60 days was associated with an increased risk of diarrhoea (IRR=1.38, 95% CI 0.98 to 1.94; p=0.065).
South Africa ³²	363 infants (HIV infected and uninfected) born to HIV-positive mothers	Prospective observational cohort study.	HIV-infected infants who received CTX had significantly lower incidence of LRTI (82%); but effect not seen in HEU infants. In HIV-infected and uninfected infants, there was a non-significant increased risk for diarrhoea in those who received CTX: in infected OR=1.58, p=0.45 and in uninfected infants OR=1.52, p=0.10.

Continued

Table 1 Continued

Country (years)	Sample size and study population	Type of study	CTX outcomes
Uganda (1997–2001) HIVNET012 (2004–2007) HIVIGLOB/ NVP ³⁶	HIVNET012—623 HIV-exposed infants, breastfeeding for at least 6 months with no CTX prophylaxis HIVIGLOB/NVP—684 HIV-exposed infants breastfeeding for 3–6 months with abrupt weaning and receiving CTX prophylaxis	Two separate RCTs to assess the effect of NVP regimens on PMTCT.	Overall rates of serious gastroenteritis events were highest in the HIVIGLOB/NVP study with CTX at 8.0 events per 1000 child-months (95% CI 6.4 to 9.8), whereas in the HIVNET012 group with no CTX there were 3.1 events per 1000 child-months (95% CI 2.1 to 4.4) which was statistically significant ($p < 0.001$).

BAN, Breastfeeding, Antiretrovirals and Nutrition; CTX, cotrimoxazole; HEU, HIV-exposed uninfected; LRTI, lower respiratory tract infections; NVP, Nevirapine; PMTCT, prevention of mother-to-child transmission; RCT, randomised control trial.

Secondary objectives

1. To compare the incidence of grade 3 and grade 4 diarrhoeal events until 12 months of age in the group receiving CTX versus no CTX.
2. To compare the incidence of grade 3 and grade 4 pneumonia events until 12 months of age in the group receiving CTX versus no CTX.
3. To compare the incidence of growth faltering events until 12 months of age in the group receiving CTX versus no CTX.
4. To compare the incidence of grade 3 and grade 4 haemoglobin (Hb) and plasma alanine aminotransferase (ALT) measurements at 6 and 12 months of age in the group receiving CTX versus no CTX.

In a subgroup of participants

1. To assess inflammation of the gut by comparing concentrations of plasma soluble CD14 (sCD14) at 6 weeks, 4 months and 6 months in infants receiving CTX versus no CTX.
2. To compare the gut microbiota profiles and gut inflammation in stool samples at 6 weeks, 4 and 6 months in infants receiving CTX versus no CTX.

Primary end point

The primary end point for the proposed study will be a composite of grade 3 or grade 4 pneumonia and diarrhoea, or all-cause mortality until 12 months of age. Pneumonia and diarrhoea are classified as follows:

- ▶ Pneumonia—the presence of cough plus fast breathing and intercostal retractions with or without central cyanosis, chest radiograph confirmation not required.
- ▶ Diarrhoea—the presence of frequent loose stool with some or severe dehydration, identification of bacterium not required.

Secondary end points

The secondary end points compare the following until 12 months of age:

- ▶ The incidence of grade 3 and grade 4 diarrhoeal events until 12 months of age in the group receiving CTX versus group not receiving CTX.
- ▶ The incidence of grade 3 and grade 4 pneumonia events until 12 months of age in the group receiving CTX versus group not receiving CTX.
- ▶ The incidence of growth faltering (weight-for-age and/or height-for-age Z-scores below -2) at 6 and 12 months of age in the group receiving CTX versus group not receiving CTX.

Grade 3 and grade 4 pneumonia and diarrhoea events are defined in [table 2](#).

Major study criteria

Inclusion criteria

- ▶ Infant born to a woman with HIV infection.
- ▶ Written informed consent to enrol infant into study obtained from the mother.
- ▶ Infant age ≤ 6 weeks (between day birth –6 weeks) at study entry.
- ▶ Infant should be breastfeeding at the time of screening and should be planning to breastfeed for at least 6 months.
- ▶ Infant HIV-negative from a screening HIV PCR laboratory test performed prior to study entry.

Table 2 Definitions of grade 3 and grade 4 diarrhoea and pneumonia events used for severity grading of adverse events

Severity grade	Grade 3	Grade 4
Pneumonia	Cough plus fast breathing and intercostal recessions without central cyanosis	Cough plus fast breathing and intercostal recessions with central cyanosis
Diarrhoea	Frequent liquid stools with some dehydration	Frequent liquid stools with severe dehydration

- ▶ Mother (and/or infant) should have received a PMTCT regimen and/or mother should be receiving lifelong ART.
- ▶ Singleton birth.
- ▶ No clinically observed genetic disorders.
- ▶ Birth weight >2000 g.
- ▶ Infant has not had any illnesses and has not received prior antibiotics or traditional medications.

Exclusion criteria

- ▶ Indeterminate HIV test result at screening.
- ▶ Receipt of antibiotics or traditional medications prior to screening visit.
- ▶ A known requirement for CTX prophylaxis prior to study entry.
- ▶ A known contraindication for CTX prophylaxis prior to study entry.
- ▶ Infants with any major serious illness (eg, heart, liver and kidney disease) or congenital malformation.

Treatment regimen

Infants will be randomised 1:1 to either arm at study entry:

- ▶ Arm 1: CTX until all exposure to HIV has ceased and the infant is confirmed to be HIV uninfected (until 6 weeks after last exposure to breastmilk); <6 months old or <5 kg: 20 mg trimethoprim/100 mg sulfamethoxazole orally and ≥6 months old or 5–15 kg: 40 mg trimethoprim and 200 mg methoxazole orally; once daily.
- ▶ CTX will be supplied from the standard clinic drug stocks.
- ▶ Arm 2: no CTX.

Participants and study visits

A cohort of 1298 infants born to HIV-infected mothers and enrolled in PMTCT follow-up and who test PCR-negative at ≤6 weeks of age will be eligible. Further eligibility criteria include that mothers are on lifelong ART and still breastfeeding at 6 weeks of age. The duration of study follow-up for all participants will be from 6 weeks until 12 months of age. Infants who test HIV-positive during the 12-month study period will be administered CTX, as per WHO guidelines and will be referred for ART and care. Infant feeding counselling will be conducted as per standard WHO and National Young Infant and Child Feeding guidelines.

The mother–infant pairs will be recruited from PMTCT programme of the Lancers Road and Cato Manor Clinics, which are under the jurisdiction of the eThekweni Municipality Health Unit, Durban and the KwaZulu-Natal Department of Health, respectively.

A prescreening standard laboratory HIV PCR test will be administered at birth or any age prior to 6-week enrolment visit. Only infants who test HIV PCR-negative will be eligible to enrol in the study. At the screening visit, the mothers sign an informed consent document. Thereafter, a detailed screening questionnaire is completed, which covers PMTCT information, infant feeding

questions and questions about the infant's health and medicinal intake. The infants 'Road to Health' booklet is also checked for any exclusionary criteria at screening and at the enrolment visit to confirm the eligibility criteria at screening and enrolment. The questionnaire is administered by the study counsellor, but the study clinician will also go through study eligibility criteria with the mother when examining the infant. At 6 weeks, participants will be randomly allocated to a study arm using a self-selected card system. Infants will undergo clinical examination and anthropometric measurements will be recorded. Blood and stool samples (only from a subgroup of participants) will be collected and then CTX will be dispensed to the intervention group. Study participants will be reviewed at the following time points: 6 weeks (enrolment and randomisation), 10 weeks, 14 weeks, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months and 12 months. They will be evaluated for interval illness, signs and symptoms of study drug toxicity, drug adherence, concomitant medications, breastfeeding status, HIV infection status and anthropometric growth. At the 6-month and 12-month visits, a blood draw will be administered to test for concentrations of ALT (a marker denoting side effects of CTX) and serum will be stored for later measurement of markers of infant immunity (antibody responses). The full schedule of evaluations being undertaken at each visit is presented in [table 3](#). Participants who acquire HIV infection on study will be referred for HIV care and treatment on site and those who are in the control arm will be started on CTX. Participants who experience a study event will continue on treatment per assignment, unless the site investigator and protocol team consider otherwise.

In a subsample of the first 100 participants who are enrolled and who give informed consent, blood and stool samples will be collected at 6 weeks, 4 months and 6 months as per [table 3](#), to conduct testing on the gut inflammation. Changes in the gut permeability can be elucidated by microbial translocation, which occurs when there is translocation of gut-derived microbes or microbial products into the systemic circulation with or without overt bacteraemia. CTX prophylaxis could increase intestinal membrane permeability, as it has been associated with increased plasma lipopolysaccharide (LPS) levels.³⁹ LPS binds to the glycoprotein CD14, which exists in a membrane-bound form and in a soluble form. CD14 then moves the LPS to the TLR4/MD-2 complex in the plasma membrane, which in turn triggers the release of proinflammatory cytokines and type I IFN.⁴⁰ Soluble CD14, therefore, is used as an alternative marker of LPS-stimulated monocyte or macrophage activation.^{41 42} Microbial translocation can, therefore, be indirectly measured by the presence of sCD14, which denotes gut inflammation. This assay uses ELISA methodology and is quantifiable, thus reproducing accurate and comparable results.⁴³ These assays will be conducted in the Department of Paediatrics and Child Health Laboratory, UKZN School of Clinical Medicine. This study will also

Table 3 Schedule of evaluations

Evaluations	Screening*	Entry†	Weeks				Months		Unscheduled visits‡	Early discontinuation of study
			10 and 14	Month 4	Month 5	Month 6	7–11	Month 12		
Socioeconomic questionnaire		X								
Interval history, Signs/Sx		X	X	X	X	X	X	X	X	X
Anthropometric measurements		X	X	X	X	X	X	X	X	X
Breastfeeding status§	X	X	X	X	X	X	X	X	X	X
Adherence questionnaire			X	X	X	X	X	X	X	X
Full blood count¶		X				X		X		X
		Only in substudy of 100								
ALT+serum storage		X				X		X		X
		Only in substudy of 100								
HIV test (DNA PCR)††	X		X			X				X
(blood spot)								X		
HIV test (antibody)¶¶									X	
Plasma for sCD14 (only in substudy of 100)		X		X		X				
Stool sample—gut inflammatory markers (only in substudy of 100)		X		X		X				

*Screening may be conducted at any time point between birth and the opening of the visit window for study entry at 6 weeks of life, with the day of birth being study day 0.

†Study entry occurs at 6 weeks of life.

‡If an event of interest to the study is reported at an unscheduled or scheduled visit, study evaluations are performed in addition to any evaluations conducted to support the diagnosis. If the date of the event-driven visit falls within 7 days of the next scheduled visit, the evaluations due at the scheduled visit should be conducted on that day. If the date of the event-driven visit falls within 7 days of the previous scheduled visit, only evaluations not performed at the previous visit are required. The events of interest for this study are:

- ▶ signs or symptoms suggestive of grade 3 or grade 4 pneumonia,
- ▶ signs or symptoms suggestive of grade 3 or grade 4 diarrhoea,
- ▶ infant death.

§Breastfeeding status refers to current exposure to breastmilk—information collected on early introduction of solids or other fluids will allow compilation of duration of exclusive breastfeeding. It is reported at each study visit through breastfeeding cessation. If breastfeeding cessation is reported, the date of cessation should be recorded.

¶Complete blood count includes haemoglobin, haematocrit, WCC, differential count and platelet count.

**If the HIV initial test is positive, confirm with a repeat test on a second sample drawn on a different day at an event-driven visit.

††Children at 54 weeks of age should have an HIV antibody ‘rapid’ test performed. If positive, this should be confirmed using DNA PCR.

compare inflammation results from plasma samples to mRNA obtained from stool samples. For the stool analysis, 8–10 g of fresh stool specimen will be collected from disposable nappies using a spatula and placed into pre-labelled cryovials with screw top lids and frozen at -70°C for later determination of gut inflammation biomarkers and microbiome testing. The technology tests biomarker expression using droplet digital PCR methodology.⁴⁴ The development of techniques for characterising the bacterial microbiome also provides an opportunity to examine changes in the microbiota of infants in a non-invasive manner. The gut microbiota will be profiled in stool samples using functional metagenomic screens. The study proposes to utilise methodologies that sequence the bacterial 16s rDNA gene. The stool investigation will be conducted at the Manary Laboratory, Washington University, St Louis, Mississippi, USA. All these measurements will be performed blinded to the CTX study arm.

CTX side effects

Although some studies have documented adverse reactions to CTX,⁴⁵ the evidence is not clear. Reported side effects include rash, anaemia and liver toxicity although these appear to be rarely documented. This study will clinically monitor infants for side effects through full blood count, Hb levels and ALT concentrations that are indicators of any drug-induced liver toxicity. Any infant showing evidence of any side effects to CTX will stop CTX prophylaxis immediately, but will continue to be followed up in the study.

Standard laboratory assays

HIV DNA PCR testing is performed prior to their 6-week visit. Owing to changes in the South African National protocol, this changed from being any time between 1 and 6 weeks of age, to being performed at birth. Routine HIV DNA PCR tests will be performed by the National Health Laboratory Systems, as per the Department of Health guidelines. For all infants, additional PCR testing will be conducted at 6 months to ensure that we are able to detect any breastfeeding HIV transmissions early and treat infants accordingly. PCR tests will also be performed at the last study visit at 12 months of age.

A complete blood count is performed at week 6 (in the substudy participants), month 6 and month 12. Alanine aminotransferase (ALT) is also measured at week 6 (in the substudy participants), month 6 and month 12 or on early discontinuation of CTX/no CTX.

Confounding variables

Information will be collected on factors that could influence the health of the infant and thereby obscure the association with CTX. These will include socioeconomic status; maternal education level; infant feeding practices; delivery and birth history and maternal health. All analyses will be conducted as 'intent-to-treat'. If any of the confounding factors are unbalanced between the groups, then secondary analysis will adjust for these

variables. Changes in PMTCT programmes and breast-feeding promotion efforts could introduce temporal trends; however, this is taken account of, to some degree, by the use of an RCT method. Since this cannot be guaranteed, we will investigate in the analysis whether or not the randomisation led to unbiased groups and will adjust in the analysis for confounders if necessary.

Sample size and statistics

A sample size of 1298 (649 per arm) was calculated to show that no CTX prophylaxis is not inferior to CTX prophylaxis (considered standard of care) in terms of preventing morbidity and mortality. This sample size will have power of 0.90 and α of 0.025.

The following assumptions were made:

The event rate of the composite primary end point will be 7% at study month 12 in the CTX arm.

This statistic is based on data of such events from two studies of breastfed HEU infants in resource-limited settings in sub-Saharan Africa, which match the expected background in the proposed study. The MASHI study⁴⁶ reported 6.7% pneumonia, 4.9% diarrhoea and 6.7% mortality at 24 months in breastfed HEU infants. The HPTN 046 trial⁴⁷ reported 5% pneumonia, 5% gastroenteritis and 4% malaria at 18 months. The majority of infants in these studies breastfed only for 6 months. In the proposed study, in line with the recent WHO recommendations, adopted throughout Africa, the investigators accept that the majority of infants will breastfeed for at least 12 months; therefore, it is likely that the event rates will be lower and thus estimated to be 7%.

Non-inferiority will be evaluated based on the difference between the two arms in the probability of a primary end point event occurring over the course of follow-up. The investigators calculate a 5 percentage-point higher end point probability in the no CTX arm compared to CTX prophylaxis arms to be an acceptable bound within the assumed ranges of event rates. The choice of delta as large as 5 percentage points is based on the need to provide clear benefits for a country administering CTX prophylaxis, which although relatively cheap for the drug itself carries the same costs for implementation of any drug intervention. It is believed that if the benefit is not at least 5% more, then the money and effort put into this programme could rather be channelled into other proven public health interventions, for example, immunisation against pneumococcal respiratory infections and rotavirus diarrhoea; and the provision of insecticide-treated bed nets.

Accrual was anticipated to take place over 2 years, allowing for 12 months of follow-up and thus, total study duration of 3 years, after the first participant enrolls, is projected. However, to date, there are considerable delays in study recruitment because of less mothers than anticipated breastfeeding. The study now proposes 3 years of recruitment together with an additional year of follow-up. The study is expected to run through until the end of 2018.

Study monitoring

Interim safety and efficacy data monitoring

This study will be reviewed by a Data and Safety Monitoring Board (DSMB) that has been set up to review the safety and efficacy data at one time point during conduction of the study, when 50% of the samples have completed follow-up.

For the DSMB review, summaries provided to the DSMB will be broken down by a blinded treatment arm. Baseline and administrative data on study conduct will be reviewed, along with safety data and, if an efficacy (full) review is scheduled, the frequency and causes of death, as well as other components to the primary end point and major secondary end points, will be reviewed. If there are serious concerns about unexpected toxicities that may have impact on the ongoing management of participants, decisions about recommending whether to continue, modify or terminate a study arm will be based primarily on the data obtained for the study's primary end point.

Monitoring guidelines

In evaluating primary end point data for efficacy, a repeated CI (RCI) approach will be used. Monitoring guidelines for DSMB review recommendations will be specified only for early evidence of inferiority. The RCI will be calculated using a Peto-Haybittle type rule, so that a recommendation for modifying or stopping the study early for inferiority may be considered if the lower limit of the two-sided 99.9% CI on the difference in probabilities of the primary end point is greater than the non-inferiority margin.

Data analysis

Primary analyses will be intent to treat, including all participants who were randomised. All available follow-up data through the end point or end of their study follow-up period will be included. For those who are lost before the end of the study, only data accrued during the study period will be utilised and data will be censored at the last visit.

Descriptive data summaries will be presented by the treatment arm and, where appropriate, also by visit week and event (reason) type. The summaries will include Kaplan-Meier plots for time-to-event variables, frequency tables for categorical variables and tables/plots for summary statistics of continuously distributed variables.

Data will also be analysed according to the duration of CTX prophylaxis: CTX for <6 months versus CTX prophylaxis 6–12 months.

A non-inferiority analysis will assess the difference in the probability of a primary end point event by the end of follow-up. Assignment to the no CTX arm will be considered non-inferior for the primary end point if the upper bound of the two-sided 95% CI for the difference in probability of a primary end point event compared to CTX prophylaxis is ≤ 0.05 . The event probability by 12 months will be estimated by the Kaplan-Meier

method, which accounts for censored event times for participants who discontinue study follow-up without a prior event before 12 months.

Supportive analyses also will consider as-treated analyses that exclude data after discontinuation of study drug. For this trial, interpretation of the primary end point comparison depends on the actual implementation of the randomised study strategies. Intent-to-treat and as-treated analyses will be very similar if the randomised strategy is not abandoned and breastfeeding continues to 12 months. Switching to the other arm may be anticonservative in an intent-to-treat analysis for a non-inferiority study. In such a case, non-inferiority may be declared despite a true difference because of the inadequate implementation of the randomised study arms. Another complication that may affect the strength of the analysis is that CTX/no CTX will be discontinued 6 weeks after breastfeeding cessation, potentially diluting differences between the groups. This switching frequency will depend on breastfeeding duration. Data on feeding practices for the study participants and the study sites will be collected to help interpret the results from intent-to-treat and as-treated analyses.

Secondary analyses will investigate adherence with CTX as well as the role of potential confounders and effect modifiers (such as maternal age and socio-economic status). These analyses will be carried out using multivariate logistic regression models and stratification as appropriate.

The full protocol will be shared online with the publication of the primary results. Anonymised individual-level data will be made available after publication of the primary results to persons who write to Professor Anna Coutsooudis with legitimate requests for scientific purposes conditional on availability of resources at the time.

Study duration

Study participants will remain on study until 12 months of age, with study entry occurring at 6 weeks of age (window: birth to 49 days of life). Screening can take place at birth and any time prior 6 weeks.

ETHICS AND DISSEMINATION

As with any clinical trial that challenges current health guidelines, there are ethical issues that need to be considered.

Concern that we will not be following WHO and national guidelines to give CTX prophylaxis from 6 weeks of age

Current WHO recommendations, namely to provide prophylactic CTX to all HIV-exposed infants irrespective of confirmed HIV status, were developed at a time when HIV transmission rates were much higher. At the same time, it was programmatically challenging to recognise young infected infants early enough to selectively offer them CTX prophylaxis to reduce the

risk of death due to PCP. There have been substantial changes in the environment since these recommendations were formulated, namely, there are fewer infants becoming infected with HIV, early infant testing rates are higher than in the past, and effective ART is now available for infants that are found to be infected. Combined with the programmatic reality that coverage of CTX prophylaxis is commonly low among all HIV-exposed infants, there is a strong case to re-examine the validity of a blanket approach advocated in WHO and national guidelines. Testing these guidelines and providing definitive evidence would strengthen the resolve of countries to scale up implementation of such guidelines, which are currently poorly implemented in most countries,⁴⁸ and the previous recommendations were developed to provide benefit to HIV-infected children by providing prophylaxis to all exposed infants. Furthermore, CDC/WHO in conjunction with researchers from Lund University⁴⁹ presented data at the IAS 2011 conference showing a considerable variation in the incorporation of WHO CTX guidelines into national guidelines and the authors concluded that this highlights the need for ongoing evidence review. Furthermore, it may be argued that it is not ethical to continue committing limited resources to a poorly implemented intervention of questionable overall public health benefit.

Concern that HIV-infected infants may be put at risk if they are allocated not to receive CTX

Only HIV-negative infants are eligible for enrolment in the study; however, there is a possibility that an infant may later become infected during the study. This probability is very low since all infants will be covered by some PMTCT prophylaxis, which is one of the eligibility criteria of the study. Furthermore, we will have frequent HIV testing and clinical vigilance (study clinicians). Any infant testing positive will immediately be discontinued off study arm and started on antiretroviral therapy as well as CTX prophylaxis.

Despite the ethical considerations, we furthermore need to consider the public health implications of the study. There are two scenarios for the main outcome of the study. Blanket CTX provides no benefit to HEU infants versus blanket CTX providing benefit for diarrhoea or pneumonia. If blanket CTX provides no benefit: this would inform the updating of CTX guidelines such that CTX would only be provided to known HIV-infected infants or those suspected of being HIV infected on clinical grounds. This will provide benefits to public health by releasing resources for other programmes; less burden for caregivers; appropriate use of antibiotics and reducing development of resistance. If blanket CTX provides benefit for diarrhoea and/or pneumonia: policymakers will now have the necessary evidence for more financial and human resource investment to scale up CTX programmes since current uptake is very low. The guidelines would also indicate

that the indication for CTX prophylaxis is for reduction in a specific morbidity and we will have an effect size on which to base this recommendation. It may provide a basis to examine the same benefits in infants who are not born to HIV-infected mothers, that is, it may not be an HIV-specific effect and may have a benefit for all infants.

Trial results will be communicated first to the Ministry of Health, and if the trial results warrant a change in policy, a government-instituted communication strategy will be implemented to inform all HIV-infected mothers that their infants who are not infected will no longer need to receive CTX prophylaxis. Furthermore, the results will be presented in scientific meetings, congresses and published in peer-reviewed journals.

Finally to summarise, in the study context, with the safeguards put in place, the research poses no greater net risk than the standard of care. This study design is required and justified to investigate this seemingly blanket antibiotic usage. Further support is highlighted in Shah and Lie's paper as situations in which research such as testing interventions which are different from standard of care are justified.⁵⁰

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Acknowledgements The authors acknowledge the assistance in the early protocol design of Lynda Stranix-Chibanda (University of Zimbabwe); Camlin Tierney (Harvard School of Public Health); Lynette Purdue (NIAID) and the Infant Health Team of the IMPAACT PROMISE P1077BF Study. Open access publication of this article has been made possible through support from the Victor Daitz Information Gateway, an initiative of the Victor Daitz Foundation and the University of Kwa-Zulu Natal.

Contributors AC conceptualised the study. All authors contributed to the design of the study. AC and BD developed the first draft of the manuscript and contributed equally. LK provided advice on trial design, randomisation and data analysis plan. All authors approved the final draft of the manuscript.

Funding This study has been funded by the HIV Prevention Unit of the South African Medical Research Council for 3 years. BD and EM-G are supported by Postdoctoral Scholarships from the UKZN College of Health Sciences.

Competing interests None declared.

Patient consent Obtained.

Ethics approval University of KwaZulu-Natal Biomedical Research Ethics Committee.

Provenance and peer review Not commissioned; externally peer reviewed.

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