Cotrimoxazole Prophylaxis in HIV-Infected Pregnant Women and their Infants: Associations with Parasitemia, Common Illnesses and Birth Outcomes

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ABSTRACT

Anna Elizabeth Dow: Cotrimoxazole Prophylaxis in HIV-Infected Pregnant Women and their Infants:
Associations with Parasitemia, Common Illnesses and Birth Outcomes
(Under the direction of Annelies Van Rie, MD, PhD)

Cotrimoxazole prophylactic treatment (CPT) is recommended by the World Health Organization for prevention of opportunistic infections in adults and children. CPT is also recommended for HIV-exposed infants while they remain at risk of HIV acquisition through breastfeeding. The benefits of CPT have been well established in adults and HIV-infected children but limited information exists among HIV-infected pregnant women and HIV-exposed, uninfected infants, including whether CPT offers protection against malaria. Using data from a longitudinal study of prevention of mother-to-child transmission of HIV, we examined the effect of CPT, initiated at six weeks of age, on adverse health outcomes during the first 36 weeks of life in HIV-exposed uninfected infants, and the effect of CPT in HIV-infected pregnant women on birth outcomes, incident malaria during pregnancy, and CD4 cell count at 24 weeks postpartum.

Among HIV-exposed, uninfected infants, CPT was associated with fewer cases of incident malaria during the first 10 weeks of CPT exposure (hazard ratio (HR) 0.35, 95% confidence interval (CI): 0.21, 0.57), but not during the remaining 20 weeks of CPT use (HR 0.93, 95% CI: 0.67, 1.29). CPT did not offer protection against other serious illness, moderate or severe anemia, or underweight. Among HIV-infected pregnant women, CPT was not associated

with a protective effect against malaria after adjustment for confounding (adjusted HR 0.66, 95% CI: 0.28, 1.52), when compared to women receiving intermittent preventive treatment during pregnancy with sulfadoxine-pyrimethamine. CPT was not associated with a protective effect in analyses of low birth weight or preterm birth. CPT was associated with a lower CD4 cell count at 24 weeks postpartum, among women receiving antiretrovirals (-77.6 cells/μL, 95% CI: -125.2, -30.1) and among women not receiving antiretrovirals (-33.7 cells/ μL, 95% CI: -8.8, -58.6).

CPT appears to offer limited protection against malaria among HIV-exposed, uninfected infants. Compared to intermittent preventive treatment administered during the first two years of the study, CPT did not offer greater protection against malaria in HIV-infected pregnant women, or against low birth weight or preterm birth. CPT was associated with a lower CD4 cell count at 24 weeks compared to women not receiving CPT.

For the people living with and affected by HIV.

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LIST OF ABBREVIATIONS

AIDS	Acquired Immunodeficiency Syndrome
AOR	Adjusted Odds Ratio
ART	Antiretroviral Treatment
BAN	Breastfeeding, Antiretrovirals and Nutrition Study
CI	Confidence Interval
CPT	Cotrimoxazole Prophylactic Treatment
DBS	Dried Blood Spot
DNA	Deoxyribonucleic Acid
HAART	Highly Active Antiretroviral Treatment
Hb	Hemoglobin
HIV	Human Immunodeficiency Virus
HR	Hazard Ratio
IgG	Immunoglobulin G
IPTp	Intermittent Preventive Therapy during Pregnancy
IRR	Incidence Rate Ratio
ITN	Insecticide Treated Net
MTCT	Mother-to-Child Transmission
NVP	Nevirapine
OR	Odds Ratio
PCP	Pneumocystis Carinii Pneumonia
PCR	Polymerase Chain Reaction
PMTCT	Prevention of Mother-to-Child Transmission

RCT	Randomized Controlled Trial
RR	Rate Ratio
SAE	Serious Adverse Event
SP	Sulfadoxine Pyrimethamine
TB	Tuberculosis
VL	Viral Load
WHO	World Health Organization

CHAPTER ONE: SPECIFIC AIMS

The World Health Organization (WHO) guidelines recommend daily cotrimoxazole prophylactic treatment (CPT) for HIV-infected pregnant and non-pregnant adults with CD4 cell count <350 cells/μL or WHO stage III or IV, HIV-infected children, and HIV-exposed infants from 6 weeks of age until cessation of risk of HIV transmission and exclusion of HIV infection.[1] These guidelines are based on results of randomized controlled trials and non-experimental studies that demonstrated decreased incidence of severe events, hospitalizations and mortality in HIV-infected non-pregnant adults and children.[2-6]

Despite the fact that the WHO recommends CPT for HIV-exposed infants, data to justify treatment of this population are limited. There is some evidence that CPT may reduce the risk of lower respiratory tract infections[7] and pneumococcal colonization rates[8] in HIV-exposed children <18 months. Data on CPT in pregnant women are also scarce, though some evidence suggests that in addition to protecting the woman against opportunistic infections, CPT may reduce risk of poor birth outcomes. A study in Zambia found reduced odds of preterm birth (odds ratio (OR) 0.49, 95% confidence interval (CI): 0.24, 0.98) and a decrease in neonatal mortality from 9% to 0% (p=0.01) after CPT was introduced for women with CD4 cell counts less than 200 cells/μL. It is not known if the same benefits would be seen in women with higher CD4 cell counts.[9]

CPT in HIV-infected adults has also been associated with reductions in incidence of malaria,[2, 4, 10] and there is evidence of a similar effect in HIV-infected children. Findings

from a study of the effects of CPT and insecticide-treated bednets (ITNs) on HIV-infected children 1-11 years of age found the use of ITNs was associated with a 43% reduction in malaria incidence (rate ratio (RR) 0.57, 95% CI: 0.46, 0.71), and the combination of CPT and ITNs was associated with a 97% reduction in malaria incidence (RR 0.03, 95% CI: 0.01, 0.10).[11] A study of older HIV-uninfected children 5-15 years of age found CPT greatly reduced episodes of clinical malaria (RR 0.005, 95% CI: 0.00, 0.04).[12] Even though children under five are at greater risk of malaria compared to older children, there are no data on the effectiveness of CPT in reducing malaria incidence in young, HIV-exposed, uninfected children.

The Breastfeeding, Antiretrovirals and Nutrition (BAN) study provides an excellent opportunity to study the effectiveness of CPT in pregnant women with higher CD4 cell counts (> 200 cells/μL) and HIV-exposed, uninfected children. Although BAN began enrolling HIV-infected women and their infants in 2004, CPT was only provided from June 2006 onwards to women with CD4 cell counts <500 cells/μL and to HIV-exposed, uninfected children (from six weeks to 36 weeks of age).

Using the BAN data, we examined the effect of CPT in two populations. We determined the effects of CPT during the first 36 weeks of life in HIV-exposed, uninfected infants on the incidence of malaria, anemia, and hospitalization or death. We also examined the impact of CPT on women with CD4 cell counts <500 cells/μL, both during and after pregnancy. These analyses broadened our understanding of the effect of CPT on incidence of malaria and other health outcomes in HIV-infected pregnant women and their infants, which will allow for more informed decision making regarding future CPT guidelines and programs.

Specific Aim 1: Effects of CPT in HIV-exposed, uninfected children

Aim 1a. To evaluate time to first episode of malaria parasitemia (dichotomous blood smear positive) by CPT exposure status (no CPT vs. CPT from 6 weeks of age) in the first 36 weeks of life.

Aim 1b. To evaluate time to first hospitalization or death, moderate or severe anemia, or malnutrition by CPT exposure status (no CPT vs. CPT from 6 weeks of age) in the first 36 weeks of life.

Rationale: Although the WHO recommends CPT for all HIV-exposed children until cessation of breastfeeding and exclusion of HIV infection, there are minimal data available on the effect of CPT on incidence of poor health outcomes, including malaria, in this age range. While CPT has been shown to decrease morbidity and mortality in HIV-infected older children and adults, it is not known if these benefits will be seen in these HIV-exposed, uninfected children less than one year of age, or whether protection through maternal antibodies will decrease the impact of CPT in this population. A better understanding of whether the benefits of CPT seen in other populations apply to HIV-exposed, uninfected children less than 1 year of age is important in a setting where breastfeeding is the main form of infant feeding, and prevention of mother to child transmission (PMTCT) programs and CPT are becoming increasingly available.

Specific Aim 2: Effects of CPT in HIV-infected women, during and after pregnancy

Aim 2a. To evaluate variation in time to malaria parasitemia, severe illness or death and moderate or severe anemia by CPT exposure status in HIV-infected women (with baseline CD4 cell counts of 200 to <500 cells/μL) during and after pregnancy.

Aim 2b. To evaluate the impact of CPT in pregnant women with CD4 cell counts of 200 to <500 cells/μL on occurrence of preterm birth and low birth weight.

Aim 2c. To evaluate the impact of CPT in women with baseline CD4 cell counts of 200 to <500 cells/μL on CD4 change from pregnancy to 24 weeks post-partum.

Rationale: The WHO recommends that CPT be given to HIV-infected pregnant women if they meet the adult criteria for treatment. Intermittent Preventive Therapy during pregnancy (IPTp), which is usually given to women during pregnancy to prevent malaria regardless of HIV status, is not given in cases where CPT is given, yet the ability of CPT to prevent malaria, as well as other adverse health and birth outcomes, has not been well studied in pregnant women. The BAN data provides an opportunity to compare the incidence of these health outcomes as well as pregnancy outcomes in women exposed and unexposed to CPT.

CHAPTER TWO: BACKGROUND AND SIGNIFICANCE

The HIV epidemic in women and children

In 2008 approximately 280,000 children died from AIDS and 430,000 children <15 years of age were newly infected with HIV, bringing the total estimate of children living with HIV to 2.1 million, 90% of whom live in sub-Saharan Africa.[13] In Malawi the estimated prevalence of HIV is 11.9%, and in 2007 there were approximately 91,000 children living with HIV/AIDS (Table 2.1).[14] More than 90% of new infections in children are transmitted during pregnancy, birth or breastfeeding. Comprehensive approaches to prevention of mother to child transmission in the developed world including antiretroviral treatment for pregnant women, elective cesarean section, and formula feeding have decreased mother to child transmission (MTCT) of HIV to < 2%. Although these measures are not currently feasible in the developing world, the use of low cost alternatives such as single dose nevirapine has decreased MTCT by 50%.[15] Prevention of mother to child transmission (PMTCT) programs providing antenatal HIV testing and counseling to pregnant women have helped to identify women in need of these interventions. Access to PMTCT interventions is increasing; in 2009, 53% of pregnant women living with HIV in low and middle-income countries received antiretrovirals to reduce the risk of HIV transmission to their infants, including antiretroviral therapy for their own health, compared with only 15% in 2005.[16] HIV will continue to be a major problem in young children until more effective interventions are universally accessible to HIV-infected pregnant women.

Association of HIV with birth and infant outcomes

HIV-infected pregnant women are at risk for the opportunistic infections experienced by the general HIV-infected adult population, as well as an increased risk of several perinatal outcomes, beyond vertical transmission of HIV, such as stillbirth and preterm birth.[17] Pregnancy in HIV-infected women has also been associated with adverse maternal outcomes including death and disease progression, although most of these findings were not statistically significant.[18, 19] Despite the increase in PMTCT programs available to pregnant women, approximately half of pregnant women identified as HIV-infected during antenatal care visits were assessed to determine whether they were eligible to receive ART for their own health.[16] In addition to the direct benefits of treatment to the health of the mother, better maternal health status is associated with better birth outcomes and infant health. A review of the literature on the effect of maternal HIV infection on perinatal outcomes found a significant increase in spontaneous abortion, still birth, infant mortality, intrauterine growth retardation, low birth weight and preterm delivery for HIV-infected women.[17] In both HIV-infected and HIV-exposed, uninfected children maternal death and low maternal CD4 have been identified as risk factors for child mortality in the first two years of life.[20-22] In summary, timely access to treatment for mothers also directly benefits the child, by enabling her to provide more comprehensive care for her infant.

The bimodal clinical course of HIV infection allows some infected children to survive early childhood without intervention,[23-25] however, the rapid disease progression seen in other children necessitates early identification of infection in order to take advantage of the limited window for effective treatment. Without access to treatment, approximately one third of infants will die before one year of age, and half will die by their second birthday.[22]

Perinatally infected children are at particular risk of death between two and six months of age.[20] Early identification of HIV-infected infants followed by antiretroviral treatment initiated at 6-12 weeks has been shown to reduce infant mortality by 76% and HIV progression by 75%.[26]

Despite the high rates or morbidity and mortality in HIV-infected infants and the established benefits of treatment, the vast majority of children are not receiving treatment. Although the number of children <15 years of age receiving antiretroviral treatment increased from 75,000 in 2005 to approximately 356,400 in 2009, only a fraction of children in need of treatment are receiving it.[16] Updated WHO guidelines issued in 2010 recommend immediate initiation of antiretroviral therapy for all infants diagnosed with HIV under two years of age.[27]

HIV testing in infants

A major obstacle preventing timely access to early treatment for HIV-infected children is the difficulty of accurately diagnosing HIV infection in infants. Maternal antibodies may be detectable in an infant's bloodstream until 18 months of age, causing commonly-used HIV antibody tests to be unreliable in this population. While an antibody test can be useful to determine exposure in the case of unknown status of the mother, a more advanced testing method, such as PCR, is required to diagnose an HIV-infected infant accurately. Unfortunately, this type of testing requires equipment and technology which is not yet available in some of the resource-poor settings where it is most acutely needed. In 2009 in low and middle income countries, only 6% of HIV-exposed children were tested before the age of 2 months.[16] In 2008, HIV DNA PCR testing of infants using dried blood

spots were being used in more than 30 low- and middle-income countries, allowing for expanded early infant diagnosis programs.[28]

Early infant testing is an important first step, but recurrent testing of HIV-exposed children is necessary to diagnosis HIV infection in a timely manner in the context of breastfeeding. A meta-analysis of MTCT through breastfeeding estimated a 4% risk of MTCT from 4 weeks to 6 months, a 9% risk from 4 weeks to 12 months, and 16% risk from 4 weeks to 18 months,[29] while another meta-analysis found an overall cumulative probability of transmission from 4 weeks to 18 months of 9.3%.[30] A study of HIV-exposed children in Zimbabwe found that among children who tested negative at 6 weeks and subsequently tested positive before 2 years of age, 19% died by 1 year of age, and 33% died by 730 days. Duration of survival following infection was shorter for infants infected before, compared with after, 6 months.[20] These mortality rates in postnatally infected infants underscore the need for recurrent testing throughout the extended HIV transmission risk period caused by breastfeeding, in order to reach infected children with ART before they fall seriously ill or die. Unfortunately, testing is often not repeated, or performed at all, until 18 months, a common testing time point and the earliest time at which antibody tests are generally considered accurate.

Until repeat testing measures are in place for all HIV-exposed infants, it is especially important to ensure that steps are being taken to link children at risk of infection to the health care system on a regular basis. This enables health care workers to identify HIV infection symptomatically as early as possible, at which point suspicion of infection can be confirmed if PCR testing is available. As in all HIV-infected patients, prevention of opportunistic

infections is important. Due to the difficulty of identifying infant infections and the importance of preventing opportunistic infections in this susceptible population, cotrimoxazole prophylaxis is routinely recommended by the WHO for all exposed infants. This approach ensures that infected infants who may not be diagnosed in a timely manner still have some level of protection against the opportunistic infections which can quickly lead to severe morbidity and mortality.

Challenges among HIV-exposed, uninfected children

The major concern for HIV-exposed, uninfected children is the risk of acquiring HIV infection, but there are many other threats to the well being of these children. The literature on morbidity in HIV-exposed, uninfected infants is limited, partly due to combined observations of infants who are HIV-exposed but uninfected with infants who are HIV-infected. A large study of morbidity among exposed, uninfected children in the first six months of life in Latin America and the Caribbean has provided insight into the burden of infectious disease morbidity in this population. Approximately 60% of infants studied experienced infectious disease morbidity in the first 6 months of life with an overall incidence rate of 4.5 infections per 100 child-weeks of observation (95% CI: 4.1, 4.7). Overall 17.5% of the 462 infants were hospitalized at least once with an infection. Infections commonly leading to hospitalization included lower respiratory tract infections (108 infections, 44 hospitalized (41%, 95% CI: 31.5, 50.6)), and systemic infections (34 infections, 16 hospitalized (47% 95% CI: 30.2, 64.6)). The most common infections were skin and mucous membrane infections, lower and upper respiratory tract infections, systemic infections and gastrointestinal infections. Although this study did not include control

children, these data provide an overall picture of the infectious disease burden experienced in this exposed, uninfected population.[31] Commonly reported symptoms and morbidities in other studies of HIV-exposed, uninfected children include fever, skin disease, cough, conjunctivitis, chronic diarrhea, lymphadenopathy, respiratory infections and failure to thrive.[12, 25] Reports of pneumonia in HIV-exposed, uninfected infants caused by *Pneumocystis jiroveci,*[32] usually considered an opportunistic pathogen of HIV infection, and higher rates of treatment failure for hospitalized cases of pneumonia compared with unexposed infants have also been noted.[33]

Mortality of HIV-exposed, uninfected children has also been examined. A review of 7 MTCT studies in Africa found that 4.9% of exposed, uninfected infants died by one year of age, and 7.6% died by 2 years of age.[22] Estimates varied by geographic region. In a cohort study of exposed, uninfected children in Zambia the estimated risk of mortality was 4.6% (95% CI: 2.8, 6.3) in the first 4 months of life.[21] Comparisons of mortality in exposed, uninfected children with unexposed children are most useful in understanding the increased risks due to HIV exposure. Although some of the earlier studies did not detect a significant difference in morbidity and mortality between these two groups,[25, 34] more recent evidence has demonstrated increased risks for exposed, uninfected children. A study of exposed and unexposed infants in Zimbabwe found that although morality in uninfected children was much lower than in infected children, exposed, uninfected infants were still 3.9 (95% CI: 3.15, 4.78) and 2.0 (95% CI: 1.2, 3.5) times as likely to die in the first and second years of life, respectively, when compared with unexposed children. The two-year mortality in exposed, uninfected children was 9.2%, compared to 2.9% in the unexposed children.[20] Similarly increased mortality in exposed, uninfected children has been reported elsewhere.[35,

[36] Recognized risk factors for infant mortality in exposed, uninfected children include maternal death,[20-22] maternal CD4 less than 200 cells/μL[20, 22] or less than 350 cells/μL,[21] low maternal hemoglobin[20, 21] and low birth weight.[20, 37] Common causes of death and hospitalization in studies of exposed, uninfected children include respiratory infections,[20, 21, 25, 34] sepsis,[21] diarrea,[20] and malnutrition.[20, 34]

There are several hypotheses to explain why HIV-exposed, uninfected children may have increased morbidity and mortality compared to HIV-unexposed children. There is evidence that passive immunity in exposed, uninfected infants may be deficient due to reduced transplacental transfer of IgG antibodies to common infections,[38, 39] and that HIV infection in the mother may interfere with development of the infant's immune system while in utero.[40, 41] HIV-exposed children may also be at greater risk of morbidity and mortality due to their living conditions and the makeup of the household. Often both parents of an exposed child are HIV infected, and the close contact with immunodeficient household members who are colonized with diverse pathogens can put infants at risk of acquiring infections to which unexposed children are less likely to be exposed (e.g., tuberculosis (TB) and *Pneumocystis jiroveci.*[31, 42-44]

Overlap of HIV and malaria epidemics

The geographical overlap of malaria and HIV is of concern especially for pregnant women and children. Malaria is the primary cause of death in children under 5 years of age in the developing world. In 2006, there were approximately 247 million episodes of malaria and

approximately 863,000 deaths due to malaria, 89% of which were in Africa, and 85% of which were in children less than five years old (Table 2.2).[45]

Malaria in children

Children less than five years of age generally experience the highest burden of malaria, and are especially vulnerable to infection during the first two years of life. The burden of disease is greater in younger children in endemic areas due to the exposure-related manner in which immunity is required. The peak age of infection is inversely related to the intensity of malaria transmission, such that infants are more affected in high transmission areas.[46] Infants have some protection through maternal antibodies resulting in decreased risk of infection in the first few months of life. Duration of protection from maternal antibodies may be greater in areas of decreased transmission intensity.[47] Infections are more common after the first two or three months of life, with increasing incidence in the remainder of infancy. Malaria infections generally reach a peak within the first one or two years of life[2, 29, 46, 48-53] especially in areas of high transmission.[54, 55]

Although malaria infection in early infancy may be asymptomatic and rapidly cleared,[56] there is evidence later in infancy of an increased risk of severe infection,[49, 51, 52, 55] severe malarial anemia,[52, 54] hospitalization,[48] and death,[52, 54] compared to older children and adults. A study of patients hospitalized with severe malaria in Tanzania found that the odds of severe malarial anemia (hemoglobin <5 g/dL) were greater in children 0-1 years of age than in older children (OR compared to children 2-4 years of age: 0.83, 95% CI: 0.72, 0.96; OR compared to children 5-15 years of age: 0.44, 95% CI: 0.27, 0.73). Respiratory distress among patients admitted to the hospital with severe malaria was also greater among infants

than children 2-4 years of age.[54] The case fatality rate was higher among children 0-1, compared with children 2-4 years of age (0-1 year: referent; 2-4 years: AOR 0.28, 95% CI: 0.18, 0.41). Infants younger than 6 months of age were also at greatest risk of death in a study of hospitalized patients with malaria in Kenya.[52] Although the relative risk of poor outcomes may be dependent on transmission intensity and other factors, the malaria-related health risks for infants and young children are considerable in any setting.

Malaria in HIV-exposed, uninfected children

Data on malaria in HIV-exposed, uninfected children are generally presented in conjunction with data on HIV-infected children, and there is often misclassification of person-time due to infrequent HIV testing. In addition, studies often present data on HIV-exposed, uninfected children together with data on HIV-unexposed children (presented as collapsed HIV-uninfected). For these reasons, there is minimal data in the literature describing the malaria experience of HIV-exposed, uninfected children. A study of HIV-exposed and unexposed infants in Uganda found that HIV-infected children had less malaria than HIV-exposed, uninfected children, while exposed-uninfected children and controls had similar rates of malaria and hospitalization due to malaria. Due to the timing of testing in this study, the person-time labeled as HIV-infected would also have included uninfected person time. This study did not find an association between occurrence of febrile illness and HIV status.[57] Another early study with probable misclassification of exposure due to timing of testing found no difference in the incidence or severity of malaria infection by HIV-exposure and infection status in children 5-9 months of age.[58]

Some studies more accurately distinguish between HIV-infected and exposed person time through more frequent testing. In HIV-exposed, uninfected infants in Kenya, malaria parasitemia was protective against infant mortality, though the association did not reach significance (HR 0.35, 95% CI: 0.10, 1.21). Among HIV-exposed, uninfected infants who died, infants who had had at least one episode of malaria parasitemia had a trend towards longer mean survival than infants who had not had an episode of malaria, but this difference was not statistically significant (263 days vs. 160 days, p=0.08). A similar trend was seen in HIV-infected infants, with an episode of malaria suggesting a protective effect against postneonatal infant mortality. Suggested explanations for this association in HIV-infected children include decreased morbidity associated with the SP used to treat malaria, benefits of a shift toward Th1-type immune response, and activation of chemokines due to malaria infection which can compete for HIV entry receptor CCR5, thereby slowing progression of HIV infection.[37]

Due to the association of both HIV and malaria with anemia in children,[59] it is important to consider anemia when examining HIV and malaria in this population. A study in Kenya of children younger than 2 years of age presenting at a hospital with acute *Plasmodium* falciparum found that relative to HIV-unexposed children, HIV-exposed children and HIV-infected children had lower hemoglobin concentrations, however, parasitemia and high density parasitemia were equivalent between the three groups. Multivariate analysis of this data demonstrated an increased risk of severe malarial anemia compared with control children for both HIV-exposed (OR 2.17, 95% CI: 1.25, 3.78) and HIV-infected (OR 8.71, 95% CI: 3.37, 22.51) children.[60] Anemia (Hb < 8 g/dL) was also found to be a statistically significant risk factor for postneonatal mortality in HIV-exposed,

uninfected infants (HR 5.03, 95% CI: 1.97, 12.81) in another study of HIV and malaria in Kenya.[37] Although the relationships between malaria, HIV-exposure and anemia are not clear, there is some evidence of a different relationship in HIV-exposed, uninfected children compared with both infected and unexposed children.

HIV and malaria co-infection pregnant women

HIV-infected adults have been found to have a significantly higher risk of parasitemia and clinical malaria compared with HIV-negative patients. The risk of clinical malaria increases with lower CD4 counts and more advanced stages of HIV infection.[61, 62] In areas of unstable transmission, HIV has been shown to increase the risk of severe or complicated malaria in adults,[63] and a study of the prevalence of severe malaria in HIV-infected and uninfected patients in South Africa found severe malaria was significantly more frequent in the HIV-infected group. The increased prevalence of severe malaria in the HIV-infected group was due to the increased frequency seen in patients who were considered non-immune to malaria, defined as born and residing in an area without stable malaria transmission.[62] Malaria has been shown to lead to a temporary decrease in CD4 in both HIV-infected and uninfected individuals which can be reversed through adequate treatment of the malarial infection.[64] Malaria is also associated with an increased plasma viral load in HIV-infected patients, although antimalarial treatment can help decrease viral load in some patients.[65, 66] Slower hematological recovery has been demonstrated in HIV-infected adults, [67] and there is evidence of an increased risk of malaria treatment failure for HIV-infected patients with low CD4 cell counts.[68, 69]

A review of the literature on co-infection with HIV and malaria in pregnant women presenting pooled data for several studies determined that HIV-infected women experienced more peripheral and placental malaria (summary relative risk: 1.58, 95% CI: 1.47, 1.71 and 1.66, 95% CI: 1.48, 1.87, respectively) compared with HIV-uninfected women. The risk of anemia is another point of concern in a setting where HIV and malaria are both present; dually-infected women are at greater risk of moderate-to-severe anemia (Hb < 8 g/dL) than those with single infections, and there is some evidence of a synergistic effect of dual infections on anemia.[70] Dual infection also increases the risk of poor birth outcomes including low birth weight and prematurity, especially in multigravidae.[71-73] Another point of concern for HIV-infected women is the decreased effectiveness of the WHO recommended, two-dose sulfadoxine-pyrimethamine intermittent preventive treatment given during pregnancy as part of a malaria prevention strategy.[74, 75]

Cotrimoxazole prophylaxis for prevention of opportunistic infections

Cotrimoxazole preventive therapy (CPT) has been identified as a valuable tool in addressing the increased morbidity and mortality associated with infections seen in HIV-infected adults and children. Cotrimoxazole, a broad-spectrum antimicrobial, is a fixed-dose combination of sulfamethoxazole and trimethoprim which targets aerobic gram-positive and gram-negative organisms, fungi and protozoa. Available both as a syrup and in solid formulations, cotrimoxazole is inexpensive, widely available and has been provided as part of standard care for prevention of PCP and toxoplasmosis for over fifteen years. Recommendations for CPT based on clinical status or CD4 cell count are shown in Table 2.3. The WHO recommends flexibility in CD4 cutoffs, suggesting a lower cutoff of 200 in some

situations, and suggests universal CPT in settings of high prevalence of HIV and limited health infrastructure.[76]

The benefits of CPT in adults are well established. The WHO CPT recommendations are based on the benefits of prophylaxis observed in HIV-infected patients with and without TB and across varying CD4 levels. CPT reduces mortality up to 46% in HIV-infected individuals not on ART in sub-Saharan Africa.[5, 6, 42, 77] Randomized controlled trials of CPT in HIV-infected adults in sub-Saharan Africa have found reductions in risk of hospitalizations,[6] adverse events such as bacterial pneumonia, acute unexplained fever,[2] and diarrhea[4] as well as reduced risk of mortality.[2, 6, 78] Similar reductions in mortality have also been observed in cohort studies among HIV-infected adults,[5, 42, 79] as well as adults co-infected with tuberculosis.[80-83] A prospective cohort study of implementation of daily CPT in HIV-infected individuals in Uganda also found a decreased annual rate of decline in CD4 cell count (77 vs. 203 cells/μL, $p<0.001$), and a decreased annual rate of increase in VL (0.08 vs. 0.90 $\log_{10}$, $p=0.01$) during the period of CPT.[10]

In addition to the direct benefits of CPT for the patient, CPT has been associated with benefits for household members of individuals receiving CPT. A prospective cohort study of CPT in HIV-infected adults in Uganda and their household members found mortality among HIV-negative household members under the age of 10 years was lowered 63% during the CPT period compared to beforehand (HR 0.37, 95% CI: 0.14, 0.95). The incidence of malaria, diarrhea and hospitalizations in household members was also significantly lower in the CPT period compared with the pre-CPT period.[42]

The benefits of CPT have also been shown in HIV-infected and uninfected children. The single randomized controlled trial in children was conducted in Zambia and included HIV-infected children 1-14 years of age. Children in the CPT had a lower risk of death (HR 0.57, 95% CI: 0.43, 0.77) an effect seen across all age groups and all baseline CD4 counts, which was attributed to decreased risk of respiratory infections, a finding supported by lower rates of antibiotic prescribing in the CPT group.[77] A trend towards lower hospital admission rates for serious bacterial infections as well as malnutrition was also seen, though this did not reach statistical significance.[84] After trial closure children previously on placebo were offered CPT, and follow-up of children found continued benefits of treatment in the CPT group, and decreased mortality and hospitalization rates in the group transferred from placebo to CPT. Mortality and hospital admission rates decreased further (approximately 6-fold and 3-fold, respectively) following availability of ART.[85] CPT has also been found to reduce the rate of nasopharyngeal pneumococcal colonization by 7%, although there was an increase in risk of colonization with cotrimoxazole-resistant pneumococci within six weeks of starting prophylaxis (RR 3.2, 95% CI: 1.3, 7.8).[8] Trials in adults and children have shown cotrimoxazole to be safe, and serious adverse reactions have been rare.[2, 77] A study of CPT in healthy children in Mali found decreased rates of gastrointestinal illness in the treatment group (rate ratio 0.68, 95% CI 0.47, 0.99), but no difference in rates of respiratory illness between treatment groups.[12]

Benefits of CPT have also been established in infants, though data is limited. A prospective cohort study of CPT in HIV-exposed infants found significantly lower incidence of lower respiratory tract infections (OR 0.18, 95% CI: 0.04, 0.77) in HIV-infected children, although the finding was not significant in HIV-exposed, uninfected children (OR 0.52, 95%

CI: 0.26, 1.05). This study also detected a non-significant increased risk of diarrhea among children receiving CPT in both HIV-infected infants and HIV-exposed, uninfected infants.[7] Analysis of two consecutive MTCT trials (Ditrame and Ditrame-Plus), one of which included CPT, found the 18-month risk of a severe event, defined as death or hospitalization >1 day, was lower in the trial offering CPT, although the difference was not statistically significant (HR 0.55, 95% CI: 0.3-1.1).[86] A study of CPT use in HIV-infected children less than two years of age hospitalized with pneumonia found children using CPT had a significantly reduced risk of PCP, compared with children not taking CPT (risk ratio 0.11, 95% CI: 0.02, 0.82).[87] A retrospective cohort study of HIV-infected infants in the U.S. found a relative risk of PCP among infants not receiving prophylaxis, relative to those receiving it, of 4.4 (95% CI: 1.2, 17), adjusted for CD4 percentage.[88] A comparison of thrice weekly or daily CPT in HIV-infected children (mean age 23 months) found that daily CPT offered greater protection against invasive bacterial disease and hospitalization, but similar protection against mortality.[89] In summary, there do appear to be benefits of CPT in infants, but few studies were designed and powered to examine the risks and benefits of CPT, and further research is needed to supplement what is currently known.

Despite WHO recommendations for CPT use in pregnant women, implementation has been slow, in part due to concerns over potential teratogenicity[90, 91] and risk of low birth weight due to the impact of CPT on folate metabolism. A recent review of the safety of CPT in HIV-infected pregnant women concluded that given the substantial benefits of CPT, it is safe to follow WHO recommendations based on the current literature. Very little research has been done in this population regarding CPT risks and benefits. In HIV-infected women in Zambia with a low CD4 cell count (<200 cells/μL), the implementation of CPT as a routine

component of antenatal care was associated with a decrease in adverse birth outcomes. The percentage of preterm births (≤34 weeks of gestation) was lower (OR 0.49, 95% CI: 0.24, 0.98) after CPT was introduced compared with beforehand, and there was a significant decrease in neonatal mortality from 9% in the pre-CPT period, to 0% in the post-CPT period (p=0.01). There was also a trend towards increased birth weight, although it did not reach significance. Nonsignificant trends towards reduced maternal mortality and hospital admissions were also seen in the post- versus pre-CPT periods. Suggested mechanisms of reduced preterm births include CPT-related decreases in bacterial and parasitic infections which may cause preterm birth, including urinary tract infections, toxoplasmosis, malaria, and pneumonia.[9]

SP given as intermittent preventive treatment during pregnancy (IPTp) is contraindicated when an HIV-infected woman is receiving CPT, however, the effectiveness of CPT in preventing malaria in this population is not known. Due to the consequences of malaria infection during pregnancy for both the mother and infant, it is important to fully understand whether forsaking IPTp for CPT is appropriate in all situations or if other considerations, such as malaria transmission intensity, should be taken into consideration before a decision is made.

The role of CPT in the context of HIV

The decreased mortality seen in patients, both adults and children, taking CPT shows the impact CPT can have on HIV-infected individuals. In HIV-exposed infants, CPT may play an especially important role in protecting health during the extended risk period while breastfeeding is ongoing, during which time they may not be tested for HIV. The

complications of HIV testing in HIV-exposed infants, combined with the continued risk of transmission through breastfeeding, often results in late diagnosis after the infant's health is already severely affected. CPT for these HIV-exposed infants may help to reduce the incidence of opportunistic infections during this high risk period. Additionally, the process of providing CPT may be a valuable tool for linking HIV-exposed children with the health system on a regular basis. This provides the opportunity for closer monitoring of the infant's health, as well as access to HIV testing and treatment when available. Despite the WHO recommendations for CPT in HIV-exposed infants, in Eastern and Southern Africa only 18% of infants in need of cotrimoxazole received it in 2009, an increase from 9% in 2008.[92]

Although access to ART is increasing for HIV-infected adults and children worldwide, the majority of HIV-infected patients in need of treatment does not have access to ART and is at risk of opportunistic infections. The enormous benefits of ART in infants has been demonstrated in South Africa through the CHER study, which found early infant testing and treatment decreased early infant mortality by 76%, and HIV progression by 75%.[26] Similar reductions were observed in Europe.[93] While access to ART for children <15 years of age increased to almost 200,000 children in 2007, the majority of children in need currently are not receiving treatment.[28] Until ART is available to HIV-infected children, CPT may help to reduce some of the morbidity and mortality experienced by this population.

CPT and malaria

One of the unintended benefits of CPT is the impact on malaria incidence. A cohort study of HIV-infected adults in Uganda found the incidence dropped from 50.8 to 9.0 episodes of malaria/100 person-years after introduction of CPT (adjusted IRR 0.24, 95% CI:

0.15, 0.38). The introduction of ART in combination with CPT further decreased incidence of malaria to 3.5 episodes per 100 person-years (adjusted IRR 0.08 (compared with baseline), 95% CI: 0.04, 0.17).[10] Benefits were also seen for HIV-uninfected family members of patients receiving CPT; HIV-uninfected household members living with patients taking CPT had a lower incidence of malaria (IRR 0.64, 95% CI: 0.50, 0.83).[94] In HIV-infected adults in a randomized controlled trial in Cote d'Ivoire, a decreased incidence of malaria was seen among patients with at least one severe adverse event (hazard ratio 0.16, 95% CI: 0.04, 0.73).[2] Among HIV-infected adults in Uganda with CD4>200 cells/μL receiving antiretroviral treatment, patients randomized to discontinue CPT had a higher rate of malaria (RR 28, 95% CI: 6, 105) compared with patients randomized to continue CPT.[4] A cohort study of CPT in HIV-infected adults in Uganda also found decreased incidence of malaria after introduction of CPT (IRR 0.31, 95% CI: 0.13, 0.72).[79]

Limited data have shown benefits of CPT on incidence of malaria in children. A prospective cohort study compared the impact of insecticide-treated bednets and CPT on the incidence of malaria between a cohort of HIV-infected children and a community-based cohort of healthy children. Although the incidence in children using CPT alone was lower than in the community-based cohort that had no intervention, the effect was not statistically significant (IRR 0.61, 95% CI: 0.25, 1.51). Use of insecticide-treated nets, however, did lead to a significantly lower incidence of malaria (IRR 0.57, 95% CI: 0.46, 0.71), and the combination of ITNs and CPT lead to a dramatically lower incidence of malaria (IRR 0.03, 95% CI: 0.01, 0.10), compared with the community-based cohort with no intervention.[11] A randomized study of CPT in healthy children 5-15 years of age in Mali investigated the impact of CPT on sulfadoxine-pyrimethamine treatment for malaria. There was only a single

episode of clinical malaria in the treatment group during 1890 person-weeks of follow-up, compared to 72 episodes in the control group during 681 person-weeks of follow-up (RR 0.005, 95% CI: 0.00, 0.04).[12] A study comparing data from two cohort studies in Uganda, found that compared to HIV-uninfected children who were not receiving CPT, CPT in HIV-infected children (mean age of 7.4 and 6.0 years, respectively) was associated with a protective efficacy of 80% (95% CI: 72, 85). The protective effect was similar in children receiving and not receiving ART.[95] In an RCT of younger children, HIV-exposed infants who continued CPT after cessation of breastfeeding and exclusion of HIV infection until 2 years of age had a 38% reduction in malaria incidence compared with infants who stopped CPT after cessation of breastfeeding. All infants in that study were also given insecticide-treated bed nets (ITNs).[96]

Antimicrobial resistance concerns and CPT

Although we do not have data on resistance in this study, it is still important to weigh concerns regarding antimicrobial resistance among bacteria and plasmodia as cotrimoxazole use expands.[97, 98] The increasingly widespread presence of drug-resistant malaria underscores the importance of carefully monitoring, in new and innovative ways, prophylactic and therapeutic treatments for malaria and their impact on resistance. A few studies have examined the association between CPT and antimicrobial resistance. A five-month study of household members of HIV-infected patients on CPT regimens did not detect a change in the proportion of cotrimoxazole-resistant diarrheal pathogens before and during CPT.[42] This study also collected blood specimens each time an episode of *P. falciparum* was diagnosed and tested them for the presence of mutations known to mediate resistance to SP

(dihydrofolate reductase (*dhfr)* Asn-108, Ile-51, and Arg-59, and dihydropteroate synthase (*dhps)* Gly-437 and Glu-540). The proportions of samples in the exposed (HIV-infected household member on CPT) and unexposed (HIV-infected household member not taking CPT) households containing double, triple and quintuple mutants was similar. Malaria incidence of household members living with an HIV-infected patient taking CPT was lower than incidence in household with a CPT unexposed HIV-infected member (IRR 0.64, 95% CI: 0.50, 0.83), and there were fewer malaria episodes due to parasites containing the *dhfr/dhps* quintuple mutation.[94] A study of CPT in HIV-infected adults with CD4 <350 cells/μL in Kenya also found that CPT prevented malaria and reduced incidence of antifolate-resistant *P. falciparum* in the HIV-infected individuals, but also resulted in relatively higher prevalence of non-susceptible pneumococcus and commensal *E. coli* resistance.[99]

CPT in HIV-exposed and unexposed Zambian children was demonstrated to reduce pneumococcal colonization by approximately 7% while increasing the risk of colonization with cotrimoxazole-resistant pneumococci within six weeks of starting prophylaxis, but authors concluded that their findings still support current WHO recommendations.[8] Studies in children and adults have not found evidence of CPT interference with the efficacy of SP in treatment of malaria.[12, 79] Although increasing antimicrobial resistance should be monitored, current data do not support refraining from CPT due to resistance concerns.

Unanswered questions

The benefits of CPT in HIV-infected adults have been well established. Benefits in prevention of some opportunistic infections in HIV-exposed and infected children have also

been seen, but further research on infants, including the impact on malaria, is still needed. As access to PMTCT interventions increases, the number of HIV-exposed, uninfected children will increase further. Therefore, the impact of CPT – a widely available, effective, safe, inexpensive therapy -- in this at-risk population may be especially important. Clearer data on the impact of CPT in HIV-infected pregnant women is also lacking. A more comprehensive understanding of the benefits of CPT on the health of HIV-infected mothers and their HIV-exposed, uninfected infants could guide future policy decisions regarding CPT and its role in protecting the health of these vulnerable populations.

Table 2.1. UNAIDS HIV/AIDS estimates for Malawi, 2007[14]

Adults and children	Women 15+	Children 0-14	Adult prevalence	AIDS deaths in adults and children
930,000 (860,000-1,000,000)	490,000 (450,000-530,000)	91,000 (81,000-100,000)	11.9% (11.0-12.9)	68,000 (59,000-77,000)

Table 2.2. WHO malaria estimates for Malawi, 2008[45]

	Estimate
Malaria cases	
All ages	4,986,779
<5 years	2,473,208
Malaria Deaths	
All ages	7,748
<5 years	4,546

Table 2.3. WHO recommendations for CPT[76]

HIV-infected adults, pregnant women, and children 5 years and older	HIV-infected children less than 1 year	HIV-infected children 1-4 years of age	HIV-exposed infants
Patients with WHO stage 2-4, if CD4 testing not available If CD4 testing available, patients with CD4<350 cells/μL OR WHO stage 3 or 4, irrespective of CD4	CPT is indicated regardless of CD4 or clinical status	WHO stage 2, 3 or 4 regardless of CD4, or any patient with CD4<25%	CPT is universally indicated, starting at 4-6 weeks, maintained until cessation of risk of transmission and exclusion of HIV infection

CHAPTER THREE: METHODS

We conducted secondary analyses using data from the Breastfeeding, Antiretrovirals and Nutrition (BAN) study. The BAN study was a randomized, controlled trial (RCT) which took place in four clinics in Lilongwe, Malawi between 2004 and 2009, designed to evaluate the following: 1) the benefit and safety of antiretroviral prophylaxis given either to the infants or to their mothers to prevent HIV transmission during breastfeeding, 2) the benefit of nutritional supplementation given to the women during breastfeeding to prevent maternal depletion, and 3) the feasibility of exclusive breastfeeding followed by early, rapid breastfeeding cessation.

DATA SOURCES

Study setting

The BAN study was based at Bwaila hospital in Lilongwe, Malawi. Malawi has a population of approximately 13.5 million. The BAN study was linked with a PMTCT program run by the UNC Project. The UNC Project-Malawi is a research, care and training facility located in the capital city of Lilongwe, established by UNC and the Malawi Ministry of Health in 1999. The mission of the UNC Project is to identify innovative, culturally acceptable and relatively inexpensive means of reducing the risk of HIV/STI and infectious disease transmission through research, and to strengthen the local research capacity through training and technology transfers.

Study background

The decision of whether to breastfeed is a complicated one for HIV-infected mothers, due to the risk of HIV transmission to the child. Breastfeeding can also lead to maternal nutritional depletion if the mother is not on an adequate diet to support her metabolic needs.[100-103] The benefits of breastfeeding for the infant are substantial and include fewer gastrointestinal and lower respiratory tract infections, and a decreased risk of developing otitis media as well as other diseases, particularly in the first six months of life.[104-106] Alternatives to breastfeeding are complicated in resource poor settings without clean water, and where the cost of formula is prohibitive.

Identification of ways to make breastfeeding safer for mothers and infants in the context of HIV has become a global health priority. In order to address the first two objectives of the BAN study, two interventions were assessed in a factorial design: 1) a 3-arm postnatal antiretroviral intervention with either additional antiretrovirals (beyond enhanced standard perinatal prophylaxis) given to the mother or infant, or nothing in addition to an enhanced standard perinatal prophylaxis, and 2) a 2-arm maternal nutritional intervention to promote maternal health with a food supplement given to half the mothers.

Recruitment and Enrollment

Recruitment for BAN took place through the PMTCT program run by the UNC Project in Lilongwe, where HIV seroprevalence in pregnant women is approximately 13%. This program offers HIV counseling and testing to all pregnant women as well as enhanced antenatal care and single-dose nevirapine to all HIV-infected women in labor and to their infants shortly after delivery. HIV testing is conducted using two simultaneous rapid tests,

Abbott Determine HIV-1/2 and Uni-Gold HIV (Trinity Biotech plc, Bray, Eire). Women identified as HIV-infected were informed of the option to participate in BAN, and if the woman was willing to participate and intended to breastfeed she signed a consent form. Primary eligibility criteria included: 1) ≤30 weeks gestation, 2) at least 18 years of age (or 14 years of age if married), 3) hemoglobin >7 g/dL, 4) CD4 count ≥200 cells/μL (increased to ≥250 during the study) , 5) no prior antiretroviral medication use, 6) normal liver function tests (<2.5 upper limit of normal), 7) no serious complications of pregnancy, and 8) not previously enrolled in BAN. Women were asked to complete 2 antenatal study visits and to deliver at Bwaila hospital, where BAN research activities are conducted. If a woman delivered elsewhere she was eligible as long as the mother and infant arrived at Bwaila for evaluation within 36 hours of birth. Antenatally women received screening for syphilis and anemia as well as iron and folate, mosquito nets (from March 2007) and tetanus toxoid. If the infant was ≥2000 grams, had no congenital malformations and there were no maternal conditions that precluded start of study drug, then mother-infant pairs were randomized to one of the six treatment arms. Infants found to be perinatally HIV-infected at birth or two weeks of life, and their mothers, were disenrolled from the study and referred for treatment. Infants who tested positive later in the study were discontinued from the treatment arm, but were encouraged to continue reporting for regular study visits.

BAN services

In addition to the full medical care provided by the UNC Project, all of the women and infants also received certain other benefits. All participants received a 2-kg bag of maize each week, and all mothers received iron and folate antenatally as well as mosquito nets and

tetanus toxoid. Pregnant women were also given sulfadoxine-pyrimethamine malaria prophylaxis at the beginning of the second and third trimesters, if they were enrolled early enough. All mothers received single dose nevirapine peripartum plus twice a day zidovudine (ZDV) 300mg and lamivudine (3TC) 150 mg during labor and for 7 days postpartum. Infants received nevirapine 2 mg/kg after delivery and also begin ZDV (12 mg) plus 3TC (6 mg) twice daily for 7 days. Post partum women received a single vitamin A supplement and counseling on exclusive breastfeeding and rapid early cessation between 24 and 28 weeks postpartum. To minimize the risks of malnutrition following early breastfeeding cessation, a locally produced ready-to-use therapeutic food (RUTF) commonly used in Malawi was provided by the study. The RUTF is made from full-cream powdered milk, peanut butter, sugar, oil and fortified with micronutrients. Infants received all routine vaccinations including BCG, polio, diphtheria, pertussis, tetanus, Haemophilus influenza, hepatitis B, and measles.

Visit schedule

There were 17 scheduled study visits: 2 antepartum screening visits, an enrollment visit soon after delivery, and postpartum visits at 1, 2, 4, 6, 8, 12, 18, 21, 24, 28, 32, 36, 42, and 48 weeks postpartum. Randomization was conducted during the enrollment visit which was usually during the delivery hospitalization unless the woman delivered elsewhere. Women who registered for the study early in their pregnancy may have had additional study visits antenatally at 28, 32, and 36 weeks' estimated gestational age. Women were also encouraged to come for a visit postnatally whenever they needed additional medical care. Data for unscheduled visits was systematically collected.

Details of nutritional and antiretroviral interventions

The nutritional supplement which half of the mothers were randomized to receive was a high-energy, high-protein, micronutrient-fortified food supplement which was supplied for 28 weeks after delivery, or until reported breastfeeding cessation, whichever came first. This supplement provided the daily energy required to support exclusive breastfeeding and 100% of the recommended dietary allowance for all micronutrients except vitamin A. The supplement was also given to women who experienced excessive weight loss, defined as 5% or more of body weight, between visits beginning 4 weeks after delivery or a body mass index that fell below 17, after examination by a clinician.

The randomization of patients after delivery to one of the 6 treatment conditions was done using a permutated block method in order to ensure a balanced allocation. Mothers and infants randomized to the antiretroviral arms were supplied with study drugs for 28 weeks after delivery or until reported cessation of breastfeeding, if earlier. Infants assigned to the ARV arm received daily nevirapine (NVP), with doses ranging from 6 mg to 26 mg, increasing as the infant ages to 28 weeks. Mothers randomized to the ARV arm received combination therapy with three drugs. At the beginning of the study, the regimen used included 300 mg zidovudine (ZDV), 150 mg lamivudine (3TC) taken orally every 12 hours for 28 weeks as well as NVP 200 mg once daily for 14 days and then 200 mg every 12 hours. Based on FDA recommendations, on January 31, 2005 the regimen was changed to ZDV/3TC and nelfinavir (NFV). On February 6, 2006 lopinavir/ritonavir replaced nelfinavir. Mothers who developed toxicity to NVP were continued on Combivir and switched to NFV initially. Mothers who developed toxicity to the ZDV component of Combivir were switched to stavudine (D4T) and 3TC. As of 27 March 2008, the arms of the study with no

antiretrovirals closed due to DSMB recommendation, and mother-infant pairs were randomized to the remaining arms.

Visit procedures and data collection

At the second screening visit, the following data was collected from the mother: demographics, current pregnancy and previous childbearing history, past medical history, concomitant medications, anthropometrics and vital signs, physical exam, current symptoms and dietary information. Mother's anthropometrics and vital signs, illness and hospitalization since last visit and current symptoms were collected at all follow up visits. Nutritional supplement and ARV adherence information was collected on a regular basis when applicable, dietary information was collected several times throughout follow-up and the concomitant medication log was updated as needed. Details of the delivery and the delivery outcome were collected at the delivery visit.

For infants an initial physical evaluation was performed at delivery, and again at 2, 6, 12, 18, 24, 28, 36 and 48 weeks. Anthropometrics, vital signs, oral exam, illness and hospitalization since last visit, and current symptoms were collected at all follow-up visits. Feeding questionnaires and dietary recall questionnaires about the infant were also administered to the mother on a regular basis over the course of follow-up.

Laboratory procedures and data collection

Laboratory analysis was conducted for the mother as follows:

- Full blood count: screening, labor and delivery, 2, 6, 12, 18, 24, 28 and 48 weeks

- Alanine aminotransferase (ALT): screening, labor and delivery, 2, 4, 6, 12, 18, 24, 28, 36, and 48 weeks
- Lipase, blood urea nitrogen (BUN), creatinine, and albumin: Screening, labor and delivery, 12, 24, and 48 weeks
- CD4 and CD8 counts: Screening, 24 and 48 weeks
- Urinalysis: screening

Laboratory analysis was conducted for the infant as follows:

- Full blood count and ALT: labor and delivery, 2, 6, 12, 18, 24, 28, 36 and 48 weeks
- Creatinine: labor and delivery
- Roche Amplicor HIV-1 DNA assay: labor and delivery, 2, 12, 28 and 48 weeks

Discontinuation of study participation

Patients were inactivated if HIV infection was detected at birth or at two weeks of age. If infection was diagnosed at a later time point, patients were encouraged to continue with regularly scheduled study visits, but were discontinued from the intervention and referred for treatment. Following a positive result on a Roche Amplicor HIV-1 DNA assay, dried blood spots stored from previous visits were tested to determine the first visit at which the patient was shown to be HIV-infected. Patients were also discontinued when they were lost to follow-up

Cotrimoxazole prophylaxis and malaria prophylaxis and treatment

CPT was not initially administered by the BAN study to participating mothers and infants. In mid-June 2006, BAN introduced cotrimoxazole as prophylaxis against opportunistic infections in accordance with the Malawi Ministry of Health and Population Guidelines. CPT was administered as follows:

1. All women participating in BAN study after the 12th week of pregnancy with a CD4 count less than 500 cells/μL, regardless of symptoms, should receive life-long prophylaxis. CD4 counts were performed at screening, 24 weeks and 48 weeks postpartum, and CPT could be started following a result of a CD4 <500 cells/ μL at any of those three time points. The dose was one tablet of single-strength (480 mg) cotrimoxazole twice daily.
2. All infants participating in the BAN Study, beginning at 6 weeks of age. Those infants found to be HIV-uninfected at 28 weeks continued the prophylaxis until age 36 weeks, while those found to be HIV-infected at any point in the study received life-long prophylaxis. Infants who failed to wean by 28 weeks continued CPT until weaning occurs and HIV infection is ruled out. The dose was a ½ tablet of single-strength (480 mg) cotrimoxazole once daily.

The implementation of CPT led to some changes in other study procedures. The routine second and third trimester doses of SP given to pregnant women were omitted in study patients on CPT in accordance with WHO recommendations. Also due to the similarities between cotrimoxazole and sulfadoxine-pyrimethamine (SP), SP was not recommended for use as first-line treatment for malaria in patients taking CPT. However, SP use was continued

until Malawi malaria treatment guidelines changed in 2007 to first line Artemether-Lumefantrine (quinine in the first trimester of pregnancy); second-line therapy was Amodiaquin-Artesunate and IVI Quinine for severe malaria.

BAN enrollment

The study's target sample size was 2418 mother-infant pairs. Enrollment of pregnant women began in April of 2004. Enrollment was slow initially but improved after community outreach activities, and reached the target rate of 15 enrollees per week in February of 2006. As of July 2006, around the time CPT was introduced, 883 mother/infant pairs had received treatment assignment, and 192 mother-infant pairs had completed the full 48 week follow-up period. As of 1 March 2009, 2,373 mother-infant pairs had received treatment assignment and 1,348 had completed the full 48 weeks of follow-up.

Adverse event reporting in BAN

Adverse events were identified through one of 4 means: 1) a participant presented for a routine visit and reported symptoms or had signs on physical exam, 2) a participant presented for an unscheduled visit and reported symptoms or had signs on physical exam, 3) a participant had an abnormal laboratory result, or 4) a participant reported an illness or hospitalization since her last study visit.

After the study nurse took a history from the participant, any illness or abnormality led to referral of the participant to the clinician for follow-up. The clinician identified the event and evaluated the event clinically, then managed the care of the participant. An SAE was defined as any experience that was fatal or life-threatening, required in-patient hospitalization,

resulted in persistent or significant disability or incapacity, was a congenital anomaly or brain defect or cancer. If the event was considered an SAE the clinician completed a Serious Adverse Event form.

Malaria

Blood smears were performed when there was clinical suspicion of malaria. Infants with a positive malaria blood smear were treated with SP regardless of symptoms and parasite density.

Note: all BAN study description is taken from the BAN study protocol, van der Horst et al.,[107] and personal communication with Yusuf Ahmed and Michael Hudgens.

METHODS

Specific Aim 1 Methods - Effects of CPT in HIV-exposed, uninfected children

Aim 1a. To evaluate time to first episode of malaria parasitemia (dichotomous blood smear positive) by CPT exposure status (no CPT vs. CPT from 6 weeks of age) in the first 36 weeks of life.

Aim 1b. To evaluate time to first severe illness or death, moderate or severe anemia, or malnutrition by CPT exposure status (no CPT vs. CPT from 6 weeks of age) in the first 36 weeks of life.

Specific Aim 1 measurements and variables

Outcomes

Malaria

Positive malaria smear: malaria smears were done when there was clinical suspicion of malaria. The lab results were recorded in a running log with the patient's ID number, date of smear, and outcome classified as one of the following: negative, one plus, two plus, three plus or four plus. For purposes of this analysis. the results were dichotomized into negative or positive (one plus, two plus, three plus and four plus combined).

Severe illness or death

SAEs were reported systematically and include the participant's ID number, type of event, date of visit, date of SAE onset, severity grade, action taken and outcome. All hospitalizations and deaths were considered to be SAEs and were included in this dataset.

Anemia

Grade 3 or 4 anemia (Table 3.1) based on routine laboratory testing was be considered an outcome of interest due to the association between malaria and anemia and the benefits of CPT on hemoglobin seen in the literature.

Underweight

Weight for age z score < -2. Underweight was classified based on weight-for-age z scores calculated using the 2005 WHO Child Growth Standards. An infant was considered undernourished if the weight-for-age z score was less than -2 after 6 weeks of age.[108]

Cotrimoxazole prophylaxis (CPT) (Main exposure)

Exposure to CPT was based on a time-point at which CPT was introduced into the BAN study. CPT was introduced on 13 June 2006. In order to allow for the lag time it may have taken for CPT to reach all study participants, data from June 13th to August 15th was not included in these analyses. In order to minimize misclassification and simplify analysis we only included children who were either never exposed to CPT or exposed from 6 weeks onwards (fully unexposed vs. fully exposed). Under the guidelines and based on this date cut-point, the following infant observations were be considered exposed/unexposed (Table 3.2). A sample of cotrimoxazole prescription files were examined which supported this classification method.

Covariables

Covariables considered in the analyses are listed in Table 3.3.

Censoring

Infants who test positive for HIV were censored at the last visit at which they tested negative (including on stored DBS). Because visits are never more than 6 weeks apart, we feel this was adequate to minimize misclassification of HIV-infected person-time. Censoring

also resulted from inactivation of study participation due to infant death (except for Specific Aim 1b), maternal death, or loss to follow-up.

Specific Aim 1 Analysis

Descriptive analysis (for all Specific Aims)

We first conducted descriptive analyses including calculation of medians, standard deviations, and frequencies of exposures, outcomes and covariates. We compared categorical proportions using chi-square test or Fisher's exact test and continuous variables using the Wilcoxon rank-sum test.[109]

General Modeling Approach

The variation in time to first or only episode of the outcomes of interest by CPT exposure status was examined in separate analyses using proportional hazards regression to model the hazard rate, based on the number of events per interval of time. While comparable to incidence rates, hazard rates are conditional on survival in the immediately-preceding time interval. The proportional hazards model is:

$$h_x(t)=h_0(t)\cdot e^{\beta x}$$

where X is a vector of explanatory variables (X_1, X_2,…X_k), $h_0(t)$ is the "baseline" hazard when X=0, and $h_x(t)$ is the hazard at X=x. When X_1 is a binary predictor variable, the interpretation of $e^{\beta 1}$ is the hazard ratio comparing those with X_1=1 to those with X_1=0 (referent) at all times t, adjusted for all other predictor variables in the model (X_2, X_3,…,X_n). This hazard ratio is assumed to be constant across time, or in other words, the hazards are

"proportional". This assumption can be relaxed through inclusion of product-interaction terms between time and individual exposures or covariables in the model [110].

Analysis began with a bivariate model containing only CPT and the outcome of interest. The proportional hazard assumption was examined graphically using log(-log(S(t))) curves and by adding interactions with time to the model (Cox test of the proportional hazards assumption). If the assumption was violated, it was relaxed by fitting interactions with categorical or continuous time.[111] Goodness of fit was assessed using deviance residuals and influence statistics. The log-rank test was used to compare the CPT-exposed and unexposed groups.

Effect measure modifiers

Identification of effect measure modifiers was performed by considering the exposure-outcome relationship at each level of a third variable (the potential effect measure modifier) by including a product interaction term between the exposure and the potential effect measure modifier.[112] This was only examined for variables for which stratified estimates would be meaningful, qualitatively. We ran a simple Cox model containing CPT exposure status, the outcome of interest, the potential effect measure modifier, and an interaction term between the CPT exposure status and the potential effect measure modifier. A p-value for the interaction term lower than $\alpha=0.10$ was be taken as evidence of substantial heterogeneity in the stratum-specific measures of effect.[113] Covariables found to be important effect measure modifiers were included in the starting multivariable model through an interaction term with CPT exposure status. Variables suspected, a priori, to be effect measure modifiers include randomization to antiretrovirals and gender.

Confounders

Confounding was not likely to be a major issue due to the 'natural experiment' scenario regarding the exposure. The bivariate distributions of potential confounders, identified through the literature, with the exposure of CPT and the various outcomes of interest were examined to determine the level of association. Potential confounders are listed in Table 3.4. Covariates that were not found to be effect measure modifiers and that led to a change in the HR by more than 10% $\ln|(HR_{unadjusted}/HR_{adjusted})|$ were considered confounders.[114]

Multivariable associations

A backward elimination modeling strategy was used to assess the joint effects of covariates. The 'fully adjusted model' contained the main exposure (CPT), potential confounders and effect measure modifiers with the appropriate interaction terms. We first assessed effect measure modification by examining the likelihood ratio test with and without the selected interaction term. Following assessment for effect measure modification, potential confounders were removed from the model in order of p-value magnitude if the estimated HR for CPT changed by more than 10% from the previous model (which contained the dropped variable).[115] For effect measure modifiers, a 10% change in estimate at any level of a given effect measure modifier was considered sufficient evidence to retain the confounder.

Specific Aim 2 Methods – Effect of CPT in HIV-infected pregnant women

Aim 2a. To evaluate variation in time to malaria parasitemia, SAEs and moderate or severe anemia by CPT exposure status in HIV-infected women (with baseline CD4 200 to <500 cells/μL) during and after pregnancy.

Aim 2b. To evaluate the impact of CPT in pregnant women with CD4 200 to <500 cells/μL on occurrence of preterm birth, low birth weight, neonatal mortality and stillbirth.

Aim 2c. To evaluate the impact of CPT in women with baseline CD4 of 250 to <500 cells/μL on CD4 change from pregnancy to 24 and 48 weeks post-partum.

Specific Aim 2 measurements and variables

Study population

Analysis for Specific Aim 2a, 2b and 2c included all women who had a CD4 count 200 to <500 cells/μL at baseline. Women who never had a CD4<500 were used to estimate seasonal/yearly variation in incidence of malaria and other outcomes through survival analysis. If the incidence of malaria by year was significantly different in these women, this was taken into consideration as a confounder in the primary analysis.

Measurements

Outcomes

Malaria

Positive malaria smear: malaria smears were done when there was clinical suspicion of malaria. The lab results were recorded in a running log with the patient's ID number, date of smear, and outcome classified as one of the following: negative, one plus, two plus, three plus or four plus. The results were dichotomized into negative or positive (one plus, two plus, three plus and four plus combined).

Severe illness or death

SAEs were reported systematically and included the participant's ID number, type of event, date of visit, date of SAE onset, severity grade, action taken and outcome. All hospitalizations and deaths were considered to be SAEs and are included in this dataset.

Anemia

Grade 3 or 4 anemia based on routine laboratory testing was considered as an outcome of interest due to the association between malaria and anemia and the documented benefits of CPT on hemoglobin.

CD4 count

CD4 count as a continuous variable measured in cells/μL at 24 weeks

Poor birth outcomes

Preterm birth defined as birth at ≤37 weeks (=1) or after 37 weeks of gestational age, low birth weight defined as birth weight <2500g (=1) or ≥2500g (=0).

Main exposure: Cotrimoxazole prophylaxis (CPT)

Exposure to CPT was based on the time-point at which CPT was introduced into the BAN study. CPT was introduced starting on June 13, 2006. In order to allow for the lag time it may have taken for CPT to reach all study participants, data from June 13th to August 15th was not included in this analysis. Under the guidelines and based on this cut-point, the following observations were considered exposed/unexposed:

Specific Aim 2 analysis

Specific Aim 2a

Analysis of first or only episode of malaria parasitemia, death, hospitalization and severe anemia was be performed as described for Specific Aim 1, using proportional hazards regression.

Specific Aim 2b

General modeling approach

For analysis of the association between CPT in pregnancy and low birth weight the multivariate log binomial regression was be used to obtain risk ratios while accounting for important interactions and controlling for important confounders. The equation for the log binomial model can be expressed as:

$$\ln(P(D|X)) = \alpha + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3$$

where D is the outcome of interest, X is the exposure of interest, and $X_{2...}X_3$ are confounders. The interpretation of $e^{\beta 1}$ for the log binomial model is the risk ratio comparing those with X_1=1 to those with X_1=0 (referent), all other covariates being equal.[116, 117] CPT exposure, the outcome of interest, potential confounders, effect measure modifiers along with the appropriate interaction terms was entered into the model. Effect measure modification was assessed by examining Wald p-values or the likelihood ratio test for the model with and without the selected interaction term. A backwards elimination process was used beginning with the potential confounder with the highest p-value to determine which covariates result in greater than a 10% change in estimate and should be retained in the model as confounders. A separate model was built for each of the outcomes of interest (low birth weight, preterm birth, and neonatal mortality), unless the frequency of these outcomes is not sufficient, in which case a combined category capturing all three poor birth outcomes may be used. If there were problems with model convergence, other model specifications such as logistic regression were explored. Goodness of fit of the model was assessed through the Hosmer-Lemeshow decile of risk test.[116]

For analysis of the association between CPT during pregnancy and preterm birth multivariate logistic regression was used following the same procedures as outlined for log binomial regression. Effect estimates of the logistic model can be interpreted as the odds ratio (OR) comparing the odds of giving birth to a low birth weight infant among women exposed to CPT to the odds of giving birth to a low birth weight infant among women unexposed to CPT.

Confounders

As with Aim 1 analyses, confounding was not a major issue due to the 'natural experiment' scenario regarding the exposure. Suspected confounders of the exposure-outcome relationships were identified through the existing literature. Bivariate distributions of these confounders with the exposure of CPT and the various outcomes of interest were examined to determine the level of association.

Effect measure modifiers

Interactions between CPT and all covariates were assessed through likelihood ratio tests for models containing the exposure, covariate and exposure*covariate term vs. a model containing only the exposure and covariate.

Specific Aim 2c

Examining the effect of CPT on CD4 count from baseline to 24 weeks after giving birth was performed by constructing separate linear regression model, stratified by ART exposure (randomization status). The linear regression model takes the form $E(Y_i)=\beta_0 + \beta_1(X_1)$, where $E(Y_1)$ is the expected response at level i of predictor variable X_1, β_0 is the intercept parameter, or mean when X=0, and β_1 is the slope of the regression line. Crude and adjusted mean difference in CD4 counts were calculated along with 95% confidence intervals. Confounders were identified by constructing models with CPT (exposure), CD4 count (outcome) and each covariate and corresponding interaction term. Effect measure modification was assessed by examining the partial F test for the model with and without the

selected interaction terms.[118] Confounding was then examined by removing covariates from the model in order of p-value magnitude if the β for CPT changes by more than 10%.

Sensitivity analysis among ineligible for CPT

Women who had a CD4 cell count of at least 500 cells/μL at screening were not eligible for CPT until at least 24 weeks postpartum, at which point they were eligible if their CD4 cell count fell below 500. We used data from this pseudo "control" group to assess unmeasured confounding, i.e. whether there were changes in the frequency of incident malaria in the study population between the time periods before and after implementation of CPT (before June 2006 and after August 2006). This is most important for outcomes such as malaria, which are known to fluctuate over time. Incidence of birth outcomes and changes in CD4 cell count are less likely to fluctuate over the time span of the BAN study. In order to assess whether there were changes in the outcome associated with the time period of participation, we assigned a time-defined exposure which coincided with the roll-out of CPT. Women with a CD4 cell count of at least 500 cells/μL who had their second prenatal study visit after August 15th, 2006 were considered to be the "exposed" group, as they were exposed to the later time period. Women who gave birth before June 13th, 2006 were considered "unexposed", as they were unexposed to the later time period. Unadjusted and adjusted HRs for the association between the time-defined exposure and malaria in pregnancy were calculated as described above. In order to quantify the changes in the frequency of malaria between the time periods before and after implementation of CPT (i.e. April 2004 to June 2006 versus August 2006 to September 2009), we calculated unadjusted and adjusted HRs. This model included both the original study population and the "control"

population. The three variables included in this model were exposure to CPT, time period (as previously defined), and CD4 cell count less than 500 cells/μL.

Table 3.1 Severity of anemia classification

PARAMETER **Hemoglobin**	**GRADE 1** **MILD**	**GRADE 2** **MODERATE**	**GRADE 3** **SEVERE**	**GRADE 4** **POTENTIALLY LIFE-THREATENING**
Adult and Pediatric ≥ 57 days (HIV NEGATIVE ONLY)	10.0 – 10.9 g/dL *1.55 – 1.69 mmol/L* OR Any decrease 2.5 – 3.4 g/dL *0.39 – 0.53 mmol/L*	9.0 – 9.9 g/dL *1.40 – 1.54 mmol/L* OR Any decrease 3.5 – 4.4 g/dL *0.54 – 0.68 mmol/L*	7.0 – 8.9 g/dL *1.09 – 1.39 mmol/L* OR Any decrease ≥ 4.5 g/dL *≥ 0.69 mmol/L*	< 7.0 g/dL *< 1.09 mmol/L*
Infant*†, 36 – 56 days (HIV POSITIVE OR NEGATIVE)	8.5 – 9.4 g/dL *1.32 – 1.46 mmol/L*	7.0 – 8.4 g/dL *1.09 – 1.31 mmol/L*	6.0 – 6.9 g/dL *0.93 – 1.08 mmol/L*	< 6.00 g/dL *< 0.93 mmol/L*

Table 3.2 Classification of CPT exposure status for infants aged 6 – 36 weeks

CPT unexposed	CPT exposed
• All relevant person-weeks of data collected before June 13, 2006	• If collected after August 15, 2006: o All relevant person-weeks of data from all infants who had their 6 week visit after August 15, 2006

Table 3.3 Classification of CPT exposure status for women

CPT unexposed	CPT exposed
• FOR AIM 2A and 2B: All women/person-time during pregnancy for women who gave birth before June 13, 2006 • FOR AIM 2C: Women who had their 24 week visit before June 13, 2006	• FOR AIM ALL AIMS: All women/person time who had their second prenatal visit after August 15, 2006:

Table 3.4 Covariates considered in the analyses, by Specific Aim (combined list for both Specific Aims)

Covariate	Definitions and Levels	1A	1B	2A	2B	2C
Infant sex	Dichotomous: male (=1) vs. female (=0)	X	X			
Low birthweight	Birthweight < 2500 grams (dichotomous)	X	X			
Infant anemia	Categorical variable: mild, moderate, severe or life threatening (Table 3.3)	X	X			
Maternal age	Age in years, continuous, and as dichotomous variable (<26 vs. ≥26 years)	X	X	X	X	X
Maternal CD4	Explored as dichotomous variable using <350, ≥ 350 and <500, ≥500	X	X	X	X	X
Maternal marital status	Dichotomous variable: married vs. other	X	X	X	X	X
Maternal education	Dichotomous variable: primary education or less vs. greater than primary education	X	X	X	X	X
Maternal Past Medical Condition	Dichotomous: Answers Yes (1) to having had any of the conditions listed or answers No (0) (conditions include TB, hepatitis, heart disease, kidney disease, asthma and STIs)	X	X	X	X	X
Rainy season	Dichotomous (Aim 2B and 2C) or time varying (Aim 1A, 1B, 2A) variable representing the rainy season; November 1-March 31*	X	X	X	X	X
Nutritional randomization	Dichotomous variable: nutritional supplement (=1), no supplement (=0)	X	X	X		x
Maternal ARV randomization	Dichotomous variable: maternal antiretroviral arm or not. *Note: potential effect measure modifier.*	X	X	X		X
Infant ARV randomization	Dichotomous variable: infant antiretroviral arm or not. *Note: potential effect measure modifier*					
First pregnancy	Dichotomous variable representing first pregnancy or greater than first pregnancy	X	X	X	X	X

Figure 3.1. The 2-by-3 factorial design and sample size for the BAN study

	No ARV	Infant ARV	Mother ARV
No nutritional supplement	333 No supp No ARV	425 No supp Infant ARV	424 No supp Mother ARV
Nutritional supplement	335 Supp No ARV	423 Supp Infant ARV	427 Supp Mother ARV

CHAPTER FOUR: The effect of cotrimoxazole prophylaxis on adverse health outcomes in HIV-exposed, uninfected infants

ABSTRACT

BACKGROUND: World Health Organization guidelines recommend cotrimoxazole prophylactic treatment (CPT) for all HIV-exposed infants until cessation of breastfeeding and exclusion of HIV infection. There are limited data regarding the effects of CPT in this population, with existing data primarily among HIV-infected infants. We examined the effect of CPT, initiated at 6 weeks of age, on adverse health outcomes in this population during the first 36 weeks of life using data from a longitudinal study of prevention of mother-to-child transmission of HIV. CPT was initiated after the first 2 years of enrollment.

METHODS: We assigned CPT exposure based on the date of initiation of CPT in the study, assigning an exposed status to infants who participated in the study after the CPT program was started. We estimated unadjusted and adjusted hazard ratios (HRs) for the effect of CPT status on time to incident malaria, severe illness or death, anemia and underweight. Infants were censored by acquisition of HIV to focus exclusively on HIV-exposed, uninfected infants.

RESULTS: The HR for the effect of CPT on incident malaria was 0.35 (95% confidence interval (CI): 0.21, 0.57) during the first 10 weeks of CPT exposure, and 0.93 (95% CI: 0.67, 1.29) for the remaining 20 weeks. CPT was not associated with the other outcomes examined.

CONCLUSION: CPT offered temporary protection against malaria in HIV-exposed, uninfected infants, but not against severe illness or death, anemia or underweight.

INTRODUCTION

In 2007 approximately 2.1 million children were living with HIV/AIDS.[13] More than 90% of new infections in children are transmitted during pregnancy, birth or breastfeeding. The use of low-cost interventions such as single-dose nevirapine can decrease the risk of mother-to-child transmission of HIV by 50%.[15] Increasing access to interventions to prevent mother-to-child transmission of HIV over the past decade has resulted in a growing number of infants who are HIV-exposed but remain HIV-uninfected; it is now estimated that up to 18% of all infants in sub-Saharan Africa are HIV-exposed and uninfected.[119] These infants face an ongoing risk of HIV acquisition through breastfeeding, and are also in close contact with immunodeficient household members who are colonized with diverse pathogens, which puts them at an increased risk of acquiring other infections.[42-44] Maternal HIV infection can negatively impact transfer of maternal antibodies,[38, 39] and may impact the development of the immune system of HIV-exposed infants *in utero*.[40, 41] All of these factors threaten the health of HIV-exposed children, leading to increased morbidity and mortality compared with HIV-unexposed children.[20, 35, 36]

The World Health Organization (WHO) recommends daily cotrimoxazole prophylactic treatment (CPT) for HIV-exposed infants from 6 weeks of age until cessation of breastfeeding and exclusion of HIV infection.[1] Due to the difficulty and cost of HIV diagnosis and testing in HIV-exposed infants, HIV is often not diagnosed until the child becomes symptomatic, therefore, the WHO CPT guideline serves to protect infants with

undiagnosed HIV infections from opportunistic infections. Cotrimoxazole, a broad-spectrum antimicrobial which targets aerobic gram-positive and gram-negative organisms, fungi and protozoa, is widely available and has been provided as part of standard care for prevention of pneumocystis carinii pneumonia and toxoplasmosis for more than 15 years in developed countries. The WHO recommendation for infants is based on results of randomized controlled trials (RCTs) and non-experimental studies that demonstrated decreased incidence of severe events, hospitalizations and mortality in HIV-infected adults and children.[2-6] Limited data are available to demonstrate the beneficial effects of CPT in HIV-exposed, uninfected infants, although there is some evidence that CPT reduces the risk of lower respiratory tract infections[7] and pneumococcal colonization rates[8] in HIV-exposed children less than 18 months of age.

CPT in HIV-infected adults and children has also been associated with reductions in incidence of malaria.[2, 4, 10-12] Even though malaria is the primary cause of death in African children under 5 years of age,[120] there are no data on the effectiveness of CPT in reducing malaria incidence in HIV-exposed, uninfected children infants.

In the present analyses we examine the effect of CPT on adverse health outcomes (malaria; severe illness due to diarrhea, malaria, meningitis, pneumonia and serious febrile illnesses; anemia; and underweight) in HIV-exposed, uninfected children during the first 36 weeks of life. A better understanding of the effect of CPT on incidence of poor health outcomes in HIV-exposed, uninfected infants will add evidence to the limited existing data on a widely recommended regimen in this extremely vulnerable population in high risk settings.

METHODS

Study design and population

All children included in these analyses were enrolled in the Breastfeeding, Antiretrovirals and Nutrition (BAN) RCT which took place at four clinics in Lilongwe, Malawi between 2004 and 2009.[107] BAN's primary findings, that the use of either a maternal antiretroviral regimen or infant nevirapine for 28 weeks was effective in reducing HIV transmission during breastfeeding, have been reported elsewhere.[121] HAART-naïve, pregnant, HIV-infected women at least 18 years of age (at least 14 years of age if married) and 30 weeks' gestation or less were eligible for enrollment if they had hemoglobin levels over 7 g/dL, CD4 cell count of at least 250 cells/μL (≥200 cells/μL before July 24, 2006), normal liver function tests (more than 2.5 upper limit of normal), and no serious pregnancy complications.

All mothers participating in the BAN study were offered single-dose oral nevirapine during labor and zidovudine and lamivudine as a single tablet (Combivir®) every 12 hours from the onset of labor to seven days after giving birth. Newborn infants received single dose oral nevirapine within 72 hours of birth followed by twice-daily zidovudine (2mg/kg) and lamivudine (4mg/kg) for 7 days. All women were counseled to exclusively breastfeed followed by rapid weaning between 24 and 28 weeks after birth.

Mother-infant pairs were randomized within one week of birth only if they met secondary eligibility criteria: infant birth weight of at least 2000 g, no signs of congenital malformations, no infant or maternal condition that would preclude the use of a study drug, mother's acceptance of the 7-day maternal and infant perinatal antiretroviral regimen, and

enrollment within 36 hours after delivery. If mother-infant pairs met all eligibility criteria, they were randomized to a two-group maternal nutritional intervention and separately to a three-group antiretroviral intervention consisting of drugs given to the mother (maternal-regimen group), infant (infant-regimen group), or neither (control group). If they did not meet the secondary eligibility criteria, they were referred for care and did not continue participating in the study. Women in the maternal-regimen group received a triple-drug antiretroviral regimen, and infants in the infant-regimen group received a daily dose of nevirapine that increased according to age. The interventions for both mothers and infants began after delivery and were continued until the cessation of breastfeeding but no longer than 28 weeks. Infants found to be perinatally HIV-infected at birth or in the first two weeks of life were disenrolled from the BAN study and referred for care. Infants who tested positive for HIV later than 2 weeks of life, which was the primary endpoint of the BAN study, were discontinued from the intervention but not disenrolled from the study, and were encouraged to continue to attend regular study visits.

Mother-infant pairs were seen for visits at delivery and at 1, 2, 4, 6, 8, 12, 18, 21, 24, 28, 32, 36, 42 and 48 weeks postpartum. Data capturing anthropometrics, vital signs, illnesses and hospitalizations since the last visit, current symptoms, and physical exam findings were collected at all follow-up visits. Blood was collected at 2, 4, 6, 12, 18, 24, 28, 36 and 48 week visits. Participants were advised to return to the clinic between visits to receive treatment if the woman or child was ill. Blood smears were performed when there was clinical suspicion of malaria. Infants with a positive malaria blood smear were treated with sulfadoxine-pyrimethamine.

In accordance with the Malawi Ministry of Health and Population Guidelines and WHO guidelines on cotrimoxazole prophylaxis,[1] CPT was initiated in the BAN study for eligible women and infants in 2006. Although CPT has been used in high-income countries for prevention of opportunistic infections for many years, WHO and UNAIDS had not published guidelines for resource-limited settings until 2006, and CPT was rarely used in these settings before release of the guidelines which gave technical and operational recommendations in the context of scaling up HIV care in resource-limited settings. Starting on 13 June 2006, CPT (240 mg once daily) was provided to all infants in the BAN study beginning at 6 weeks of age. Infants who stopped breastfeeding by 28 weeks of age and were also HIV-uninfected at 28 weeks continued CPT until age 36 weeks. Infants who did not wean by 28 weeks continued CPT until weaning occurred and HIV infection was ruled out. CPT for children who were HIV-infected was intended to be life-long, and was provided for the duration of participation by the BAN study. CPT was also initiated at this time for mothers who had a CD4 cell count less than 500 cells/μL, as measured during pregnancy or at 24 weeks post partum.

For the purposes of these analyses, we excluded infants who did not present for a visit between 6-8 weeks of age and who did not have at least one follow-up visit after that time. Infants diagnosed with HIV at or before the 6 week visit were never included in the analyses.

Ethical review

The BAN study's protocol was approved by the Malawi National Health Science Research Committee and the institutional review boards at the University of North Carolina at Chapel Hill and the U.S. Centers for Disease Control and Prevention. This secondary

analysis of the BAN study data was reviewed and approved by the institutional review board of the University of North Carolina at Chapel Hill.

Statistical Analysis

All statistical analyses were performed using SAS (version 9.2, SAS Institute, Cary, NC).

Descriptive analyses included calculation of medians, standard deviations, and frequencies of exposures, outcomes and covariables. Categorical proportions were compared using chi-square test and continuous variables were assessed using the Wilcoxon rank-sum test.[109]

We estimated unadjusted and adjusted hazard ratios (HRs) and 95% confidence intervals (CIs) for the effect of CPT status on time to (1) first infection with malaria, (2) first severe illness or death, (3) anemia, or (4) underweight. For each of these four outcomes, we began with separate bivariate models containing only CPT and the outcome of interest. Multivariable models were then constructed which included covariables associated with CPT or the outcome of interest. We examined the proportional hazard assumption graphically using log-log plots and by adding interactions with time to the model.[122] If the assumption was violated, it was relaxed by fitting interactions with categorical or continuous time.[111] We explored rainy season, antiretroviral regimen, age and first pregnancy as modifiers of the association between CPT and the outcomes of interest. We evaluated these variables as possible effect measure modifiers by comparing the magnitude and precision of the main association within each level of each possible modifying covariable, and made qualitative assessments about the value of presenting stratified estimates. In order to identify effect measure modifiers we considered the exposure-outcome relationship at each level of a third

variable (the potential effect measure modifier) by including a product interaction term between the exposure and the potential effect measure modifier.[112] We ran a simple Cox model containing CPT exposure status, the outcome of interest, the potential effect measure modifier, and an interaction term between the CPT exposure status and the potential effect measure modifier. A p-value for the interaction term lower than α=0.10 was taken as evidence of substantial heterogeneity in the stratum-specific measures of effect.[113] Covariables found to be important effect measure modifiers were included in the starting multivariable model through an interaction term with CPT exposure status. To construct final models, we used a manual, backward elimination, change-in-estimate strategy. Potential confounders were removed from the preliminary full model in order of p-value magnitude (covariables with the highest *p*-values were removed first). If the CPT-outcome association changed by less than 10% overall or in any stratum of an interacting variable, a given covariable was not retained. [115]

For time-to-event analyses, children who tested positive for HIV were censored at the last visit at which they tested negative. Infants were also censored at death, maternal death, or loss to follow-up.

Definitions

Because the CPT guidelines were initiated 2 years into the BAN study, this created a unique opportunity for analysis similar to a natural experiment, with one CPT-unexposed period and one CPT-exposed period. While this was not a randomized treatment, the only factor dictating whether an infant received treatment was the time period during which the infant participated in the study. Therefore, for the purpose of this analysis, exposure to CPT was based on the 2006 time-point at which standardized CPT was implemented in the BAN

study. To minimize misclassification of CPT, inclusion in our analysis was restricted according to three criteria. First, in order to account for any lag time between the decision to administer CPT and the routine implementation of this practice, person-time from infants presenting for a visit between 6-8 weeks of age between 13 June 2006 (the date the first infant was started on CPT) and 15 August 2006 was not included in these analyses. Second, infants were only included if they presented between 6-8 weeks of age, to ensure that all infants were started on CPT within a 2 week age window. Finally, to avoid mixed exposure, analyses only included children who were either never exposed to CPT (fully unexposed, age 36 weeks by June 13, 2006), or children who were exposed to CPT from 6-8 weeks of age onward (fully exposed, age 6 weeks on or after August 15, 2006).

HIV infection was established using the Amplicor 1.5 DNA polymerase chain reaction (PCR) assay (Roche Molecular Systems). Positive specimens were confirmed by testing a specimen obtained at the next visit. If an infant was lost to follow-up or died before a confirmatory test was obtained, then a second specimen from the same day was tested at the reference laboratory at the University of North Carolina at Chapel Hill.

Malaria was defined as the first episode of malaria after 6 weeks of age and was diagnosed as a positive direct blood smear in a child presenting with symptoms of malaria. We excluded children who had a malaria diagnosis before 6 weeks of age. Severe illness was defined as any event of diarrhea, malaria, meningitis, pneumonia, or serious febrile illness, after 6 weeks of age that was fatal or life-threatening, required in-patient hospitalization, or resulted in persistent or significant disability or incapacity. Anemia was defined as a hemoglobin level below 7g/dl from 6 weeks to 8 weeks of age, or below 9 g/dl after 8 weeks of age, corresponding to grade 3 or higher anemia according to toxicity tables from the

Division of AIDS at the National Institute of Allergy and Infectious Diseases, as revised in March 2006. Malnutrition was classified based on weight-for-age z scores calculated using the 2005 WHO Child Growth Standards. An infant was considered undernourished if the weight-for-age z score was less than -2 after 6 weeks of age.[108]

Rainy season, defined as November through March and analyzed as a time-varying covariable, was evaluated both as an effect measure modifier and a confounder in the analysis for each outcome.

RESULTS

Baseline characteristics

After excluding 387 infants with mixed CPT exposure, and 19 infants who were diagnosed with HIV at or before the 6 week visit, 1522 mother-infant pairs were eligible for analysis (Table 4.1). Mothers of infants exposed to CPT were slightly older (26 vs. 25 years) and more likely to be married (92.6 vs. 89.1%). Mothers of CPT-exposed infants had a non-significantly higher CD4 cell count (441.0 vs. 431.0 cells/μL).

Effect of CPT on time to adverse health outcomes

Thirty-four infants had malaria before 6 weeks of age and were not included in the time to event analysis examining the association between CPT and incident malaria. We observed 311 cases of infant malaria prior to 36 weeks of age (and prior to censoring due to HIV infection, death or loss to follow-up) (Table 4.2) (Figure 4.1). Seventy occurred in CPT-unexposed children and 241 in CPT-exposed children. The unadjusted HR for the effect of CPT exposure on time to incident malaria was 0.71 (95% CI: 0.55, 0.93) (Table 4.3). As evidenced by the log-log plot and the statistical significance of a continuous interaction term

with time in the multivariable model, the effect of CPT appeared to change over time, and therefore, a categorical time interaction term at 70 days after initiation of CPT, corresponding to 16 weeks of age, was included in the model. This time-stratified analysis yielded an HR of 0.35 (95% CI: 0.21, 0.57) for the effect of CPT on incident malaria from 6 to 16 weeks of age, and an HR of 0.94 (95% CI: 0.68, 1.30) from >16 weeks to 36 weeks of age. Additional covariables did not meet the *a priori* criteria set for inclusion in the final model and thus our final model included only CPT, a categorical interaction term at 70 days after initiation of CPT, and malaria (Table 4.3).

We observed 169 severe illnesses or deaths among eligible infants: 34 in CPT-unexposed and 135 in CPT-exposed infants (Table 4.2) (Figure 4.1). Pneumonia was the most common (33.1% of all events) followed by diarrhea (27.2%). The unadjusted HR for the effect of CPT exposure on time to severe illness was 0.92 (95% CI: 0.63, 1.34) (Table 4.3). None of the covariables considered met our criteria for effect measure modification or confounding for this outcome.

Anemia was documented in 49 CPT-unexposed infants and 166 CPT-exposed infants (Table 4.2) (Figure 4.1). The unadjusted HR for the effect of CPT exposure on time to anemia was 0.78 (95% CI: 0.58, 1.05) (Table 4.3). No covariable met our criteria for inclusion in the final model.

Underweight occurred in 51 CPT-unexposed and 213 CPT-exposed infants (Table 4.2) (Figure 4.1). The HR for the effect of CPT exposure on time to underweight was 0.97 (95% CI: 0.72, 1.32). With adjustment for baseline weight (at 6 weeks of age), the direction of the association was reversed (adjusted HR 1.15, 95% CI: 0.83, 1.59) (Table 4.3) but

continued to be weak and not statistically significant. No other covariable met our criteria for inclusion in the final model.

DISCUSSION

Despite global recommendations for CPT for all HIV-exposed infants until cessation of breastfeeding and exclusion of HIV infection, little is known about the effect of CPT in HIV-exposed, uninfected infants. Our results contribute to the scant literature on CPT in HIV-exposed, uninfected infants. We assessed the effect of CPT from 6 to 36 weeks of age on adverse health outcomes in HIV-exposed, uninfected infants. We found that CPT may provide temporary protection against malaria in infancy but not against anemia, underweight and severe illness or death.

CPT protects against malaria in HIV-infected and HIV-uninfected adults,[4, 10, 79] and older HIV-uninfected children (age 5-15 years of age).[12] CPT also reduces the incidence of malaria in HIV-exposed, uninfected infants after cessation of breastfeeding to 2 years. In one RCT, HIV-exposed infants who continued CPT after cessation of breastfeeding and exclusion of HIV infection until 2 years of age had a 38% reduction in malaria incidence compared with infants who stopped CPT after cessation of breastfeeding. All infants in that study were also given insecticide-treated bed nets (ITNs).[96] Our findings extend and clarify the time period during which CPT may be effective: we found that CPT was associated with a 65% reduction in time to malaria between 6 and 16 weeks of age, but we did not observe a protective effect after 16 weeks of age.

The effect of CPT in infants, particularly during the first months of life, may be complex. Malaria is more commonly seen after the first 2 or 3 months of life, with increasing incidence in the remainder of infancy generally reaching a peak within the first

two years,[2, 46, 48-53] especially in areas of high transmission.[54, 55] Lower incidence in the first months of life is generally attributed to the protective effect of maternal antibodies.[47] Maternal HIV infection has been demonstrated to negatively impact the transfer of some maternal antibodies,[38, 39] and is also believed to impact the development of the immune system *in utero*.[40, 41] These factors may cause HIV-exposed infants to be more susceptible to malaria and other infections during the first few months of life, when passive immunity usually provide protection, and when the infant's immune system is still developing. CPT may help protect these children during this time period. Alternatively, there may be a synergistic interaction between CPT and existing maternal antibodies during the first few months of life, resulting in additional protection against malaria for the infant. This effect may fade as the infant's maternally acquired antibodies against malaria wane.

CPT was not significantly associated with grade 3 or 4 anemia, though we observed a protective trend in these Malawian infants. Observed associations between CPT and anemia in the literature are mixed. Protection against anemia was observed in healthy children (5-15 years old) receiving CPT, where CPT was also found to be highly protective against malaria, potentially contributing to the decrease in anemia.[12] In HIV-infected adults with a CD4 cell count of at least 500 cells, CPT had no effect on hemoglobin.[123] If CPT does protect against anemia, it is possible that this effect would be weaker in breastfeeding infants, who are born with iron stores that are generally sufficient for the first 4-6 months of life.[124] These infants also receive iron through breastmilk, particularly in the first few months when iron in breastmilk is most bioavailable.[125]

In our analyses CPT did not protect against severe illness or death. In contrast, other studies have reported that CPT in HIV-infected children protects against death and

respiratory infections and is associated with somewhat lower admission rates for bacterial infection.[77] In some analyses CPT also protects against respiratory infections in HIV-infected infants.[7, 88] In older healthy children (age 5-15 years), CPT decreased rates of gastrointestinal illness, but did not protect against respiratory illnesses.[12] Similar trends have been observed in HIV-exposed infants. In contrast to those benefits, a trend towards an increased risk of diarrhea in HIV-infected and HIV-exposed, uninfected infants has also been observed, but this association did not reach statistical significance.[7] We did not observe an effect of CPT on severe illness and death, but due to the small number of events, we were unable to assess associations between CPT and specific infections. Breastfeeding, particularly in the first 6 months, is associated with protection against infectious disease in infants,[126] therefore, it is possible that CPT would offer more protection when provided to infants who breastfeed for longer time periods, or in the context of mixed feeding. Benefits of CPT may also be more apparent when assessing mild instead of severe infections.

CPT also did not appear to be associated with underweight, even after adjustment for weight at 6 weeks of age. Because of the exclusive breastfeeding recommendations of the parent BAN study, our analysis population may exhibit less variation in nutritional outcomes than what is observed outside of a clinical trial setting. In addition, following cessation of breastfeeding mothers were counseled to feed infants a lipid-based nutrient supplement (LNS). This energy-dense, micronutrient-fortified supplement, provided for free until 12 months of age, was designed to fulfill infant micronutrient requirements and replace the energy and protein that would have been provided by breastmilk.[107] This type of supplement and support to ensure sufficient nutritional intake is unusual in resource-poor settings, and clearly affects the generalizability of our findings on CPT and malnutrition.

Our analyses have important limitations which are similar to other observational studies. First, exposure status was based on presumed exposure using the 2006 date that CPT was initiated as a general guideline for classifying all infants, rather than using pharmacy and adherence records. A review of a random sample of patient pharmacy records showed good adherence to the CPT guidelines (data not shown). It is possible some children classified as exposed, may, in fact, have not taken cotrimoxazole, which could bias our results. If this is the case, our observed estimates are likely weaker than the true estimates of the effect of CPT on the outcomes of interest. Second, unmeasured changes in the general population over the 5 year period of observation from 2004-2009 may have coincided with the roll-out of CPT (mid-2006). Temporal changes in disease incidence occurring during the study period cannot be separated from the effects of CPT. For example, starting in March of 2007, ITNs were distributed to 3500 HIV-infected pregnant women in Lilongwe, including some of the women participating in the BAN study. Ownership and use of ITNs was not monitored by BAN, and thus we could not adjust for it in our analysis. If malaria decreased due to the use of ITNs or other factors, the protective effect in the first 16 weeks of life suggested by our analysis may be an artifact of ITN use or other changes in disease incidence unrelated to CPT. CPT in HIV-infected adults has been shown to offer some health benefits to other household members,[42] therefore, it is also possible that the simultaneous initiation of CPT in BAN mothers with a CD4 cell count less than 500 cells/μL could have contributed to the protective effect against malaria demonstrated in our analyses.

As noted earlier, suboptimal adherence likely occurred in our study as we did not monitor adherence. Suboptimal adherence would also occur outside of a study setting. Our

study may thus approximate the "normal" conditions of varied adherence observed in routine roll-out of CPT, which strengthens the generalizability of our findings.

The primary rationale for CPT in HIV-exposed infants is to reduce the morbidity and mortality of opportunistic infections in HIV-infected infants in whom the HIV infection status has not yet been determined. Following current WHO guidelines, it is no longer ethical to conduct an RCT examining the effect of CPT in HIV-exposed, uninfected infants, thus observational studies such as this one provide valuable insight into the effects of CPT in HIV-exposed, uninfected infants. Our findings indicate that CPT in this population may be limited to a temporary protection against malaria in the first 16 weeks of life, without a meaningful effect on anemia, malnutrition, or severe illness or death. Continued monitoring of CPT and its effects in this population of HIV-exposed infants, including adverse effects and cotrimoxazole resistance, will help to inform future guidelines as interventions in the prevention and treatment of HIV continue to develop. Given the limited protection offered by CPT in HIV-exposed, uninfected infants, when more accurate infant testing for HIV becomes widely available and the incidence of acquisition of HIV is reduced, an in depth assessment of the CPT guidelines may be necessary in order to ensure that CPT is the best use of extremely limited resources available to address the multitude of factors threatening the health of this vulnerable population.

Table 4.1. Baseline Characteristics of 1522 Infants and their Mothers

Characteristic	CPT-unexposed* (N = 283)	CPT-exposed* (N = 1239)	Total population (N=1522)	Missing	P value†
Infants					
Gender (male) %	53.6	50.2	50.9	N=6	0.32
Low birth weight (< 2500 g) (%)	5.7	5.9	5.9	N=9	0.91
Infant ARV arm (%)	33.9	38.6	37.7		0.14
Mothers					
Age (yr)					
Median	25.0	26.0	26.0		0.02
Interquartile range	22.0-29.0	23.0-30.0	23.0-29.0		
CD4 at baseline (cells/μL)†					
Median	431.0	441.0	439.0		0.31
Interquartile range	328.0-576.0	332.0-577.0	332.0-576.0		
Maternal education (>primary)	34.3	35.5	35.3	N=2	0.69
Married (%)	89.1	92.6	91.9		0.05
Mother's first pregnancy (%)	12.7	11.2	11.5		0.48
Nutritional supplement arm (%)	50.9	49.9	50.1		0.76
Maternal ARV arm (%)	32.2	37.0	36.1		0.13

* Infants were considered CPT-unexposed if > 36 weeks of age by June 13, 2006; infants were considered CPT-exposed if they reached 6 weeks of age on or after August 15, 2006

† P-values based on Wilcoxon rank-sum test for continuous variables and chi-square test for binary variables, comparing CPT-exposed and CPT-unexposed groups

Table 4.2. Frequency of Outcomes of Interest from 6 to 36 Weeks of Age in CPT-Exposed and Unexposed Infants.

Outcome	CPT-unexposed infants* (N=283)	CPT-exposed infants* (N=1239)	Total (N=1522)
Malaria	70 (24.7%)	241 (19.5%)	311 (20.4%)
Anemia	49 (17.3%)	166 (13.4%)	215 (14.1%)
Severe illness or death	34 (12.0%)	135 (10.9%)	169 (11.1%)
Diarrhea	11 (3.9%)	35 (2.8%)	46 (3.0%)
Malaria	10 (3.5%)	14 (1.1%)	24 (1.6%)
Meningitis	1 (0.4%)	9 (0.7%)	10 (0.7%)
Pneumonia	5 (1.8%)	51 (4.1%)	56 (3.7%)
Febrile illness	6 (2.1%)	22 (1.8%)	28 (1.8%)
Vomiting	0 (0.0%)	2 (0.2%)	2 (0.1%)
Death	1 (0.4%)	2 (0.2%)	3 (0.2%)
Underweight (weight for age < -2)	51 (18.0%)	213 (17.1%)	264 (17.3%)

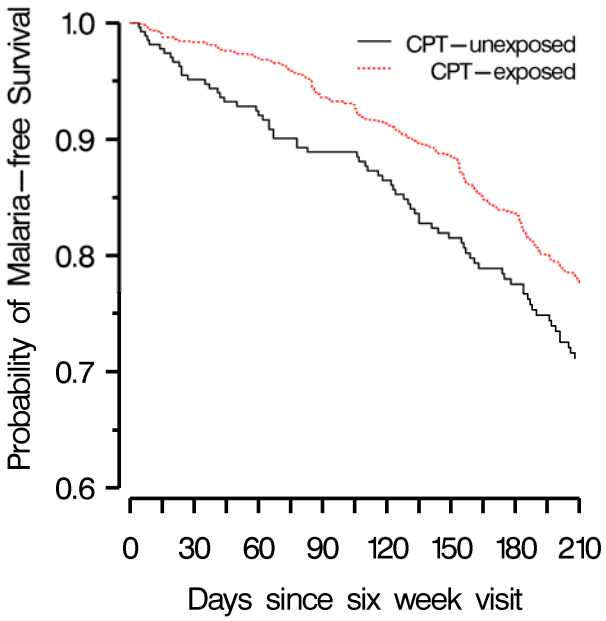
* Infants were considered CPT-unexposed if > 36 weeks of age by June 13, 2006; infants were considered CPT-exposed if they reached 6 weeks of age on or after August 15, 2006

Table 4.3. Hazard Ratios for Exposure to Cotrimoxazole Prophylactic Treatment Administered from 6 to 36 Weeks of Age on Adverse Health Outcomes from 6 to 36 Weeks of Age.

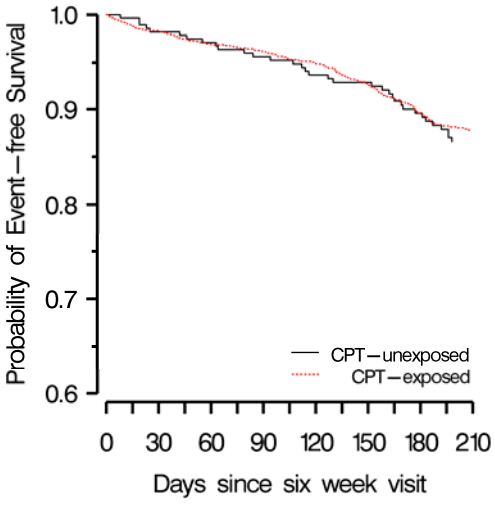
	Unadjusted Hazard Ratio (95% CI)	**Adjusted Hazard Ratio (95% CI)**
Malaria	0.71 (0.55, 0.93)	0.35 (0.21, 0.57) from 6 weeks to 16 weeks of age 0.94 (0.68, 1.30) from > 16 weeks to 36 weeks of age
Severe illness or death	0.92 (0.63, 1.34)	0.92 (0.63, 1.34)
Anemia (grade 3 or 4)	0.78 (0.58, 1.05)	0.78 (0.58, 1.05)
Underweight (Weight for age < -2)	0.97 (0.72, 1.32)	1.15 (0.83, 1.59) *Adjusted for baseline weight*

Figure 4.1 – Kaplan-Meier curves illustrating the probability of (A) Malaria, (B) severe illness or death, (C) moderate or severe anemia, and (D) weight-for-age z score <-2. Red represents infants exposed to CPT, black represents infants unexposed to CPT.

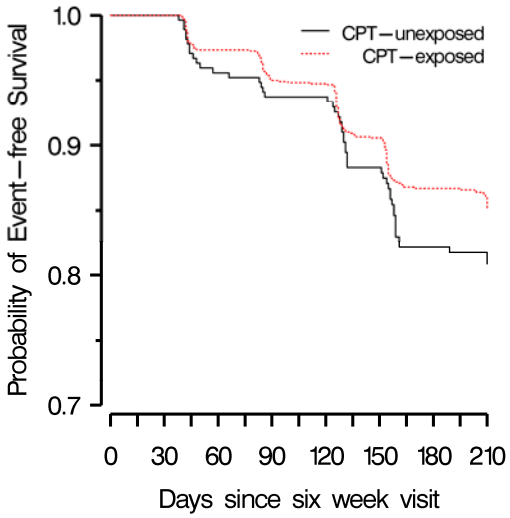
A) Malaria



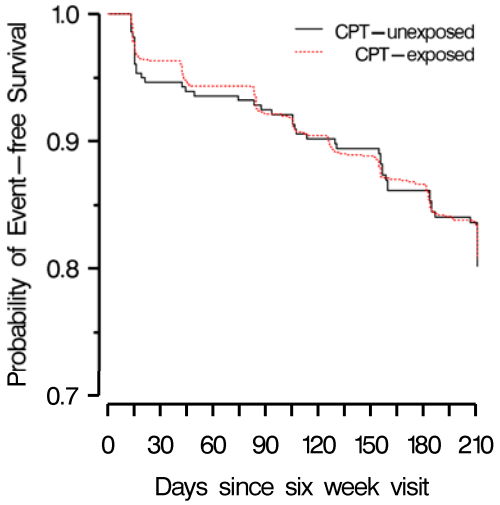
B) Severe illness



C) Anemia



D) Underweight (weight for age z score < -2)



CHAPTER FIVE: Effect of Cotrimoxazole Prophylactic Treatment on Malaria, Birth Outcomes and CD4 cell count in HIV-infected Pregnant Women

ABSTRACT

BACKGROUND: HIV-infected pregnant women receive cotrimoxazole prophylactic treatment per the guidelines established by the World Health Organization for HIV-infected adults. However, there are limited data regarding the effects of CPT in pregnant women, including how CPT protects against malaria compared to the standard malaria prophylaxis of intermittent preventive treatment with sulfadoxine-pyrimethamine, which is contraindicated in women receiving CPT. We examined the effect of CPT in HIV-infected pregnant women with a CD4 count between 200 and 500 cells/μL, on adverse maternal and infant outcomes.

METHODS: Using data from a large prevention of mother-to-child transmission study, we assigned CPT exposure based on the date of initiation of CPT in the study, assigning an exposed status to mothers who participated in the study after the CPT program was started. We examined unadjusted and adjusted hazard ratios, odds ratios and risk ratios for the effect of CPT status on time to infection with malaria, low birth weight and preterm birth, respectively. We used linear regression to assess the effect of CPT on CD4 cell count at 24 weeks post-partum.

RESULTS: After adjustment for changes in disease incidence over time, CPT had no effect on time to malaria (adjusted Hazard Ratio: 0.66, 95% Confidence Interval (CI), 0.28, 1.52).

CPT was not associated with preterm birth or low birth weight. CPT was associated with a lower CD4 cell count at 24 weeks postpartum in women not receiving antiretrovirals (-77.6 cells/μL, 95% CI: -125.2, -30.1) and women receiving antiretrovirals (-33.7 cells/μL, 95% CI: -58.6, -8.8), compared to women not receiving CPT.

CONCLUSIONS: Compared to intermittent preventive treatment with sulfadoxine-pyrimethamine, CPT does not appear to provide additional protection against malaria in HIV-infected pregnant women, nor does it offer protection against preterm birth or low birth weight. Women receiving CPT had a lower CD4 cell count at 24 weeks post-partum compared to women not receiving CPT, regardless of antiretroviral treatment status.

INTRODUCTION

Cotrimoxazole prophylaxis has been shown to reduce morbidity and mortality in HIV –infected adults and children.[2-6] The World Health Organization (WHO) guidelines recommend daily cotrimoxazole prophylactic treatment (CPT) for HIV-infected adults and HIV-infected pregnant women with CD4 cell count less than 350 cells/μL or WHO clinical stage III or IV.[1] In settings with high prevalence of HIV and limited healthcare infrastructure, the WHO suggests consideration of broader access to CPT, including universal access for anyone with confirmed HIV infection.[1] The guidelines are based on results of randomized controlled trials (RCTs) and non-experimental studies that demonstrated decreased incidence of severe events, hospitalizations and mortality in HIV infected adults and children. Data on CPT in HIV-infected pregnant women are scarce, though there is some evidence suggesting that CPT may reduce the risk of poor birth outcomes in addition to reducing morbidity and

mortality due to opportunistic infections. This data came from an observational study in Zambia which found that compared to historic controls, CPT provision to women with a CD4 cell count less than 200 cells/μL resulted in reduced odds of preterm birth and a decrease in neonatal mortality.[9] It is not known if the same benefits would be seen in women with higher CD4 cell counts.

CPT in HIV-infected adults has also been associated with a reduction in malaria incidence.[2, 4, 10] HIV-infected pregnant women in particular would benefit from reduced risk of malaria, as these women experience more peripheral and placental malaria compared with HIV-uninfected pregnant women.[70] Dual infection with HIV and malaria in pregnant women is particularly concerning due to the associated increase in the risk of poor birth outcomes, including low birth weight and prematurity, especially in multigravidae.[71-73] Due to similarities between cotrimoxazole and sulfadoxine-pyrimethamine (SP), SP-based Intermittent Preventive Therapy during pregnancy (SP-IPTp) for malaria, which is usually given to women during pregnancy regardless of HIV status, is not given in cases where CPT is given.[1] However, the ability of CPT to prevent malaria has not been well studied in pregnant women.

In the present analyses we examined the effect of CPT initiated during pregnancy in women with a CD4 cell count between 200 and 500 cells/μL on adverse maternal and infant outcomes, and on CD4 cell count at 24 weeks postpartum. These analyses on CPT will add evidence to the limited knowledge on this component of HIV care which is widely used in highly vulnerable populations.

METHODS

Study design and population

All women included in this analysis were enrolled in the Breastfeeding, Antiretrovirals and Nutrition (BAN) RCT which took place at 4 clinics in Lilongwe, Malawi between 2004 and 2009.[107] BAN's primary findings, that the use of either a maternal antiretroviral regimen or infant nevirapine for 28 weeks was effective in reducing HIV transmission during breastfeeding, have been reported elsewhere.[121] HAART-naïve, pregnant, HIV-infected women at least 18 years of age (at least 14 years of age if married) and at least 30 weeks' gestation were eligible for enrollment if they had hemoglobin levels over 7 g/dL, CD4 count of at least 250 cells/μL (≥200 cells/μL before July 24, 2006), normal liver function tests (more than 2.5 upper limit of normal), and no serious pregnancy complications. Depending on the estimated gestational age at screening, women were asked to return for follow-up prenatal care at approximately 28, 32 and 36 weeks' gestation.

All mothers participating in the BAN study were offered single-dose oral nevirapine during labor and zidovudine and lamivudine as a single tablet (Combivir®) every 12 hours from the onset of labor to 7 days after giving birth. Newborn infants received single dose oral nevirapine within 72 hours of birth followed by twice-daily zidovudine (2mg/kg) and lamivudine (4mg/kg) for seven days. All women were counseled to exclusively breastfeed followed by rapid weaning between 24 and 28 weeks after birth.

Mother-infant pairs were randomized within one week of birth if they met secondary eligibility criteria: infant birth weight of at least 2000 g, no signs of congenital malformations, no infant or maternal condition that would preclude the use of a study drug,

mother's acceptance of the 7-day maternal and infant perinatal antiretroviral regimen, and enrollment within 36 hours after delivery. If mother-infant pairs met these criteria, they were randomized to a two-group maternal nutritional intervention and separately to a three-group antiretroviral intervention consisting of drugs given to the mother (maternal-regimen group), infant (infant-regimen group), or neither (control group). Women in the maternal-regimen group received a triple-drug antiretroviral regimen which initially consisted of Combivir® and nevirapine. Nevirapine was replaced after the first 39 women were randomized with nelfinavir, which was later replaced with lopinavir/ritonavir. Infants in the infant-regimen group received a daily dose of nevirapine that increased according to age. The interventions for both mothers and infants began after delivery and were continued until the cessation of breastfeeding but no longer than 28 weeks. Infants found to be perinatally HIV-infected at birth or in the first two weeks of life were disenrolled from the BAN study and referred for care. Infants who tested positive for HIV later than two weeks of life, which was the primary endpoint of the BAN study, were discontinued from the intervention but not disenrolled from the study, and were encouraged to continue to attend regular study visits.

Mother-infant pairs were seen for visits at delivery and at 1, 2, 4, 6, 8, 12, 18, 21, 24, 28, 32, 36, 42 and 48 weeks postpartum. Data capturing anthropometrics, vital signs, illnesses and hospitalizations since the last visit, current symptoms, and physical exam findings were collected at all follow-up visits. Blood was collected at 2, 4, 6, 12, 18, 24, 28, 36 and 48 week visits. Patients were advised to return to the clinic between visits to receive treatment if the woman or child was ill. Blood smears were performed when there was clinical suspicion of malaria.

In accordance with the Malawi Ministry of Health and Population Guidelines and WHO guidelines on cotrimoxazole prophylaxis,[1] CPT was initiated in the BAN study for eligible women and infants in 2006. Although CPT has been used in high-income countries for prevention of opportunistic infections for many years, WHO and UNAIDS had not published guidelines for resource-limited settings until 2006, and CPT was rarely used in these settings before release of the guidelines which gave technical and operational recommendations in the context of scaling up HIV care in resource-limited settings. Starting on 13 June 2006, CPT (480 mg twice daily) was provided after the 12th week of pregnancy to all participating women with a CD4 cell count less than 500 cells/μL, regardless of symptoms. CD4 cell counts were performed at screening, 24 weeks and 48 weeks postpartum. CPT could be started based on a CD4 cell count less than 500 cells/μL at any of those time points. The routine second and third trimester doses of SP given to pregnant women were omitted in women receiving CPT in accordance with WHO recommendations.[1] Due to similarities between cotrimoxazole and SP, SP was not recommended for use as first line treatment for malaria in women taking CPT, however, SP as treatment for malaria was continued until the Malawian Department of Health malaria guidelines changed in 2007 to first line Artemether-Lumefantrine (quinine in first trimester of pregnancy), second line Amodiaquin-Artesunate, and IV Quinine for severe malaria. Once initiated, CPT was intended to be life-long, and was provided for the duration of participation by the BAN study.

Ethical review

The BAN study's protocol was approved by the Malawi National Health Science Research Committee and the institutional review boards at the University of North Carolina

at Chapel Hill and the U.S. Centers for Disease Control and Prevention. This secondary analysis of the BAN study data was reviewed and approved by the institutional review board of the University of North Carolina at Chapel Hill.

Statistical Analysis

All statistical analyses were performed using SAS (version 9.2, SAS Institute, Cary, NC).

Descriptive analyses included calculation of medians, standard deviations, and frequencies of exposures, outcomes and covariates. Categorical proportions were compared using chi-square test and continuous variables were assessed using the Wilcoxon rank-sum test.[109]

We estimated unadjusted and adjusted hazard ratios (HRs) and 95% confidence intervals (CIs) for the effect of CPT status on time to malaria infection. Because SP-IPTp was given to women before CPT was introduced, this analysis amounted to a comparison between the two regimens. We began with a bivariate model containing only CPT and the outcome of interest. We then constructed multivariable models to include covariates associated with CPT or the outcome of interest. The proportional hazard assumption was examined graphically using log-log plots and by adding interactions with time to the model. If the assumption was violated, it was relaxed by fitting interactions with categorical or continuous time.[111] The log-rank test was used to compare the CPT-exposed and unexposed groups.[122]

We explored rainy season, age and first pregnancy as modifiers of the association between CPT and the outcomes of interest. We evaluated these variables as possible effect

measure modifiers by comparing the magnitude and precision of the main association within each level of each possible modifying covariable, and made qualitative assessments about the value of presenting stratified estimates. In order to identify effect measure modifiers we considered the exposure-outcome relationship at each level of a third variable (the potential effect measure modifier) by including a product interaction term between the exposure and the potential effect measure modifier.[112] We ran a simple Cox model containing CPT exposure status, the outcome of interest, the potential effect measure modifier, and an interaction term between the CPT exposure status and the potential effect measure modifier. A p-value for the interaction term lower than α=0.10 was taken as evidence of substantial heterogeneity in the stratum-specific measures of effect.[113] Covariables found to be important effect measure modifiers were included in the starting multivariable model through an interaction term with CPT exposure status. To construct final models, we used a manual, backward elimination, change-in-estimate strategy. Following assessment for effect measure modification, potential confounders were removed from the preliminary full model in order of *p*-value magnitude (covariates with the highest *p*-values were removed first). If the CPT-outcome association changed by less than 10% overall or in any stratum of an interacting variable, a given covariate was not retained.[115]

To assess the association between CPT during pregnancy and low birth weight we estimated odds ratios (ORs) and 95% confidence intervals (95% CIs) using multivariate logistic regression. CPT exposure, the outcome of interest, potential confounders and effect measure modifiers along with the appropriate interaction terms were entered into the model. Effect measure modification was assessed by examining Wald p-values or the likelihood ratio test for the model with and without the selected interaction term. A manual backward

elimination process was used beginning with the potential confounder with the highest p-value to determine which covariates resulted in greater than a 10% change in estimate and should be retained in the model as confounders.

For analysis of the association between CPT during pregnancy and preterm birth multivariate log binomial regression was used, due to higher frequency of the outcome (23.6%),[117] following the same procedures as outlined for logistic regression.

The effect of CPT on change in CD4 cell count at 24 weeks postpartum was assessed by constructing separate linear regression models, stratified by maternal antiretroviral regimen status (randomization status). Crude and adjusted CD4 cell counts at 24 weeks were calculated along with 95% confidence intervals. Effect measure modification was assessed by examining the partial F test for the model with and without the selected interaction terms.[118] As with the other analyses, a manual backward elimination process was used to finalize the model.

Definitions

Because the CPT guidelines were initiated 2 years into the BAN study, this created a unique opportunity for analysis similar to a natural experiment, with one CPT-unexposed period and one CPT-exposed period. While this was not a randomized treatment, the only factor dictating whether a woman received treatment was the time period during which she participated in the study. Therefore, for the purpose of this analysis, exposure to CPT was based on the 2006 time-point at which standardized CPT was implemented in the BAN study. To minimize misclassification of CPT, inclusion in our analysis was restricted according to 2 criteria. First, in order to account for any lag time between the decision to administer CPT and the routine implementation of this practice, person-time from women

presenting for their second prenatal visit (median time of 12.7 weeks before delivery) between 13 June 2006 (the date the first mother was started on CPT) and 15 August 2005 was not included in these analyses. Second, analyses only included women who were either never exposed to CPT (women who gave birth before 13 June 2006, or for the CD4 outcome, women who had their 24 week visit before 13 June 2006), or women who were exposed from their second prenatal visit onwards (fully exposed, second prenatal visit after 15 August 2006).

Malaria was defined as the first episode of malaria after the second prenatal visit, based on the finding of parasites on a blood smear performed when there was clinical suspicion of malaria. Because of the short time period of observation, we excluded women who had a diagnosis of malaria at or before the time of CPT initiation. Preterm birth was defined as birth before 37 weeks of gestation, based on the date of the last reported menstrual period. Low birth weight was defined as a birth weight less than 2500 grams.

Sensitivity analysis among women ineligible for CPT

Women who had a CD4 cell count of at least 500 cells/μL at screening were not eligible for CPT until at least 24 weeks postpartum, at which point they were eligible if their CD4 cell count fell below 500. We used data from this pseudo "control" group to assess unmeasured confounding, i.e. whether there were changes in the frequency of incident malaria in the study population between the time periods before and after implementation of CPT (before June 2006 and after August 2006). This is most important for outcomes such as malaria, which are known to fluctuate over time. Incidence of birth outcomes and changes in CD4 cell count are less likely to fluctuate over the time span of the BAN study. In order to

assess whether there were changes in the outcome associated with the time period of participation, we assigned a time-defined exposure which coincided with the roll-out of CPT. Women with a CD4 cell count of at least 500 cells/μL who had their second prenatal study visit after 15 August 2006 were considered to be the "exposed" group, as they were exposed to the later time period. Women who gave birth before 13 June 2006 were considered "unexposed", as they were unexposed to the later time period. Unadjusted and adjusted HRs for the association between the time-defined exposure and malaria in pregnancy were calculated as described above. In order to quantify the changes in the frequency of malaria between the time periods before and after implementation of CPT (i.e. April 2004 to June 2006 versus August 2006 to September 2009), we calculated unadjusted and adjusted HRs. This model included both the original study population and the "control" population. The three variables included in this model were exposure to CPT, time period (as previously defined), and CD4 cell count less than 500 cells/μL.

RESULTS

After excluding 850 women with a CD4 greater than 500 at screening, and 197 women with mixed CPT exposure, 1236 mother-infant pairs were eligible for analysis (Table 5.1). Median CD4 cell count at the screening visit was slightly lower in CPT-unexposed women. This difference was statistically significant but unlikely to be clinically relevant.

There were 91 low birth weight infants, 36.3% were born to CPT-unexposed mothers and 62.6% were born to mothers who were CPT-exposed. Median birth weight among these infants was 2300 grams (interquartile range: 2140, 2400). The unadjusted OR for the effect of CPT vs SP-IPTp on giving birth to a low birth weight infant was 1.08 (95% CI: 0.70,

1.69). None of the covariates explored met the criteria for inclusion in the final model as an effect measure modifier or confounder.

Date of last menstrual period was available for 624 (50.5%) of the women considered in this analysis, due to a change in the data collected in the BAN study. Women who did not have this information available were less likely to have completed more than primary school level of education (p=0.002) and were more likely to give birth to a low birth weight infant (p=0.02). Among the women included in this analysis, 147 women had a preterm birth, 40.1% of these women were unexposed to CPT and 59.9% were exposed to CPT. The RR for the effect of CPT exposure on preterm birth was 1.00 (95% CI: 0.75, 1.34) (Table 5.3). None of the covariates explored met the criteria for inclusion in the final model as an effect measure modifier or confounder.

Linear analysis of CD4 cell count at 24 weeks postpartum was performed using two separate models; one for women randomized according to BAN Study procedures to the maternal antiretroviral arm, and one for the women randomized to either the infant antiretroviral regimen group or the control group. CD4 cell count at screening (conducted at a median time of 14.3 weeks (interquartile range: 9.7, 18.6) before delivery) was included in both linear models. Among the 810 eligible women, the median time between the two CD4 measurements was 38.4 weeks (interquartile range, 34.0, 42.7). There were 514 women eligible for analysis who did not receive the maternal antiretroviral regimen, of whom 358 were CPT-exposed and 156 were CPT-unexposed, and 296 who did receive the antiretroviral regimen, of which 225 were CPT-exposed and 71 were CPT-unexposed (Figure 5.2). The mean CD4 cell counts at 24 weeks were higher than mean CD4 cell counts at screening for all groups, as is expected due to the physiological hemodilution that occurs during

pregnancy.[127, 128] Overall, CPT appeared to be associated with lower CD4 cell counts at 24 weeks post-partum. Among women not receiving the antiretroviral regimen, CD4 cell count at 24 weeks postpartum was 33.7 cells/μL (95% CI: 8.8. 58.6) lower among those who received CPT, compared to women who did not receive CPT, after adjustment for CD4 cell count at screening. Similarly, among women who received the antiretroviral regimen, CD4 cell count at 24 weeks postpartum was 77.6 cells/μL (95% CI: 30.1, 125.2) lower among those who received CPT, compared to women who did not receive CPT, adjusted for CD4 cell count at screening. CD4 cell count at screening was the only covariate that met the criteria for inclusion as a confounder or effect modifier in either of the final models.

Among the pregnant women included in the analysis, 54 women were diagnosed with malaria during the time period between the second prenatal study visit (at which time CPT was started if eligible; visit occurred at a mean of 12.7 weeks before delivery) and delivery (Table 5.2) (Figure 5.1). Of these 54 women, 61% were in the CPT-unexposed group and 38.9% were in the CPT-exposed group. The unadjusted HR for the effect of CPT vs SP-IPTp on incident malaria was 0.35 (95% CI: 0.20, 0.60). None of the covariates explored met the criteria for inclusion in the final model as an effect measure modifier or a confounder.

Sensitivity analyses among women ineligible for CPT

There were 700 pregnant women with a CD4 cell count of at least 500 cells/μL who comprised the population for the sensitivity analysis to assess the effect of potential unmeasured confounders of the association between malaria and CPT.

First, we developed a model to assess the association between malaria and participation during the later time period, as described earlier. Among the 700 women with

CD4 cell count of at least 500 cells/μL, 38 women were diagnosed with malaria during pregnancy: 55.3% were diagnosed during the earlier time period (between April 2004 and June 2006) and 44.7% were diagnosed during the later time period (between August 2006 and September 2009). The unadjusted HR for the association between malaria and time period of participation was 0.51 (95% CI: 0.27, 0.97). None of the covariates explored met the criteria for inclusion in the final model, indicating the likelihood of unmeasured confounding.

Next, we developed a model including both women with a CD4 cell count of at least 500 cells/μL (the sensitivity analysis population described above) and women with a CD4 cell count less than 500 cells/μL (our original study population) in order to quantify the effect of CPT on malaria during pregnancy, adjusted for time period of participation. The HR for the association between participation in the later time period and malaria, adjusted for CPT, was 0.52 (95% CI 0.27, 0.98). The HR for the effect of CPT on malaria, adjusted for time period of participation was 0.66 (95% CI: 0.28, 1.52).

DISCUSSION

HIV-infected women receive CPT for prevention of opportunistic infections following the WHO guidelines for HIV-infected adults. Although there are unique health concerns and considerations for HIV-infected pregnant women, little is known about the effects of CPT in this specific population. We assessed the effect of CPT, initiated during pregnancy, on malaria, low birth weight, preterm birth and CD4 cell count at 24 weeks postpartum. Among HIV-infected women with CD4 cell counts between 200 and 500 cells/μL, we observed no meaningful effect of CPT on birth outcomes or malaria, after

adjustment for time period of participation. In addition, we observed that exposure to CPT, independent of maternal antiretroviral regimen status, may limit the rebound usually seen in CD4 cell count during the postpartum period, as measured at 24 weeks postpartum.

The WHO CPT recommendations for HIV-infected adults are based on the benefits of prophylaxis, including reduced hospitalizations, morbidity and mortality in HIV-infected patients across varying CD4 levels.[2, 4-6, 42, 77-79] CPT also offers protection against malaria in both HIV-infected and uninfected adults and children.[4, 10, 12, 79] In our population of pregnant women in Lilongwe, Malawi, CPT started at a median of 12.7 weeks before birth appeared to protect against malaria during pregnancy. However, after consideration of overall trends in malaria incidence during the study, it appears the observed effect of CPT may in fact be due to an overall decrease in malaria during the later part of the study (and thus be unrelated to CPT). Our ability to assess the effect of time period through inclusion of both study women (women with CD4 cell count below 500 cells/μL) and "control" women (who had higher CD4 cell counts and were therefore never eligible for CPT), was an important strength of our analysis. This sensitivity analysis allowed us to address confounding that was unmeasured in our primary study population, a major limitation in most observational studies. The analysis demonstrated that, despite our initial findings from traditional analyses, CPT did not in fact meaningfully affect malaria incidence. Of course, we also made multiple assumptions when conducting this sensitivity analysis and thus the findings must be interpreted cautiously. For example, the model assumes the effect of time period is the same regardless of CD4 cell count, that the effect of CPT is the same across time periods, and that the effect of time period is the same across CPT groups.

Another important consideration when interpreting our results is the replacement of the SP prophylaxis with CPT. Due to similarities in action between cotrimoxazole and SP, the WHO recommends that women receiving CPT should not concurrently receive SP-IPTp. Therefore, the routine second and third trimester doses of SP given to pregnant women in the BAN study were omitted in study patients who received CPT. Comparison of women who received CPT after August 2006 to women in the BAN study before that time equates to a comparison of SP-IPTp and CPT. Through manual comparison of the HRs for our study population and the "control" population, and through examination of the model including both populations, it appears there may be a trend towards a protective effect of CPT (hence, a benefit of CPT vs. SP IPT), however, this effect did not reach significance in our analysis.

In our analysis CPT was not associated with an effect on preterm birth or low birth weight. Women in whom we could not assess preterm birth status, due to missing LMP, were more likely to have a low birth weight infant, however, since there was no difference in distribution of exposure by between women missing and not missing LMP, it is unlikely that our results are substantially biased by the missing data. In HIV-infected women in Zambia with a CD4 cell count less than 200 cells/μL, CPT was associated with a decrease in risk of birth at or before 34 weeks of gestation (OR 0.49, 95% CI 0.24, 0.98) and a trend towards increased birth weight, though this association did not reach significance.[9] The authors of the Zambian study suggest a CPT-related decrease in bacterial and parasitic infections as a mechanism for the reduction in preterm birth, a mechanism which may be less likely to impact birth outcomes in women in our study, who had higher CD4 cell counts. A large RCT of antibiotics in HIV-infected and uninfected pregnant women in Africa found that a short course of erythromycin and metronidazole given at 24 weeks of gestation and metronidazole

and ampicillin given during labor did not reduce the rate of preterm birth or increase birth weight, despite reducing the rate of vaginal infections.[129] The authors suggest that the failure of this regimen to reduce the rate of histologic chorioamnionitis may explain why the antibiotics failed to reduce preterm birth. Additional data on infections during pregnancy in HIV-infected women across a range of CD4 cell counts may be necessary to elucidate the relationship between CPT and birth outcomes.

Regardless of ART and CPT status, there was an increase in median CD4 cell count at 24 weeks postpartum compared with CD4 cell count in pregnancy in our study population. While CD4 cell counts usually decline over time in HIV-infected patients, in pregnant women CD4 cell count increases in the months after giving birth, following a decline during pregnancy from physiological hemodilution.[127, 128] Through separate analyses by maternal antiretroviral regimen status, we found CPT was associated with a lower CD4 cell count at 24 weeks postpartum. The effect of CPT on CD4 cell counts in pregnant women has not been well studied. Results in HIV-infected adults have been mixed. The annual mean rate of decline of CD4 cell count was lower during CPT than before CPT (77 versus 203, $p<0.001$) in a cohort of HIV-infected patients with a range of CD4 cell counts at baseline in Uganda.[3] In another study of HIV-infected patients in Uganda, CPT was only associated with an effect on CD4 cell count among patients with an initial CD4 cell count of at least 500 cells/μL, in whom CPT was associated with a mean decrease of 22.3 cells/μL (95% CI: 3.7, 42.0).[123]

Our analysis provides much needed data on the effects of CPT in HIV-infected pregnant women with CD4 cell counts of 200 to 500 cells/μL. Although these results expand our understanding CPT in this population, several limitations should be noted. Data on potential confounders which were unmeasured for the analysis of malaria, including use of

insecticide-treated nets (ITNs), would enhance our analysis. ITNs were provided to some women in the BAN study for a period of time beginning in 2007, however, the number provided is not known and there are no data on use of these ITNs by the women included in the analysis. The lack of a true control group should also be noted. Although we used women with a CD4 cell count of at least 500 cells/μL to assess temporal changes in malaria, these women differ immunologically from our study population, and therefore, changes in disease incidence in these "control" women may not be a true representation of changes in disease incidence in our study population. While incidence of the other outcomes is more stable, there may also have been unmeasured changes in these which were unrelated to CPT and could confound our results.

We found that CPT in HIV-infected pregnant women with CD4 cell counts between 200 and 500 cells/μL does not affect malaria incidence during pregnancy (as compared to SP-IPTp), preterm birth, or low birth weight. CPT may reduce the increase in CD4 cell count seen 24 weeks after birth, however, the duration and any clinical implications of this reduction in CD4 increase was not assessed by this study. Assessment of a control group of women with a CD4 cell count less than 350 cells/μL is not ethically feasible due to WHO guidelines for CPT, however, an RCT may be able to fully address the effects of CPT in women with CD4 cell counts above 350 cells/μL, and could be used to enhance our understanding of the effect of CPT in pregnancy on CD4 in the context of varying access to antiretrovirals. Due to the consequences of malaria infection during pregnancy for both the woman and the fetus,[70-73] it is important to fully understand whether forsaking SP-IPTp for CPT is appropriate in all settings. RCTs in women with higher CD4 cell counts should also address remaining questions about the comparative effectiveness of CPT versus SP-IPTp

across varying CD4 cell counts and malaria transmission intensities. HIV-infected pregnant women face a vast array of health threats, and it is important to consider all of these threats when integrating new regimens into routine care and treatment. Additional data about CPT in pregnant women is necessary to enhance our understanding of the effects of CPT beyond its primary effect on opportunistic infections, in order to develop the most beneficial and comprehensive prophylactic treatment for this highly vulnerable population.

Table 5.1. Baseline characteristics of 1236 pregnant women by CPT exposure status

Characteristic	CPT-unexposed* (N = 468)	CPT-exposed* (N = 768)	Total population (N=1236)	P value†
Age (yr)				
Median	25	26	26	0.40
Interquartile range	(22-29)	(23-30)	(23-30)	
CD4 at screening (cells/μL)				
Median	350	362	357	<0.01
Interquartile range	(276-421)	(303-429)	(295-427)	
Maternal education (% >primary) ‡	38.5	35.3	36.5	0.26
Married (%)	91.7	92.5	92.2	0.62
Mother's first pregnancy (%)	12.4	12.4	12.4	0.99

* Women were considered CPT-unexposed if they gave birth before 13 June 2006; women were considered CPT-exposed if they had their second prenatal visit after 15 August 2006

† P-values based on Wilcoxon rank-sum test for continuous variables and chi-square test for binary variables, comparing CPT-exposed and CPT-unexposed groups

‡ Level of education was missing for one mother

Table 5.2. Frequency of Outcomes of Interest and Effect Estimates in CPT-Exposed and CPT-Unexposed Pregnant Women

Outcome	CPT-unexposed women*	CPT-exposed women*	Total	Effect Estimate† (95% CI)
Malaria during pregnancy	7.2% (33/457)	2.8% (21/751)	4.5% (54/1208)	HR: 0.35 (0.20, 0.60)
Preterm Birth	23.5% (59/251)	23.6% (88/373)	23.6% (147/624)	OR: 1.08 (0.70, 1.69)
Low birth weight‡	7.1% (33/467)	7.6% (58/762)	7.4% (91/1229)	RR: 1.02 (0.76, 1.36)

Note: totals for each outcome differ based on data available which met inclusion criteria for the individual outcomes of interest

* Women were considered CPT-unexposed if they gave birth before June 13, 2006; women were considered CPT-exposed if they had their second visit after August 15, 2006

† Effect estimates are unadjusted as no confounders or effect measure modifiers met criteria for inclusion in final models

‡ Data were missing for 7 infants

Figure 5.1. Kaplan-Meier curves illustrating the probability of malaria in HIV-infected pregnant women before and during cotrimoxazole prophylaxis periods for (A) the women with a CD4 less than 500 cells/μL at screening (study population), log rank p<0.0001 and (B) women who had a CD4 of at least 500 cells/μL at screening ("controls"), log rank p=0.0353. Red represents women exposed to CPT, black represents women unexposed to CPT.

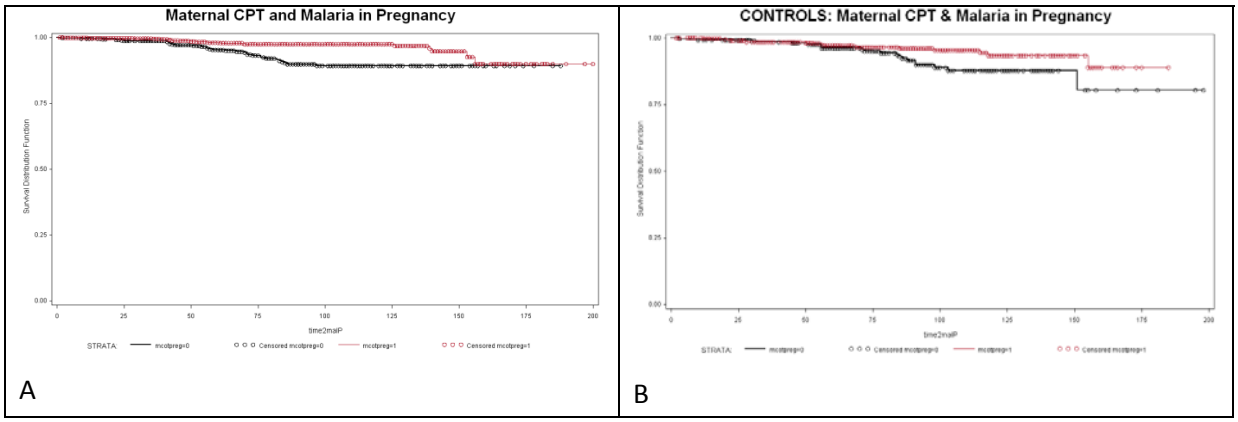
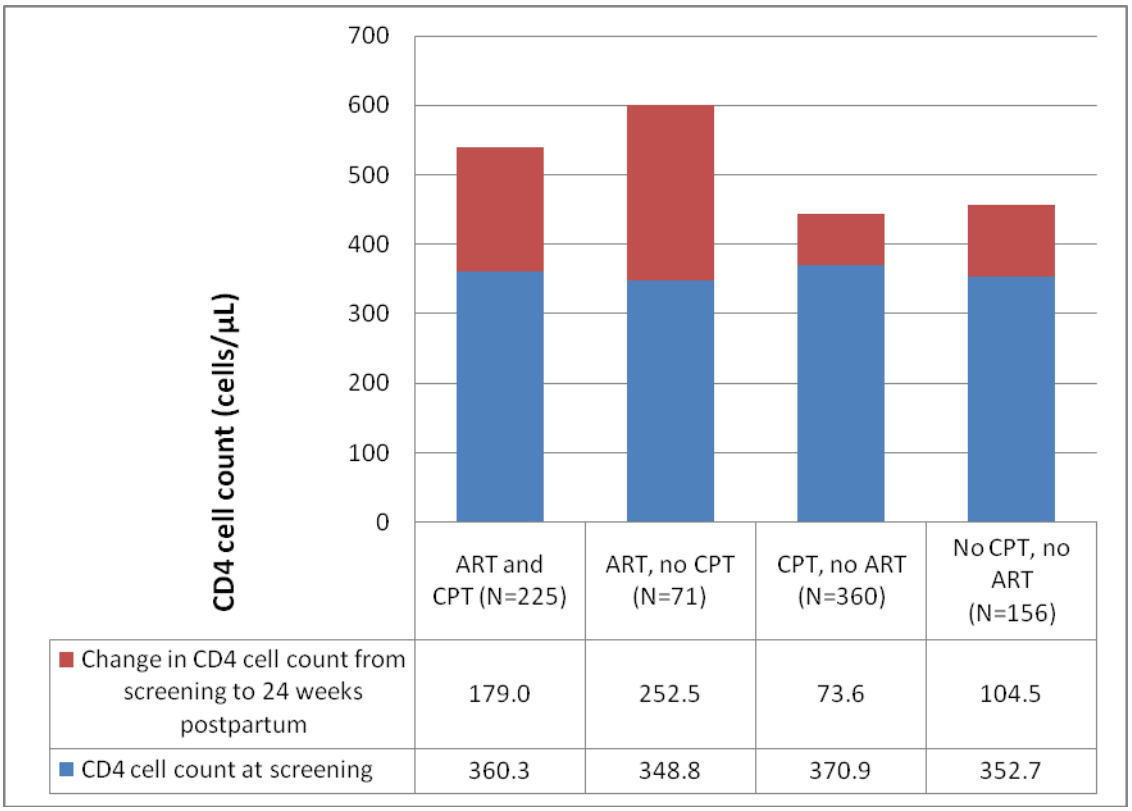


Figure 5.2. Mean CD4 cell count during pregnancy and mean change in CD4 cell count from screening to 24 weeks postpartum in HIV-infected women



	ART and CPT (N=225)	ART, no CPT (N=71)	CPT, no ART (N=360)	No CPT, no ART (N=156)
Change in CD4 cell count from screening to 24 weeks postpartum	179.0	252.5	73.6	104.5
CD4 cell count at screening	360.3	348.8	370.9	352.7

CHAPTER SIX: DISCUSSION

Scarce resources and an inadequate healthcare infrastructure contribute to the suboptimal care of the large HIV-infected population in sub-Saharan Africa (SSA). Further exacerbating the situation, poverty and a host of other infectious diseases, including TB and malaria, threaten the health of this vulnerable population. In settings of high HIV prevalence, these factors combine with a compromised immune system, creating an extremely precarious situation which often results in preventable loss of life. For these reasons, low-cost prophylactic measures and treatments are particularly important. Cotrimoxazole, long-used in developed countries as part of a treatment plan to protect against opportunistic infections, has proven to be a useful tool in protecting HIV-infected patients against opportunistic infections in resource-poor settings. The WHO guidelines for cotrimoxazole prophylactic treatment (CPT) are based on data from observational studies and clinical trials in HIV-infected adults and children. Data to guide CPT in two particularly vulnerable populations, HIV-infected pregnant women, and the infants born to these women, are scarce. A clear understanding of the risks and benefits of any health intervention is always valuable, but this is especially true in a setting where there are so many health issues to consider, when limited resources require careful prioritization of therapies, and where widespread resistance can easily derail efficacy of treatment plans for entire populations. In these dissertation analyses, we explored the effect of CPT on health outcomes in HIV-infected pregnant women and their HIV-exposed infants.

Summary of findings

In our first specific aim we explored the effect of CPT administered from 6 to 36 weeks of age on adverse health outcomes in HIV-exposed, uninfected infants, in whom CPT is a precautionary measure in case of undiagnosed mother-to-child transmission (MTCT) of HIV. CPT provided temporary protection against malaria in infancy, but did not protect against anemia, underweight and severe illness or death in our population. Protection against malaria was only seen for a 10-week period following the start of CPT at 6 weeks of age. We were limited by small numbers in our analyses of severe illnesses, and were unable to examine illnesses separately. Additionally, unmeasured confounding by insecticide-treated net (ITN) use and temporal changes in disease incidence could not be adjusted for in our analyses.

In our second aim we assessed the effect of CPT initiated during pregnancy in HIV-infected women with a CD4 cell count between 200 and 500 cells/μL on malaria, birth outcomes and CD4 cell count at 24 weeks. After adjustment for time period (temporal changes in disease incidence), CPT did not offer protection against malaria during pregnancy as compared to intermittent preventive treatment during pregnancy with sulfadoxine-pyrimethamine (SP-IPTp). CPT also did not offer protection against low birth weight or preterm birth. In analyses of CD4 cell count at 24 weeks postpartum, CPT was associated with a lower CD4 cell count, compared to CPT-unexposed women, regardless of antiretroviral treatment regimen, even after adjustment for CD4 at baseline (measured during pregnancy).

Interpretation

In HIV-exposed infants, we found some benefit of CPT for the prevention of malaria, and no increased risks of the other poor health outcomes explored. CPT in this population is a unique intervention: this preventative measure is primarily given to protect the minority of infants who acquire HIV infection. The majority of infants, those who do not acquire HIV, still represent a highly vulnerable population and have been shown to be at increased risk of infant mortality compared to HIV-unexposed infants.[20, 22, 35] CPT has been shown to be a relatively safe prophylactic therapy, and our findings in a population of HIV-exposed infants reinforce those previous findings. The benefit in prevention of malaria, while short-lived, is still noteworthy in a setting where malaria results in significant loss of life in children younger than 5 years of age.[120] The protection against malaria that we observed is not as straightforward as the effect seen in both HIV-infected and uninfected adults and children.[10-12] The only published analyses comparable to ours found a protective effect of CPT among somewhat older children in the period following cessation of breastfeeding.[96] The short term protection we observed is likely due to a combination of factors including presence and strength of maternal antibodies, and the age-related manner in which immunity against malaria is acquired.

The lack of effect against anemia may be due to the age range of our study, and the exclusive breastfeeding strategy which was in place for these mother-infant pairs. Additionally, the food supplement given to infants upon weaning was designed to prevent malnutrition and likely provided additional protection for these infants against grade 3 or 4 anemia. CPT did not protect against severe illness or death in these HIV-exposed infants. The

primary indication for CPT is prevention of opportunistic infections, which often lead to severe illness or death in HIV-infected individuals. Among HIV-uninfected infants, who have stronger immune systems, the benefit of CPT to prevent infections is less plausible, particularly in a setting of regular interaction with a healthcare facility (through BAN), where infections and illness were likely diagnosed and treated before they led to hospitalization or death.

In our analyses of HIV-infected pregnant women, we found no protection of CPT against malaria in pregnancy as compared to SP-IPTp, which was in place during the earlier time period before CPT was implemented. This result is not unexpected, given the established effectiveness of SP-IPTp in pregnant women, although SP-IPTp has been shown to be less effective in HIV-infected pregnant women compared to women who do not have HIV.[74, 75] Our efforts to control for unmeasured confounding led us to conclude that the protective effect of CPT against malaria observed in our unadjusted findings was in fact due to confounding by time period and the changes in malaria incidence over the five-year period of the study.

We saw no protective effect of CPT against low birth weight or preterm birth (birth before 37 weeks' gestation), as opposed to a study which found that CPT provided protection against preterm birth (before 34 weeks' gestation).[9] The different definition of preterm birth between ours and the previous study, and the fact that the previous study was among women with lower CD4 cell counts compared to ours, could explain the different results regarding effect of CPT. Immunocompromised women may be at greater risk of infections associated with poor birth outcomes, which may be prevented by CPT. A recent study of antibiotics during pregnancy found no benefits of antibiotics with regard to birth outcomes,[129] which is

more in line with our findings. We did not examine specific causes of preterm birth or low birth weight, therefore, we cannot assess whether the effectiveness of CPT in preventing poor birth outcomes would vary depending on the specific causes responsible for poor birth outcomes.

The effect of CPT on CD4 cell count at 24 weeks postpartum is not what we had expected to find, and is somewhat harder to interpret. The hemodilution which takes place in pregnancy means that CD4 cell count is less stable in the pregnancy and the postpartum period than it usually is in HIV-infected adults.[127, 128] The lower CD4 cell count in women receiving CPT is not what was expected based on the literature in HIV-infected adults, in whom CPT has been associated with an increase in CD4 or no effect.[3, 123] The mechanism of this effect is not currently clear, and further research is needed to explore this in depth.

Public Health Significance

The HIV epidemic in SSA has been, and continues to be, a huge challenge in the face of efforts to improve the health of an already vulnerable population. Particularly high-risk populations include HIV-infected pregnant women and their infants, who are at risk of acquisition of mother-to-child transmission of HIV. Low-cost, uncomplicated interventions are desperately needed to protect these populations against a host of factors that threaten their wellbeing. While better HIV prevention programs and more accessible HIV treatment programs are the strategies that are likely to lead to global control of the HIV epidemic, small steps to limit morbidity and mortality are valuable during the slow process of establishment and expansion of these more comprehensive programs. Cotrimoxazole prophylactic treatment is a prime example of a low-cost, logistically feasible intervention that can protect HIV-

infected individuals from preventable illnesses that exacerbate their health problems. While CPT is a valuable tool in the fight against HIV, the effects of this prophylactic treatment have not been well examined in all populations covered by the WHO guidelines.

HIV-exposed children receive CPT in case they become HIV-infected. Therefore, the primary indication of CPT, prevention of opportunistic infections, does not apply to these children, because the majority will not acquire HIV. Because this population is still at risk of poor health outcomes compared to HIV unexposed children, it is important to understand the effect of a CPT intervention applied to this population. Our finding that CPT provides some protection against malaria in this population is noteworthy, given the threat that malaria poses to children younger than 5 years of age. Protection against malaria is valuable, however, age-related patterns of malaria immunity vary widely by malaria transmission intensity, and CPT may have a varying impact based on the malaria context in which it is used. Key issues to monitor in decisions about CPT in this population are the effectiveness of local prevention of mother-to-child HIV transmission (MTCT) programs, access to and frequency of infant HIV testing, and cotrimoxazole resistance. If MTCT falls to levels seen in developed countries, and if HIV-exposed infants can be tested for HIV on a regular basis such that their infections are diagnosed and treated early, then funding currently used for CPT for all HIV-exposed infants may be better suited for other health interventions needed for this vulnerable population. Additionally, if CPT resistance becomes more widespread, minimizing the benefits of CPT and rendering it less useful for treatment as well as prophylaxis, CPT guidelines will need to be revised. In the meantime, it appears that CPT may offer some benefits for HIV-exposed infants who are receiving it, though these benefits are not as strong as those seen in the true target population - HIV-infected infants.

HIV-infected pregnant women receive CPT under the general guidelines for HIV-infected adults. It appears that withholding SP-IPTp in the context of CPT does not substantially impact malaria in HIV-infected pregnant women with a CD4 cell count between 200 and 500 cells/μL, however, the effect of CPT on malaria may vary by intensity of malaria transmission. The inability of CPT to protect against poor birth outcomes among women with a CD4 cell count between 200 and 500 cells/ μL means that interventions to improve birth outcomes in this population are still needed. The implications of finding that women receiving CPT had lower CD4 cell counts at 24 weeks postpartum are difficult to understand without further examination of the clinical consequences. The hemodilution which occurs during pregnancy complicates our understanding of the effect of CPT on CD4 cell counts. Since opportunistic infections are most common in individuals with lower CD4 cell counts, this decrease in CD4 cell count should be explored to determine what the clinical implications are, and to make sure that the decrease in CD4 does not negate the benefits of CPT.

We found limited benefits of CPT in our analyses. CPT is a relatively cheap and easy prophylactic intervention, but it is still a financial burden and an added strain on the already-overextended healthcare system of many countries in SSA. Therefore, analyses demonstrating the limits of the benefits of CPT are as important as those demonstrating the depth of the benefits. This is particularly true when the true targets of an intervention, in this case HIV-infected infants and HIV-infected adults with lower CD4 cell counts, represent only a fraction of the population receiving the intervention. When assessing the CPT guidelines, it is important to consider the costs in terms of time spent by health care workers to assess and distribute cotrimoxazole, as well as the actual costs of the medication,

particularly if the benefits of CPT are not overwhelming, or if these benefits could be achieved through other simple interventions. For example, if ITNs could provide similar protection against malaria among infants, or if SP-IPTp could offer similar protection against malaria among pregnant women, and if these interventions place less of a burden on the patients and the healthcare system, they may be more appropriate, particularly in areas of lower malaria transmission intensity. We did not observe benefits of CPT for the other outcomes assessed, which may be due to our study population being healthier than some of the populations where the benefits of CPT has previously been established. In settings where CPT is being broadly distributed, it will continue to be important to assess the larger picture of the role and importance of CPT, and to prioritize its use appropriately in the context of other health interventions.

Future Research

This dissertation substantially increases our knowledge on the effects of CPT in HIV-infected pregnant women and HIV-exposed, uninfected infants; however, the results also raise questions which need to be addressed in future studies. Unanswered questions about CPT in infants include the effect of CPT on health outcomes in the context of mixed feeding regimens, as well as examination of the effect of CPT for longer than the 36 weeks assessed in the current analyses. Because breastfeeding duration in much of SSA is longer than in our study, and because duration of CPT is dependent on duration of breastfeeding, more data are needed on health outcomes after longer periods of CPT and as the child ages and susceptibility to various pathogens changes. Also, because breastfeeding offers protection against some infectious pathogens,[126] the effect of CPT may differ among infants who are

receiving mixed feeding, where they may be more susceptible to a variety of infections due to reduced acquisition of maternal antibodies through breastmilk and increased exposure to pathogens through consumption of potentially contaminated food and water. It would be valuable to assess whether CPT protects against specific infections which lead to hospitalization, such as diarrhea and pneumonia, which we were unable to do due to limited sample size, and for which there are limited published data available. Also, CPT may protect against milder illnesses which require treatment or a clinic visit but not hospitalization. The BAN study collected data on milder illnesses but these were not available for the current analyses.

Although an RCT of CPT in HIV-exposed infants is not ethical under current WHO guidelines, several interesting research questions can still be addressed. One such question is disease incidence, particularly incidence of malaria, in HIV-exposed, uninfected children after CPT is stopped. It is possible that there will be a rebound effect after CPT is stopped in these children, particularly due to the age-related acquisition of immunity associated with malaria. This effect may vary depending on the duration of treatment. Additionally, comparison of disease incidence in CPT-exposed, HIV-exposed, uninfected infants and CPT-unexposed, HIV-unexposed infants could provide a better understanding of the benefits of CPT for this population. Depending on available data, it may be possible to adjust for differences between the HIV-exposed and HIV-unexposed infants in order to quantify the effects of CPT.

One ongoing study of CPT is the PROMISE (Promoting Maternal-Infant Health Everywhere) study, a large, multinational PMTCT clinical trial which began in January, 2010. The CPT component of this study involves randomization of HIV-exposed, uninfected,

weaned infants under one year of age to either continue receiving CPT or to receive a placebo through age 18 months. The objective is to determine whether continuing CPT in this population from the time of cessation of breastfeeding through 18 months decreases their risk of illness and death without causing side effects or generating bacterial resistance to cotrimoxazole.[130] While this study will not provide information on the benefits provided during breastfeeding when CPT is recommended by the WHO, information on the effects of CPT on illnesses after cessation of breastfeeding and on cotrimoxazole resistance in this population will be valuable and will shed light on the overall benefits of this prophylactic measure in uninfected infants and young children.

Regarding the effects of CPT on malaria in HIV-infected pregnant women, the primary need is for data across a range of malaria transmission intensities and among women with a range of CD4 cell counts, in order to assess whether forsaking SP-IPTp for CPT is beneficial across all levels of CD4 and malaria transmission intensity. There are opportunities to compare various health and birth outcomes in HIV-infected women by CPT status due to the flexibility of the WHO guidelines regarding the recommended CD4 values for initiation of CPT.[1] This allows for RCTs of the effects of CPT in women with higher CD4 counts in order to assess the value of CPT in these women. Given the increasing access to ART, it is also important to assess whether the value of CPT changes once women are receiving more comprehensive treatment for HIV.

Conclusion

CPT has proven to be an important prophylactic measure in the HIV-infected population. This intervention is particularly valuable as access to more comprehensive care is lacking across much of SSA. This dissertation adds to the limited literature on CPT in HIV-exposed, uninfected infants, and demonstrates limited benefits of CPT through protection against malaria. CPT also appears to be an adequate substitute for SP- IPTp in HIV-infected pregnant women. The analyses described are valuable in order to clarify the impact of this intervention in these highly vulnerable populations, where resources are extremely limited and where it is crucial to have a comprehensive understanding of any intervention used to protect against the multitude of health threats plaguing the region bearing the brunt of the HIV epidemic.

APPENDIX A. Aim 1B Using a Broader Definition of Anemia

A broader definition of anemia was used to explore whether CPT had an effect on time to anemia when including milder anemia. For this analysis, anemia was defined as a hemoglobin level below 10g/dl after six weeks of age, corresponding to grade 2 or higher anemia according to toxicity tables from the Division of AIDS at the National Institute of Allergy and Infectious Diseases (NIAID), as revised in March 2006.

Anemia was seen in 170 CPT-unexposed patients and 659 CPT-exposed patients (Table 2). The unadjusted HR for anemia was 0.85 (95% CI, 0.72, 1.01). The effect of CPT appeared to change over time, based on the log-log plot and the statistical significance of a continuous interaction term entered into the model, assessed through comparison of likelihood ratio test comparing a model with and without the interaction term. A categorical interaction term was fit to the model corresponding to 20 weeks of treatment (at 26 weeks of age). Inclusion of this time interaction resulted in an HR in the time period up to 20 weeks or 0.94 (95% CI, 0.77, 1.15), and an HR from 20 weeks to 36 weeks of 0.64 (95% CI, 0.46, 0.88). The covariates examined did not meet the criteria for inclusion in the final model.

The timing of this protection coincides with the time when women were counseled to rapidly wean their infants from breastfeeding. Infants are born with iron stores which are generally sufficient for the first 4-6 months of life,[124] and while iron is bioavailable in breastmilk, it decreases over time.[125] Following cessation of breastfeeding infants can be susceptible to anemia if they are not ingesting foods with sufficient iron content.[131-133] It is unknown if the observed association is causal, and, if causal, through which mechanism CPT could protect HIV- exposed, uninfected non breastfeeding infants from anemia.

APPENDIX B. Additional analyses for Aim 2

Additional analyses were performed to assess malaria not only during pregnancy but through the entire follow-up period. We also examined anemia and first hospitalization and death in these women.

The baseline time for analysis of malaria was the second visit during pregnancy, at which point, following June 2006, CPT was started in women who met CPT initiation criteria based on CD4 at the screening visit. The baseline time for anemia was two weeks postpartum. This time point was chosen because following the blood collection at the screening visit, blood was not routinely drawn until birth. Due to the gynecological issues which can cause anemia at that timepoint, which we did not feel were likely to be affected by CPT, we chose to begin analysis of anemia at the two weeks after the mother gave birth. Baseline time for SAEs was birth, since this is when serious adverse events were routinely monitored.

There were 56 women diagnosed with malaria in the CPT-unexposed group, and 150 in the CPT-exposed group. The HR for the effect of CPT on malaria was 0.57 (95% CI 0.42, 0.77). None of the covariates explored met the criteria for inclusion in the final model as an effect measure modifier or confounder. As was done with the analysis of malaria during pregnancy, a sensitivity analysis including women with a CD4 cell count greater than 500 was performed. The HR for the effect of CPT on malaria, adjusted for period of participation, was 0.91 (95% CI, 0.55, 1.50).

Analysis of anemia after birth was performed based on the 9 anemia events among CPT-unexposed women and 23 events among CPT-exposed women. The unadjusted HR for the effect of CPT on incident anemia after birth was 0.63 (95% CI, 0.29, 1.37). None of the

covariates explored met the criteria for inclusion in the final model as an effect measure modifier or confounder.

There were 75 hospitalizations or deaths eligible for inclusion in the analyses among women. Of these, 17 were among CPT-unexposed women, 58 were among CPT exposed women. The unadjusted HR for the effect of CPT on severe illness was 0.86 (95% CI, 0.49, 1.49). None of the covariates explored med the criteria for inclusion in the final model as an effect measure modifier or confounder.

The analyses of malaria produced very similar results to those seen when focusing on the period of pregnancy. The sensitivity analyses led us to the conclusion that after adjustment for period of participation, CPT did not offer a protective effect against incident malaria.

CPT also was not associated with a protective effect against moderate or severe anemia after birth, or against severe illness over the time period examined in these analyses.

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