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**MATERNAL ANTIBODY TRANSFER IN
HIV-1 INFECTED WOMEN AND IMPACT
ON INFANT HEALTH -
THE ROLE OF ANTIRETROVIRAL
PROPHYLAXIS AND BREASTFEEDING
PRACTICES**

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Maternal antibody transfer in HIV-1 infected women and
impact on infant health – the role of antiretroviral
prophylaxis and breastfeeding practices
THESIS FOR DOCTORAL DEGREE (Ph.D.)

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To my family and friends

There's no limit to what your mind can achieve, what your heart can accomplish, and what your soul can realize...The sky's dangers offer the eagle more opportunities than the nest's comfort.

- Matshona Dhliwayo

I will forever praise this God who didn't close His heart when I prayed and never said no when I asked Him for help.

- Psalms 66:20 TPT

ABSTRACT

Children born to human immunodeficiency virus type 1 (HIV-1) infected women are more vulnerable to infections and are more likely to die even when they are not HIV-1 infected. These adverse outcomes may blunt any gains made in escaping HIV-1 infection and could be ameliorated by improving maternal antibody transfer to the baby. The overall aim of this thesis was to study the role of antiretroviral prophylaxis and breastfeeding practices on maternal antibody transfer to their children and on the morbidity these children experience in the first year of life .

Studies I and II examined the influence of antiretroviral treatment on antibody levels during pregnancy and delivery, in cord blood and breast milk. We used data from a randomized clinical trial in which HIV-1 infected pregnant women with CD4 counts between 200 and 500 were randomized to short-course zidovudine (ZDV) or triple antiretroviral therapy (ART) during pregnancy for prevention of HIV-1 transmission from mother to child. Antibody levels against measles, pneumococcus and rotavirus were measured in maternal plasma, infant cord blood and breast milk and compared between the trial arms. We found that maternal levels in plasma (Study I) and breast milk (Study II) were comparable between the two groups. Compared to women on short-course ZDV, women on triple ART transferred higher amounts of antibody via the placenta.

In Study III, we compared infant morbidity, hospitalization, and mortality during the first year of life of HIV-1 exposed uninfected (HEU) children born to the women in Study I and II. Our morbidity outcomes of interest were those that cause significant mortality in these children namely diarrhea, pneumonia, and lower respiratory tract infection, and a composite measure of any infectious morbidity. We found important predictors for mortality in these children; however, we found no effect of the mother's ART treatment regimen for any of these outcomes.

Study IV used a real world setting in which HIV-1 infected women were enrolled during pregnancy into a clinic-level, before-after breastfeeding counseling intervention study. Women in the intervention arm were offered three counseling sessions that promoted exclusive breastfeeding (EBF), explained breastfeeding techniques and described its benefits. EBF prevalence was comparable between the two arms at 14 weeks postpartum. We found no differences between the groups for 6-week HIV-free survival or 14-week infant survival for the children born to these women.

The results of this thesis show some benefit for maternal triple ART compared to short-course ZDV in passive antibody transfer via the placenta and that high EBF rates are attainable. The non-significant findings for impact on morbidity and mortality outcomes among HEU children highlight the complexity of unravelling the mechanisms that underlie the higher vulnerability that has been observed in these children. The findings from this thesis may be used to inform the design of future studies so that ultimately the health and survival of HEU children can be secured.

LIST OF SCIENTIFIC PAPERS

- I. Bosire R, Farquhar C, Nduati R, Broliden K, Luchters S, Van De Perre P, De Vincenzi I, Merkel M, Wachuka V, Mbori-Ngacha D, John-Stewart G, Lohman-Payne B, Reilly M. Higher transplacental pathogen specific antibody transfer among women randomized to triple antiretroviral treatment versus shortcourse zidovudine. *Pediatr Infect Dis J* 2018;37:246-252.
- II. Bosire R, Farquhar C, Ndiaye A, Nduati R, Broliden K, Luchters S, De Vincenzi I, Ichugu CG, Wachuka V, John-Stewart G, Reilly M, Lohman-Payne B. Effect of pregnancy and postpartum antiretroviral prophylaxis on longitudinal breast milk IgA levels. (*Manuscript*).
- III. Bosire R, Ndiaye A, Reilly M, Nduati R, Luchters S, De Vincenzi I, Mbori-Ngacha D, John-Stewart G, Lohman-Payne B, Farquhar C. Morbidity, hospitalization and mortality among infants born to women randomized to triple antiretroviral therapy vs shortcourse zidovudine in Kenya. (*Manuscript*).
- IV. Bosire R, Betz B, Aluisio A, Hughes JP, Nduati R, Kiarie J, Chohan BH, Merkel M, Lohman-Payne B, John-Stewart G, Farquhar C. High rates of exclusive breastfeeding in both arms of a peer counseling study promoting exclusive breastfeeding among HIV-infected Kenyan women. *Breastfeed Med* 2016;11(2) 56-63.

The papers will be referred to throughout the text by their Roman numerals.

Related Articles (not included in the thesis)

Odeny BM, Pfeiffer J, Farquhar C, Igonya EK, Gatuguta A, Kagwaini F, Nduati R, Kiarie J, Bosire R. The stigma of exclusive breastfeeding among HIV-positive and HIV-negative women in Nairobi, Kenya. *Breastfeed Med* 2016;11:252-8

Aluisio AR, Bosire R, Betz B, Gatuguta A, Kiarie JN, Nduati R, John-Stewart G, Farquhar C. Male partner participation in antenatal clinic services is associated with improved HIV-free survival among infants in Nairobi, Kenya: A prospective cohort study. *J Acquir Immune Defic Syndr* 2016;73(2):169-76

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LIST OF ABBREVIATIONS

AIDS	Acquired immune deficiency syndrome
ANC	Antenatal care
ANOVA	Analysis of variance
ART	Antiretroviral therapy
ARV	Antiretroviral
DNA	Deoxyribonucleic acid
ELISA	Enzyme linked immunosorbent assay
FcR	Fc receptor
GEE	Generalized estimating equations
HEU	HIV exposed uninfected
HIV	Human immunodeficiency virus
HUU	HIV unexposed uninfected
ICD	International classification of diseases
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IQR	Interquartile range
IRR	Incidence rate ratio
KDHS	Kenya Demographic and Health Survey
LRTI	Lower respiratory tract infection
MTCT	Mother to child transmission
MCV	Measles vaccination coverage
NVP	Nevirapine
OD	Optical density
OR	Odds ratio
PCP	Pneumococcal capsular polysaccharides
PCR	Polymerase chain reaction
PMTCT	Prevention of mother to child transmission
RCT	Randomized clinical trial
RNA	Ribonucleic acid
RR	Relative risk

SIA	Supplemental immunization activities
SSA	Sub-Saharan Africa
3TC	Lamivudine
ULN	Upper limit of normal
UNAIDS	Joint United Nations Programme on HIV/AIDS
WHO	World Health Organization
ZDV	Zidovudine

1 INTRODUCTION

As a result of life-saving antiretroviral therapy (ART) and a better understanding of the transmission dynamics of maternally acquired human immunodeficiency virus type 1 (HIV-1), the pediatric HIV-1 burden has significantly and elimination of vertical transmission may be within reach. From a peak of more than 40% of children born to HIV infected women becoming infected in the pre-ART era[1], increased access to and uptake of interventions to prevent mother to child transmission (PMTCT) of HIV-1 have resulted in transmission rates below 1% and about 1% in non-breastfeeding and breastfeeding populations, respectively[2, 3]. With continuing expansion of PMTCT coverage, the number of infected children is shrinking. However, the population of children exposed to HIV-1 and to antiretroviral (ARV) medication but born uninfected is growing.

While initial efforts were rightly focused on prevention of pediatric HIV, it is increasingly being recognized that HIV-exposed uninfected (HEU) children merit special attention due to their susceptibility to infectious morbidity and mortality that blunt the gains achieved by averting HIV-1 infection[4-7]. Epidemiological studies of the contribution of HIV exposure in this HEU infant population pose a number of challenges. Earlier in the HIV epidemic, definitive HIV-1 infection status in either mother or child was challenging because 1) of lack of repeated testing of women who initially tested negative antenatally, and 2) antibody based HIV diagnosis in children, making it difficult to measure the contribution of HIV exposure with precision. In more contemporary studies, it is impossible to disentangle the effects of dual exposures to HIV and ART. In both eras, challenges to the interpretation of data collected from studies include a lack of a suitable unexposed uninfected comparison group, differences in breastfeeding uptake, intensity and duration, and incomplete consideration of the underlying web of environmental, behavioral, socioeconomic and health system factors that universally affect child survival, but may have a greater impact for HEU children[5, 8].

In spite of these challenges, we sought to understand the increased morbidity and mortality among HEU children in this thesis. In particular, we focused on the contribution of improved suppression of maternal HIV-1 to infant health. We compared immunologic outcomes of infants born to women who received triple ART administered during pregnancy and breastfeeding in comparison to the then standard of care comprising of short course monotherapy administered during pregnancy and delivery. In addition, we investigated whether breast milk benefits could be increased via improved breastfeeding practices.

2 BACKGROUND

2.1 PREVALENCE OF HIV-1 GLOBALLY, IN SUB-SAHARAN AFRICA AND KENYA

HIV-1 infection is one of the greatest health challenges the world has faced. However, due to concerted global prevention and treatment efforts, remarkable gains have been made in decreasing HIV-1-related deaths and new infections. Annual HIV-related deaths have reduced by half from the peak in 2004 to about 940,000 in 2017, and new infections have almost halved from the peak in 1996 to 1.8 million in 2017[9]. This public health success of a fast decline in HIV related deaths with a slower decline in new infections has resulted in more people living with HIV. In 2017, there were 36.9 million people living with HIV in 2017, 53% of whom live in sub-Saharan Africa (SSA). Unlike outside SSA where the majority of adults living with HIV are men, in SSA, the majority of HIV-positive adults are reproductive-age women who continue to disproportionately account for a large percentage of new adult infections (59% in 2017). Adolescent girls and young women are particularly vulnerable, with three out of every four new infections in adolescents aged 15-19 years in SSA being girls, and compared to young men, 15-24 year old young women are twice as likely to be living with HIV[9].

Kenya is a success story in the remarkable progress it has made in its response to HIV. However, the disease burden is still among the highest in the world with an adult prevalence of 4.9%[10]. Its profile mirrors that of SSA in that it is a generalized epidemic with higher adult prevalence among women. Of the 1.5 million Kenyan adults living with HIV-1 in 2017, 62% were women, and a third of all new infections in adults occurred in 15-24 year old adolescent girls. Within the country there are regional differences, with some counties having an adult prevalence >20% while it is <0.1% in other areas. Eight of the 47 counties contribute to more than 50% of people living with HIV-1 in the country[10]. Nairobi and Mombasa, where the studies reported in this thesis were conducted, are among the 8 counties with a large HIV-1 burden. Although their adult HIV-1 prevalence decreased between the time the studies were conducted until 2017, going from 8.8% and 8.1% to 6.1% and 4.1%, respectively, it still remains high[10].

2.2 MOTHER TO CHILD TRANSMISSION OF HIV-1 GLOBALLY AND IN KENYA

The high HIV-1 burden among reproductive age women results in a large number of children exposed to HIV-1 in utero, intra-partum and post-natally through breastfeeding.

More than 1 million children are born to these women every year. However, with the remarkable success in improved access to interventions such as ARVs, either as prophylaxis to prevent transmission of HIV-1 from mother to child, or treatment for their own health, the number of infections occurring among children born to these women have reduced dramatically from about 300,000 in 2010 to about 110,000 in 2017[9, 11]. In 2011, targets to eliminate pediatric HIV were set by the World Health Organization (WHO). A few countries have already been certified as having eliminated mother to child transmission (MTCT) of HIV-1 (defined as transmission rates below 5% in breastfeeding populations and below 2% in non-breastfeeding populations) (Figure 1), and many are working to achieve the 2030 elimination targets set by WHO[12].

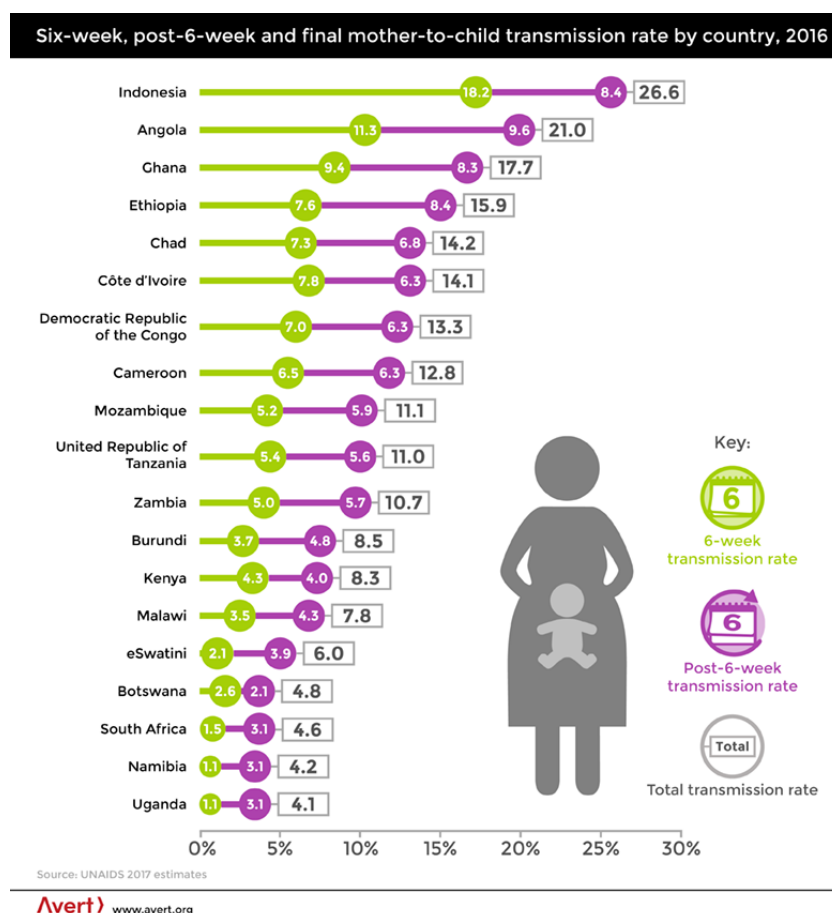


Figure 1: Mother to child HIV-1 transmission rates for select countries in 2016

With a majority of low and middle income countries now offering lifelong antiretroviral treatment (ART) to HIV infected pregnant and breastfeeding women as soon as the HIV-1 diagnosis is made, a majority of their infants will not become infected (Figure 2). However, this progress means that there is an increasingly large number of children exposed to HIV and ARVs who remain uninfected. Although they have escaped HIV infection, these children have special health and developmental needs.

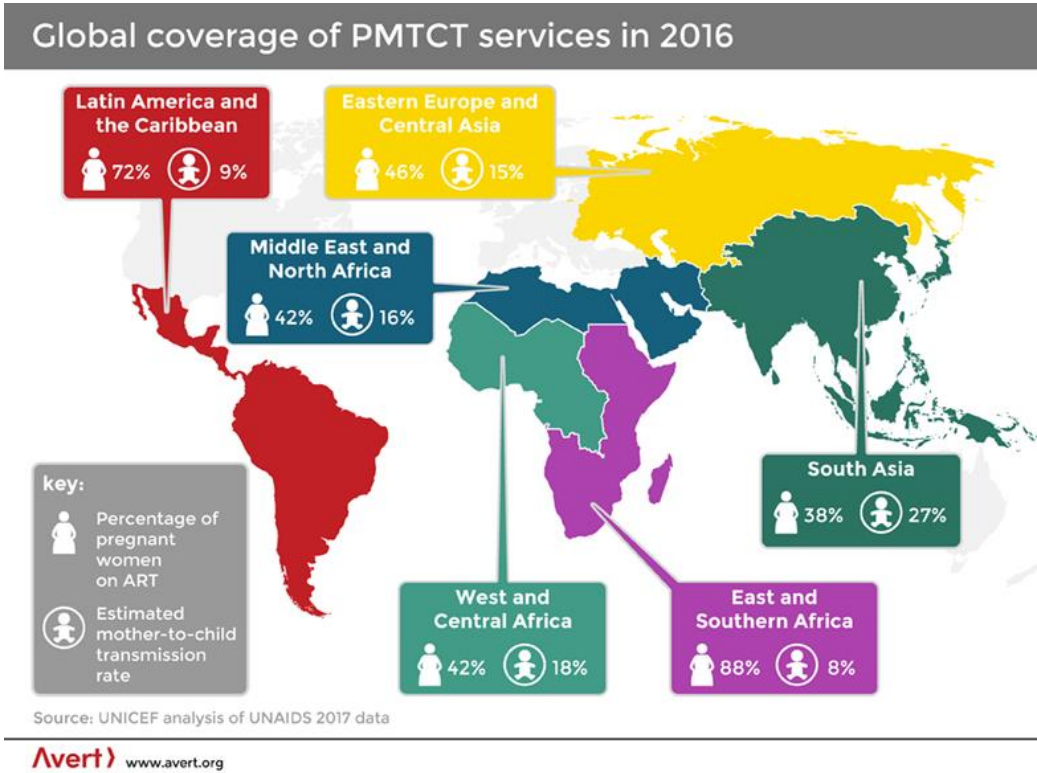


Figure 2 Global PMTCT coverage and estimated MTCT in 2016

2.3 MORTALITY AND MORBIDITY AMONG HIV-1 EXPOSED UNINFECTED CHILDREN

Although some studies have reported no increase in mortality risk[13, 14], most evidence points to a higher morbidity and mortality risk for HEU children compared to HIV unexposed uninfected children (HUU)[15-20]. HEU children appear to be at a higher risk for infection and death in the presence of advanced maternal HIV-1 characterized by low CD4 count, high HIV-1 viral load, and symptomatic HIV-1 disease factors which have been demonstrated in several studies to be associated with worse infant outcomes[21-24]. The mortality risk among these children is especially high in the first year of life compared to children born to (HIV-1) uninfected women, with HEU children having up to a 4-times higher mortality[15, 23, 25-28]. HEU children in low-resource settings are at an even higher risk for infectious morbidity and mortality outcomes[29-32]

While multiple factors have been proposed as explanations for the higher morbidity and mortality risk from infectious causes, including social and behavioral factors, it is thought that immunological alterations due to HIV-1 (and possibly ARV) exposure in utero have a part to play (Figure 3).

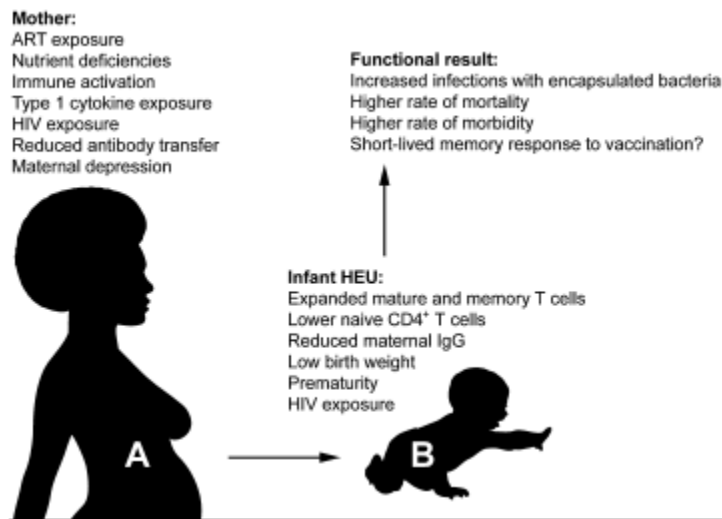


Figure 3 Morbidity and mortality vulnerability cascade in HEU children[4]

In these HIV-1-exposed infants, the commonest causes of death are the same as in any young children, namely pneumonia, diarrhea, sepsis and other invasive bacterial and viral infections [25, 33] in which acute respiratory infections and diarrheal disease account for a large proportion of these deaths (Figure 4). These are conditions for which a child may receive some protection from passive immunity obtained via trans-placental and breast milk transfer of maternal antibodies [34, 35]. However, in HIV-1 infected individuals, there is defective memory B cell function resulting in suboptimal antigen-specific antibody production[36]. In addition, abnormal immunoglobulin G (IgG) production by hyperactivated naïve B cells produces a hyper-gammaglobulinemic state. The combination of these and other factors such as placental malaria, and infant factors such as gestational age and birthweight result in inefficient transfer of pathogen specific antibodies across the placenta[37-40], while large amounts of non-specific antibodies are transferred. Therefore, although HEU infants have higher immunoglobulin levels compared to HUU infants, among infants born to antiretroviral naïve women with high maternal systemic viral load, pathogen specific antibody levels are found to be lower [41]. These low levels of pathogen specific antibodies may explain some of the high mortality experienced during infancy by HEU children and particularly in early postnatal life before they build their own immunity to pathogens in their immediate environment.

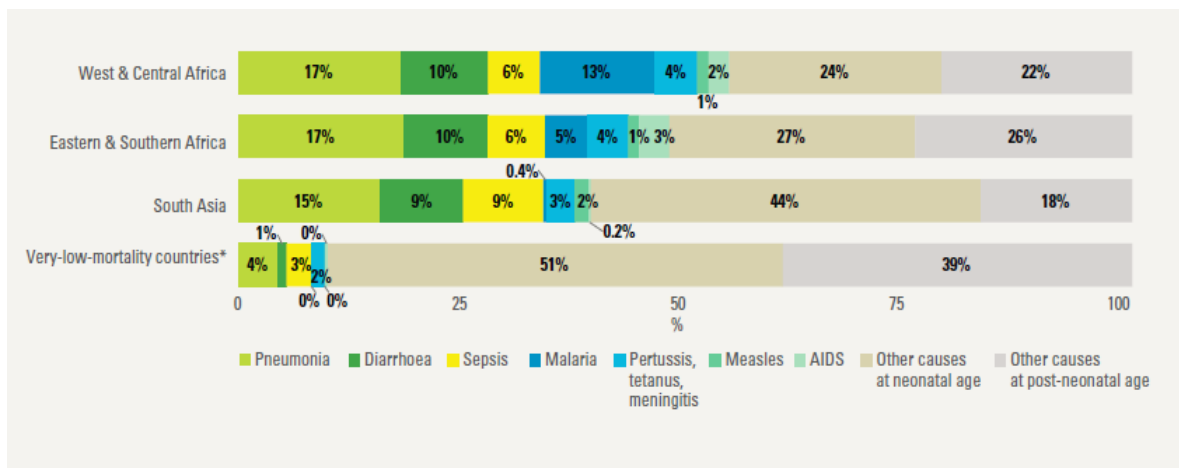


Figure 4 Distribution of causes of death in children below 5 years of age by region in 2015[42]

In infants born to antiretroviral naïve HIV-1 infected women, low levels of specific antibodies have been demonstrated for several pathogens, including tetanus, measles virus, *Streptococcus pneumoniae*, and others [40, 43-46]. A recent pooled analysis in which 80% of the women did not receive ARVs, or received mono or dual prophylaxis, suggests that maternal ART and breastfeeding are significant contributors to survival in HEU children[20]. However, it is not known whether providing triple ART to HIV-1 infected pregnant women becomes a game changer for their children [47, 48] particularly with regard to pneumonia, diarrhea, and sepsis which cause the most deaths during infancy. In our studies, we focused on measles, *S. pneumoniae* and rotavirus due to their significant direct and indirect contribution to diarrhea and pneumonia morbidity and mortality among young children.

2.3.1 Streptococcus pneumoniae disease

Streptococcus pneumoniae is an important cause of bacterial pneumonia, meningitis, and sepsis in children and is estimated to cause 14.5 million cases worldwide and 700,000 to 1 million deaths in children under the age of 5 each year [49]. Of the different clinical illnesses caused by this bacteria, pneumonia is the most common form (96% of cases) of serious pneumococcal disease, and accounts for 17% of child deaths in developing countries, making it a leading cause of death among young children [42].

For a long time, the burden of disease due to *S. pneumoniae* in developing countries went unrecognized. However, a meta-analysis conducted to determine the global burden of disease due to *S. pneumoniae* found that between 1980 and 2005, Africa had both the largest number of total deaths from pneumococcal disease and the highest rate of pneumococcal

mortality as a result of both a high incidence rate (3627 per 100,000), and the highest overall case-fatality rate (399 per 100,000)[50]. The magnitude of this problem is further borne out by the fact that only ten countries, all in Africa and Asia, account for 61% of all pneumococcal deaths worldwide and a similar number of countries also in Africa and Asia, account for 66% of all pneumococcal cases. Although these deaths occur via any of the three serious clinical syndromes, namely pneumonia, meningitis, and non-pneumonia non-meningitis invasive disease, almost all the deaths (90%) are due to pneumococcal pneumonia [50]. The incidence of pneumococcal meningitis among all children below 5 years of age is low (0.7% of pneumococcal disease compared to 96% for pneumococcal pneumonia). However, the case fatality is high, especially in Africa where it is 73%, making it an important cause of death in this age group.

In addition to primary infections, *S. pneumoniae* is a common secondary infection being the most commonly identified organism found in 30% to 50% of all microbiologically confirmed tests in measles-related pneumonia deaths [51, 52] as well as a common secondary bacterial infection in unvaccinated children infected with respiratory viruses[53]. The burden of pneumococcal disease and adverse outcomes is of special significance for HEU infants who are more likely to experience at least one episode of pneumonia in the first two years of life than HUU infants [54, 55], and are more likely to experience treatment failure and death following a pneumonia episode, especially in the first 6 months of life [32]. It is therefore likely that HEU children disproportionately contribute to the high mortality from pneumonia in African children under 5 years of age. Although there are other pathogens involved in childhood pneumonia among HEU infants, the existence of a safe and effective vaccine makes prevention of morbidity and mortality due to *S. pneumoniae* a meaningful target. Prevention of pneumococcal disease would greatly accelerate progress towards achieving the ambitious global target of reducing the mortality rate in children younger than 5 years to 25 or fewer deaths per 1,000 live births by 2030[42].

2.3.2 Rotavirus disease

Acute gastroenteritis caused by rotavirus infection is a significant cause of childhood morbidity and mortality, resulting in ~ 220,000 deaths of young children below 5 years of age in SSA each year[56, 57]. Although the greatest burden of rotavirus disease and mortality occurs in Asia, SSA has the highest rates of rotavirus-associated mortality in young children,

and rotavirus is thought to account for a third of diarrheal hospital admissions in African infants [58, 59]. In a study in Kilifi District, Kenya, group A rotavirus alone was responsible for 38% of hospital admissions of infants for diarrhea with an incidence of 1,431 cases per 100,000 person-years[60].

Although the incidence of rotavirus infection is highest in children aged 6 months to 2 years of age, infection has been known to occur in neonates and infants younger than 2 months[61, 62]. In developing countries in Africa and Asia, rotavirus infection and disease occur early in life and 75% of infants will acquire rotavirus infection during the first year of life, with 81% of children hospitalized for rotavirus diarrhea being less than 1 year of age [63, 64]. Rotavirus is thought to be the single most common cause of diarrhea, contributing to as much as a quarter of all diarrheal cases in both outpatient and inpatient African children. There is substantial overlap between high HIV disease burden and diarrheal case fatality, with the countries most heavily burdened by HIV experiencing the highest diarrheal case fatality[65]. In addition, both HIV-infected and HEU infants are at a higher risk of developing diarrhea and of having poorer outcomes following diarrheal episodes[24, 66]. A prospective cohort study of infants in the Democratic Republic of Congo demonstrated that HIV-1-infected infants had an increased risk of death due to diarrhea when compared with HIV-1-uninfected infants and HEU infants had a greater risk of persistent diarrhea when compared with HUU infants[24].

2.3.3 Measles disease

Despite recent progress in measles control in the developing world due to mass immunizations and supplemental immunization activities (SIAs), measles remains a significant cause of morbidity and mortality worldwide, particularly in SSA which accounted for 59% of the approximately 454,000 global deaths due to measles in 2004[67]. Furthermore, regular transmission of measles continues in many of the countries where the HIV-1 disease burden is high. Infants born to HIV-1-infected women are at a higher risk for measles infection compared to HUU infants, and are susceptible at a younger age [68], before administration of the live attenuated measles vaccine at 9 months of age. In these children, infection is associated with a greater severity of measles that is more likely to result in death, regardless of the infant's own HIV-1 infection status [68-71]. This is thought to be due to the lower levels of measles-specific trans-placental antibody transfer demonstrated in these infants[72, 73].

Concerns about inadequate transfer of passive immunity to HIV-1-exposed infants

and the inability of HIV-1-infected infants to mount a protective immune response against measles, suggest that HIV-1 may be a potential barrier to the elimination of measles[74, 75]. This is of particular concern as HIV-1 infected children have been shown to have prolonged measles virus RNA shedding, and with improved survival due to increased access to pediatric antiretroviral therapy, these children could form a large pool of infectious individuals with potential for causing measles outbreaks[76, 77]. One study demonstrated that even when viral suppression has been achieved through use of ART, responses to revaccination waned over time, with the proportion of children with detectable antibody dropping from 98% to 60% at one and 24 months post-revaccination, respectively[78]. Indeed the resurgence of measles outbreaks in 28 (61%) of 46 SSA countries in 2009-2010 compared to 9 in 2008, despite improved and sustained measles vaccination coverage (MCV) (Figure), show fragility of gains made towards eliminating measles on the continent and it is thought that this may be linked to the HIV-1 epidemic[75, 79, 80]. Leveling off of MCV since 2010 is of concern, as it implies a slackening of progress towards elimination of measles. Global measles outbreaks have occurred in the past 2 years in all WHO regions, with SSA experiencing a 100% increase in measles incidence. Whereas the largest increases occurred in Europe and the US, there are concerns that transmissibility of the virus, sub-optimal measles vaccination coverage, and increasing vaccine hesitancy may wipe out progress made in the past 15 years and slow down or reverse any gains made towards the global elimination of measles[81, 82].

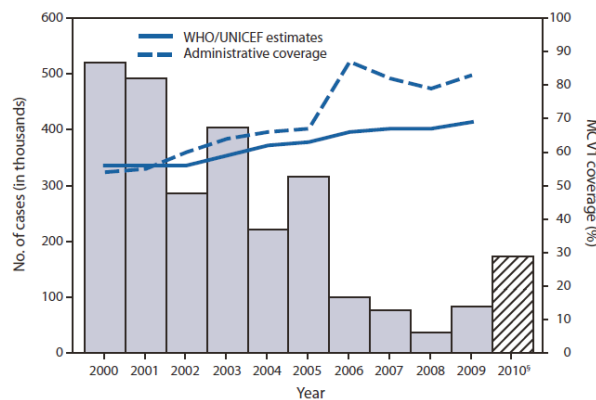


Figure 5 Number of reported measles cases and coverage with the first dose of measles containing vaccine in children below 1 year, WHO African region 2000-2010[83]

2.4 PASSIVE TRANSFER OF MATERNAL ANTIBODIES

Before mounting their own immunity to pathogens commonly occurring in a child's

environment, infants are protected by maternal antibodies transferred across the placenta and through breast milk. The efficiency of transplacental transfer is affected by multiple factors, including maternal immunization status and antibody levels, infections such as HIV-1 and placental malaria, and infant gestational age and birthweight[39, 40, 84]. In HIV-1-infected individuals, memory B cell function that is important in antigen-specific antibody production is defective[36]. This, coupled with hypergammaglobulinemia due to abnormal immunoglobulin G (IgG) production by hyperactivated naïve B cells, may result in insufficient and inefficient maternal antibody transfer across the placenta[37].

Although HEU infants have higher immunoglobulin levels compared to HUU infants, pathogen specific antibody levels are found to be lower, particularly in the presence of high systemic viral load in the mother[41]. This has been demonstrated for several pathogens including tetanus, measles virus, and *S. pneumoniae*[40, 43-46]. Therefore, infants born to HIV-1-infected women represent a vulnerable population with increased risk of infectious disease morbidity and mortality, and this vulnerability is thought to contribute to the up to 4-fold higher mortality they experience in the first year of life[15, 23, 47, 48]. HIV-1 viral load and HIV-1-specific IgG antibody levels are inversely associated with placental transfer of anti-measles IgG, thus suggesting that advanced maternal disease may compromise placental transfer[41]. There is evidence that some humoral immune reconstitution occurs following initiation of antiretroviral therapy particularly if initiated early in HIV disease[36], and this benefit could be transferred via the placenta.

Besides the effect on antibody transfer via the placenta, HIV-1 disease may decrease the ability of breast milk to protect infants against infections. HIV-1 infection results in rapid and persistent depletion of gut-associated CD4+ T cells[85] and has been associated with decreases in immunoglobulin A (IgA) producing B cells in the intestines[86]. A reduction in these cells may affect the levels of breast milk IgA, as IgA production is thought to occur in B cells with homing profiles similar to that of B cells found in the gut associated lymphoid tissue (GALT). Should this be the case, the immunological resources in breast milk could therefore be compromised. Studies that have demonstrated no association between mode of infant feeding and morbidity or mortality in infants born to HIV-infected women[87, 88] support the hypothesis that breast milk from HIV-infected women may not confer adequate protection against infections. However, this finding was not confirmed in a recent study[89], where B cells with homing markers suggesting gut origin were equally identified in breast milk of HIV-infected and uninfected women, suggesting that HIV does not diminish the immune profile of breast milk. Likewise, a comparison of breast milk from HIV-infected and

uninfected women[28] found that *Haemophilus influenzae*, *Campylobacter jejuni*, *Helicobacter pylori*, and *S. pneumoniae* IgG and IgA levels were similar in the two groups, while in another study HIV-infected women had higher total IgG levels, but similar total IgA levels to uninfected women[90].

Infant feeding recommendations for HIV-1 infected women have evolved over time alongside interventions to initially decrease and now eliminate MTCT of HIV. As breastfeeding is a key infant survival strategy in resource limited settings, women are advised to practice exclusive breastfeeding (EBF) during the first 6 months of an infant's life to minimize HIV-1 transmission risk and maximize breast milk benefits to the baby. Potential mechanisms for reduced HIV-1 transmission associated with EBF include 1) a reduction in dietary antigens and enteric pathogens that may maintain the integrity of the intestinal mucosal barrier, thus limiting inflammatory responses to the gut mucosa, and 2) promotion of beneficial intestinal micro flora that may increase resistance to infection and modulate the infant's immune responses[91]. Either mechanism may limit the passage of breast milk virus from the gut to the systemic and lymphatic circulation. In addition, continuous complete emptying of the breast in women practicing EBF contributes to maintenance of mammary epithelial integrity. This reduces the likelihood of both clinical and sub-clinical mastitis, which are associated with a 2-4 fold increased HIV-1 transmission risk due to altered breast milk cellular composition, increased breast milk virus and altered local defenses within the breast [92],[93, 94]. EBF is especially attractive in this era when efficacious antiretroviral regimens are available and recommended for all HIV-1 infected women regardless of their immunological status, in order to diminish transmission risk during pregnancy and breastfeeding.

Some studies have demonstrated important immunological breast milk benefits for HEU infants in resource limited settings with a reduction of pneumonia incidence and all-cause mortality[95-98]. However, there are no studies to date of the effect of ARVs on immunological components of breast milk.

Despite recommendations to exclusively breastfeed, EBF rates remain low in many parts of the world[99]. Data from the Kenya Demographic and Health Survey (KDHS) 2013 shows that there had been an improvement since previous surveys. However, the EBF rate of 84% at 1 month dropped rapidly to 63% at 3 months and 7.6% at 6 months, with a median EBF duration of 3.3 months[100]. Therefore, besides the need to continue to promote EBF uptake, further work is needed to investigate the effect of maternal ART on pathogen-specific

specific IgA levels in breast milk and the impact this would have on infant morbidity and mortality, taking into account potential confounding factors such as intensity and duration of breastfeeding, and infant vaccination and HIV infection status.

3 AIMS

The broad goal of this thesis was to determine the contributions of antiretroviral prophylaxis and breastfeeding practices on transfer of maternal antibodies and their impact on infant health. We hypothesized that 1) by restoring maternal immune function and reducing HIV-1 viral load through triple ART compared to standard short course treatment, systemic and breast milk pathogen specific antibody levels and their passive transfer will increase 2) infants born to mothers who receive triple ART will have less morbidity and mortality and this advantage will be dependent on the intensity of breastfeeding 3) providing HIV-1 infected women with intensive counseling will increase the proportion who practice exclusive breastfeeding. To answer our research questions, we developed the following aims:

Study I. To compare changes between antenatal enrolment and delivery in total and pathogen specific IgG levels (measles, Rotavirus and *S. pneumoniae*) and in trans-placental transfer between women who received triple ART prophylaxis and those who received a short-course regimen.

Study II. To compare breast milk IgA levels in women randomized to triple ART versus the standard short-course regimen at 2 weeks after delivery and longitudinally.

Study III. To determine association of maternal treatment with infant morbidity and mortality.

Study IV. To investigate effectiveness of intensive counseling on breastfeeding practices among HIV-1 infected women at 3 months post-partum.

4 RESEARCH APPROACH

4.1 STUDY DESIGNS

We answered our research questions using 2 studies conducted in Kenya. The first 3 aims, were nested within the Kesho Bora study[101], which was a multi-site randomized clinical trial (RCT) conducted between 2004 and 2009. HIV-1-infected pregnant women identified prior to 32 weeks gestation were recruited and followed up together with their infants for 12-18 months after delivery. The main study had two sites in Kenya, one in Burkina Faso, and two in South Africa. The three studies reported in this thesis used data and samples from the Kenyan sites, Nairobi and Mombasa.

For the last aim, we used data from a before-after study conducted in Nairobi, to determine whether providing intensive breastfeeding counseling to HIV-infected pregnant women would improve the proportion who practice exclusive breastfeeding.

We intentionally developed these two studies to meet the needs of our research questions. Thus, the participants in the RCT were a healthy and select group who received care in well-equipped research facilities with highly trained health personnel. On the other hand, the before-after study took place in a real-world setting and participants received care that was not different than that which they would have received if they had not participated in a research study.

4.2 INCLUSION CRITERIA, PARTICIPANT RECRUITMENT AND FOLLOW UP

Besides confirmed HIV diagnosis, willingness to provide informed consent, and to be randomized, additional criteria for study participation in the RCT included

- No evidence of clinically significant conditions (obstetric, cardiac, respiratory [including active tuberculosis], hepatic, gastrointestinal, endocrine, renal, haematologic, psychiatric, neurologic, or allergic) requiring care which could interfere with study interventions
- Not taking ARVs at the time of presentation for possible enrolment
- Capacity and willingness to participate in all follow-up visits, clinical examinations and agreement to venipuncture for themselves and their children
- Residency in site catchment area until two years after delivery
- Willingness and no contraindications to receive ARVs, namely:
 - Severe anemia (hemoglobin <7gm/dl)

- Severe neutropenia (neutrophil count $<750 \times 10^6$ cells/l)
- Blood alanine amino transferrase >5 times upper limit of normal (ULN)
- Amylase >2 times ULN
- Blood creatinine >3 times ULN
- Known allergy to one of study ARVs or to benzodiazepines
- Treatment with anticoagulants, benzodiazepines, rifampicin, magnesium sulphate, corticosteroids for more than 7 days at the time of planned enrolment.

Women were randomized to one of two MTCT prophylaxis regimens: (1) triple prophylaxis using 300 mg Zidovudine (ZDV), 150 mg lamivudine (3TC), and 400 mg/100 mg lopinavir/ritonavir (Kaletra) given twice daily beginning at 28-36 weeks gestation until six months postpartum, or (2) a short-course regimen consisting of 300 mg ZDV given twice daily from 28-36 weeks gestation until the onset of labor, when one dose each of 600 mg ZDV and 200 mg Nevirapine (NVP) were administered. All infants received one dose NVP (2 mg/kg oral suspension) preferably within 72 hours of birth, but no later than 7 days after birth. In addition, infants born after December 2006 received twice daily zidovudine in the first week of life.

After initiation of treatment/prophylaxis, follow-up visits occurred every week until delivery. Three to five days after delivery, the women received a home visit. Women and their infants were then followed up in clinic every two weeks until eight weeks postpartum, then every month until one year postpartum and quarterly thereafter. At each visit, questionnaires were administered to obtain socio-demographic and clinical information and clinical examinations were conducted by medical doctors. In addition, maternal and infant blood, cord blood, and breast milk specimens were collected over the course of follow-up. Maternal interviews and medical records were utilized to determine morbidity and mortality in infants. In the study overall, cumulative 12 month follow-up was 92% in the triple ART arm and 90.5% in the short-course arm.

For the before-after study, women attending antenatal clinics were routinely offered counseling and testing for HIV-1. After counseling on available PMTCT interventions, women identified as HIV-1-infected women chose freely how they planned to feed their infants. After receiving information about the study, those who planned to breastfeed their infants, met the following criteria and were willing to participate and provided informed consent, were enrolled into the study:

- Age \geq 18 years (minimum legal age for informed consent in Kenya)
- Gestation by dates less than 36 weeks
- Absolute CD4 count greater than 200 cells/mm³
- Planning to deliver in Nairobi and to stay in the study area for 3 months postpartum

At enrolment, a questionnaire was administered to obtain sociodemographic information and women were seen weekly until delivery. Thereafter, they were seen together with their infants at 1, 6, and 10 weeks, and again at 3 months, at which times questionnaires were administered and clinical examinations were conducted by nurse counselors. The week 6 and week 10 visits were timed to coincide with times when the mother would be bringing her baby to clinic for immunization or coming to access family planning services. Women in the control (before) group received standard of care while those in the intervention (after) group received additional breastfeeding counseling antenatally, and at 1 and 6 weeks postpartum by peer counselors (HIV-infected women with experience in exclusive breastfeeding) who were not involved in questionnaire administration or clinical examination.

4.3 APPROACHES TO STATISTICAL ANALYSIS

Linear Regression

Linear regression is a suitable analytic approach for a quantitative continuous outcome variable. The use of a linear regression model assumes that the relationship between the outcome and the explanatory variable is linear, that the observations are independent, and that for any value of the explanatory variable, the distribution of the outcome variable is normal. The variance of the outcome is also assumed to be constant across different values of explanatory variables. After fitting the model, it is appropriate to formally test whether these assumptions hold. In some cases, data transformations may be necessary to achieve normality.

Kaplan Meier estimator

The Kaplan-Meier estimator curve is a popular non-parametric method used to estimate and display survival function for time-to event variables, in the presence of censoring. The use of the Kaplan Meier method assumes that censoring is independent of the likelihood of failure (i.e. that the event of interest happens) and that the survival probabilities are comparable in the individuals in the sample. Once the survival times have been estimated

and the survival curves drawn for different groups of subjects, they can be formally compared using the log-rank test.

Cox proportional hazards regression

This approach is useful for time-to event data when we want to assess the effect of risk factors on the survival time. It models the “hazard” which is the instantaneous incidence rate. It is only valid under the proportional hazards assumption, which states that the hazard ratio between the two groups compared is constant over time, and that the survival times between individuals in the sample are independent. The proportionality assumption should be formally checked to see if it holds, for example, by using Schoenfeld residuals.

Poisson/negative binomial regression

When the outcome variable is a count, Poisson regression is the classical method used to assess the association with risk factors. A variable with Poisson distribution has a mean equal to its variance. Where over-dispersion is present (the mean is significantly different from the variance), it is best to use alternative approaches such as the negative binomial regression or the Quasi-poisson, which are more robust.

Log Binomial regression

A commonly used measure of association for binary outcomes in epidemiological investigations is the relative risk or risk ratio (RR). It measures the probability of disease among the exposed individuals relative to the probability of disease among the unexposed. When the outcome is rare, logistic regression can be used, as the odds ratio (OR) so obtained closely approximates the RR. However, when the response outcome is common (where incidence >10%), there is wide divergence between the OR and the RR. In such cases, the log-binomial regression is more suitable. However, it is important to bear in mind that the log-binomial can produce narrower confidence intervals (thus exaggerating the certainty of the point estimate) and the iterative method of implementation can fail to converge.

Repeated measures

When we have several measures of the same individual, or different measures of different individuals within the same cluster, we have to account for the between-subject variability as well as for the within-subject variability. Ignoring dependence between the observations will underestimate the standard errors of the time-dependent explanatory variables. Standard regression models are therefore not applicable. Instead, specific methods

such as the repeated-measures analysis of variance (ANOVA), generalized estimating equations (GEE), or linear mixed models are used.

4.4 ETHICAL CONSIDERATIONS

It would currently be unethical to conduct the RCT that is providing the data for studies I-III. This is because the findings from the main study indicated that triple ART is superior to short-course zidovudine in preventing transmission of HIV from the mother to her child. The current practice, therefore, is to put all HIV-1-infected women on triple antiretroviral therapy as soon as the HIV diagnosis is made, both for their own health and that of their children. However, these data provide us with a unique opportunity to answer an important question concerning the immunological effect of this treatment and impact on infant health. Nevertheless, there are some concerns that ARV exposure in utero may contribute to some of the vulnerability of HEU children, and the long-term effect of ARV exposure on the child's immune system development. This is a question that will not be answered easily; while it may be possible to examine effects of ARV exposure by comparing children exposed to ARV drugs with those born to ARV naïve women using historical cohorts, it is not possible to tease out the independent effect of exposure to HIV on the child's immunological development. We are therefore left to wait for data to accrue on the long term effects of ARVs on the child's health.

In conducting the breastfeeding study whose data we have used for study IV, there are 3 ethical issues that arose. One was the involvement of the male partners. The majority of the women did not want their male partners involved, as this could disclose their HIV status to their partners, and they cited concerns about intimate partner violence or relationship dissolution due to economic dependence on the male partner. However, this meant that the woman forfeited partner support for negotiating management of her infection and protecting her child from becoming infected. Male partner support has been shown (and was found in a related publication) to be associated with better infant outcomes, therefore there are infants who either became HIV infected or died who might have survived HIV-free. In addition, non-disclosure puts the woman's sexual partner(s) at risk for becoming HIV infected.

Secondly, there is a perception by the communities that EBF is for HIV infected women. Therefore, HIV-positive women risk inadvertent disclosure of HIV status by their commitment to exclusively breastfeed their infants, or may jeopardize their infants' health should they give into the pressure to mix feed. On the other hand, in an effort to prove to their friends, neighbors, and family that they are not HIV-infected, many HIV-negative women living within these environments practice mixed feeding. This is unfortunate, as it puts their children at risk for poor growth and development, and increased morbidity and

mortality. In addition, because these women are at risk for HIV infection themselves, it puts their infants at a higher risk of becoming HIV infected, should the mother contract HIV later in pregnancy or during the breastfeeding period.

Thirdly, there are high levels of stigma associated with a positive HIV diagnosis. This necessitated HIV-positive women to make difficult and burdensome choices to succeed in meeting their breastfeeding goals. These included changing their residency in order to not go against community norms to mix feed, avoiding social or extended family gatherings which may put them under undue pressure to mix feed, and never going anywhere without their child, as any other caregiver could potentially violate the feeding practices even when there was a sufficient quantity of expressed breast milk for the child. These had consequences for the family including the woman's inability to earn income and forfeiting social support that would be beneficial to her coping with her HIV diagnosis and providing optimum care to her child.

5 RESULTS

In this population of HIV-infected women with moderate immunosuppression, we found non-significant declines in pathogen-specific maternal serum antibody levels during pregnancy. IgG levels were comparable at delivery between the two arms. In a subset of the women who had cord blood available, women in triple ART transferred higher levels of pathogen specific antibody levels. Women who were more immunosuppressed, as indicated by higher total immunoglobulin levels, transferred lower levels of pathogen specific antibodies. Antibody transfer was positively correlated with pathogen specific antibody levels in maternal plasma.

We found no effect of treatment on breast milk levels of IgA to measles and rotavirus in transitional milk obtained at two weeks post-partum. In addition, there was no effect of treatment on IgA levels in mature milk obtained at 14 weeks and at 5 months postpartum. Women with higher serum measles IgG were more likely to have high measles IgA levels with a trend for more immunosuppressed women to have lower levels. Women with higher serum rotavirus IgG levels, and who took antiretroviral prophylaxis for longer duration prior to delivery, had higher rotavirus IgA levels. The strongest association with rotavirus IgA levels was primigravida which was inversely associated with rotavirus IgA levels.

We found no effect of treatment on diarrhea, pneumonia, hospitalization, or death. Triple ART was associated with a 2-fold increased risk for acute lower respiratory infection. For all three morbidity outcomes, we found a higher risk for male infants, and low birthweight infants had an almost 4-fold increased risk for pneumonia. Breastfeeding and being on ARV for 30 days or more during pregnancy were protective for acute infectious morbidity.

We found high levels of EBF among HIV-infected women. While the levels were not different between control and counseling intervention groups, they were higher than the Kenyan general population averages.

6 DISCUSSION AND CONCLUSIONS

Our finding of no effect of treatment on diarrhea, pneumonia, hospitalization or mortality while not being the result we hoped for is nevertheless an important finding. First, slow immune reconstitution following initiation of ARV use necessitates being on treatment for longer periods of time to reverse the effects of HIV infection and realize the beneficial effect of treatment. In addition, the narrow range of CD4 count of the women in the study and their overall good health may have resulted in a group of women in whom it is more difficult to detect an effect of treatment. There is a paucity of data on IgG transfer levels for uninfected women, making interpretation of our findings challenging. However, it is encouraging that levels of measles IgG that were transferred are within the range of what has been reported for uninfected women. We could therefore postulate that this beneficial effect may extend to the other immunoglobulins for which we do not have general population data for comparison.

The finding that socioeconomically advantaged primigravid women had lower rotavirus IgA levels than multiparous women is an interesting finding that may be reflective of the immunological integration that occurs between a mother and her child during breastfeeding in order to provide targeted protection to environmental exposures. It may be for this reason that breast milk is an important child survival strategy in resource limited settings, where it is thought that more children have died for lack of breastfeeding than from HIV infection. Therefore, maximizing breast milk benefits by improving maternal health and nutrition, and practicing safe breastfeeding practices is important for HEU children.

The high levels of exclusive breastfeeding rates among HIV-infected women is an important finding. It shows that with support, women are amenable to change with regard to infant feeding practices. Extension of this benefit to all women irrespective of their HIV status could be achieved by reducing EBF stigma, and providing other targeted interventions to mitigate EBF challenges including increasing male partner involvement and support.

7 FUTURE PERSPECTIVES

Optimizing the health of the growing population of HEU children remains an important pursuit. Larger prospective studies involving HEU children from diverse backgrounds will help better understand whether their vulnerability to infectious disease morbidity and mortality was more a function of untreated HIV infection in the mother or whether it is still a concern in the era of efficacious ART especially when initiated prior to conception. These studies will need to include unexposed uninfected children from similar socioeconomic environments in order to make suitable comparisons in their health outcomes. Beyond early childhood, there is a need to understand long term growth and developmental effects of HIV and ART exposure.

In addition, we still don't know that much about breast milk and mucosal immunity. Future studies may need to take a more holistic approach to breast milk characterization linked to infant outcomes to better understand its role and mechanism in infant immune system development. Alongside this there is need to understand contextual barriers to EBF practice and to continue promoting safer breastfeeding practices among all women irrespective of their HIV-infection status. Such a universal approach would create a more supportive environment for women to meet their breastfeeding goals.

As pediatric HIV disease transitions from one with high incidence and mortality to one with low incidence, there is need to continue to investigate patient, health provider and health system factors that contribute to the excess mortality experienced by HEU children. Studies that take into account regional differences in these factors are necessary in order to design more effective interventions to optimize the health of all children.

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