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Higher Transplacental Pathogen-Specific Antibody Transfer Among Pregnant Women Randomized to Triple Antiretroviral Treatment Versus Short Course Zidovudine

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Abstract

Background—HIV-1 infection may impair transplacental antibody transfer to infants. The impact of highly active antiretroviral treatment (ART) given during pregnancy on transplacental antibody transport is unknown.

Methods—HIV-1 infected pregnant women with CD4 counts between 200 – 500 were randomized to short-course zidovudine (ZDV) or triple ART at 32 weeks gestation for prevention of mother-to-child HIV-1 transmission. Levels of maternal antibody against measles, pneumococcus and rotavirus at delivery, and antibody transfer to the baby through cord blood, were compared between trial arms.

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Results—Overall, 141 and 148 women were randomized to triple ART and ZDV respectively; cord blood was available for a subset (n=20 in triple ART and n=22 in ZDV). Maternal antibody levels to all pathogens during pregnancy and at delivery were not significantly different between arms. Within each arm antibody levels at delivery were lower than at enrolment. For all antibodies, a woman's levels prior to delivery were an important predictor of amount transferred to her infant. Women on triple ART transferred higher levels of pathogen-specific antibodies when compared to women on short course zidovudine.

Conclusions—Women on triple ART transferred higher levels of pathogen-specific antibodies compared to women on zidovudine alone.

Introduction

In sub-Saharan Africa where prevalence of human immunodeficiency virus type-1 (HIV-1) among reproductive age women is high, children face the double burden of high background infant mortality and HIV-1 infection. HIV-1 exposed uninfected (HEU) children have more than a 3-fold increased risk of dying in the first 2 years of life compared to children born to HIV-1 uninfected women^[1–5]. This mortality attenuates gains in HIV-free survival from interventions to prevent mother-to-child transmission (PMTCT) of HIV-1 and is not fully explained by differences in socioeconomic status, maternal survival or infant birthweight^[5]. The most common causes of death in HEU children are bacterial and viral infectious diseases such as pneumonia, diarrhea, and sepsis. Infants receive protection against these infections from antibodies obtained via the placenta and breast milk^[6]. Lower levels of these protective antibodies may be one explanation for increased mortality among HEU children.

Several studies have observed reduced transplacental transfer of antibodies against important infant pathogens including measles virus, *Streptococcus pneumoniae*, and tetanus among HIV-1 infected untreated women compared to HIV-1 uninfected women^[7–11]. Abnormal maternal antibody transfer is thought to be as a result of impaired function of maternal B cells, and less efficient active transport of IgG across the placenta in HIV-1 infected women^[12]. During untreated HIV-1 infection, major perturbations in the B-cell compartment occur, including hypergammaglobulinemia, B-cell exhaustion and impaired antigen responses.^[13] Initiation of antiretroviral therapy (ART) can restore physiologic levels of serum IgG by reducing polyclonal B cell activation, increasing absolute B cell lymphocyte count (13–15) and by stabilizing CD4+ T helper function and unbalanced cytokine profiles associated with B-cell dysfunction. However, it is unknown whether initiation of ART during pregnancy improves pathogen-specific antibody levels in the infant.

We postulated that ART initiation as PMTCT will have the added benefit of reducing production of non-specific maternal immunoglobulin, which in turn, will restore the relative levels of antigen specific immunoglobulins available for passive transfer. Using archived samples and data from a randomized clinical trial (RCT) comparing triple ART to ZDV for PMTCT^[14], we examined the effect of treatment on maternal and transplacentally transferred IgG antibodies specific for measles virus, pneumococcus and rotavirus, three pathogens that cause significant under-5 morbidity and mortality.

Methods

Study design, subject randomization and follow up procedures

Our study was conducted between 2013 and 2015 using samples from participants enrolled in the Kenyan sites (Nairobi and Mombasa) of the Kesho Bora study^[14]. Trial eligibility criteria and study methods are explained in detail elsewhere^[14]. In brief, HIV-1-infected pregnant women aged 18 years and older with CD4 counts between 200 and 500 cells/ μ L who had no contraindications to ART were randomized at 28–36 weeks gestation to one of two prophylaxis regimens for prevention of MTCT: triple ART (ZDV/lamivudine [3TC]/lopinavir/ritonavir [Kaletra]) given twice daily until cessation of breastfeeding, or short-course ZDV (twice daily until delivery and single-dose nevirapine [NVP] at the onset of labor followed by ZDV/3TC twice daily for 1 week postpartum). Socio-demographic, obstetric and clinical information were obtained at enrollment, and follow-up visits occurred every 2 weeks until delivery. At each follow-up visit, questionnaires were administered to obtain clinical information and physical examinations were conducted. For our study, we used all available peripheral blood obtained at enrollment and delivery from women in the study and infant cord blood obtained only at the Nairobi site (see Figure, Supplemental Digital Content1).

Sample processing, lymphocyte subset analysis and viral load assay

Blood samples were processed the same day on site and plasma stored at -80° Celsius. T-cell subsets were measured at each site using a FACScan flow cytometer (Becton Dickinson, Mountain View, USA) and the CD4 counts were used to select subjects for randomization. Maternal plasma HIV-1 RNA was determined centrally using the Gen-Probe HIV-1 viral load assay (Gen-Probe Incorporated, San Diego, USA) by laboratory personnel who were unaware of the participants' treatment assignment.

Total and pathogen-specific IgG assays

Total serum IgG was measured using a commercial kit (ICL Laboratories, Newberg, USA) which was re-calibrated to determine the optimal sample dilution factor (1:400,000) that was subsequently used for all measurements according to manufacturer's instructions. Measles IgG levels were measured using the Enzygnost kit (Siemens, Marburg, Germany) and calibrated against an international reference preparation of measles antibody against Edmonston strain to convert the results into milli-International Units per milliliter (mIU/ml). Results for each sample were classified as seropositive (IgG titre >350 mIU/ml), seronegative (IgG titre <150 mIU/ml) or equivocal (IgG titre 150–350 mIU/ml) using the manufacturer's cut-off values for the corrected optical density (OD). Pneumococcal IgG levels were determined using the VaccZyme kit (The Binding Site, Birmingham, UK) that measures immune responses to 23 pneumococcal capsular polysaccharides (PCP) that represent 80% of the virulent subtypes (1–5, 6B, 7F, 8, 9N, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F, 33F) that are commonly encountered. Rotavirus IgG levels against the rhesus rotavirus (RRV) strain were measured using an in-house assay whose read out gives a dilution factor as the measure of concentration as previously reported^[15]. For all antibody determinations, absorbance was read using an EMax[®] endpoint microplate ELISA reader

with **SoftMax® Pro** data acquisition and analysis software (Molecular Devices, Sunnyvale, USA).

Statistical analyses

Study participants were compared for baseline characteristics using T-tests or Wilcoxon signed-rank tests for numerical variables, and Chi-squared tests for categorical variables. Because antibody responses were highly skewed, Wilcoxon signed-ranks tests were used to compare the two arms for antibody levels at enrollment, delivery and in cord blood. The effect of antiretroviral prophylaxis regimen, maternal immune status and viral load on antibody levels was examined using linear regression models, fitted separately for each antibody, to assess differences between the treatment arms and to account for differences between the study sites if necessary. Assuming a linear trend in antibody levels between enrolment and delivery, and because antibody transfer occurs throughout pregnancy, particularly in the third trimester^[16, 17], we used the mother's enrolment and delivery levels of antibody to estimate the average level in the six weeks prior to delivery. The pathogen-specific levels in cord blood were then compared to the maternal level using a difference and a ratio as a measure of transfer. For each pathogen specific antibody, regression models were run to assess how the difference, ratio or cord blood levels depended on mother's pathogen specific levels and mother's total IgG. The effect of viral load, maternal age, parity, gestational age, and sex of the baby that have previously been reported to be important determinants of antibody transfer were also assessed. All analyses were conducted using STATA/IC (version 11.2; StataCorp, College Station, TX).

Ethical considerations

Mothers provided informed consent for themselves and their child for participation in the parent study and future studies that would use their data and samples. Ethical approval to conduct this sub-study was obtained from the University of Washington Institutional Review Board, the Kenyatta National Hospital Ethical and Research Committee, and the Stockholm Regional Ethics Committee.

Results

Participant characteristics

Overall 141 women were randomized to triple ART and 148 to short course ZDV. The mean age was 28 years and a majority (86%) was married. Women were enrolled at a mean gestation of 32 weeks. Women in the two arms were similar for socio-demographic, clinical and virological characteristics at baseline (Table 1). Although gestational age at enrollment was similar between the two arms, women in the triple ART arm delivered at an earlier gestation compared to women in the short course ZDV arm, (38.6 weeks vs 39.2 weeks, $p=0.02$). There were some differences between women from the two study sites: women from Mombasa were more likely to be employed, 33% (81/245) compared to 16% (7/44) and had a higher mean body mass index, 26.4 vs 24.9, with $p=0.02$ for both comparisons; Nairobi women had higher hemoglobin levels, 11.9 g/dL vs 9.3 g/dL, $p<0.0001$ (data not shown).

Duration of treatment and adherence

Information on duration of treatment between enrollment and delivery was available for almost all women, 139/141 and 146/148 in the triple ART and short course ZDV arms, respectively. Two women in each arm were lost to follow up during pregnancy. Overall, women in the triple ART arm had a shorter exposure to their assigned prophylaxis before delivery compared to women in the short course ZDV arm (mean 36 days vs 40 days, $p=0.05$). While there was no difference in duration of exposure to assigned prophylaxis at the Mombasa site, at the Nairobi site women on triple ART took their prophylaxis for a significantly shorter period of time compared to women in ZDV arm (mean 25 days vs 34 days, $p=0.03$). There was a trend for a higher proportion of women in the triple ART arm to be fully adherent to their treatment compared to women in the short course ZDV arm (85% vs 74%, $p=0.06$). Women from Mombasa took their prophylaxis for a longer period prior to delivery, for a mean of 40 days compared to 30 days in Nairobi ($p=0.001$).

Antibody levels at enrollment and delivery

Total IgG levels—Total IgG levels did not differ significantly between the two arms at enrollment (43 g/L vs 51 g/L, $p=0.1$) or at delivery (19g/L vs 18g/L, $p=0.4$) (Table 2, top panel). Women in both arms exhibited a decline in total IgG levels in the time between enrollment and delivery. However, women in the triple ART arm experienced a smaller decline in total IgG compared to women in the short course ZDV arm, a median of 5.9g/L [IQR 24, -9] vs 27g/L [IQR 63, 6], $p<0.001$. Declines were similar in the two arms when analyses were restricted to women who took their PMTCT regimen for at least 30 days prior to delivery. Levels at delivery were positively correlated with levels at enrollment and with viral load (data not shown).

Pathogen-specific IgG levels at enrollment and delivery—Maternal pathogen-specific IgG levels were also comparable between arms at enrollment and at delivery. However, for each pathogen specific antibody, levels were lower at delivery than at enrollment, but the decline did not differ significantly between randomization arms (Table 2). Due to differences in some characteristics of women from the two sites and since only Nairobi women contribute to the analysis of cord blood levels, analyses of antibody levels stratified by study site are also presented (Table 2). At each site, we found similar antibody levels in the two arms, with the exception of higher rotavirus antibody levels at delivery in the triple ART arm in Nairobi ($p=0.01$), likely due to their somewhat higher levels at enrolment ($p=.07$).

Correlates of pathogen-specific IgG levels at delivery—For all pathogens, maternal pathogen-specific IgG level at enrolment was the most significant determinant of antibody levels at delivery, accounting for 55%, 39% and 68% of the variation in measles, pneumococcal and rotavirus IgG levels, respectively. We found higher maternal HIV-1 viral load to be a significant predictor for lower pathogen-specific IgG levels, but we did not find any significant association with treatment or CD4 count for any of the antibodies (Table 3). On further adjustment for characteristics that have been reported to be associated with antibody levels during pregnancy such as maternal age, parity, and gestational age (see Table, Supplemental Digital Content 2), we found maternal age and parity to be positively

associated with measles antibody levels at delivery ($p=0.006$ and $p=0.04$, respectively). On including these in our multivariate model, only gestational age was an independent significant predictor ($p<0.001$) and the effect of treatment remained non-significant. For the other pathogen-specific antibodies, there was a weak negative association of parity with pneumococcal antibody levels ($p=0.06$) and there was a trend for a positive association of parity with rotavirus antibody levels ($p=0.09$). For all analyses, we included study site as a potential confounder, but none of these adjusted analyses changed the results.

Pathogen-specific antibody transfer

Data on transfer across the placenta were available for the Nairobi site only. Higher median rotavirus antibody levels were transferred, 31748 (IQR 12051, 50019) vs 7563 (IQR 4381, 23630) among women on triple ART ($p=0.01$), and there was a weak trend for higher measles antibody transfer, 5440 mIU/mL (IQR 873, 6959) vs 1943 mIU/mL (IQR 1018, 4999), $p=0.1$. Women in the two arms transferred similar amounts of pneumococcal antibody, 29 g/mL (IQR 11, 106) vs 29 g/mL (IQR 12, 58), $p=0.7$. When expressed as a ratio to the mother's levels at delivery, these cord blood levels correspond to a higher mean ratio of all antibodies in the triple ART arm (measles 5.9 vs. 1.5, PCP 1.1 vs. 0.97, rotavirus 2.2 vs 1.2) but none of these were statistically significant. For multivariable analysis, linear regression models of the ratio or difference between cord blood levels and maternal levels provided a very poor fit, whether using a linear or log scale. However, models of the cord blood levels for measles and pneumonia, and the log-transformed levels for rotavirus, provided very good fit, indicating that maternal pathogen-specific and total IgG levels, together with treatment arm, accounted for at least 72% of the variation in cord-blood levels. The levels of pathogen-specific antibody levels in cord blood were positively correlated with maternal pathogen-specific levels, and for rotavirus and pneumococcus, were inversely correlated with maternal total IgG (Table 4). Adjusting for these measures of maternal immune status, there was an independent effect of treatment on cord blood levels, with infants in the Triple ART arm having significantly higher levels of antibodies against measles and pneumococcus ($p=.05$ for both) and a trend for higher levels of rotavirus antibodies ($p=.06$). We found no independent effect of viral load on antibody transfer.

We examined other factors that have been known to predict transfer across the placenta and we found that taken individually, parity and gestational age at delivery were significant predictors of pneumococcal antibody levels in cord blood ($p<0.01$ for both) (data not shown). Due to our finding that women on triple ART delivered at an earlier gestation than women on short course ZDV, we restricted the multivariable analysis to women who delivered at term and did not find evidence of an independent contribution of parity or gestational age to pneumococcal antibody transfer. For measles or rotavirus antibody transfer, none of the factors studied was associated with cord blood levels.

Discussion

In this study comparing women randomized to either triple ART or ZDV for PMTCT, we found declines in total and pathogen-specific IgG levels between enrollment (median 32 weeks gestation) and delivery with minimal differences in maternal antibody levels between

women in the two groups at delivery. The antibody levels in cord blood were positively correlated with maternal pathogen-specific levels, inversely correlated with maternal levels of total IgG and positively associated with triple ART. This evidence of more efficient trans-placental transport of antibodies for women who were on triple ART suggests that although treatment does not seem to affect delivery levels in the mother, it may impact on the transfer to the baby.

HIV-positive pregnant women who have not received anti-retroviral treatment have been reported to transfer lower levels of pathogen-specific antibodies compared to HIV-negative women^[7, 9, 18–20]. Although one study demonstrated a decline in IgG levels among HIV infected Caucasian women over the course of the pregnancy^[21], we found no such study from African women, and results from hemo-dilution studies of HIV-uninfected pregnant women are inconsistent depending on race, pre-pregnancy body size, parity, infant birthweight^[22], with some finding a decline while others report an increase or no change^[23, 24]. We observed a decline in maternal antibody levels in the course of pregnancy, with similar levels for women in the two groups at delivery, but differences in the cord blood levels that indicate a (maternal) treatment benefit for the infants. Another important finding of our study is that measles antibody levels transferred by women on triple ART are comparable to what has previously been reported for HIV-uninfected women^[7] and are within the range of what has been described as post-immunization protective levels^[8, 25, 26]. Thus these data demonstrate an additional benefit to infants of the current World Health Organization (WHO) guidelines that have been adopted for use in clinical care settings in Kenya that recommend Option B+ with ART for all HIV-1 infected mothers irrespective of CD4 count^[27].

Maternal pathogen-specific antibody levels at enrollment were a strong correlate of the delivery levels, which were also dependent on the viral load at enrollment. We had hypothesized that treatment would contribute to an increase in pathogen-specific IgG and a decline in total IgG due to proportionate reduction in non-specific antibody production, more pronounced in triple ART compared to monotherapy. Contrary to our expectations, we observed pathogen-specific antibody decline alongside a decline in total IgG. However, reversal of the loss and decrease in function of memory B-cells important in antigen response and serological memory following ART has been shown to be slow, incomplete or absent resulting in little or no improvement in pathogen-specific antibody production^[28]. This may explain our inability to find increases in pathogen-specific antibody levels during pregnancy or significant differences in levels between the two arms.

Transplacental antibody transfer is dependent on an active mechanism involving neonatal Fc receptors (FcRn) on the surface of trophoblast cells. This takes place with better efficiency for transfer of thymus dependent antibodies such as measles and rotavirus antibodies compared to non-thymus dependent antibodies such as pneumococcal antibody^[7, 29]. Various factors have been shown to affect efficiency of antibody transfer via the placenta. Independent of viral load, HIV-1 infection negatively affects frequency and magnitude of Fc receptor expression^[30], induces morphological and inflammatory changes in the placenta^[31–33] that affect the placental immune regulatory activity^[18] and produces a hypergammaglobulinemic state that inhibits antibody binding to FcRn^[34]. It is possible that

some of these changes are reversed in the presence of ART resulting in better antibody transfer. ART corrects some B-cell dysregulation due to HIV-1 infection and would be expected to reduce HIV-1 associated hypergammaglobulinemia^[35, 36] and has been shown to decrease HIV-induced inflammatory changes in the placenta^[37] both of which could result in improved antibody transfer. On the other hand, total IgG levels which are known to remain high for months after initiation of ART^[35, 38] would likely exert some inhibitory effect on antibody transfer by saturating the available receptors^[39]. We observed such an effect in our study where pathogen specific antibody levels in cord blood were inversely correlated with maternal levels of total IgG. This supports earlier findings that HIV infection exerts an inhibitory effect on antibody transfer through multiple mechanisms which are not fully understood.

Our study has some limitations. First, we were unable to directly determine the contribution of hemo-dilution to the changes in antibody levels during pregnancy as we did not measure albumin levels. However, this likely had minimal impact on our findings as randomization resulted in similar groups of women in the two arms at enrollment and adjusting for factors such as parity and infant birthweight that are known to be predictive of hemo-dilution did not change our findings. In addition, plasma volume expansion is thought to plateau at 32–34 weeks gestation^[22], and would therefore be unlikely to impact the changes observed in our study. Second, placental malaria which has been reported in some studies to impact antibody transfer across the placenta^[9, 34] was not ascertained in our study. However, our analysis of placental antibody transfer was limited to women who resided in a low malaria endemicity zone. It is therefore unlikely that placental malaria was a major determinant of antibody transfer among this population of women. Third, lack of a significant effect of the triple ART regimen on antibody levels at delivery might be due to the slow and incomplete immune reconstitution process following initiation of therapy^[40, 41] and the short duration of exposure to the drugs, as only slightly more than half of the women took the treatment for at least 30 days. Our study was not designed to study effects of timing and duration of treatment, and further studies are necessary to examine optimal timing and duration of treatment to maximize benefits to mother and baby. Fourth, we did not measure subclass levels of total IgG or subclass transfer or FcR expression, which hampered our ability to more precisely describe total IgG changes and differential antibody transfer in the presence of ART.

This study demonstrates the complexity of the maternal-fetal immunological relationship and the potential benefits of anti-retrovirals to not only prevent mother-to-child transmission of HIV-1 but also to improve the mother's immunological profile and consequently that of her infant. Next steps would include investigation of functional changes in these antibodies in the presence of triple antiretroviral therapy and their subsequent impact on infant morbidity and mortality. Examining mortality and morbidity due to diarrheal and respiratory illnesses in relation to antibody transfer would provide greater understanding of immunological processes and help improve clinical care approaches in our endeavors to increase survival of all HIV-1 exposed children.

In the era of MTCT elimination with widely accessible antiretroviral therapy for HIV infected pregnant women, our findings are important for the growing population of HIV

exposed uninfected (HEU) infants. These infants are a vulnerable subgroup in light of the re-emergence of vaccine preventable infections to which these children are particularly at risk^[42, 43]. Maternal vaccination to protect the infant in the first few months of life is an important strategy that has been used successfully for tetanus^[44] and would be especially useful in these infants. Our finding of enhanced transfer of antibody to pathogens in the mother's environment following maternal ART also suggests a benefit to infants from treatment of their mothers. Thus current WHO recommendations to offer ART to all HIV positive pregnant women and to offer measles vaccination to HEU as early as 6 months of age^[45] in regions with high incidence of both HIV and measles may act as synergistic strategies to counter infections that have high morbidity and mortality early in postnatal life among children born to HIV-1 infected women, to help improve survival of these infants. This study provides support that such strategies could be especially useful in sub-Saharan Africa which has a high infectious disease burden and where HIV-1 prevalence in women of reproductive age remains high.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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APPENDIX 1: The Kesho Bora Study Group

Study sites

1. Nairobi, Kenya (University of Nairobi/Network for AIDS Researchers in East and Southern Africa): Ruth Nduati (Principal Investigator), Judith Kose (Study Coordinator), Ephantus Njagi (Laboratory Coordinator), Peter Mwaura (Data Manager).
2. Mombasa, Kenya (International Centre for Reproductive Health / Coast Provincial General Hospital): Stanley Luchters, Marcel Reyners (Principal Investigators), Eunice Irungu (Study Coordinator), Christine Katingima, Mary Mwaura and Gina Ouattara (Investigators), Kishor Mandaliya, Sammy Wambua (Laboratory Coordinators), Mary Thiongo (Data Manager);

Supporting institutions

1. Agence Nationale de Recherches sur les SIDA et les Hépatites Virales, France: Brigitte Bazin, Claire Rekacewicz (Sponsor Representatives);

2. Centers for Disease Control and Prevention, USA: Allan Taylor, Nicole Flowers, Michael Thigpen, Mary Glenn Fowler, Denise Jamieson (Sponsor Representatives and Co-Investigators);
3. *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, USA: Jennifer S. Read (Sponsor Representative and Co-Investigator);
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Table 1

Characteristics of study participants

	Triple ART n=141	Zidovudine n=148
	Mean (SD) or n (%)	
Study site participants		
Nairobi	20 (14)	24 (16)
Mombasa	121 (86)	124 (84)
Baseline demographic and clinical		
Age (years)	27.8 (4.4)	27.7 (5.4)
Parity	2 (1.5)	2 (1.4)
Gestational age (weeks)	32.3 (1.7)	32.0 (1.9)
Education level		
Tertiary	7 (5)	10 (7)
Secondary	56 (40)	48 (32)
Primary	73 (52)	81 (55)
None	5 (3)	9 (6)
Baseline laboratory		
Hemoglobin level (g/dL)	9.7 (1.6)	9.7 (1.8)
CD4 count (cells/ μ L)	373 (72)	355 (85)
Viral load (log ₁₀ copies per ml)	4.2 (1.3)	4.2 (1.1)
Delivery characteristics		
Duration of PMTCT regimen (days)	36 (17.1)	40.6 (17.8)
Duration of PMTCT regimen 30 days	94 (68)	105 (72)
Adherence to PMTCT		
Fully adherent [§]	114 (85)	104 (74)
Missed at most 1 dose	130 (97)	129 (92)
Gestational age at delivery (weeks)	38.7 (2.2)	39.0 (2.2)
Female infant	70 (50)	76 (52)

[§]Took 100% of their doses

Antibody levels (median and interquartile range) at enrollment and delivery stratified by treatment group

Table 2

	Enrollment antibody levels				Delivery antibody levels				Difference in antibody level between enrollment and delivery			
	Triple anti-retroviral	Zidovudine	p ^a	Triple anti-retroviral	Zidovudine	p	Triple ART	Zidovudine	p	Triple ART	Zidovudine	p
All Women	n=134	n=136		n=121	n=128		n=120	n=125				
Total IgG (g/L)	42.7 (32.3, 77.1) ^b	51.4 (29.1, 87.7)	0.1	19 (12.5, 29.5)	17.8 (12.2, 28.5)	0.4	-5.9 (-24, 9)	-27 (-63, -6)	<0.001			
Measles IgG (mIU/mL)	2349 (1096, 5897)	2592 (1159, 5343)	0.3	1614 (676, 4462)	1858 (644, 4340)	0.9	-88 (-831, 93)	-263 (-1022, 59)	0.2			
<i>S. pneumoniae</i> IgG (mg/L)	45 (26, 78)	50 (28 - 80)	0.9	36.5 (20, 67)	39 (18, 65.5)	0.8	-7 (-20, 0)	-9 (-20, 0)	0.8			
Rotavirus IgG (U/mL)	12580 (6609, 26022)	12444 (7166, 21937)	0.9	11688 (6650, 23736)	11806 (6883, 21668)	0.8	-531 (-4950, 3293)	-343 (-3996, 2905)	0.7			
Mombasa site	n=115	n=115		n=103	n=107		n=102	n=105				
Total IgG (g/L)	36 (28, 43) ^b	32 (25, 42)	0.3	18 (12, 25)	16 (11, 25)	0.4	-7.2 (-24, 8)	-34 (-77, -12)	<0.001			
Measles IgG (mIU/mL)	2081 (1114, 5853)	2700 (1158, 5348)	0.2	1456 (697, 4274)	1986 (663, 4354)	0.6	-88 (-779, 107)	-263 (-964, 84)	0.3			
<i>S. pneumoniae</i> IgG (mg/L)	43 (26, 81)	50 (30, 80)	0.7	36 (20, 68)	41 (18, 68)	0.9	-7 (-20, 0)	-9 (-18, 0)	0.96			
Rotavirus IgG (U/mL)	10645 (5996, 21995)	12035 (7031, 21192)	0.4	10918 (6013, 22015)	11608 (6393, 25368)	0.5	-478 (-4376, 2639)	-343 (-3996, 3346)	0.7			
Nairobi site	n=19	n=21		n=18	n=21		n=18	n=20				
Total IgG (g/L)	38 (27, 49)	26 (17, 42)	0.1	36.7 (29, 45)	32 (23, 40)	0.6	-4 (-20, 9.8)	3.2 (-7.6, 10.8)	0.5			
Measles IgG (mIU/mL)	3158 (799, 6638)	2102 (1246, 4529)	0.5	3339 (560, 6026)	1511 (469, 3900)	0.3	-88 (-1071, 79)	-312 (-1164, -53)	0.5			
<i>S. pneumoniae</i> IgG (mg/L)	50.5 (22, 64)	44.5 (24, 81)	0.9	39.5 (20, 61)	38 (17, 52)	0.4	-7 (-18, -1)	-10 (-24, -1)	0.7			
Rotavirus IgG (U/mL)	25489 (11963, 79631)	14245 (8107, 18843)	0.07	20336 (11521, 50145)	11952 (8168, 16795)	0.01	-2315 (-12093, 5209)	-135 (-5227, 1513)	0.8			

^a All p-values from Wilcoxon signed-rank test.^b Excludes 2 plates due to group imbalance in assay set up.

Table 3

Correlates of maternal antibody levels at delivery: coefficient estimates from linear regression with 95% confidence intervals and p-values

	Measles IgG	<i>S. pneumoniae</i> IgG	Rotavirus IgG ^a	
Crude Estimate (Triple ART only)	135 (-541, 810)	p= 0.7	-4.2 (-16.1, 7.8)	p= 0.5
Adjusted Estimates				
Triple ART	81.3 (-311, 474)	p= 0.7	-4.3 (-13.2, 4.7)	p= 0.4
Baseline HIV-1 viral load (log ₁₀ copies/ml)	-175.4 (-303, -47.5)	p=0.007	-3.6 (-7.2, 0.03)	p=0.05
Baseline IgG levels	0.6 (0.5, 0.7)	p <0.001	0.6 (0.4, 0.7)	p<0.001
			0.88 (0.78, 0.98)	p<0.001

^aAnalyses conducted on the log scale.

Correlates of cord blood levels of pathogen-specific IgG antibodies: coefficient estimates from linear regression, with 95% confidence intervals and p-values.

Table 4

	Measles IgG	<i>S. pneumoniae</i> IgG	Rotavirus IgG ^a	
Crude Estimate (Triple ART only)	1947 (−67, 3962)	0.06	29 (−6.5, 64)	0.1
Adjusted Estimates				
Triple ART	1312 (28, 2595)	0.05	17.7 (0.08, 35.4)	0.05
Maternal IgG levels	0.97 (0.7, 1.2)	<0.001	0.92 (0.7, 1.14)	<0.001
Maternal total IgG levels	−25.9 (−62.7, 10.8)	0.2	−0.5 (−0.99, 0.08)	0.09
			−0.02 (−0.04, −0.005)	0.02

^aAnalyses conducted on the log scale.