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Delayed HIV detection among infants exposed to postnatal antiretroviral prophylaxis during breastfeeding

Caroline C. King[a], **Athena P. Kourtis**[a], **Deborah Persaud**[b], **Julie A.E. Nelson**[c], **Carrie Ziemniak**[b], **Michael G. Hudgens**[d], **Gerald Tegha**[e], **Charles S. Chasela**[f], **Denise J. Jamieson**[a], and **Charles M. van der Horst**[g]

[a]Division of Reproductive Health, National Center for Chronic Disease Prevention and Promotion, U.S. Centers for Disease Control and Prevention, Atlanta, Georgia [b]Department of Pediatrics, Johns Hopkins University, Baltimore, Maryland [c]Department of Microbiology and Immunology, School of Medicine, North Carolina, USA [d]Department of Biostatistics, Gillings School of Global Health, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA [e]UNC Project-Malawi, Lilongwe, Malawi [f]Division of Epidemiology and Biostatistics, University of Witwatersrand, Johannesburg, South Africa [g]Division of Infectious Diseases, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

Abstract

Objective—The objective of this study is to determine whether detection of HIV infection was delayed in infants exposed to antiretroviral prophylaxis to prevent HIV transmission during breastfeeding.

Design—The Breastfeeding, Antiretrovirals and Nutrition (BAN) study was a randomized trial of 2369 mother–infant pairs conducted from 2004 to 2010. In addition to an intrapartum regimen, all mother–infant pairs were randomly assigned to three antiretroviral intervention arms during 28 weeks of breastfeeding: no further antiretroviral prophylaxis (control arm); infant-daily nevirapine (nevirapine arm); and maternal zidovudine, lamivudine and either nevirapine, nelfinavir or lopinavir-ritonavir (maternal arm). After breastfeeding cessation counselling and stopping the antiretroviral interventions by 28 weeks, 28 infant HIV infections occurred.

Methods—To determine whether these infections occurred during the breastfeeding and antiretroviral intervention phase but had delayed detection on the antiretroviral arms, we

Correspondence to Athena P. Kourtis, Centers for Disease Control and Prevention, 4770 Buford Highway, NE, MS F-74, Atlanta, GA 30341, USA. Tel: +1 770 488-5216; apk3@cdc.gov.

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Conflicts of interest

No other authors have any potential conflicts of interest to report.

performed ultrasensitive (droplet digital PCR) HIV testing on infants with stored peripheral blood mononuclear cell (PBMC) specimens at 24 weeks (n = 9).

Results—Of the nine infants, all three on the infant nevirapine arm had detectable HIV DNA at 24 weeks, compared with two of four on the maternal antiretroviral arm and one of two on the control arm. For infants with detectable HIV at 24 weeks, the median delay in detection between the ultrasensitive and standard assays was 18.3 weeks for the nevirapine arm, 15.4 weeks for the maternal arm and 9.4 weeks for the control arm.

Conclusion—The prolonged inability to detect HIV with standard assays in the context of postnatal antiretroviral prophylaxis suggests that early antiretrovirals may restrict HIV replication sufficiently to lead to missed diagnosis among infected infants. Therefore, repeat virologic testing is warranted beyond the WHO-recommended point of testing at 6 weeks after breastfeeding cessation.

Keywords

antiretroviral; breastfeeding; detection; HIV; infant

Introduction

Early detection and treatment of HIV type 1 infection among infants is essential to reduce their high risk of disease progression and mortality [1,2]. Very early initiation of combination antiretroviral therapy (ART) in the infant leads to rapid virologic control, limited seeding of replication-competent HIV reservoirs, reduced HIV quasispecies diversity, and in some cases, prolonged absence of HIV DNA detection, negative HIV serology and absence of HIV-specific humoral and cell-mediated immune responses [3–10]. However, early HIV detection depends on duration of the infection, assay sensitivity, specimen type, and potentially, the recommended use of antiretroviral prophylaxis by the HIV-infected mother or exposed infant [11]. There have been reports of delayed HIV DNA detection in nonbreastfeeding infants induced by 4–6 weeks of infant prophylaxis [12,13]. To our knowledge, no studies have assessed the effect of longer antiretroviral prophylaxis type or extent for breastfeeding infants on timing of HIV diagnosis. The WHO-recommended duration of antiretroviral prophylaxis in settings in which breastfeeding is practiced extends to 1 week after breastfeeding cessation; breastfeeding is recommended for 12 months for HIV-infected mothers in settings in which formula feeding is associated with an increased risk of morbidity and mortality [14].

Previously, we conducted a clinical trial of 2369 mother–infant pairs randomized to infant nevirapine, maternal antiretrovirals or neither during 28 weeks of breastfeeding [the Breastfeeding, Antiretrovirals and Nutrition (BAN) Study] [15]; both the infant and maternal prophylaxis significantly reduced HIV transmission during breastfeeding [16,17]. After breastfeeding cessation counselling and stopping the antiretroviral intervention by 28 weeks postpartum, 28 infant infections were first detected during follow-up from 29 to 48 weeks [17]. Although not statistically significant, more infections occurred on the infant nevirapine and maternal antiretroviral arms than on the control arm. To assess whether these infections occurred during the breastfeeding and antiretroviral intervention phase, but had

delayed detection on the antiretroviral intervention arms, we performed ultrasensitive HIV testing on stored peripheral blood mononuclear cell (PBMC) specimens.

Materials and methods

The BAN study was a randomized, controlled, clinical trial of 2369 mother–infant pairs enrolled in Lilongwe, Malawi, during the period 25 March 2004 to 28 January 2010 to investigate antiretroviral prevention of mother-to-child transmission of HIV-1 during breastfeeding (www.ClinicalTrials.govnumberNCT00164736) [15–17]. All mothers in labour and their newborn infants received a single dose of oral nevirapine and 7 days of Combivir (zidovudine, 300 mg, along with lamivudine, 150 mg) twice daily from the onset of labour for the mothers, and zidovudine (2 mg per kilogram of body weight) and lamivudine (4 mg per kilogram) twice daily for 7 days for the infants. Mother–infant pairs were randomly assigned to three antiretroviral intervention arms: no further antiretroviral prophylaxis (control arm); infants received a daily dose of nevirapine that increased according to age, ranging from10 mg daily during the first 2 weeks to 30 mg daily for weeks 19 through 28; and mothers received Combivir (twice daily) and either nevirapine (200 mg) once daily for 2 weeks and twice daily through week 28, or nelfinavir (1250 mg) or Kaletra (lopinavir, 400 mg, along with ritonavir, 100 mg) twice daily through week 28. All antiretroviral interventions were stopped at 28 weeks postnatal or after reported breastfeeding cessation, whichever occurred first. In March 2008, the data safety monitoring board (DSMB) halted enrolment to the control arm after 668 of the planned 806 mother–infant pairs had received control-arm assignment because the HIV transmission rate was significantly higher among the control group than one of the antiretroviral groups.

By using a standardized protocol derived from the WHO's Breastfeeding Counselling: A Training Course [18], all mothers were individually counselled to breastfeed exclusively for the first 24 weeks postpartum, and then to wean from 24 to 28 weeks. Mothers were provided with breast milk replacement food for the infants. Internal validity checks for visits and forms were used to estimate a date of weaning for each mother–infant pair.

Mother–infant pairs were followed at 1, 2, 4, 6, 8, 12, 18, 21, 24, 28, 32, 36, 42 and 48 weeks postpartum. The BAN Study protocol called for infant HIV testing on whole blood collected at birth, 2, 12, 28 and 48 weeks with Roche Amplicor HIV-1 Qualitative DNA assay, version 1.5 (Roche Molecular Systems, Pleasanton, California, USA). The test uses 200 μl of infant blood and detects five copies of provirus 92% of the time [19]. Positive results were confirmed by tests of a second specimen. The window of HIV transmission was later narrowed by testing dry blood-spot (DBS) specimens from all interim visits for HIV DNA by using the Roche Amplicor DNA assay, or RNA by using the Gen-Probe Aptima HIV-1 assay, both performed at the University of North Carolina. Two 6 mm punches of DBS were used for an estimated volume of 40 μl for testing; the Roche DNA assay is 98.8% sensitive, and the Aptima RNA assay is 96.5% sensitive above 400 copies/ml in DBS [20]. PBMCs were collected at five infant visits and were cryopreserved at −80°C; midway through the study, such collection stopped to redirect resources [15].

The BAN study was approved by the Malawi National Health Science Research Committee and institutional review boards at the University of North Carolina at Chapel Hill and the U.S. Centers for Disease Control and Prevention (CDC). All women provided written, informed consent for specimen storage and laboratory studies.

To determine whether the infant HIV infections after 28 weeks occurred during breastfeeding but had delayed detection on the antiretroviral arms, we performed ultrasensitive HIV testing on available infant PBMC specimens at 24 weeks. This time was chosen, as, per protocol, it represented the last study visit when the infant was still being exclusively breastfed. To exclude known ongoing exposure to HIV after 28 weeks, we excluded five infants whose reported breastfeeding cessation date was past the end of the antiretroviral intervention at 28 weeks. Of the remaining 23 infants, nine had available PBMC specimens at 24 weeks: three on the nevirapine arm, four on the maternal antiretroviral arm and two on the control arm. These nine HIV-infected infants, as well as three infants (one from each study arm) who remained HIV-negative by the end of the study follow-up at 48 weeks were blindly tested for HIV by using an ultrasensitive droplet digital PCR assay [21]. HIV and genomic DNA were extracted from frozen PBMC pellets by using QIAamp DNA Blood Mini Kit (Qiagen, Valencia, California, USA). The median number of cells per specimen was 376 200 (interquartile range: 188 650–502 250). HIV DNA was measured in six replicates by using ultrasensitive droplet digital PCR (ddPCR) (limit of detection ranging from 1.1 to 9.6 copies per million cells) [21]. Proviral burden was expressed as HIV DNA copies per million PBMCs.

The cumulative risk of infant HIV infection in BAN was estimated by an extension of the Kaplan–Meier method to account for the competing risk of infant death [22]. Infants who did not test HIV-positive or die were right censored at their last negative HIV test. Cumulative risk from 2 weeks to 28, 32, 36, 42 and 48 weeks was compared among arms on the basis of a two-sided *P* value for a *Z* statistic equal to the difference between the cumulative risk estimates at each time point divided by the corresponding estimated standard error. The median time between the ultrasensitive and standard assay's detection of HIV DNA, as well as the median maternal viral load across trial arms, are presented. The exact Kruskal–Wallis test was used to estimate *P* values for the comparison of medians across trial arms despite low statistical power. For analyses limited to infants with detectable HIV DNA on the ultrasensitive assay, the minimal attainable *P* value based on a power calculation was 0.1 [23].

Results

In BAN, 28 incident infant HIV infections were identified from 29 to 48 weeks, after completion of the antiretroviral prophylaxis and breastfeeding period, as per the study protocol. Although not statistically significant, more infections were identified after 28 weeks on the infant nevirapine (13/852) and maternal antiretroviral (nine of 849) arms than on the control arm (six of 668) (Fig. 1). However, the significantly increased cumulative risk of HIV infection at 28 weeks among infants on the control arm compared with the intervention arms reported previously [5.7% compared with 1.7% on the infant nevirapine arm ($P<0.001$) and 2.9% on the maternal antiretroviral ($P = 0.02$)] [17] remained

significantly increased at 32, 36, 42 and 48 weeks of life (Table 1). The largest incremental increase in cumulative incidence estimates of infant HIV risk after 28 weeks occurred from 32 to 36 weeks on the infant nevirapine arm, with a 1.1% increase during this 4-week period compared with an incremental increase of 0.3 and 0.4% during the same 4-week period occurring on the maternal antiretroviral and control arms, respectively.

Of the nine infants with infections after 28 weeks tested with the ultrasensitive assay, all those on the infant nevirapine arm (three of three) had detectable HIV DNA at 24 weeks, compared with half of those on the maternal antiretroviral (two of four) and control (one of two) arms (Table 2). None of the three infants who remained HIV-negative by BAN testing through 48 weeks had a positive ddPCR test result. For the infants with detectable HIV at 24 weeks, the median delay in detection between the ultrasensitive assay and the first positive BAN assay was 18.3 weeks on the nevirapine arm, 15.4 weeks on the maternal arm and 9.4 weeks on the control arm ($P = 0.267$).

For the six infants with detectable HIV at 24 weeks, we tested a PBMC specimen from their 12-week visit. However, four assays failed because of insufficient cells ($n = 3$) or failed ddPCR droplet formation ($n = 1$). Of the remaining two infants, one on the infant nevirapine arm had detectable DNA at 12 weeks (12.5 copies per million cells) and the other on the maternal antiretroviral arm did not. Including these two infants, the median delay in detection between the ultrasensitive and standard assays was 22.0 weeks on the infant nevirapine arm compared with 15.4 weeks on the maternal arm. Because of only having six infants to compare, the median delay in detection did not differ significantly by antiretroviral arm ($P = 0.1$).

There were no significant differences between the antiretroviral arms in adherence to the antiretroviral intervention or maternal viral load. All three mothers on the nevirapine arm reported complete adherence to the nevirapine regimen at all their reporting time points from 1 to 21 weeks of age, and all four mothers on the maternal antiretroviral arm reported complete adherence to the HAART regimen at their reporting time points at 21 and 28 weeks postpartum. There was no significant difference between the three study arms in median maternal log viral load at 24 weeks: 5.2 copies/ml on nevirapine arm, 3.7 copies/ml on the maternal antiretroviral arm and 5.1 copies/ml on the control arm ($P = 0.76$).

The infants and their mothers were evaluated for antiretroviral resistance mutations in HIV extracted from specimens collected at 48 weeks postpartum. Only one infant who was on the nevirapine arm had a resistance mutation, and it was the same as the mother's (K103N), suggesting that the mutated virus was transmitted to the infant (Table 2). For the one infant on the control arm with detectable HIV at 24 weeks, we were unable to sufficiently amplify virus from dried blood spot specimens at 48 weeks to test for resistance mutations.

Discussion

By using an ultrasensitive assay, extremely low HIV DNA concentrations were detected in six of nine (66%) infants up to 31 weeks earlier than HIV detection by standard HIV testing. The delay between HIV detection on the ultrasensitive and standard assays was longest for

infants exposed to 28 weeks of infant nevirapine (median of 22 weeks, including the 12-week positive result), of intermediate duration for infants on the maternal antiretroviral arm (15 weeks) and shortest for infants on the control arm (9 weeks). By using standard assays, other studies among nonbreastfed infants have reported shorter delays in HIV detection with use of 4–6 weeks of perinatal antiretroviral prophylaxis [12,13]. In one study, timing of HIV detectability in nonbreastfed infants was dependent on both the infant's and mother's receipt and duration of zidovudine prophylaxis [12]. Mothers with longer duration of antenatal zidovudine (7.5 weeks or more) were 2.7 times as likely to have an infant with an HIV infection only detectable after birth as at birth. If the mother received a shorter course of antenatal zidovudine, then infant zidovudine prophylaxis of 4 weeks or more was associated with HIV DNA detection at a median of 43 days postpartum compared with 11 days for infants on a short regimen of 3 days or less. In a pooled analysis of nonbreastfed HIV-infected infants from several cohorts, the delay in HIV DNA positivity increased with potency of perinatal antiretrovirals [13]. The cumulative probabilities of the perinatally infected infants not being HIV DNA positive by 42 days were 6% for infants not on antiretrovirals, 9% for a single nucleoside reverse transcriptase inhibitor, 4% for single-dose nevirapine with and without zidovudine, and 21% for infants on three or more antiretrovirals [13]. In comparison, by 6 weeks from reported breastfeeding cessation in our study, all three infants on the nevirapine arm and three of four infants on the maternal antiretroviral arm were still HIV negative by the standard HIV assays. These findings suggest that the delay in HIV detection with prolonged antiretroviral prophylaxis during breastfeeding is longer than with perinatal prophylaxis.

The WHO recommends virologic testing of all HIV-exposed breastfeeding infants at 6 weeks or more after breastfeeding cessation [11]. Testing at 6 weeks only would have failed to capture seven of the nine infants (78%) whose HIV infection was detected more than 6 weeks after reported breastfeeding cessation by standard assays. Ultrasensitive testing or repeat virologic testing more than 6 weeks after breastfeeding and antiretroviral cessation may be warranted. Given the benefit of early diagnosis and treatment of infant HIV infection [7–9], some investigators have advocated for the use of combination ART for newborns of mothers with inadequately controlled HIV replication at delivery [9]. Although this approach may be ideal for infants who are HIV-infected, its potential for prolonged inability to detect HIV DNA with standard assays may complicate determination of infant HIV status and necessitate the use of ultrasensitive testing protocols or testing on larger blood volumes [24] before halting such prophylaxis. The current cost of the ultrasensitive assay and the fact that it is performed only in specialized laboratories restrict its clinical utility. However, as the ultrasensitive assay becomes more automated, we expect that it will become more widely used and clinically validated for diagnosis of HIV infection.

Our findings demonstrate much earlier HIV acquisition for many infants than that detected by standard testing. The fact that the delay in detecting HIV is longer when the infant received prophylaxis (either maternal or infant) suggests that the use of antiretrovirals may restrict HIV seeding of reservoirs and reduce reservoir size. Particular properties of antiretroviral drugs (such as half life and pharmacokinetic properties such as passage into breast-milk) may have different effects on the extent of such delay. The currently recommended by WHO first-line antiretroviral regimen during breastfeeding includes

efavirenz [14], whose pharmacokinetic properties differ from those of a protease inhibitor, used in our study. It is unknown whether the delays in infant HIV diagnosis may be even more prolonged with the currently recommended regimen.

The lack of de-novo development of nevirapine resistance in all three infants on the nevirapine arm who had HIV detected while continuing NVP prophylaxis for 4–17 weeks suggests limited active viral replication and possibly HIV 'latency'. Together with the reports of viral rebound among adults and infants, even after prolonged viral suppression after antiretroviral discontinuation (e.g. the recent Mississippi infant) [25,26], these data indicate that HIV infection can remain latent for months and activate with the appropriate stimuli. Of interest, one infant on the control arm not receiving any antiretrovirals for prophylaxis had HIV detected 9 weeks before detection on the standard assay. Host and viral factors may contribute to the outcomes among different individuals. Our findings concur with those of other researchers who have shown that early initiation of ART restricts, but does not eliminate, latent infection of resting $CD4^+$ T cells [5,27].

Another implication of our findings relates to the accuracy of reporting breastfeeding cessation by the mothers. Although in the absence of a biologic marker of breastfeeding, infant HIV infections acquired past the time of reported breastfeeding cessation might be attributed to continued unreported breastfeeding, our findings of earlier HIV detection during known breastfeeding challenge this assumption.

The primary limitation of this analysis is the small number of infants infected after 28 weeks with available cryopreserved PBMC at appropriate time points for testing with the ultrasensitive assay. Another limitation is blood volume restrictions among young infants, which limits the number of cells for our virus assay. This precluded amplification and sequencing of the virus to verify genetic linkage between the virus detected on ultrasensitive assay and that detected by standard BAN testing. Despite this, it seems unlikely that contamination occurred because all assays were performed in a CLIA-compliant laboratory, and the detection or absence of HIV DNA was consistent across repeat ultrasensitive assays on separate PBMC samples, when available (n = 6 infants). Another limitation to the interpretation of our study findings is that the estimates of delayed detection are interval censored because of HIV testing at study visits scheduled 4–6 weeks apart. Despite this, only one of the infants (on the infant nevirapine arm) was missing a single interim HIV assay between their last negative and first positive BAN assay. Furthermore, the study HIV tests were performed on whole blood only at 2, 12, 28 and 48 weeks, with HIV testing at the remaining interim visits performed on DBS. However, the frequent occurrence of first HIV detection on DBS (seven out of nine infants) and the short time frame between the detection of some infant HIV infections on DBS and last HIV-negative test on whole blood (4 weeks for those detected at 32 weeks) suggests that there was not much loss in HIV sensitivity of the assay on the basis of the type of specimen tested.

In conclusion, in the context of postnatal antiretroviral prophylaxis, the prolonged inability to detect HIV DNA on standard assays suggests that antiretroviral prophylaxis may keep HIV infection 'latent', in some cases for months, but not necessarily prevent its establishment. In addition, this conclusion may apply to other categories of preexposure

prophylaxis, beyond infancy. Our findings also suggest that repeated virologic testing beyond the WHO-recommended earliest point for HIV testing at 6 weeks after breastfeeding cessation is warranted to detect HIV infections in infants. Although the benefit of extending the duration of antiretroviral prophylaxis longer past cessation of HIV exposure, or intensifying antiretroviral prophylaxis during exposure, may need to be tested for their potential to augment prevention of transmission, our data suggest that antiretroviral approaches may not be enough to achieve elimination of transmission. Other novel strategies targeting the rare cellular reservoirs or boosting the immune system's response may be necessary to achieve this goal. Ultrasensitive testing may allow for earlier antiretroviral treatment, which might modify establishment of HIV reservoirs, and its role in future testing strategies will need to be evaluated with advances in its automation and performance [5].

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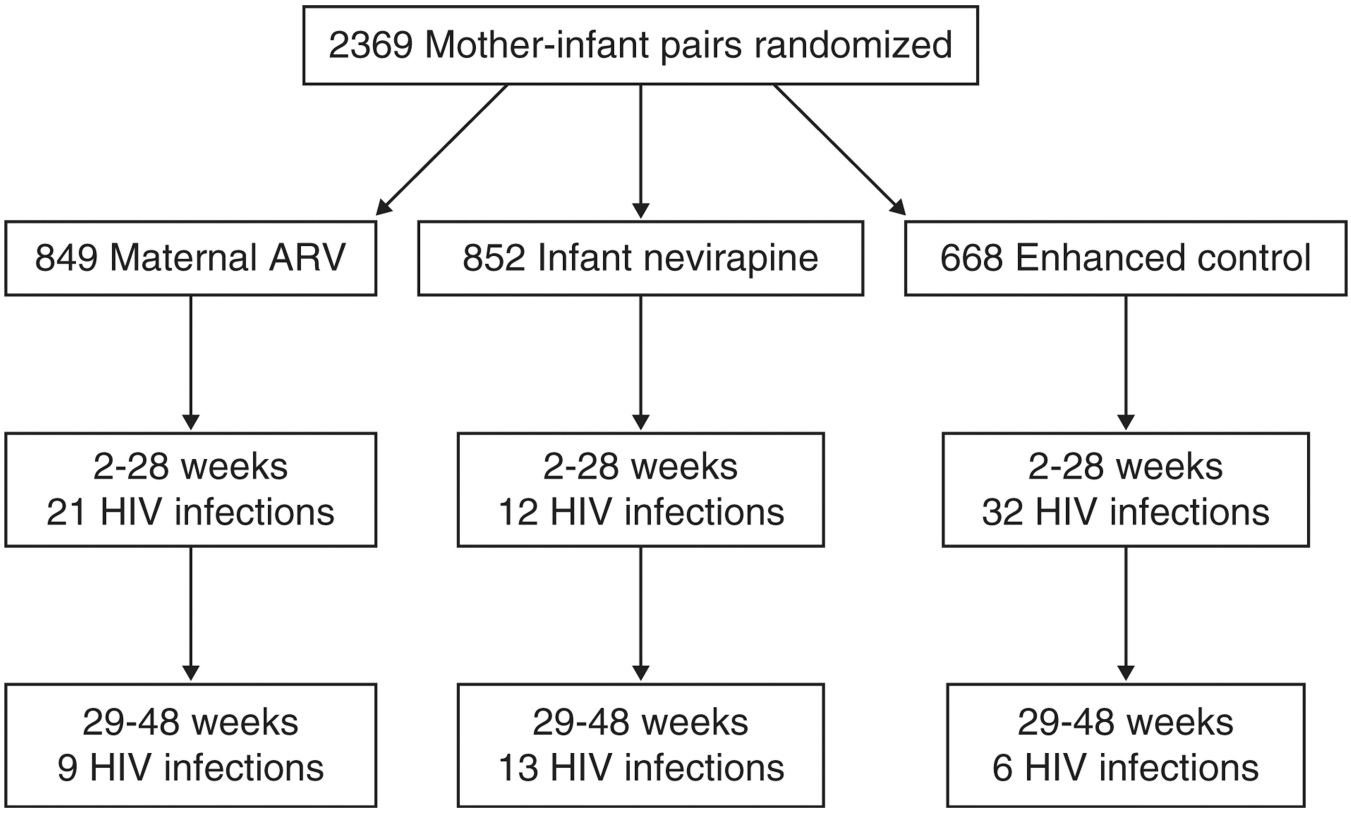

Fig. 1. Antiretroviral interventionsa, HIV testing time pointsb and infant HIV infection outcomes in the Breastfeeding, Antiretrovirals and Nutrition Study, Malawi 2004–2010

[a]Mother–infant pairs were randomized to 3 antiretroviral intervention arms: Maternal ARV [zidovudine, lamivudine and either nevirapine (*n* = 39), nelfinavir (*n* = 146) or lopinavir-ritonavir (*n* = 664)]; infant nevirapine (daily infant nevirapine with dosage increasing with age); and enhanced control (mothers and infants received only the 7-day intrapartum regimen provided to all BAN participants). [b]The BAN Study protocol called for infant HIV testing on whole blood collected at birth, 2, 12, 28 and 48 weeks. The window of seroconversion was later narrowed by testing dried blood spots from all interim visits shown. Infants infected by 2 weeks of age were excluded from counts of HIV infections to minimize counting of in-utero/peripartum infections.

Table 1

Cumulative risk of HIV infection among infants alive and HIV-negative at 2 weeks by antiretroviral intervention arm in the Breastfeeding, Antiretrovirals and Nutrition Study.

	Maternal triple-drug prophylaxis		Infant nevirapine		Control		*P* maternal	*P* infant
Infant age (weeks)	Number of HIV infections	Probability of infection (%) (95% CI)	Number of HIV infections	Probability of infection (%) (95% CI)	Number of HIV infections	Probability of infection (%) (95% CI)	ARV vs. control[a]	nevirapine vs. control
28	21	2.9% (1.7–4.1)	12	1.7% (0.7–2.6)	32	5.7% (3.8–7.6)	0.02	<0.001
32	24	3.3% (2.0–4.6)	14	1.9% (0.9–2.9)	33	5.9% (3.9–7.8)	0.03	<0.001
36	26	3.6% (2.3–5.0)	21	3.0% (1.7–4.2)	35	6.3% (4.3–8.3)	0.03	0.006
42	27	3.8% (2.4–5.2)	23	3.3% (2.0–4.6)	38	6.9% (4.8–9.1)	0.02	0.004
48	30	4.3% (2.8–5.8)	25	3.6% (2.2–5 0.0)	38	6.9% (4.8–9.1)	0.05	0.01

Cumulative risk of HIV estimated on the basis of extension of the Kaplan–Meier estimator to allow for competing risks. CI, confidence interval.

[a]Two-sided *P* value for *Z* statistic of the difference in risk at given time point; all *P* values comparing maternal ARV vs. infant nevirapine were higher than 0.05 (not shown).

Table 2

Ultrasensitive HIV DNA assay results of 9 infants who had reported breastfeeding cessation by 28 weeks and first tested HIV-positive by sensitive assay after stopping the postnatal antiretroviral intervention in the Breastfeeding, Antiretrovirals and Nutrition Study.

ID	Arm	Age last breast- fed (days)	BAN HIV-negative results starting at 12-week visit[a] (age in days)	BAN first HIV-positive Result[a] (age in days)	Detectable vs. undetectable HIV DNA on ultrasensitive assay of PBMC collected at 12-week or 24-week study visits (for detectable HIV DNA, pol copy per 10^6 PBMCs)		Delay in detection (days)	Resistance testing of mother and infant (48-week visit)	Maternal log VL at 24 weeks (copies/ml)
					12-week visit	24-week visit			
1	Infant NVP	203	84, 203, 232, 260, 263	302	Detectable at 84 days 12.5 copies	Detectable at 179 days 6.1 copies	218	No mutations	3.88
2	Infant NVP	196	85, 196, 254	321	Insufficient cell number[b]	Detectable at 167 days 4.1 copies 5.1 copies	154	K103N[c]	5.24
3	Infant NVP	199	85, 199, 227, 255	299	Droplet formation failure[d]	Detectable at 171 days 20.1 copies	128	No mutations	5.22
4	Maternal cARV	196	84, 196, 224, 252, 293	321	Not tested[e]	Undetectable at 167 days	–	No mutations	Not available
5	Maternal cARV	204	85, 204, 262	303	Insufficient cell number[b]	Detectable at 176 days 23.5 copies 15.4 copies	127	No mutations	3.42
6	Maternal cARV	197	84, 197	230	Not tested[e]	Undetectable at 169 days	–	No mutations	5.39
7	Maternal cARV	168	84, 196, 224	257	Undetectable at 84 days	Detectable at 168 days 2.1 copies 7.5 copies	89	No mutations	3.79
8	Control	202	90, 202	240	Insufficient cell number[b]	Detectable at 174 days 20.0 copies 32.7 copies	66	Not tested[f]	4.88
9	Control	172	88, 196, 225, 256	293	Not tested[e]	Undetectable at 168 days	–	No mutations	5.21

Control, control arm receiving no extended antiretrovirals to either the mother or infant; infant NVP, infant nevirapine arm; maternal cARV, maternal combination antiretrovirals.

[a] Infant HIV testing during the BAN Study follow-up was done on whole blood collected at birth, 2, 12, 28 and 48 weeks with a qualitative Roche Amplicor 1.5 DNA PCR assay (Roche Molecular Systems, Pleasanton, California, USA). For positive infants, the window of HIV transmission was later narrowed by testing dried blood-spot specimens from interim visits for HIV DNA by Roche DNA assay or RNA using Gen-Probe Aptima HIV-1 assay.

[b] Insufficient cells in sample to complete ultrasensitive assay.

[c] Mutation seen in mother and infant.

[d] Failed PCR droplet formation so unable to complete ultrasensitive assay.

[e] Infants were not tested at 12 weeks if HIV DNA was undetectable on the ultrasensitive assay at 24 weeks.

[f] Unable to sequence virus from infant due to failed amplification from dried blood spot specimens.