MAJOR ARTICLE

Late Postnatal Transmission of HIV-1 in Breast-Fed Children: An Individual Patient Data Meta-Analysis

The Breastfeeding and HIV International Transmission Study Group[a]

(See the editorial commentary by Bulterys et al., on pages 2149–53.)

Background. We analyzed individual patient data to determine the contribution of late postnatal transmission to the overall risk of mother-to-child transmission of human immunodeficiency virus type 1 (HIV-1) and the timing and determinants of late postnatal transmission.

Methods. Eligible trials were conducted where breast-feeding was common; included ⩾2 HIV-1 tests by 3 months, and, if follow-up continued, ⩾2 tests at 3–12 months; and regularly assessed infant-feeding modality. Data on children born before January 2000 were analyzed.

Results. Of 4085 children from 9 trials (breast-fed singletons for whom HIV-1 testing was performed), 993 (24%) were definitively infected (placebo arms, 25.9%; treatment arms, 23.4%; $P = .08$). Of 539 children with known timing of infection, 225 (42%) had late postnatal transmission. Late postnatal transmission occurred throughout breast-feeding. The estimated hazard function for time to late postnatal transmission was roughly constant. The cumulative probability of late postnatal transmission at 18 months was 9.3%. The overall risk of late postnatal transmission was 8.9 transmissions/100 child-years of breast-feeding and was significantly higher with lower maternal $CD4^+$ cell counts and male sex.

Conclusions. Late postnatal transmission contributes substantially to overall mother-to-child transmission of HIV-1. The risk of late postnatal transmission is generally constant throughout breast-feeding, and late postnatal transmission is associated with a lower maternal $CD4^+$ cell count and male sex. Biological and cultural mechanisms underlying the association between sex and late postnatal transmission should be further investigated. Interventions to decrease transmission of HIV-1 through breast-feeding are urgently needed.

Mother-to-child transmission of HIV-1 can occur in utero, during delivery, and postnatally through breast-feeding [1]. In settings where breast-feeding is the norm, a significant proportion of mother-to-child transmission of HIV-1 occurs through breast-feeding [2]. However, more information is needed with regard to the risk and timing of transmission through breast-feeding and potential risk factors for such transmission.

To inform the development of appropriate interventions to prevent transmission through breast-feeding in areas of the world where complete avoidance of breast-feeding is not, generally, feasible, we conducted an individual patient data meta-analysis of transmission of HIV-1 through breast-feeding. The objectives were the following: to estimate the contribution of late postnatal transmission of HIV-1 to the overall risk of mother-to-child transmission of HIV-1 and to characterize the timing and determinants of late postnatal transmission.

SUBJECTS AND METHODS

Participating trials. Eligibility criteria for randomized, placebo-controlled trials of HIV-1–infected women and their children were the following: trial was

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[a] The persons and trials participating in the Breastfeeding and HIV International Transmission Study are listed after the text.

completed (or ongoing, if enrollment was completed), was conducted among populations in which breast-feeding was common, and scheduled regular assessments of children's feeding modality and ⩾2 HIV-1 diagnostic tests by 3 months of age (and, if follow-up continued, 2 additional tests between 3 and 12 months of age). Trials were identified by computerized searches of the medical literature in English, by use of Medline, and through discussions with experts. Inclusion criteria for the study population, as well as selection and coding of meta-analysis variables, were agreed on during a meeting of representatives of eligible trials.

Definitions and variables. Uniform definitions (HIV-1 infection status of children, timing of transmission, and duration of breast-feeding) were applied across all trials (see appendix). Other variables, selected on the basis of the anticipated availability and comparability of data among trials, were transmitted to the data management center (see appendix).

Study population. Data on all mothers and children enrolled in the trials were transferred to the data management center, except for data on the following: mothers who were lost to follow-up or who died before delivery, mothers whose pregnancy did not end in a live birth, and children not born by 1 January 2000. The meta-analysis incorporated data obtained at scheduled study visits on breast-fed singletons for whom HIV-1 diagnostic testing was performed. Detailed analyses of late postnatal transmission included breast-fed children with negative HIV-1 assays at 4 weeks of age, some of whom eventually acquired HIV-1 infection through late postnatal transmission.

Statistical analysis. Univariate techniques were used to describe the study population and to quantify the contribution of late postnatal transmission to the overall rate of mother-to-child transmission. Survival or time-to-event analyses were used to examine the relationship of breast-feeding to late postnatal transmission. Children were considered to be at risk for acquisition of HIV-1 infection only as long as they continued breast-feeding [3]. Definitions of the period of risk for late postnatal transmission, for both HIV-1–infected and HIV-1–uninfected children, are shown in the appendix.

The Peto-Turnbull method [4–5], as implemented by use of SAS [6–7], was used to estimate the probabilities that time to late postnatal transmission will exceed specified times, such as 3 or 6 months. The Cox proportional hazards model, used to assess the effect of covariates on time to event, yields hazard ratios comparing the risk of late postnatal transmission in 1 group to the risk in another. Since this model does not allow for interval-censoring of the response variable, a simulation approach similar to but less elaborate than the method developed by Pan [8] was used to correct for imprecision in the P values (resulting from imprecise knowledge of the event times) through multiple imputation [9–10]. Homogeneity of the relationship of breast-feeding to late postnatal transmission among the participating clinical trials and among treatment arms within trials was assessed by use of models including interactions tested by a multiple-*df* Wald test, corrected for imputation.

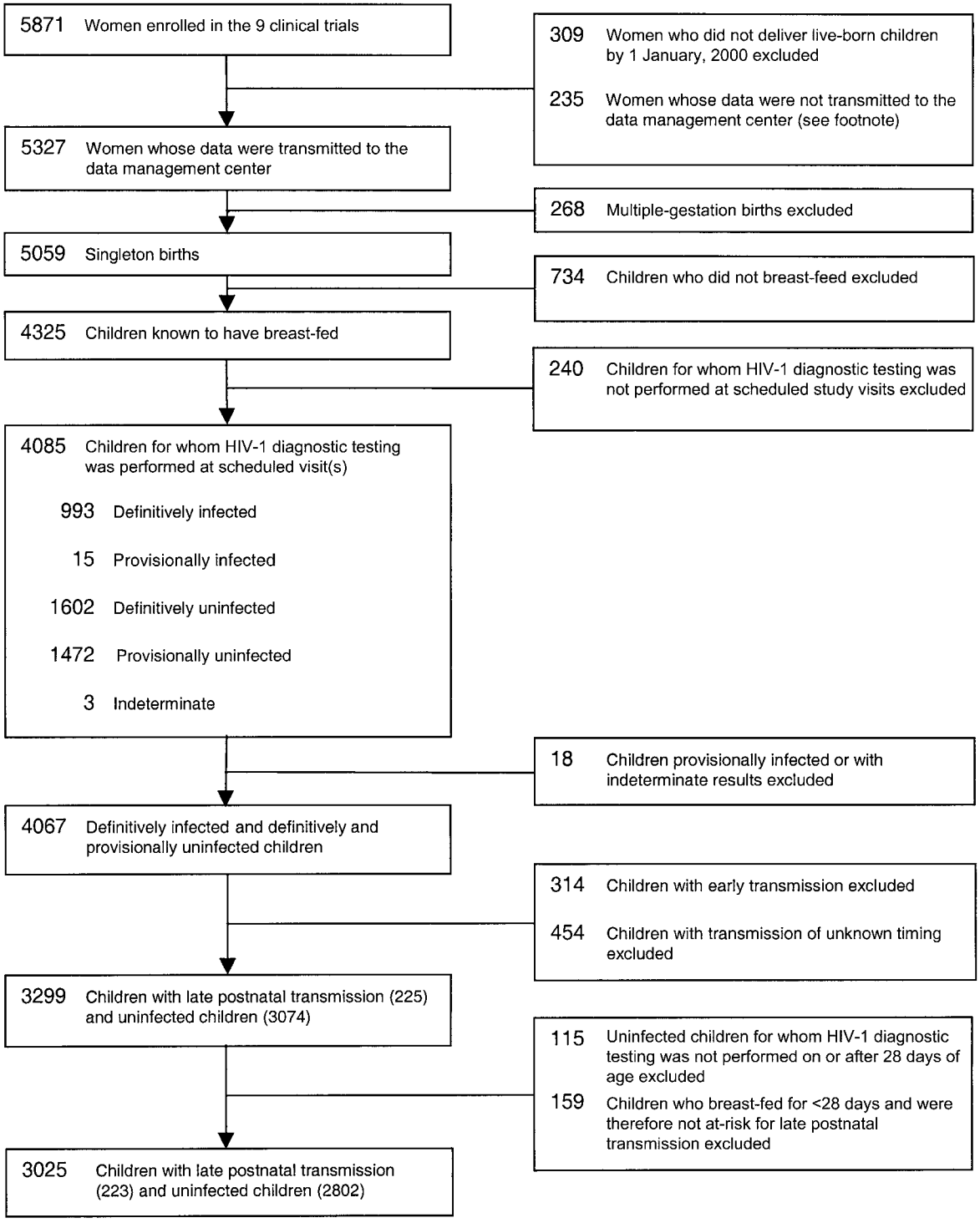
The survival distribution function is included in the article as the primary means for displaying the time-to-event data. Starting at 100% at time zero, the plot shows the probability that the event, late postnatal transmission, has not yet occurred at each subsequent time point. Since the survival function can obscure important features of the exposure-outcome relationship, the estimated hazard function also was examined and is described in the text. The SAS SMOOTH macro, which implements a kernel smoother, was used to generate the hazard function [11]. The detailed shape of the estimated hazard function depends on a smoothing parameter that controls how much data on either side, before and after the time of a late postnatal transmission event, is used in estimating the hazard (the slope of the survival curve on the log scale) at that time point. Smoothing over too narrow an interval yields noisy hazard estimates that track every variation in the data.

Sensitivity analyses were conducted to assess the stability of the final model. These analyses assessed the effect of including provisionally HIV-1–infected children in the analysis and the effect of possible informative censoring (resulting from children's deaths occurring before determination of HIV-1 infection status), on the basis of the approach of Allison [11].

RESULTS

Participating trials. Representatives of 9 of 10 eligible trials agreed to participate in the meta-analysis. Details regarding the study designs, data collection, and study populations of the participating trials have been reported elsewhere [2, 12–19]. All protocols were approved by the respective institutional review boards, and all subjects provided informed consent for participation in the respective trials. Only preliminary data from the remaining trial, a trial of vitamin A supplementation in Zimbabwe, have been presented [20].

Study population. Of 5871 women enrolled in the clinical trials, data on 5327 women and their children were transferred to the data management center (figure 1). Of these children, 4085 singletons were breast-fed and had HIV-1 testing performed. Of these 4085 children, 993 (24%) were definitively infected (99% of the infected children were identified by use of HIV-1 polymerase chain reaction [PCR] assays); similar proportions of children enrolled in placebo or treatment arms were definitively infected (379/1461 [25.9%] and 614/2624 [23.4%], respectively; $P = .08$). Early acquisition of infection occurred in 314 children (32%) (122 had a positive HIV-1 result at birth), and 225 children (23%) had late postnatal transmission. The timing of transmission was unknown for 454 children (46%)



The PETRA trial did not transmit data for 235 mother-child pairs who were enrolled into the intervention arms of the trial after the placebo arm was discontinued in February 1998.

Figure 1. Flow diagram of derivation of population

(343 without a prior negative HIV-1 assay and 111 with a prior negative HIV-1 assay before 28 days of age). By assuming that the timing of transmission among these 454 children was similar to that for children with known timing of infection, it can be theorized that 58% of definitively infected children acquired infection early and 42% acquired infection through late postnatal transmission. The population evaluated in detailed analyses of late postnatal transmission included 3025 children, all with negative HIV-1 results at 4 weeks of age and were breast-fed through at least 28 days of age; 223 had late postnatal transmission, and 2802 remained uninfected.

Late postnatal transmission. Characteristics of these 3025 children and their mothers are described in table 1. The duration of breast-feeding ranged from 0.9 to 37.0 months (median, 10.0 months; interquartile range, 4.7–17.1 months). The length of follow-up and further information regarding the duration of breast-feeding are shown in the table.

The overall estimated risk of late postnatal transmission was 8.9 transmissions/100 child-years of breast-feeding (95% confidence interval [CI], 7.8–10.2 transmissions/100 child-years of breast-feeding). Cases of late postnatal transmission continued to occur throughout the duration of breast-feeding, with the cumulative probability of late postnatal transmission at 18 months being 9.3% (figure 2*A*).

Because of the relatively small number of late postnatal transmission cases by trial and by treatment arm, trials were grouped according to geographic region (see appendix), with South Africa being the reference group for this analysis. Since the time enrolled in the study, for the proportional hazards analysis, was determined on the basis of the period of risk for late postnatal transmission through breast-feeding (see definitions of "Period of risk for late postnatal transmission" in the appendix), breast-feeding was not included as an independent variable in the model. Interactions of trials (grouped according to geographic region) with treatment arm were not statistically significant ($P = .88$). The main effect of treatment did not contribute significantly to the model ($P = .08$), nor did trials (grouped geographically) ($P = .36$). The model-based plot of late postnatal transmission–free survival (not shown) was very similar in appearance to the Peto-Turnbull plot (figure 2*A*). Therefore, with breast-feeding represented by the time enrolled in the study, the model including the main effects of the trials served as the basis for assessing the contribution of covariates.

Determinants of late postnatal transmission. A number of covariates potentially affecting the relationship between breast-feeding and late postnatal transmission of HIV-1 were evaluated, including both maternal variables (age, parity, and $CD4^+$ cell count) and child variables (birth weight and sex). Since $CD4^+$ cell counts can vary considerably over time, only data on $CD4^+$ cell counts obtained close to delivery (no more than 3 months before or 1 month after delivery) were analyzed (2030 mothers). The following covariate analyses were based on this subset of the study population.

For each covariate, a model was fit to the data that included grouped trials and the single covariate (data not shown). Neither maternal age, parity, nor birth weight made significant contributions to the model. The final model fit to the data included trials grouped geographically, maternal $CD4^+$ cell counts, and child's sex. Maternal $CD4^+$ cell counts and child's sex were found to make significant independent contributions to the model, but trials did not; the trial indicator variables were, nevertheless, retained in the model, since this is a meta-analysis of pooled data. There was no significant interaction of sex and maternal $CD4^+$ cell count ($P = .38$).

To explore the sex effect further, we examined the timing of transmission of HIV by sex, to attempt to assess whether more girls than boys had early transmission of HIV-1, possibly reducing the pool of girls susceptible to late postnatal transmission. There was no statistically significant difference between the proportions of girls and boys with early transmission of HIV-1 ($P = .24$).

The estimated hazard ratios and 95% CIs for the final model, including trials, maternal $CD4^+$ cell counts, and child's sex are shown in table 2. Similar results were obtained when this model was fitted to the data on all individuals for whom data on $CD4^+$ cell counts were available, regardless of when the measure was obtained relative to the timing of delivery (data not shown).

A Peto-Turnbull plot (figure 2*B*), illustrating the independent main effects of maternal $CD4^+$ cell count and child's sex, shows that late postnatal transmission–free survival declined most rapidly for boys breast-fed by mothers with $CD4^+$ cell counts <200 cells/mm^3, followed by boys breast-fed by mothers with $CD4^+$ cell counts of 200–499 cells/mm^3 and, then, girls breast-fed by mothers with $CD4^+$ cell counts <200 cells/mm^3. The pattern of late postnatal transmission–free survival, by group, observed in the model-based plot (not shown) was very similar to that of the Peto-Turnbull plot (figure 2*B*), suggesting that the proportional hazards model with random assignment of timing of late postnatal transmission provided a good fit to the data.

The distributions of duration of breast-feeding by sex and by maternal $CD4^+$ cell count were examined (data not shown). Duration of breast-feeding was similar among boys (median, 10.0 months) and girls (median, 9.7 months) ($P = .5$). When duration of breast-feeding was evaluated according to maternal $CD4^+$ cell count around the time of delivery (categorized as <200, 200–499, and ≥ 500 cells/mm^3), modest but highly significant differences in duration of breast-feeding were noted (median, 8.8, 9.2, and 11.3 months, respectively) ($P < .0001$). Mothers with lower $CD4^+$ cell counts breast-fed for shorter durations.

The estimated hazard function for time to late postnatal transmission was roughly constant (plot not shown). In other words,

Table 1. Characteristics of the study population, overall and by trial ($N = 3025$).

Characteristic	Trial									Overall
	MB	CHL	ANRSA	ANRSB	RETRO-CI	HIVNET	VITA	PETRA	MICRO	
Children										
Sample size	130	321	285	74	197	484	251	705	578	3025
Planned length of follow-up, months	24	3.5	18	18	24	18	24	18	24	
Observed length of follow-up, mean (median), months	17.1 (18.6)	2.7 (3.2)	15.3 (17.9)	15.2 (17.0)	18.9 (24.0)	16.8 (18.0)	12.3 (14.5)	15.9 (18.0)	19.9 (24.1)	15.3 (18.0)
Observed duration of breast-feeding, mean (median), months	14.8 (15.0)	2.7 (3.2)	11.8 (9.7)	12.5 (11.5)	14.7 (15.1)	9.3 (8.3)	7.3 (6.9)	9.8 (9.4)	17.1 (18.8)	10.9 (10.0)
LPT status, no. (%)										
Infected	13 (10.0)	3 (0.9)	17 (6.0)	6 (8.1)	16 (8.1)	20 (4.1)	12 (4.8)	73 (10.4)	63 (10.9)	223 (7.4)
Uninfected	117 (90.0)	318 (99.1)	268 (94.0)	68 (91.9)	181 (91.9)	464 (95.9)	239 (95.2)	632 (89.6)	515 (89.1)	2802 (92.6)
Gestational age, weeks										
Mean (median)	39.9 (40.0)	38.6 (39.0)	38.9 (39.0)	39.2 (39.0)	39.6 (40.0)	39.4 (39.0)	39.5 (40.0)	39.6 (39.0)	38.5 (39.0)	39.2 (39.0)
No. of subjects for whom data were not available	49	38	37	4	0	0	18	6	0	152
Birth weight, g										
Mean (median)	3197.5 (3200.0)	3006.0 (3000.0)	2925.9 (2920.0)	2930.4 (2890.0)	3016.8 (3000.0)	3154.4 (3150.0)	3146.1 (3080.0)	3130.2 (3145.0)	3064.0 (3000.0)	3080.9 (3050.0)
No. of subjects for whom data were not available	11	11	3	4	3	12	3	13	53	113
Sex, no. (%)										
Female	59 (47.2)	160 (49.8)	133 (46.7)	29 (39.7)	96 (48.7)	236 (48.8)	130 (53.1)	337 (47.9)	264 (45.7)	1444 (48.0)
Male	66 (52.8)	161 (50.2)	152 (53.3)	44 (60.3)	101 (51.3)	248 (51.2)	115 (46.9)	366 (52.1)	314 (54.3)	1567 (52.0)
No. of subjects for whom data were not available	5	0	0	1	0	0	6	2	0	14
Oral candidiasis reported at any time, no. (%)										
Yes	29 (22.3)	21 (6.5)	44 (15.4)	9 (12.3)	33 (16.8)	84 (17.4)	31 (12.4)	118 (16.7)	167 (28.98)	536 (17.7)
No	101 (77.7)	300 (93.5)	241 (84.6)	64 (87.7)	164 (83.2)	400 (82.6)	220 (87.6)	587 (83.3)	411 (71.1)	2488 (82.3)
No. of subjects for whom data were not available	0	0	0	1	0	0	0	0	0	1
Vital status,[a] no. (%)										
Alive at last known follow-up	119 (91.5)	317 (98.8)	269 (94.4)	67 (90.5)	189 (95.9)	465 (96.1)	247 (98.4)	649 (92.1)	496 (85.8)	2818 (93.2)
Died while enrolled in trial/during follow-up	11 (8.5)	4 (1.2)	16 (5.6)	7 (9.5)	8 (4.1)	19 (3.9)	4 (1.6)	56 (7.9)	82 (14.2)	207 (6.8)
No. of subjects for whom data were not available	0	0	0	0	0	0	0	0	0	0
Mothers										
Sample size	130	321	285	74	197	484	251	705	578	3025
Age, years										
Mean (median)	24.2 (24.0)	24.2 (23.0)	25.0 (24.0)	24.7 (23.0)	26.6 (26.0)	24.4 (24.0)	26.0 (25.0)	26.4 (26.0)	24.9 (24.0)	25.3 (25.0)
No. of subjects for whom data were not available	0	0	0	0	0	2	0	4	0	6
Education, no. (%)										
No formal education	4 (3.1)	11 (3.4)	136 (47.7)	35 (47.3)	85 (43.2)	20 (4.2)	8 (3.2)	38 (5.4)	41 (7.1)	378 (12.6)
Primary school	60 (46.2)	184 (57.7)	95 (33.3)	24 (32.4)	81 (41.1)	290 (60.5)	47 (19.0)	354 (50.6)	475 (82.2)	1610 (53.5)
Some secondary school	40 (30.8)	45 (14.1)	51 (17.9)	14 (18.9)	29 (14.7)	0	121 (48.8)	140 (20.0)	61 (10.6)	501 (16.6)
Complete secondary school	19 (14.6)	79 (24.8)	2 (0.7)	1 (1.4)	0	165 (34.5)	55 (22.2)	70 (10.0)	0	391 (13.0)
Postsecondary school	7 (5.4)	0	1 (0.4)	0	2 (1.0)	4 (0.8)	17 (6.9)	98 (14.0)	1 (0.2)	130 (4.3)
No. of subjects for whom data were not available	0	2	0	0	0	5	3	5	0	15

Gravidity										
Mean (median)	2.4 (2.0)	2.4 (2.0)	3.2 (3.0)	3.8 (3.0)	3.7 (3.0)	3.2 (3.0)	2.3 (2.0)	4.2 (4.0)	2.9 (2.0)	3.2 (3.0)
No. of subjects for whom data were not available	0	0	0	41	0	0	0	4	11	56
Parity										
Mean (median)	2.2 (2.0)	2.2 (2.0)	2.8 (2.0)	2.9 (2.0)	3.5 (3.0)	NA	1.2 (1.0)	3.0 (3.0)	2.5 (2.0)	2.6 (2.0)
No. of subjects for whom data were not available	0	0	0	0	0	484	0	4	11	499
$CD4^+$ cell count, cells/mL										
Mean (median)	437.3 (407.5)	NA	602.7 (584.5)	637.5 (619.0)	603.4 (575.0)	503.1 (478.0)	493.0 (465.0)	510.8 (470.0)	440.0 (420.0)	510.0 (475.0)
No. of subjects for whom data were not available	8	321	5	1	0	2	26	5	30	398
When $CD4^+$ cell count was obtained, no. (%)										
>3 months before delivery	2 (1.6)		0	0	0	0	63 (26.7)	1 (0.1)	500 (90.7)	566 (21.4)
Between 3 months before delivery and delivery	118 (96.7)	NA	280 (100)	71 (97.33)	192 (97.5)	482 (100)	168 (71.2)	680 (96.5)	51 (9.3)	2042 (77.2)
Between delivery and 1 month after delivery	2 (1.6)	NA	0	2 (2.7)	5 (2.5)	0	1 (0.4)	2 (0.3)	0	12 (0.5)
>1 month after delivery	0		0	0	0	0	4 (1.7)	22 (3.1)	0	26 (1.0)
No. of subjects for whom data were not available	8	321	5	1	0	2	15	0	27	379
DROM, h										
Mean (median)	4.0 (1.0)	2.3 (1.0)	4.2 (0.8)	4.2 (1.0)	5.8 (1.2)	2.7 (0.3)	6.0 (2.0)	4.7 (2.1)	4.8 (1.5)	4.2 (1.1)
No. of subjects for whom data were not available	3	14	34	13	1	22	75	92	83	337
Mode of delivery, no. (%)										
C-S, before labor and before delivery	0	0	3 (1.1)	0	0	15 (3.3)	0	62 (8.9)	0	80 (2.7)
C-S, other	12 (9.3)	0	6 (2.1)	1 (1.5)	3 (1.5)	43 (9.3)	67 (26.9)	137 (19.6)	29 (5.1)	298 (10.0)
Vaginal delivery	117 (90.7)	321 (100)	273 (96.8)	67 (98.5)	194 (98.5)	403 (87.4)	182 (73.1)	499 (71.5)	542 (94.9)	2598 (87.3)
No. of subjects for whom data were not available	1	0	3	6	0	23	2	7	7	49
Tuberculosis diagnosis, no. (%)										
Yes	8 (6.2)	NA	2 (1.6)	2 (2.7)	1 (0.5)	13 (100)	3 (1.2)	16 (2.3)	NA	45 (3.0)
No	122 (93.8)	NA	123 (98.4)	72 (97.3)	196 (99.5)	0	248 (98.8)	689 (97.7)	NA	1450 (97.0)
No. of subjects for whom data were not available	0	321	160	0	0	471	0	0	578	1530
Mastitis reported at any time, no. (%)										
Yes	11 (8.5)	0	1 (0.4)	0	0	4 (100)	1 (3.7)	11 (1.6)	0	28 (2.3)
No	118 (91.5)	0	284 (99.67)	73 (100)	0	0	26 (96.3)	680 (98.4)	0	1181 (97.7)
No. of subjects for whom data were not available	1	321	0	1	197	480	224	14	578	1816
Breast abscess reported at any time, no. (%)										
Yes	8 (6.2)	3 (1.0)	6 (2.1)	0	0	2 (100)	3 (11.1)	0	16 (2.8)	38 (2.7)
No	121 (93.8)	309 (99.0)	279 (97.9)	73 (100)	0	0	24 (88.9)	0	559 (97.2)	1365 (97.3)
No. of subjects for whom data were not available	1	9	0	1	197	482	224	705	3	1622
Cracked or bleeding nipples reported at any time, no. (%)										
Yes	18 (14.0)	5 (1.6)	6 (2.1)	2 (5.0)	0	2 (100)	0	25 (3.6)	0	58 (3.9)
No	111 (86.0)	306 (98.4)	279 (97.9)	38 (95.0)	0	0	27 (100)	666 (96.4)	0	1427 (96.1)
No. of subjects for whom data were not available	1	10	0	34	197	482	224	14	578	1540

NOTE. C-S, Cesarean section; DROM, duration of ruptured membranes; LPT, late postnatal transmission; NA, not available.

[a] The determination of children's vital status includes children with no additional follow-up beyond the date of birth.

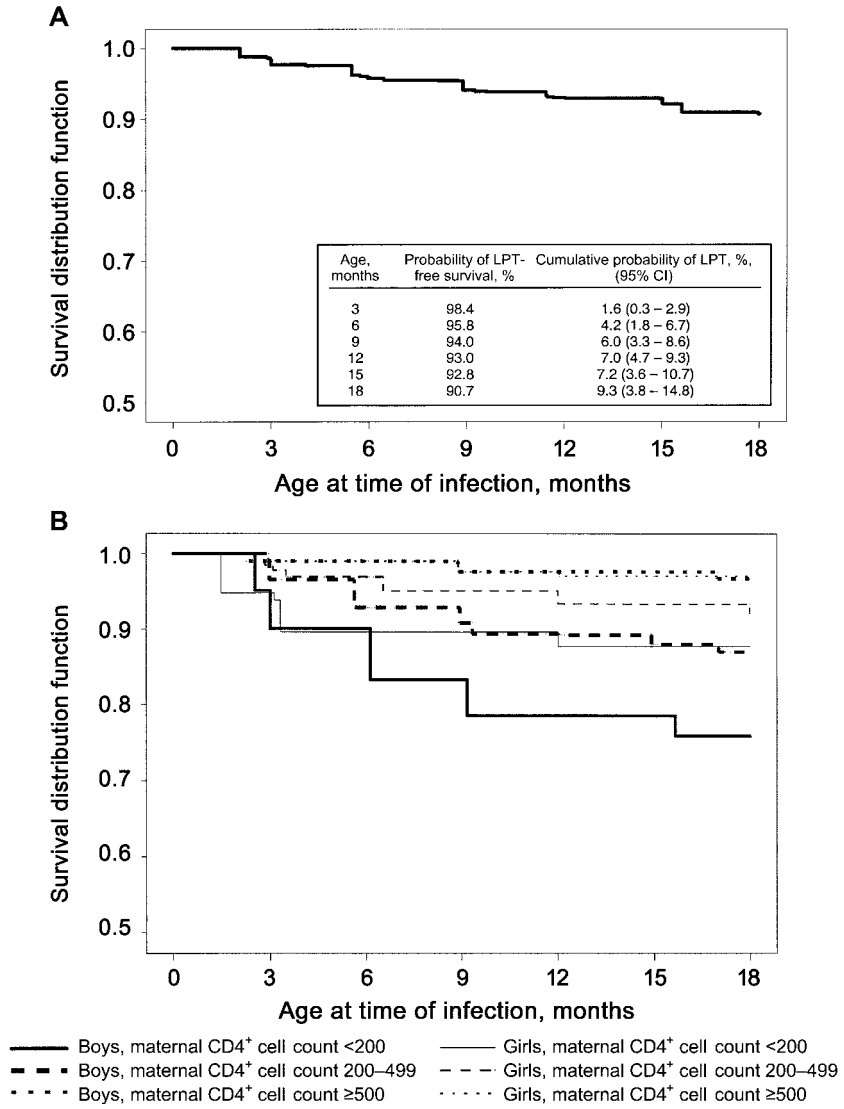


Figure 2. *A,* Peto-Turnbull plot of late postnatal transmission (LPT)–free survival, overall ($N = 3025$). *B,* Peto-Turnbull plot of LPT-free survival, by child's sex and maternal $CD4^+$ cell count (cells/mm^3) ($N = 2030$). Although gaps (intervals with no estimate) are a feature of Peto-Turnbull estimates of the cumulative time-to-event distribution for interval censored data, the lines are displayed in these figures without gaps. CI, confidence interval.

the incremental risk of late postnatal transmission through continued breast-feeding did not change significantly over time.

Sensitivity analyses. Sensitivity analyses were conducted to examine the effect of including provisionally HIV-1–infected children, children whose first positive HIV-1 diagnostic assay result occurred after cessation of breast-feeding, and of informative censoring (possibly resulting from deaths occurring among children before final HIV-1 infection status could be determined). The results were qualitatively similar to those obtained originally.

DISCUSSION

Our analyses indicate that late postnatal transmission represents at least 24%, and possibly as much as 42%, of overall mother-to-child transmission of HIV-1. Children of HIV-1–infected mothers have a nearly constant risk of late postnatal transmission throughout the period of breast-feeding. Consequently, a longer duration of breast-feeding is associated with a greater cumulative risk of late postnatal transmission of HIV-1. Lower maternal $CD4^+$ cell counts and male sex are independently associated with an increased risk of late postnatal transmission.

This individual patient data meta-analysis provides a better assessment of the risk and timing of late postnatal transmission of HIV-1 among breast-fed children than previously possible, for several reasons, including the large size of the pooled data set (by far the largest database to evaluate transmission of HIV-1 through breast-feeding ever compiled) and concomitantly enhanced statistical power, and the application of time-to-event

Table 2. Estimated hazard ratio (HR) and 95% confidence interval (CI) of late postnatal transmission, obtained from proportional hazards regression modeling with timing of infection randomly imputed ($N = 2030$).

Model	HIV infected	No. of subjects	Unadjusted models			Adjusted model		
			Coefficient (SE)	*P*	HR (95% CI)	Coefficient (SE)	*P*	HR (95% CI)
Grouped trial								
West Africa	39	549	−0.16 (0.31)	.32	0.9 (0.5–1.6)	0.05 (0.31)	.44	1.1 (0.6–1.9)
East Africa	87	1131	0.07 (0.29)	.40	1.1 (0.6–1.9)	−0.002 (0.29)	0.50	1.0 (0.6–1.8)
South Africa	14	350			1.00			1.00
$CD4^+$ cell count around the time of delivery, cells/mL								
<200	32	183	2.07 (0.26)	<.001	7.9 (4.8–13.0)	2.08 (0.26)	<.001	8.0 (4.8–13.3)
200–499	78	844	1.30 (0.22)	<.001	3.7 (2.4–5.6)	1.30 (0.22)	<.001	3.7 (2.4–5.6)
⩾500	30	1003			1.00			1.00
Sex								
Female	51	970	−0.46 (0.18)	.014	0.6 (0.4–0.9)	−0.46 (0.18)	.014	0.6 (0.4–0.9)
Male	89	1060			1.00			1.00

NOTE. SE, standard error.

analyses, allowing for interval-censoring of HIV-1 infection. In addition, selection bias is unlikely, since identification of eligible trials was not based solely on previous publications. Uniform definitions were applied across trials, and repeated assessments of children's HIV-1 infection status while breast-feeding (almost always by use of HIV-1 PCR assays) were incorporated, as was covariate adjustment. Potential bias resulting from deaths of enrolled children during follow-up is unlikely, on the basis of the results of sensitivity analyses assessing the potential effect of informative censoring. However, the estimation of the duration of breast-feeding and of the timing of acquisition of HIV-1 infection was not without some degree of imprecision. Calculation of the duration of breast-feeding was dependent on information acquired through interview of the mothers during study visits, without means of confirmation, and intervals between study visits during which HIV-1 diagnostic testing of children was performed were sometimes weeks to months apart. Related in large part to these intervals, categorization of the timing of transmission was not possible for 46% of the HIV-1–infected children. Any imputation of the timing of infection for these children requires assumption(s), and we addressed the issue by making one relatively conservative assumption, to provide a reasonable estimate of the timing of transmission for these children. Although of interest because of previously reported associations with transmission through breast-feeding, covariates such as maternal breast abnormalities [21, 22], children's oral candidiasis [22], and type of feeding (exclusive breast-feeding vs. mixed breast-feeding) [23] could not be examined, since they were not collected systematically in all participating trials. Finally, all of the participating trials in this meta-analysis were conducted in urban settings, whereas most of the population of sub-Saharan Africa resides in rural areas where breast-feeding continues for a much longer period. Thus, the risk of mother-to-child transmission of HIV-1 attributed to late postnatal transmission in this meta-analysis may represent an underestimate.

Early estimates of the risk of transmission of HIV-1 through breast-feeding, on the basis of small sample sizes [24–27], were relatively imprecise. Other early studies, which depended on serologic or clinical evidence of infection [28] or estimated the additional risk of HIV-1 infection among breast-fed children by comparing infection rates among children ever breast-fed versus children never breast-fed [29], were unable to accurately assess the timing of acquisition of HIV-1 infection during breast-feeding. Consistent with our findings, earlier studies have suggested that a longer duration of breast-feeding was associated with an increased risk of transmission of HIV-1 [22, 30–32]. The results of the present meta-analysis also are consistent with those of the randomized clinical trial of breast-feeding versus formula feeding conducted in Kenya, in which 44% of mother-to-child transmission of HIV-1 in the breast-feeding arm was attributable to breast-feeding (both late postnatal transmission and earlier postnatal transmission), and the estimated rate of transmission through breast-feeding, at 24 months, was 16.2% (95% CI, 6.5%–25.9%) [2].

Although point estimates of the risk of late postnatal transmission through breast-feeding in our analyses differ from those reported elsewhere by use of observational data [31, 33], methodological differences make detailed comparisons between our meta-analysis and these smaller studies difficult. For example, whereas other studies defined late postnatal transmission as occurring after 6 weeks [33] or after 2.5 months [31] of age, we defined late postnatal transmission as occurring after 4 weeks of age, thereby increasing the number of possible late

postnatal transmissions. In the discussion of their results, Leroy et al. [31] acknowledge that their reported incidence of late postnatal transmission underestimates infections occurring through breast-feeding, since infections acquired before 2.5 months of age were excluded. Interestingly, there was a substantial overlap in the CIs formed around the cumulative probability of late postnatal transmission at 18 months (6.3%; 95% CI, 3.9–9.95) reported by Leroy et al. [31], on the basis of a subset of the data, with information regarding the timing of acquisition of HIV-1 infection, and that reported in this meta-analysis (9.3%; 95% CI, 3.8–14.8).

The essentially constant risk of HIV-1 transmission per person-month of breast-feeding in our meta-analysis contrasts with the declining hazard of late postnatal transmission with longer duration of breast-feeding observed in 1 study [33]. In this latter study [33], the hazard function was estimated on the basis of unadjusted analyses that grouped survival data into intervals of 5–6 months. However, such broad grouping can increase bias (the systematic deviation between the estimated hazard and the true population value) while lowering variance; that is, the estimate can be precise but may not be accurate. A narrower grouping, such as 1 month, decreases bias at the cost of increased variance. There are 2 ways to possibly evade this difficult trade−off between bias and variance: (1) use a more complex estimate of the hazard function, an estimate that is smooth without much bias; or (2) increase the sample size. In our analyses, we estimated the hazard by use of proportional hazards regression, and the sample size was very large. The hazard function was estimated by use of a model that adjusted for the effects of maternal $CD4^+$ cell count around the time of delivery and child's sex, both of which contributed significantly to the model. The "roughly constant" description indicates that the plotted line was not perfectly flat but also that it did not correspond to an obvious decrease or increase in the hazard over time, indicating that each additional day of breast-feeding is associated with the same risk of late postnatal transmission as the days that preceded it.

Although previously identified as risk factors for transmission of HIV-1 through breast-feeding [33], neither maternal age nor parity was independently associated with transmission of HIV-1 in this meta-analysis. In our analyses, birth weight was not a significant risk factor for late postnatal transmission, although it has been associated with in utero or intrapartum transmission in studies of predominantly formula-feeding populations [34]. Our results confirm earlier reports suggesting that more-advanced maternal disease stage, as manifested by low $CD4^+$ absolute lymphocyte counts, represents a risk factor for postnatal transmission of HIV-1 [21, 22, 32, 35]. Lower maternal $CD4^+$ cell counts possibly reflect higher virus loads in maternal blood and breast milk, both of which are associated with a higher risk of mother-to-child transmission of HIV-1 [21, 35–37], and a higher plasma virus load is associated with a higher probability of transmission through breast milk [32, 37].

The child's sex has not previously been associated with late postnatal transmission, although it has been associated with human T lymphotropic virus type 1 seropositivity in children [38] and with mother-to-child transmission by 3 months of age [39]. Potential mechanisms for an association between child's sex and transmission of HIV-1 through breast-feeding could be cultural, biological, or a combination of the 2. Cultural mechanisms would presume that boys have more exposure to HIV-1–infected breast milk (e.g., through being breast-fed more frequently or for longer periods of time than girls). In addition, the type of feeding (exclusive breast-feeding vs. mixed breast-feeding) could vary according to sex, with girls more likely to be exclusively breast-fed; by assuming that mixed breast-feeding is associated with a greater risk of transmission of HIV-1 through postulated mechanisms [23, 40], it could be theorized that this could increase the risk of transmission in boys, compared with girls. We found no difference between boys and girls in the duration of breast-feeding, but data were not available on details such as the frequency of breast-feeding, the total daily volume of milk ingested, or the type of feeding (exclusive breast-feeding vs. mixed breast-feeding). Biological mechanisms for a sex difference in transmission of HIV-1 through breast-feeding would presume that, for a given exposure to HIV-1–infected breast milk, boys are more likely to acquire HIV-1 infection, compared with girls. Along these lines, previous studies have shown differences in peripheral blood virus load [41] and cellular immune markers [42] between untreated HIV-1–infected girls and boys. Finally, the finding that girls were at lower risk of acquiring HIV-1 infection through late postnatal transmission could merely reflect a greater vulnerability of girls than boys to early transmission of HIV-1 (i.e., infection in utero, around the time of labor and delivery, or during the first 27 days of life). If true, postnatally, the "pool" of susceptible girls would be relatively depleted, perhaps leaving fewer uninfected girls vulnerable to late postnatal transmission, compared with the pool of boys, who had not experienced this same depletion. We examined the timing of transmission of HIV by sex but found no statistically significant difference in the proportions of girls and boys who had early transmission.

The results of this meta-analysis should inform the development of appropriate interventions to prevent transmission of HIV-1 through breast-feeding in areas where complete avoidance of breast-feeding is not, generally, feasible. First, because the risk of transmission through breast-feeding is much higher among women with lower $CD4^+$ cell counts, interventions aimed at ameliorating HIV-1–infected mothers' disease (such as highly active antiretroviral therapy) should be vigorously pursued. Indeed, a wide range of institutions and organizations are working to expand access to antiretroviral drugs in resource-

poor settings around the world. Second, the potential biological and cultural mechanism(s) of the association of child's sex with the risk of transmission of HIV-1 through breast-feeding should be further investigated. Finally, the substantial proportion of mother-to-child transmission of HIV-1 attributable to breast-feeding highlights the urgency of developing effective interventions to prevent transmission through breast-feeding, to complement the progress made with prevention of mother-to-child transmission during the antepartum and intrapartum periods. Such potential interventions that are currently being evaluated include the following: early weaning; decreasing virus load in breast milk, by chemical or heat treatment of the milk or by antiretroviral drug(s) administered to the breast-feeding mother; preventing or treating factors related to facilitation of transfer of HIV-1 from mother to child (e.g., by preventing or treating maternal breast abnormalities and infant candidiasis or by avoidance of mixed breast-feeding during the first 4–6 months of life); and improving infant defenses against HIV-1 infection, by active or passive immunization or by antiretroviral prophylaxis to breast-feeding children [43]. An important implication of our analyses is that, since children of HIV-1–infected mothers have a consistent and substantial risk of acquisition of HIV-1 throughout the period of breast-feeding, to be most effective, interventions to prevent transmission through breast-feeding should be continued until the cessation of breast-feeding.

BREASTFEEDING AND HIV INTERNATIONAL TRANSMISSION STUDY (BHITS)

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APPENDIX

HIV-1 infection status

- Definitively infected: Single positive RNA or DNA polymerase chain reaction (PCR) result at any age; or positive serologic test result at 18 months of age (or negative serologic test result followed by positive serologic test result), with subsequent confirmation of the positive serologic test result
- Provisionally infected: Positive serologic test result at 18 months of age (or negative serologic test result followed by positive serologic test result), without subsequent confirmation of the positive result
- Definitively uninfected: Negative DNA or RNA PCR result $\geq$1 month or negative serologic test result $\geq$2 months after cessation of breast-feeding
- Provisionally uninfected: Negative diagnostic result obtained either while still breast-feeding or <1 (DNA or RNA PCR) or <2 (serologic testing) months after cessation of breast-feeding
- Indeterminate: HIV-1 diagnostic testing performed, but results either not reported or reported as indeterminate

Timing of HIV-1 transmission

- Early transmission (in utero, intrapartum, or neonatal): Positive HIV-1 result(s) before 4 weeks (28 days) of age
- Late postnatal transmission: Negative HIV-1 result(s) at or after 4 weeks (28 days) of age, followed by positive test result(s)
- Unknown timing of infection: Positive test result(s) at or after 4 weeks (28 days) of age, but either no prior negative result or the last negative result was before 4 weeks (28 days) of age

Duration of breast-feeding

- Length of time between birth until the date of cessation or, if still breast-feeding at the end of the trial, the date of the last study visit
- If breast-feeding ceased before the end of the trial but the date of cessation was unknown, duration determined from birth to the midpoint between the date of the study visit when last breast-feeding and the date when no longer known to be breast-feeding

Period of risk for late postnatal transmission

- HIV-1–infected children: From birth to the date of the first positive HIV-1 diagnostic test; children ceasing breast-feeding before the last negative diagnostic test defining the infection interval were considered to be censored (uninfected) at the time of cessation
- HIV-1–uninfected children: From birth to the date of cessation or, if still breast-feeding at the end of the study, to the date of the last available negative HIV-1 diagnostic test

Other variables

- Trial
  - Short-course antiretroviral prophylaxis trials
    - Zidovudine versus placebo

    - ANRSA: ANRS 049a (Cote d'Ivoire and Burkina Faso)
    - RETRO: RETRO-CI Study (Cote d'Ivoire)
  - Nevirapine versus placebo and zidovudine versus placebo (note that the trial was redesigned and placebo was eliminated after enrollment initiated)
    - HIVNET: HIVNET 012 (Uganda)
  - Zidovudine/lamivudine versus placebo
    - PETRA: PETRA Study (South Africa, Tanzania, Uganda)
- Cervicovaginal and/or infant cleansing trials
  - Benzalkonium chloride
    - ANRSB: ANRS 049b (Cote d'Ivoire and Burkina Faso)
  - Chlorhexidine
    - CHL: Chlorhexidine Intervention Study (Kenya)
- Infant formula versus breast-feeding trial
  - MB: Mother-Baby Study (Kenya)
- Micronutrient supplementation trials
  - VITA: South Africa Vitamin A Study (South Africa)
  - MICRO: Tanzania Micronutrient Study (Tanzania)
- Geographic region
  - West Africa: ANRSA, ANRSB, RETRO
  - East Africa: CHL, HIVNET, MB, MICRO, and PETRA sites in Uganda and Tanzania
  - South Africa: VITA and PETRA sites in Durban and Johannesburg
- Treatment arm of trial

Maternal variables

- Maternal age determined at time of delivery or enrollment
- Maternal education (highest level of education completed)
- Gravidity assessed as of date of index delivery
- Parity assessed as of date of index delivery
- $CD4^+$ lymphocyte count ($CD4^+$) closest to enrollment and date obtained
- Mode of delivery
- Duration of ruptured membranes
- Last known vital status and date of death (if deceased)
- Tuberculosis diagnosed at enrollment or while enrolled in the study, with diagnosis date
- Breast abnormalities (mastitis, abscess, and cracked and/or bleeding nipples), with date and method of diagnosis

Child variables

- Birth weight
- Sex
- Gestational age
- Last known vital status and date of death (if deceased)
- Oral candidiasis, with date and method of diagnosis

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