Role of breastfeeding cessation in mediating the relationship between maternal HIV disease stage and increased child mortality among HIV-exposed uninfected children

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Background   Maternal CD4 count predicts child mortality in HIV-uninfected children born to HIV-infected women.

Methods   To explore the mediating role of breastfeeding cessation in this relationship, we compared marginal structural models of maternal CD4 count on child death with and without adjustment for breastfeeding.

Results   In crude analyses, children of mothers with CD4 < 200 during pregnancy were 3.2 times more likely to die by 18 months (CI 1.3–8.1) as children whose mothers had CD4 > 500. Earlier breastfeeding cessation was also associated with low CD4 (HR 1.8; CI 1.2–2.7). After adjusting for breastfeeding and low birth weight using a marginal structural model, the low CD4 count-child mortality association through 18 months was reduced 17%. The change was overestimated using a traditional Cox proportional hazards model (35% reduction in HR from 3.4 to 2.5).

Conclusions   Our analysis suggests that only a small part of the effect of low vs high CD4 count on child mortality through 18 months is mediated through breastfeeding cessation. Our results must be taken into account when deciding whether or not to recommend breastfeeding for infants of HIV-infected mothers.

Keywords   HIV, infant mortality, direct and indirect effects, marginal structural model, Africa, breastfeeding

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Most infants born to HIV-infected mothers escape HIV infection during childhood. Evidence suggests that HIV-exposed (i.e. born to an HIV-infected woman) but uninfected infants have higher morbidity and mortality than infants born to HIV-uninfected mothers.[1–5] Some hypothesize that the increased risk reflects behavioural factors affecting a mother's ability to care for her sick child, though there is currently little evidence to support this. Several studies have suggested an HIV-infected mother's degree of immunosuppression adversely affects survival in her uninfected infant.[6–8] This was established more firmly in a cohort of HIV-exposed but confirmed uninfected infants who were significantly more likely to die by

4 months of age if their mother's CD4 count near birth was <350.[9] Given the apparent significance of maternal health in the survival of her uninfected infant, a logical next step is to investigate potential causal mechanisms through which maternal CD4 count could affect infant survival in order to seek ways to mitigate the harmful impact on the infant.

One possible explanation for increased mortality among HIV-exposed infants is that the general debility associated with advanced maternal AIDS leads to a decrease or complete cessation of breastfeeding.[10,11] Numerous studies have established the immunological and nutritive benefits of breast milk, including the reduction of both major morbidity[12,13] and mortality.[14] While likely underestimating the total effect in developing countries, odds ratios for mortality comparing breastfeeding to non-breastfeeding ranged from 4.2 during the first months of life to 1.4 in the 9th though 11th months of life in a pooled analysis.[14] Given the link between breastfeeding duration and infant health, it is possible that increased infant mortality associated with a lower maternal CD4 count may be mediated through breastfeeding.

This analysis uses longitudinal data collected from a well-defined cohort of pregnant women and their infants enrolled in a breastfeeding intervention trial in Zambia to evaluate the mediating role of breastfeeding cessation in the relationship between maternal CD4 count and child mortality.

Materials and methods

Study design and population
The Zambia Exclusive Breastfeeding Study (ZEBS) was a randomized controlled trial designed to assess the impact of abrupt weaning at 4 months on overall HIV-free survival. Details of the study have been described previously.[15,16] Briefly, between May 2001 and September 2004, 1435 HIV-infected pregnant women, were enrolled into a randomized controlled trial of a breastfeeding intervention to prevent mother-to-child transmission of HIV at two clinics in Lusaka, Zambia at antenatal clinic visits. At the time of this study highly active antiretroviral therapy (HAART) was not yet available in Zambia. ZEBS was approved by institutional review boards at Boston University, Childrens Hospital of Los Angeles, Columbia University and the University of Zambia Research Ethics Committee.

All enrolled women were intensively counselled to exclusively breastfeed through 4 months of age. At 1 month post-delivery, women were randomized to either: (i) abrupt breastfeeding cessation at 4 months; or (ii) exclusive breastfeeding through 6 months with weaning as they normally would. Mothers and infants were followed for 2 years. The current analysis includes all live born, singleton infants in ZEBS randomized to usual breastfeeding behaviour (i.e. the control group who were counselled to stop breastfeeding when they felt it was appropriate) who

did not test HIV-positive at any time during follow-up. Thus for all mothers included in the current analysis, breastfeeding behaviour was not under the control of the investigator and was not influenced by the study team. This analysis is limited to the first 18 months of follow-up as there were only two child deaths after this time.

Analytic variables
At baseline all mothers had blood drawn for CD4+ T-cell lymphocyte counts (FACSCount system, BD Immunocytometry Systems, San Jose, CA). Maternal disease stage as indicated by CD4 count during pregnancy at enrollment was categorized into three groups (<200, 200–499, >500).

All mothers were scheduled for clinic visits at 1 week, then monthly through 6 months, then every 3 months. For any child who died, the date and circumstances of the death were elicited from the mother or family members and hospital records when available. Infant follow-up time was defined as the period from birth to the date of the earliest of: (i) completion of 18 months of follow-up; (ii) infant death; and (iii) last visit if lost to follow-up.

We defined breastfeeding cessation to have occurred at the child's actual as reported by the mother on her first report of stopping all breastfeeding. We assumed children who died were breastfed up to their date of death unless records specifically reported that breastfeeding stopped prior to the start of the illness immediately preceding the child's death. Breastfeeding time was defined as the period from birth to the date of the earliest of: (i) first visit a mother reported stopping all breastfeeding; (ii) infant or maternal death; (iii) completion of 18 months; and (iv) last visit before loss to follow-up.

Statistical methods
To assess the mediating role of breastfeeding in the maternal CD4 count-child mortality relationship, we first examined the relationship between maternal CD4 count and child mortality. Next, we explored the association between maternal CD4 count and breastfeeding cessation. Finally, we created a model with child mortality as the outcome and maternal CD4 count as the exposure and then added breastfeeding cessation as a covariate to look for changes in the CD4-child mortality relationship when adjusting for breastfeeding.[17]

We calculated crude rates of child mortality and breastfeeding cessation (per 100 person-years) and incidence rate ratios within each CD4 category. To identify confounders for multivariable proportional hazards models, we used a two step process. We first conducted univariate comparisons by child vital status of each variable that could plausibly confound the CD4-child mortality relationship. For those variables with a chi-square test of independence $P < 0.20$, we then used a modified forward step-wise procedure[18]
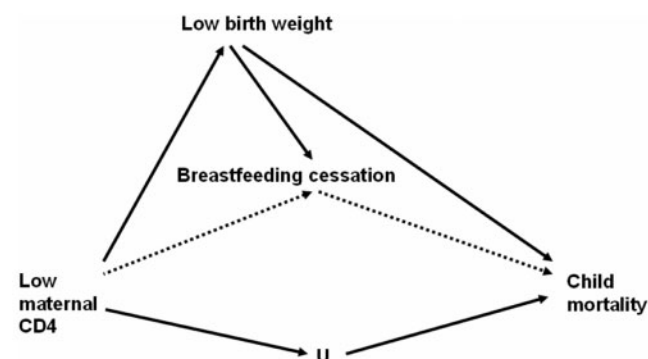
to identify covariates that changed the hazard ratio (HR) for maternal CD4 count. The covariate producing the largest change >10% was retained and a new percentage change was calculated with each remaining variable until no remaining variable resulted in a change >10%. We did not adjust for any factor that was affected by maternal CD4 count, such as maternal death.

Causal intermediate

We hypothesize that some of the effect of maternal CD4 count on infant mortality is mediated through breastfeeding cessation (Figure 1); that is, mothers with lower CD4 counts near birth have a reduced ability to adequately maintain breastfeeding, which in turn leads to increased child mortality. To test this, we employed an approach described in reference[17] (p. 184). We created two distinct but similar regression models, both of which had maternal CD4 count as the independent variable and child mortality as the dependent variable. Model 1 also included confounders of the CD4 count-child mortality relationship. If there were no bias or random error in our study, model 1 would reflect the total causal effect of maternal CD4 count on infant mortality (some of which we believe is mediated through breastfeeding cessation).

Model 2 included the covariates in model 1, but also included breastfeeding cessation as a time dependent covariate and any confounders of the breastfeeding–child mortality relationship.[19] Because model 2 contains a variable affected by the exposure (i.e. we hypothesize women with lower CD4 counts breastfeed for shorter duration), it represents the effect of maternal CD4 count on child mortality remaining after controlling for breastfeeding. What remained would be the effect of maternal CD4 count on child mortality through other pathways (e.g. low birth weight—LBW).

Figure 1 shows the proposed causal pathways for the effect of maternal CD4 count on child survival. The dotted line represents the potential indirect effect of maternal CD4 count on infant survival mediated through duration of breastfeeding. Similarly, an indirect effect is likely mediated through LBW (e.g. mothers with low CD4 counts are more likely to have a LBW child, which likely, in turn affects the child's risk of death). Because birth weight is affected by maternal CD4 count, including LBW as a covariate in a standard regression model would provide biased results. However, we believe LBW is also a confounder of the breastfeeding cessation–child mortality relationship.[20] LBW may cause changes in breastfeeding duration and independently increase infant mortality. Thus, any model intended to identify the indirect effect of breastfeeding cessation that did not adjust for LBW would also be biased.

To adjust for LBW, we employed a proportional hazards marginal structural model (MSM).[21,22] MSMs weight each subject in the dataset proportional to the inverse of the probability of receiving the exposure they actually received given their covariates. In this analysis this would be the probability of being breastfed the duration a child was actually breastfed given their birth weight and other covariates. Because we weight subjects we use robust standard errors to prevent unduly small confidence intervals (CI).

Changes in the estimate of effect of CD4 count on infant mortality from models 1 to 2 are expressed as percentage change:

$$100\left[\left(\frac{HR_1 - HR_2}{HR_1 - 1}\right)\right]$$

where HR_x is the HR from model x. Any decrease in HR from models 1 to 2 with adjustment for breastfeeding would be consistent with a portion of the effect of maternal disease stage on child mortality being mediated through breastfeeding cessation.

Results

Of the 1435 ZEBS children, 357 met the eligibility criteria for this analysis. Two children were excluded because we had no data on the mother's enrollment CD4 count. Maternal CD4 counts largely fell in the 200–500 range (54.6%, $N = 194$) with fewer in the >500 category (27.6% $N = 98$) or <200 CD4 range (17.7% $N = 63$). There were 14 maternal deaths (3.9%) during follow-up and 45 infant deaths (12.7%) of which 17 were in the first 6 months. The median follow-up time was 548 days. By Kaplan-Meier estimate the median duration of breastfeeding was 487 days, but was considerably shorter in the low CD4 group (372 days) than either the intermediate or high groups (487 and 517 days, respectively).

Crude analyses

Crude analyses revealed few independent predictors of child death (Table 1). High socioeconomic score predicted lower risk of child death (RR 0.55;

Figure 1 Causal diagram of the effect of maternal CD4 count on child mortality through breastfeeding cessation. U represents other unknown variables

* U represents other unknown variables

Table 1 Crude relative risks for predictors of child mortality through 18 months among 355 HIV-uninfected infants born to HIV-infected mothers in Zambia between May 2001 and September 2004

Factor	Level	Child death (%)	No child death (%)	Total	Relative risk (95% CI[a])
Mother died	No	40 (11.7)	301 (88.3)	341	1.0
	Yes	5 (35.7)	9 (64.3)	14	3.0 (1.4–6.5)
Number of previous	0	2 (4.7)	41 (95.3)	43	1.0
pregnancies	1–3	33 (13.7)	208 (86.3)	241	2.9 (0.73–11.8)
	>3	10 (14.1)	61 (85.9)	71	3.0 (0.70–13.2)
Mother's age	<20	4 (11.8)	30 (88.2)	34	1.0
	20–29.9	33 (13.9)	205 (86.1)	238	1.2 (0.45–3.1)
	30+	8 (9.6)	75 (90.4)	83	0.82 (0.26–2.5)
SES > 0[a]	No	11 (20.4)	43 (79.6)	54	1.0
	Yes	34 (11.3)	267 (88.7)	301	0.55 (0.30–1.0)
Has a water tap in house	No	43 (12.8)	292 (87.2)	335	1.0
	Yes	2 (10.0)	18 (90.0)	20	0.78 (0.20–3.0)
Electricity in house	No	29 (13.4)	187 (86.6)	216	1.0
	Yes	16 (11.5)	123 (88.5)	139	0.86 (0.48–1.5)
Ran out of food in previous 30 days	No	29 (10.6)	245 (89.4)	274	1.0
	Yes	16 (19.8)	65 (80.2)	81	1.9 (1.1–3.3)
Mother employed	No	41 (12.3)	292 (87.7)	333	1.0
(full-time)	Yes	4 (18.2)	18 (81.8)	22	1.5 (0.58–3.7)
Low birth weight[b]	No	34 (10.8)	281 (89.2)	315	1.0
	Yes	10 (33.3)	20 (66.7)	30	3.1 (1.7–5.6)
Child is male	No	25 (14.5)	148 (85.5)	173	1.0
	Yes	20 (11.0)	161 (89.0)	181	0.76 (0.44–1.3)
Child born at home	No	36 (11.6)	275 (88.4)	311	1.0
	Yes	9 (20.5)	35 (79.5)	44	1.8 (0.91–3.4)

[a]CI, confidence interval; SES, socio-economic status. SES defined as sum of: owns a fridge, owns electricity, completed >8 years of education, employed and had food security for the previous month.
[b]Low birth weight defined as <2500 g.

95% CI 0.30–1.0), while food insecurity in the prior month (RR 1.9; 95% CI 1.1–3.3), LBW (RR 3.1; 95% CI 1.7–5.6) and maternal death (RR 3.0; 95% CI 1.4–6.5) increased the likelihood of child death.

Table 2 shows incidence rates of infant mortality by time period stratified by CD4 group. Children whose mothers had low CD4 counts were at markedly increased risk of death compared with those whose mothers had high CD4 count through 18 months (HR 3.2; 95% CI 1.3–8.1). Over 18 months, earlier breastfeeding cessation was clearly associated with advanced maternal disease stage (HR 1.8; 95% CI 1.2–2.7) (Table 2). Little difference was seen between the intermediate and high CD4 groups.

Adjusted analysis

The proportional hazards marginal structural model regression (Table 3) shows the effect of adjustment for SES on child mortality up to 18 months. Model 1 is not adjusted for cessation of breastfeeding or LBW and represents the estimate of the total effect of CD4 count on child mortality. After adjusting for SES, infants of mothers with CD4 counts below 200 were at substantially increased risk of death through 18 months compared with those with mothers with CD4 above 500 (HR 3.4; 95% CI 1.3–8.4). Little difference was seen between the intermediate and high CD4 groups.

The second column of Table 3 (model 2) shows the analysis further adjusted for breastfeeding cessation and LBW. This model estimates the effects of CD4 count on child mortality *not* mediated through breastfeeding cessation. In this model breastfeeding cessation was predictive of child mortality through 18 months (HR 2.0; 95% CI 1.0–3.7).

The percentage change in the hazard ratio from models 1 to 2 is shown in column 3. This value represents an estimate of the indirect effect of maternal CD4 count on mortality mediated through breastfeeding cessation. After adjusting for breastfeeding and LBW (model 2), the association between low CD4 count (<200) and child mortality through 18 months

Table 2 Crude incidence rate ratios of the association between maternal CD4 count and child mortality and breastfeeding cessation through 18 months among 355 HIV-uninfected infants born to HIV-infected mothers in Zambia between May 2001 and September 2004

| Maternal CD4 count group | N | Child death | | | | Breastfeeding cessation | | | |
		Events	PY	Rate/100 PY	IRR 95% CI	Events	Breastfeeding PY	Rate/100 PY	IRR 95% CI
>500	98	7	123.0	5.7	Reference	49	107.2	45.7	Reference
200–500	194	25	233.4	10.7	1.9 (0.81–4.4)	113	187.4	60.3	1.3 (0.94–1.8)
<200	63	13	70.9	18.3	3.2 (1.3–8.1)	39	47.6	81.9	1.8 (1.2–2.7)

PY, Person-years; IRR, Incidence Rate Ratio.

Table 3 Marginal structural hazards ratios of the relationship between maternal CD4 count and child mortality through 18 months among 355 HIV-exposed but uninfected children in Zambia between May 2001 and September 2004[a]

Factor	Model 1[b]	Model 2[b]	Percent Change Models 1 to 2[c]
Maternal CD4 > 500	Reference	Reference	
Maternal CD4 200–500	2.0 (0.87–4.6)	1.9 (0.80–4.3)	10.0
Maternal CD4 < 200	3.4 (1.3–8.4)	3.0 (1.2–7.9)	16.7
SES 0[d]	Reference	Reference	
SES 1–2	0.43 (0.21–0.89)	0.43 (0.21–0.89)	0.0
SES > 2	0.57 (0.24–1.3)	0.49 (0.21–1.2)	−19.0
Breastfeeding cessation	N/A	2.0 (1.0–3.7)	

[a]Model adjusted for low birth weight (defined as <2500 g) using marginal structural model.
[b]Both models 1 and 2 show hazard ratios of infant mortality and 95% CIs adjusted for all other variables in the model.
[c]Percent change from models 1 to 2 defined as $(HR_2 - HR_1)/(HR_1 - 1)$.
[d]SES, Socio-economic status; SES defined as sum of: owns a fridge, owns electricity, completed >8 years of education, employed and had food security for the previous month.

was reduced compared with model 1 (from 3.4 to 3.0), a reduction in estimate of effect of 17%. This suggests that only a small part of the effect of low vs high CD4 count on child mortality through 18 months is mediated through breastfeeding cessation. For comparison, we note that adjustment for breastfeeding cessation using a conventional Cox proportional hazards model showed an even greater effect. The high-low CD4 comparison was reduced by 35% (from 3.4 to 2.5); however this approach is simultaneously adjusting for the intermediate effects of both breastfeeding cessation and low birth weight and is therefore likely biased.

Discussion

Our results build upon the earlier finding in this cohort (9) which showed that uninfected infants of HIV-infected mothers with CD4 counts <350 were 2.9 times more likely to die by 4 months than infants of mothers with CD4 count >350. The current analysis in the same population found that after adjusting for socioeconomic status, children born to mothers with CD4 count <200 had 3.4 times the risk of death through 18 months as did infants born to mothers with high CD4 counts. A similar finding was observed

in a cohort of uninfected children of HIV-infected mothers in Kenya, where children of mothers with a CD4 count <200 had 2.8 times the risk of death compared with all other CD4 counts.[7] Our results differ from a pooled analysis which found a small, non-significant effect of a mother's CD4 count <200 compared with a CD4 count >500.[23] They found a strong effect of maternal death on infant mortality and these variables likely both measure maternal disease stage; additionally, their results were adjusted for breastfeeding cessation which, according to our biologic model, would adjust away some of the effect of CD4 count.

We also found that breastfeeding cessation occurred earlier among immunocompromised mothers compared with healthier mothers near the time of the birth of their child. Our results agree with an earlier finding by Sedgh et al.[24] in Tanzania where women with a CD4 count <200 breastfed on average 3.8 months less than women with a CD4 count ⩾500 near the birth of her child. They are also consistent with data from Botswana which showed an increased risk of morbidity in children of HIV-infected mothers if the mother had stopped breastfeeding.[5]

Unlike previous studies, we assessed the mediating role of breastfeeding in the CD4 count–child mortality relationship. Our analysis supports the hypothesis

that breastfeeding cessation accounts for some of the effect of maternal CD4 count on child death among HIV-exposed but uninfected infants. However, most of the CD4 count–child mortality association remained after adjusting for breastfeeding, suggesting other factors also explain the observed association. These may include increased exposure to infectious diseases, low birth weight and poor growth, or a general inability to care for the child.

The current study employed a marginal structural model to adjust for confounding by LBW as it is likely both a confounder and causal intermediate. When using a conventional Cox proportional hazards model, we found a substantially larger change in the estimate of effect of CD4 count on child mortality through 6 months when adjusting for LBW. This suggests that some of the effect of maternal CD4 count may be mediated through birth weight and that conventional approaches to adjusting for LBW will be biased.

While we identified no other study of breastfeeding cessation as a mediator of the CD4 count–infant mortality relationship, the reduction in effect of maternal CD4 count and child survival seen in one previous study after adjusting for breastfeeding cessation is consistent with breastfeeding cessation being a mediator.[23] In their crude analyses, CD4 <200 was associated with 2.5-fold increased risk of child death compared with children of mothers with CD4 count >500. After adjusting for breastfeeding and other covariates their odds ratio was reduced to 1.7 (95% CI 0.9–3.2); this is in agreement with our finding that some of the effect is being mediated through breastfeeding cessation. However, as their analysis was not designed to assess the mediating role of breastfeeding it is not clear whether this change reflects adjustment for the mediator or adjustment for other confounders in their model.

Considerable debate still exists regarding the possibility and optimal approach for separating direct and indirect effects. The parsing of direct and indirect effects requires certain assumptions:[19,25–28] there must be no residual confounding between the exposure and the outcome, or between the causal intermediate and the outcome, and there must be no interaction between the exposure and the intermediate. While our study was observational and therefore subject to confounding, we identified few confounders of the CD4 count–child mortality relationship and we adjusted for LBW, the only confounder identified for the breastfeeding cessation–child mortality relationship.

Some feel these assumptions are overly restrictive[28,29] while others feel the conditions for deconstructing total effects rarely occur.[27] Some[27] argue that the no interaction requirement is unlikely to be satisfied. However, others[25] note that, 'in the presence of interaction, it is the potentially estimable parameter—the fraction of the exposure effect that could be eliminated by control of the intermediate—that will usually be the parameter of public health interest'. Thus, even if our assumption of no interaction between CD4 count and breastfeeding has been violated, our results still imply that a portion of the relative increase in child deaths through 6 months attributable to advanced maternal disease stage could be prevented by increasing breastfeeding duration.

This study should be considered in light of several limitations. First, there could have been residual confounding in our analysis. However, because maternal CD4 count was measured only during pregnancy, and most of our data was collected after the exposure, we had few candidate variables for adjustment. While it is possible that important residual confounding remained, as we could identify no confounders in this analysis other than low birth weight (either statistically or through substantive knowledge) we consider this scenario unlikely.

Second, time of breastfeeding cessation could have been misclassified. Because the misclassification is of the intermediate variable, if it is non-differential with respect to the exposure and the outcome, it would be expected to reduce the ability to adjust for the intermediate. This implies that any reductions seen in the CD4 child–mortality relationship after adjusting for breastfeeding cessation would not completely control for the intermediate, so that the indirect effect observed would be expected to be an underestimate.[30] Thus our results likely represent an underestimation of breastfeeding as a causal intermediate.

Finally, we did not have data on cause of death for the current analysis, and therefore we cannot exclude the possibility that some of the child deaths were unrelated to breastfeeding (e.g. deaths due to injury). The impact of including these deaths is unclear, and future research on the topic should seek to refine the outcome.

In conclusion, we found that uninfected children born to HIV-infected mothers with more advanced immunosuppression were breastfed for shorter duration and had higher mortality though 18 months of life compared with children of mothers who were not immunocompromised. If confirmed, our results must be taken into account when deciding whether or not to recommend breastfeeding for infants of HIV-infected mothers. Our results suggest it is particularly important for mothers with low CD4 counts near delivery to persist in providing breast milk to their uninfected infants, though this risk must be balanced with the increased risk of HIV transmission with longer duration of breastfeeding. The more widespread availability of highly active antiretroviral therapy should also allow breastfeeding mothers the opportunity to continue to prolong breastfeeding without substantial increased risk of HIV transmission and therefore provide their uninfected infants the added protection and benefits of breast milk during the critical first months of life.[31]

Funding

Acknowledgements

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