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HIV-1 disease is not made worse by breastfeeding in non-immuno-compromised HIV-1 infected mothers participating in the ANRS12174 clinical trial

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Abstract

Objective

We have assessed disease progression of mothers in relation to exclusive or any breastfeeding duration among breastfeeding HIV1-positive women participating in the ANRS12174 trial (clinical trial no NCT0064026).

Methods

The analysis was carried out on 203, 212, 272 and 529 HIV-1 negative infants born to HIV-1-positive women with CD4 count >350 cell/µl from Burkina Faso, South Africa, Uganda and Zambia, respectively. The trial compared Lamivudine and Lopinavir/Ritonavir as a peri-exposure prophylaxis. A multiple logistic regression model was also run with the HIV-1 disease progression as the dependent composite end-point combining the mothers' weight, CD4 count and HIV-1 clinical stage as per WHO classification. Exclusive or predominant breastfeeding (EPBF) duration and duration of any breastfeeding were the key explanatory variables.

Results

In the adjusted model, the associations between EPBF duration and weight change, CD4 cell count and the HIV-1 viral load were consistently insignificant. The CD4 cell count was associated with a significantly higher mothers' body mass index (BMI; a mean increase of 4.9 (95% CI: 2.1; 7.7) CD4 cells/µl per each extra kilogram per square meter of BMI) and hemoglobin concentration (19.4 (95% CI: 11.4; 27.4) CD4 cells/µl per each extra gram per decilitre of hemoglobin concentration).

There was no significant association between EPBF duration and HIV-1 disease progression. However, randomization to the lopinavir/ritonavir arm was related to a significant acceleration of HIV-1 disease progression (adjusted odd ratio of 1.3 (95% CI: 1.0; 1.6; p=0.04) at the multivariate mode)l.

Conclusion

Breastfeeding was not a risk factor for the HIV-1 infected mother's weight, CD4 cell count and HIV-1 viral load change or HIV-1 disease progression in this cohort that had a baseline CD4 cell count of >350 cells/µl.

Keywords: HIV-1 infection; breastfeeding; Sub Saharan Africa

Strengths and limitations of this study

- Our study has been implemented in 4 Countries in Africa, namely Burkina Faso (West), South Africa and Zambia (South), and Uganda (East), which made our sample typically representative of the wider Sub-Sahara African population.
- The data were collected in the context of a rigorous clinical trial, which minimized the lost to follow-up, the missing data as well as other data collection errors, and therefore improved the quality of our data.
- However, the selection associated with the environment of a clinical trial, usually quite different from a routine environment, may have slightly biased our findings.
- Nonetheless, our end-points (mother's weight, CD4 cell count and HIV-1 viral load) were sufficiently robust and had a high validity.

INTRODUCTION

In 2015, 36.7 [34.0-39.8] million people were infected with HIV. Among them 17.4 [16.1-20.0] million were women of childbearing age [1 2]. HIV-1 prevalence is estimated between 5.3 and 6.5% among pregnant women in Sub-Saharan Africa [3]. Because of the almost irreversible immune activation involved, HIV-1 infection creates a condition of metabolic stress that may result in wasting and immune depression [4-7]. Ten per cent weight loss and a CD4 count of <350 cells/µl in the context of HIV-1 infection have been recognized as major criteria of the diagnosis of AIDS [8-12]. This weight loss is also associated with a higher risk of mortality in HIV-1-infected patients [13]. Furthermore, HIV-1 is a major cause of maternal mortality in affected countries in Southern Africa. About 25% of pregnancy-related deaths in Sub-Saharan Africa are attributable to HIV [14], and 88% of deaths among pregnant and postpartum women with HIV infection are attributable to the virus [15].

In women, pregnancy is, though a physiological condition, a period of increased metabolic activities and synthesis requiring a supplement of energy and nutrients. After delivery, breastfeeding prolongs the increased metabolic demands. In spite of this, WHO still recommends HIV-1-infected women to breastfeed as the best choice for the infant and the mother [16] in contexts where replacement feeding is not safe.

There have been conflicting results on assessment of the impact of breastfeeding in HIV-1-infected mothers. Some studies found that breastfeeding was harmful to HIV-positive mothers by either accelerating HIV disease progression assessed by the mother's weight loss, a decrease in CD4 cells count, or even an increased risk of maternal mortality, suggesting that metabolic, immunologic or hormonal changes associated with breastfeeding may accelerate HIV-1 disease progression in postpartum mothers [17-19]. Others found no

effect on the mothers' health assessed by death, development of a low CD4 cell count, anemia or excessive weight loss [20 21] . Some studies have found breastfeeding protective, allowing weight gain in HIV-1 infected breastfeeding mothers [19 22-26].

In the ANRS12174 trial, we assessed mothers' HIV-1 disease progression (measured by the change in weight, CD4 cells count and HIV-1 disease stage as per WHO classification) in relation to exclusive breastfeeding or duration of any breastfeeding during the infant first 6 months of life and until week 50 post-partum.

METHODS

Study design

The ANRS 12174 clinical trial in Ouagadougou (Burkina Faso), East London (South Africa), Mbale (Uganda) and Lusaka (Zambia) was conducted from 2009 to 2013. The protocol and the main outcome have been published [27 28]. Briefly, HIV-1 infected, pregnant women, at the time not eligible for highly active antiretroviral therapy because CD4 count was >350 cells/µl, aged 18 or above, planning to breastfeed were identified from ante-natal clinics between 28 and 40 weeks of amenorrhea. As part of the HIV post-test counselling session, they were informed on the different feeding options for their babies. Only women intending to breastfeed were referred to the research clinic for further assessment of the inclusion criteria during the antenatal period and again with their child within 6 days after birth, for an enrolment and randomisation at day 7 postpartum. From 28 weeks of pregnancy to day 7 after birth, programmatic mother to child transmission prophylaxis was implemented with antepartum zidovudine, intrapartum single dose nevirapine and zidovudine-lamivudine for mothers and nevirapine for infants for 7 days postpartum. Twins and triplets, infants with positive HIV-1 DNA PCR test result at day 7 (+/- 2 days) postpartum, low birth-weight or ill babies (ranked grade II or above of the ANRS classification for adverse events) were excluded [29]. The intervention provided an infant prophylaxis in the breastfeeding period plus one week from day 7 to 50 weeks of age with either lopinavir/ritonavir or lamivudine.

Data management and analysis

Data was collected on a paper case-report form or directly entered online using the Electronic Data capture system: OpenClinica™ (www.openclinica.com). Twenty-four h and one week breastfeeding recalls were collected during the enrolment visit at day 7±2 days after birth and the 13 monthly-scheduled follow-up visits that started at week 2. During these visits, mothers were asked in particular if they gave their infants other foods/liquids as well as breastmilk. Prelacteal feeding data - defined as any food item except mothers' milk given to infants before initial breastfeeding - were also collected at the enrolment visit.

The mothers at each visit were categorized into the following groups: 1) exclusive breastfeeding, EBF (only breastmilk being given to the infant without any other food or liquid, except medically prescribed drugs or vitamins); 2) predominant breastfeeding, PBF (breastmilk with some liquid-based food, such as juice, tea, sugar-water and salt-water, including glucose without any kind of formula, or animal milk); and 3) mixed feeding, MF

(breastmilk with other solid or liquid-based food, including other kinds of milk). We thereafter combined EBF and PBF into one group called “exclusive or predominant breastfeeding” (EPBF)) as PBF presented few cases and was assessed as having much the same risk as EBF, at least with regard to postnatal HIV transmission [30].

During the follow-up visits, the mothers underwent a clinical assessment, including weight measurement and HIV-1 infection staging at the first screening visit, day 7 post-partum, weeks 26 and 50; CD4 cell count analysis at screening one, weeks 26 and 50; and HIV-1 viral load at screening one, day 7, weeks 6, 14, 26, 38 and 50. The dependant variables were mothers’ weight, CD4 cell count and HIV-1 viral load considered separately. We generated a new variable called “weight loss”, which was calculated as the mothers’ weight at W26 (mothers ‘weights were not available for week 50) minus the baseline weight at day 7 postpartum, which was compared to the baseline weight to assess if the loss had reached 10%. Furthermore, we combined CD4 cell count, mothers’ weight loss and HIV disease stage as per WHO classification to create the composite endpoint called “HIV-1 disease progression”. HIV-1 disease progression was accelerated when CD4 cell count decreased to<350 Cells/µl, or the HIV-1 infection was assessed by the trial physician at stage 3 or above, or the mothers lost >10% of their weight; otherwise, HIV-1 disease progression was deemed absent or slow. Our main independent variable was EPBF (until week 26 post-partum) or any breastfeeding (until week 50 post-partum) duration.

We first ran linear mixed-effect models that considered separately the mothers’ weight, CD4 cell count and HIV-1 viral load changes as dependant variables, and EPBF or any breastfeeding as key independent variables. When the inter-country variability was not significant, a linear multivariate regression analysis was run. We ran a logistic regression regarding the composite endpoint. Adjustment covariates included the mother’s baseline BMI, education level, marital status, hemoglobin concentration, mode of delivery, breastfeeding initiation time and the baby’s gender, and the trial arm. These multivariate analyses were run taking all participants together and also as 2 strata comprising South African mothers (stratum 1) and Burkina Faso, Uganda and Zambia together (stratum 2) because South Africa presented important socio-economic, cultural and demographic differences compared with the other countries. STATA/SE 13.1 statistical software has been used for the analyses.

Ethics

Prior to enrolment, the mothers signed a written informed consent and assent form for themselves and their children, respectively. The trial was conducted according to the sponsor (ANRS) ethic charter, Good Clinical Practices and the principles of the Helsinki declaration.

The protocol had obtained approval from the relevant ethical committees, including the Ethical Committee for Health Research in Burkina Faso, the Biomedical Research Ethics Committee in Zambia, the Uganda National Council for Science and Technology, the Stellenbosch University ethical committees and the Medicines Control Council in South Africa.

RESULTS

In the ANRS 12174 trial, 1,273 mother-infant pairs were randomized and 6 were excluded due to protocol violations. Of the remaining 1,267 participants, 204 were from Ouagadougou, 222 from East London, 278 from Mbale and 563 from Lusaka. In all, 42 were excluded from analysis due to lack of breastfeeding data after inclusion, 7 due to inaccurate feeding duration data and 2 women had no data on weights. The analysis included 1,216 subjects. The mean baseline weight, the percentage of educated and employed women was highest, and the mean EPBF and any breastfeeding durations shortest in South Africa where the HIV-1 viral suppression was also most important (Table 1).

Table 1:

Table 1a: Baseline characteristics (continuous variables)

	Burkina Faso	**South Africa**	**Uganda**	**Zambia**	**All sites**
	N=203	N=212	N=272	N=529	N=1216
	Mean (95% CI)	mean (95% CI)	mean (95% CI)	mean (95% CI)	mean (95% CI)
Mean duration of AZT regimen post-delivery (days)	6.6 (6.5; 6.8)	7 (7.0; 7.0)	6.8 (6.7; 6.9)	7.0 (6.9; 7.0)	6.9 (6.8; 7.0
Mean duration 3TC regimen post-delivery (days)	6.6 (6.5; 6.8)	Data not available	6.7 (6.6; 6.8)	7.0 (6.9; 7.0)	6.8 (6.8; 6.9)
Mean baseline CD4 count*10^2cel/µl	5.6 (5.4; 5.8)	5.5 (5.3;5.7)	5.6 (5.4; 5.8)	6.0 (5.8;6.2)	5.8 (5.7; 5.9)
Mean baseline viral load*10^3 copies/µl	23.0 (7.3; 38.7)	13.5 (7.5; 19.6)	34.9 (19.7; 50.0)	29.1 (21.5; 36.6)	26.4 (21.1; 31.8)
Baseline mothers' weight (kg)	62.9 (61.4; 64.5)	72.1 (70.0; 74.1)	58.1 (57.0; 59.2)	62.0 (61.0; 62.9)	63.0 (62.3; 63.7)
Mean EPBF duration	6.3 (6.2; 6.4)	4.8 (4.7; 4.9)	5.6 (5.5; 5.7)	6.0 (5.9; 6.1)	5.8 (5.7; 5.9)

(months)					
Mean breastfeeding duration (months)	10.5 (10.4; 10.6)	6.7 (6.6; 6.8)	8.4 (8.3; 8.5)	8.4 (8.3; 8.5)	8.4 (8.3; 8.5

Table 1b: Baseline characteristics (categorical variables)

	Burkina Faso	**South Africa**	**Uganda**	**Zambia**	**All sites**
	N=203	N=212	N=272	N=529	N=1216
	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)
Mother's age group					
Below 25 years	26.2 (20.5; 32.6)	34.4 (28.3; 41.1)	39.3 (33.7; 45.3)	37.8 (33.8; 42.0)	35.6 (33.0; 38.3)
25 – 30 years	36.9 (30.6; 43.8)	31.2 (25.2; 37.7)	35.7 (30.2; 41.5)	33.1 (29.2; 37.2)	34.0 (31.3; 36.7)
30 and above	36.9 (30.6; 43.8)	34.4 (28.3; 41.1)	25.0 (20.2; 30.5)	29.1 (25.4; 33.1)	30.4 (27.9; 33.1)
HIV stage 1	93.1 (88.7; 95.9)	98.6 (95.7; 99.5)	92.3 (88.4; 94.9)	99.8 (98.7; 100.0)	96.8 (95.6; 97.6)
Education					
Uncomplete primary school	68.5 (61.7; 74.5)	8.5 (5.4; 13.1)	48.5 (42.6; 54.5)	28.2 (24.5; 32.2)	36.0 (33.4 ; 0.38.8)
Completed primary school	7.4 (4.5; 11.9)	0.5 (0.1; 3.3)	15.8 (11.9; 20.6)	18.5 (15.4; 22.1)	12.9 (11.1; 14.9)
Secondary school and more	24.1 (18.7; 30.5)	91.0 (86.4; 94.2)	35.7 (0.30.2; 41.5)	53.3 (49.0; 57.5)	51.1 (48.2; 53.9)
Marital status (married)	90.6 (85.8; 94.0)	39.1 (32.8; 45.9)	82.0 (76.9; 86.1)	88.7 (85.7; 91.1)	78.9 (76.5; 81.1)
Occupation (employed)	8.9 (5.6; 13.6)	41.5 (35.0; 48.3)	35.3 (29.8; 41.2)	17.0 (14.0; 20.5)	24.0 (21.7; 26.5)
Primipara	21.7 (16.5; 27.9)	33.5 (27.4; 40.1)	18.0 (13.9; 23.0)	20.6 (17.4; 24.3)	22.4 (20.2; 24.9)
Vaginal delivery	93.6 (89.3; 96.2)	65.1 (58.4; 71.2)	93.4 (89.7; 95.8)	96.2 (94.2; 97.5)	89.7 (87.9; 91.3)

Breastfeeding initiation time (within one hour)	6.9 (4.1; 11.3)	51.4 (44.7; 58.1)	55.9 (49.9; 61.7)	80.7 (77.1; 83.9)	57.7 (54.9; 60.5)
Lamivudine arm	49.7 (42.9; 56.6)	51.9 (45.1; 58.6)	49.6 (43.7; 55.6)	50.3 (46.0; 54.5)	50.3 (47.5; 53.1)
Female baby	41.9 (35.2; 48.8)	49.1 (42.4; 55.8)	52.9 (46.0; 58.8)	48.4 (44.1; 52.7)	48.4 (45.6; 51.2)

Overall in the adjusted model, the association between EPBF duration and weight change was negative and insignificant. Mothers who completed secondary school had a significant mean increase of 1.1 kg compared to those who did not complete primary school (Table 2). There was no weight change at univariate and multivariate analysis considering any breastfeeding duration (Table 3).

Table 2: Mother's weight, CD4 cells count and HIV-1 viral load change and HIV disease progression according to EPBF duration adjusted to different covariates: stratification presenting South Africa Vs the other sites and pooled analysis

	South Africa		Burkina Faso, Uganda and Zambia		Pooled analysis	
	Unadjusted coefficient (95% CI[a])	Adjusted coefficient (95% CI[a])	Unadjusted coefficient (95% CI[a])	Adjusted coefficient (95% CI[a])	Unadjusted coefficient (95% CI[a])	Adjusted coefficient (95% CI[a])
Dependant variable=mother's weight						
EPBF duration (months)	0.1 (-0.7; 0.9)	-0.2 (-0.6; 0.1)	-0.1 (-0.5; 0.3)	0.1 (-0.0; 0.3)	-0.2 (-0.5; 0.2)	-0.1 (-0.2; 0.1)
Baseline BMI[b]	2.5 (2.4; 2.7)	2.4 (2.3; 2.6)	2.5 (2.4; 2.6)	2.4 (2.3; 2.5)	2.5 (2.4; 2.6)	2.4 (2.3; 2.5)
Mother's age	0.8 (0.5; 1.2)	0.1 (0.0; 0.3)	0.5 (0.4; 0.6)	0.1 (0.1; 0.2)	0.5 (0.4; 0.7)	0.1 (0.1; 0.2)
HIV disease stage						
HIV stage 1						
HIV stage >1	12.4 (-4.5; 29.4)	6.7 (0.4; 13.1)	-2.8 (-6.4; 0.8)			
Education						
Not completed primary school			1	1	1	1
Completed primary school			3.9 (2.0; 5.9)	0.2 (-0.7; 1.1)	4.4 (2.2; 6.5)	0.6 (-0.3; 1.5)
Secondary school and more			2.7 (1.2; 4.1)	0.7 (0.1; 1.4)	3.1 (1.5; 4.6)	1.1 (0.4; 1.8)
Marital status						
Married/cohabiting	1				1	

mothers						
Single mothers	-3.1 (-7.2; 0.9)				-1.2 (-3.0; 0.5)	
Delivery						
Vaginal delivery	1		1		1	1
C-section delivery	3.7 (-0.4; 7.9)		4.5 (1.5; 7.6)		4.4 (2.1; 6.7)	-1.1 (-2.1; -0.1)
Parity						
Primipara	1		1		1	-
Multipara	6.1 (1.9; 10.3)		3.1 (1.4; 4.7)		3.9 (2.3; 5.4)	-
Trial arm						
Lamivudine arm	1	1	1	1	1	1
Lopinavir/ritonavir arm	2.0 (-2.0; 6.1)	0.8 (-0.7; 2.3)	-0.1 (-1.4; 1.3)	-0.3 (-0.9; 0.2)	0.3 (-1.0; 1.6)	-0.1 (-0.7; 0.4)
Dependent variable=CD4 count						
EPBF duration (months)	-1.0 (-8.9; 7.0)	-6.4 (-18.6; 5.8)	9.3 (2.3; 16.3)	7.9 (-4.2; 20.1)	5.4 (-0.1; 10.9)	4.5 (-6.2; 15.1)
Baseline BMI[b]			4.9 (2.4; 7.3)	5.9 (2.5; 9.2)	3.3 (1.3; 5.3)	4.9 (2.1; 7.7)
Mother's age	-3.1 (-7.5; 1.2)		-4.9 (-7.3; -2.5)	-6.2 (-8.6; -3.8)	-4.7 (-6.9; -2.6)	-6.2 (-8.4; -4.1)
Hemoglobin concentration	33.3 (12.7; 53.8)	34.8 (14.4; 55.1)	15.2 (7.8; 22.6)	12.9 (4.6; 21.2)	19.3 (12.3; 26.4)	19.4 (11.4; 27.4)
Breastfeeding initiation time						
Breastfeeding initiation within 1 h	1	1	1	1	1	-
Breastfeeding initiation	-56.2 (-94.9; -	-39.9 (-90.3;	-40.5 (-60.1; -20.9)		-42.5 (-61.1; -	-

after 1 h	17.4)	10.6)			23.9)	
Child's gender						
Male babies	1	1	1			
Female babies	-53.1 (-104.7; -1.6)	-52.9 (-103.0; -2.9)	21.8 (-3.9; 47.4)			
HIV disease stage						
HIV stage 1			1	1	1	1
HIV stage >1			-85.8 (-131.5; -40.2)	-86.5 (-147.1; -26.0)	-70.2 (-115.5; -25.0)	-83.7 (-144.1; -23.4)
Education						
Non completed primary school			1		1	1
Completed primary school			29.2 (-8.6; 67.1)		25.1 (-12.6; 62.9)	24.4 (-12.9; 61.6)
Secondary school and more			1.0 (-26.8; 28.9)		-7.8 (-34.9; 19.3)	-9.3 (-36.8; 18.2)
Marital status						
Married/cohabiting mothers			1	1	1	1
Single mothers			-34.5 (-73.2; 4.2)	-44.6 (-83.1; -6.03)	-24.6 (-55.9; 6.6)	-29.7 (-61.0; 1.6)
Delivery						
Vaginal delivery			1	1		

C-section delivery			71.6 (11.7; 131.4)	71.1 (11.1; 131.2)		
Trial arm						
Lamivudine arm	1	1	1	1	1	1
Lopinavir/ritonavir arm	-33.4 (-69.2; 2.3)	-65.3 (-116.4; -14.1)	-12.8 (-31.6; 6.1)	-12.9 (-38.2; 12.4)	-15.8 (-32.6; 1.0)	-19.2 (-41.9; 3.6)
Dependent variable=viral load (coefficient * 10^3)						
EPBF duration (months)	4.5 (-3.4; 12.4)	-3.6 (-11.5; 4.4)	5.4 (-7.1 18.0)	2.0(-11.3 15.4)	6.2 (-2.5; 14.9)	1.7 (-7.3; 10.8)
Baseline BMI[b]	-7.7 (-10.9; -4.6)	-14.5 (-17.9; -11.0)	-4.7 (-8.5; -1.0)	-5.7 (-9.8; -1.6)	-6.5 (-9.2; -3.8)	-8.0 (-11.0; -4.9)
Mother's age	-2.7 (-5.3; -0.1)	-2.7 (-5.5; 0.1)	-1.9 (-4.6; 0.8)	-4.5 (-7.7; -1.4)	-2.1 (-4.3; 0.1)	-4.5 (-7.0; -2.0)
Breastfeeding initiation time						
Breastfeeding initiation<1h	1	1				
Breastfeeding initiation>1h	70.5 (41.3; 99.7)	45.1 (13.5; 76.7)				
Child's gender						
Male babies	1	1	1	1	1	1
Female babies	-49.1 (-79.4-18.7)		-19.5 (-48.5; 9.4)	-36.5 (-66.0 -7.2)	-25.3 (-49.2; -1.4)	-35.2 (-59.2; -11.1)

Education						
Non completed primary school			1	1	1	1
Completed primary school			-10.2 (-53.9;33.5)	2.9 (-41.4; 47.2)	-5.0 (-45.1; 35.2)	13.4 (-26.9; 53.8)
Secondary school and more			-76.7 (-108.1; -45.3)	-73.4 (-105.9; -41.0)	-72.7 (-98.7; -46.7)	-62.0 (-89.7; -34.3)
Marital status						
Married/cohabiting mothers	1	1				
Single mothers	55.6 (21.6; 89.5)	127.9 (92.8 163.0)				
Delivery						
Vaginal delivery	1	1	1	1	1	1
C-section delivery	118.5 (86.4; 150.5)	143.2(108.8; 177.5)	72.6 (6.8; 138.4)	84.2 (17.6;150.7)	90.8 (49.8; 131.8)	105.5 (65.2; 145.7)
Parity						
Primipara	1	1	1	1	1	1
Multipara	66.5 (34.8; 98.2)	125.9 (90.5; 161.2)	47.7 (12.1; 83.2)	56.7 (15.1; 98.2)	54.8 (26.7; 83.0)	65.1 (32.8; 97.4)
Trial arm						
Lamivudine arm	1	1	1	1	1	1
Lopinavir/ritonavir arm	-48.4 (-77.6; -	-37.6 (-67.5; -	39.9 (12.4; 67.4)	47.0 (17.9;	22.6 (-0.0; 45.2)	31.1 (7.1; 55.0)

	19.2)	7.6)		76.1)		
Birthweight	0.0 (0.0 ; 0.1)	0.1 (0.0; 0.1)	0.0 (0.0; 0.1)	0.1 (0.0; 0.1)		
HIV disease progression						
	Unadjusted odd ratio (95% CI[a])	Adjusted odd ratio (95% CI[a])	Unadjusted odd ratio (95% CI[a])	Adjusted odd ratio (95% CI[a])	Unadjusted odd ratio (95% CI[a])	Adjusted odd ratio (95% CI[a])
EPBF duration (months)	1.0 (0.9; 1.1)		1.1 (1.0 1.2)	1.0 (0.9;1.1)	1.1 (1.0; 1.1)	1.1 (1.0; 1.2)
Mother's age			1.0 (1.0; 1.1)	1.0 (1.0; 1.1)		1.0 (1.0; 1.0)
Child's gender						
Male babies			1		1	1
Female babies			0.8 (0.6; 1.0)		0.8 (0.6; 1.0)	0.8 (0.6; 1.0)
HIV disease stage						
HIV stage 1			1	1		
HIV stage >1			4.0 (2.5; 6.2)	4.2 (2.6; 6.5)		
Marital status						
Married/cohabiting mothers			1	1	1	1
Single mothers			1.6 (1.1; 2.2)	1.8 (1.3; 2.6)	1.5 (1.2; 1.9)	1.6 (1.3; 2.1)
Trial arm						
Lamivudine arm			1	1	1	1
Lopinavir/ritonavir arm			1.3 (1.0; 1.6)	1.3 (1.0; 1.7)	1.3 (1.0; 1.6)	1.3 (1.0; 1.6)
Birthweight			0.9 (0.8; 1.0)			

[a]Confidence interval

[b]Body mass index

Table 3: Mother's weight, CD4 cell count and HIV viral load change and HIV disease progression according to any breastfeeding duration adjusted to different covariates: stratification presenting South Africa Vs the other sites and pooled analysis

	Unadjusted coefficient (95% CI[a])	Adjusted coefficient (95% CI[a])	Unadjusted coefficient (95% CI[a])	Adjusted coefficient (95% CI[a])	Unadjusted Odd Ratio (95% CI[a])	Adjusted odd ratio (95% CI[a]
	South Africa		**The other 3 countries**		**Pooled analysis**	
		Weight				
Any breastfeeding duration	0.3 (-0.2; 0.8)	-0.1 (-0.3; 0.0)	-0.0 (-0.3; 0.2)	0.1 (0.0; 0.3)	-0.0 (-0.3; 0.2)	-0.0 (-0.2; 0.1)
Baseline BMI[b]	2.5 (2.4; 2.7)	2.5 (2.3; 2.6)	2.5 (2.4; 2.6)	2.4 (2.3; 2.5)	2.5 (2.4; 2.6)	2.4 (2.3; 2.5)
Mothers' age	0.8 (0.5; 1.2)	0.2 (0.0; 0.3)	0.5 (0.4; 0.6)	0.1 (0.1; 0.2)	0.5 (0.4 0.7)	0.1 (0.1; 0.2)
HIV disease stage						
HIV stage 1	1	1	1			
HIV stage>1	12.4 (-4.5; 29.4)	6.4 (0.0; 12.7)	-2.8 (-6.4; 0.8)			
Education						
Non completed primary school			1	1	1	1

Completed primary school			3.9 (2.0; 5.9)	0.3 (-0.6; 1.1)	4.4 (2.2; 6.5)	0.6 (-0.3; 1.5)
Secondary school and further			2.7 (1.2; 4.1)	0.9 (0.2; 1.5)	3.1 (1.5; 4.6)	1.0 (0.4; 1.7)
Marital status						
Married/cohabiting mothers	1				1	
Single mothers	-3.1 (-7.2; 0.9)				-1.2 (-3.0; 0.5)	
Delivery						
Vaginal delivery			1		1	
C-section delivery	3.7 (-0.4; 7.9)	-1.6 (-3.2; 0.0)	4.5 (1.5; 7.6)		4.4 (2.1; 6.7)	-1.2 (-2.1; -0.2)
Parity						
Primipara	1	1	1	1	1	
Multipara	6.1 (1.9; 10.3)		3.1 (1.4; 4.7)		3.9 (2.3; 5.4)	
Trial arm						
Lamivudine arm	1	1	1	1	1	
Lopinavir/ritonavir arm	2.0 (-2.0; 6.1)	0.7 (-0.8; 2.2)	-0.1 (-1.4; 1.3)	-0.3 (-0.9; 0.3)	0.3 (-1.0; 1.6)	0.1 (-0.7; 0.4)
		CD4 cells count				
Any breastfeeding duration	0.4 (-6.8; 7.6)	-2.4 (-9.5; 4.7)	1.2 (-5.8; 8.3)	9.8 (-2.1; 21.8)	1.5 (-3.9; 7.0)	5.7 (0.4; 10.9)
Baseline BMI[b]			4.9 (2.4; 7.3)	5.7 (2.4; 9.1)	3.3 (1.3; 5.3)	4.2 (1.5; 6.9)
Mother's age	-3.1 (-7.5; 1.2)		-4.9 (-7.3; -2.5)	-6.5 (-8.9; -4.1)	-4.7 (-6.9; -2.6)	-6.2 (-8.4; -

						4.1)
Hemoglobin concentration	33.3 (12.7; 53.8)	33.9 (13.5; 54.3)	15.2 (7.8; 22.6)	15.7 (7.2; 24.3)	19.3 (12.3; 26.4)	16.7 (9.0; 24.4)
Breastfeeding initiation time						
Within 1 hour			1		1	
After 1 hour	-56.2 (-94.9; -17.4)		-40.5 (-60.1; -20.9)		-42.5 (-61.1; -23.9)	
Child's gender						
Male babies	1	1	1			
Female babies	-53.1 (-104.7; -1.6)	-54.1 (-104.3; -3.6)	21.7 (-3.9; 47.4)			
HIV stage						
HIV stage 1			1	1	1	1
HIV stage>1			-85.8 (-131.5; -40.2)	-91.5 (-152.9; -30.1)	-70.2 (-115.5; -25.0)	-88.8 (-148.3; -29.3)
Education						
Non completed primary school			1		1	1
Completed primary school			29.2 (-8.6; 67.1)		25.1 (-12.6; 62.9)	19.9 (-16.7; 56.5)
Secondary school and further			1.0 (-26.8; 28.9)		-7.8 (-34.9; 19.3)	-17.4 (-43.7; 9.5)

Marital status						
Married/ cohabiting mothers			1	1	1	1
Single mothers			-34.5 (-73.2; 4.2)	-43.1 (-81.5; -4.6)	-24.6 (-55.9; 6.6)	-43.3 (-72.8; -13.7)
Delivery						
Vaginal delivery			1	1		
C-section delivery			71.6 (11.7; 131.4)	71.8 (11.9; 131.7)		
Parity						
Primipara			1			
Multipara						
Trial arm						
Lamivudine arm	1	1	1	1	1	1
Lopinavir/ritonavir arm	-33.4 (-69.; 2.3)	-58.7 (-109.6; -7.8)	-12.8 (-31.6; 6.1)	-13.4 (-38.6; 11.8)	-15.8 (-32.6; 1.0)	-19.2 (-41.9; 3.6)
		HIV Viral load (coefficient*10^3) copies/µl				
Any breastfeeding duration	11.2 (6.8; 15.6)	7.7 (3.4; 12.1)	5.9 (-1.5; 13.2)	2.5 (-5.2; 10.2)	9.8 (4.9; 14.7)	6.1 (1.0; 11.2)
Baseline BMI[b]	-7.7 (-10.9; -4.6)	-14.0 (-17.4; -10.6)	-4.7 (-8.5; -1.0)	-5.7 (-9.8; -1.5)	-6.5 (-9.2; -3.8)	-7.6 (-10.7; -

						4.5)
Mother's age	-2.7 (-5.3; -0.1)	-3.4 (-6.2; -0.6)	-1.9 (-4.6; 0.8)	-4.6 (-7.8; -1.4)	-2.1 (-4.3; 0.1)	-4.8 (-7.3; -2.3)
Breastfeeding initiation time						
Within 1 hour	1	1				
After 1 hour	70.5 (41.3; 99.7)	34.2 (2.7; 65.7)				
Child's gender						
Male babies	1		1	1	1	1
Female babies	-49.1 (-79.4; -18.7)		-19.5 (-48.5; 9.4)	-37.2 (-66.6; -7.9)	-25.3 (-49.2; -1.4)	-36.2 (-60.2; -12.2)
Education						
Non completed primary school			1	1	1	1
Completed primary school			-10.2 (-53.9; 33.5)	4.7 (-39.8; 49.3)	-4.9 (-45.1; 35.2)	16.6 (-23.8; 57.0)
Secondary school and further			-76.7 (-108.1; -45.3)	-70.7 (-104.4; -37.1)	-72.7 (-98.7; -46.7)	-54.5 (-82.8; -26.1)
Marital status						
Married/ cohabiting mothers	1	1			1	
Single mothers	55.6 (21.6; 89.5)	124.9 (89.9; 160.0)				
Delivery						

Vaginal delivery	1	1	1	1	1	1
C-section delivery	118.5 (86.4; 150.5)	137.0 (102.7; 171.2)	72.6 (6.8; 138.4)	84.4 (18.0; 150.7)	90.8(49.8; 131.8)	104.7 (64.5; 144.9)
Parity						
Primipara	1	1	1	1	1	1
Multipara	66.5 (34.8; 98.3)	125.0 (89.8; 160.3)	47.7 (12.1; 83.2)	57.2 (15.6; 98.8)	54.8 (26.7; 83.0)	65.0 (32.7; 97.3)
Trial arm						
Lamivudine arm	1	1	1	1	1	1
Lopinavir/ritonavir arm	-48.4 (-77.6; -19.2)	-35.0 (-64.8; -5.3)	39.9 (12.4; 67.4)	47.6 (18.6; 76.7)	22.6 (-60.2; 45.2)	31.9 (8.0; 55.8)
		HIV disease progress				
Any breastfeeding duration			1.1 (1.0; 1.2)	1.0 (0.9; 1.1)	1.0 (0.9; 1.0)	1.0 (0.9; 1.0)
Baseline BMI[b]			1.0 (1.0; 1.1)			
Mother's age				1.0 (1.0; 1.1)	1.0 (0.9; 1.0)	
Breastfeeding initiation time						
Within 1 h	1					
After 1 h						
Child's gender						
Male babies			1		1	
Female babies			0.8 (0.6; 1.0)		0.8 (0.6; 1.0)	

HIV stage						
HIV stage 1			1	1	1	1
HIV stage>1			4.0 (2.5; 6.2)	4.2 (2.6; 6.6)	4.4 (2.8; 6.7)	4.6 (2.9; 7.3)
Education						
Non completed primary school			1			1
Completed primary school						1.4 (0.9; 2.0)
Secondary school and further						0.7 (0.5; 0.9)
Marital status						
Married/cohabiting mothers			1	1		
Single mothers			1.6 (1.1; 2.2)	1.8 (1.2; 2.6)	1.5 (1.2; 1.9)	
Trial arm						
Lamivudine arm			1	1	1	1
Lopinavir/ritonavir arm			1.3 (1.0; 1.6)	1.3 (1.0; 1.7)	1.3 (1.0; 1.6)	1.3 (1.0; 1.6)

[a]Confidence interval

[b]Body mass index

The association between CD4 cell count and EPBF duration was insignificant (5.4 (95% CI:-0.1; 10.9) and 4.5 (95% CI:-6.2; 15.1) CD4 cells/µl increase per month of EPBF duration at univariate and multivariate analysis, respectively. The association was significantly positive between the mothers' baseline BMI, hemoglobin concentration and CD4 cell count yielding a mean increase of 4.9 (95% CI: 2.1; 7.7) CD4 cells/µl per BMI unit and 19.4 (95% CI:11.4; 27.4) CD4 cells/µl per each additional hemoglobin gram/dl throughout the EPBF period (Table 2). Regarding any breastfeeding, there was a significant mean increase of 5.7 (95% CI: 0.4; 10.9) CD4 cells/µl per month and a mean decrease of -43.3 (95% CI: -72.8; -13.7) CD4 cells/ µl in single mothers compared to married ones (Table 3).

There was no significant association between HIV-1 viral load and EPBF duration. The heavier and older mothers, those who delivered female babies, and the best educated women group had a significantly lower mean viral load in the multivariate analysis. The mothers allocated to the lopinavir/ritonavir group had a significantly higher mean viral load than the ones in the Lamivudine arm (Table 2). Any breastfeeding duration was also associated with a significantly higher mean viral load (Table 3).

We found no significant association between EPBF duration and HIV-1 disease progression. However, randomization to the lopinavir/ritonavir arm or being single mother led to a significantly adjusted odd ratios (AOR) of 1.3 (95% CI: 1.0; 1.6; p=0.04) and 1.6 (95% CI: 1.3; 2.1), respectively (Table 2). Analysis with any breastfeeding pattern showed exactly the same associations (Table 3).

In the stratified analysis, we found that EPBF duration had no influence on mothers' weight, their CD4 count, or their HIV viral load, whatever the stratum. HIV disease progression was not accelerated either by EPBF duration. In stratum 2, C-section delivery was associated with an increase in CD4 cell count, whereas delivering a female baby and being educated beyond secondary school were associated with a decrease in HIV-1 viral load.

In South Africa, initiating breastfeeding one h post-delivery and being a single mother were related to an increase in HIV-1 viral load. In both strata, C-section delivery and multiparity were related to this increase in HIV-1 viral load. In South Africa situation, randomisation to the lopinavir/ritonavir arm was associated with a decrease, whereas it was associated in stratum 2 to an increase in HIV-1 viral load. Nevertheless, in stratum 2 and with respect to the HIV-1 disease progression, being in the lopinavir/ritonavir group prompted a border-line significant hazard ratio of 1.3 (Table 2). There was no association between any breastfeeding and the mothers' weight, CD4 count and HIV-1 disease progression, whatever the stratum. However, any breastfeeding duration tended to increase the HIV-1 viral load in South African women.

DISCUSSION

Considered separately, there appeared to be no variations in the mothers' weight, CD4 cell count and HIV-1 viral load related to EPBF or any breastfeeding. The same conclusion applied to these outcomes combined in a composite endpoint representing HIV-1 disease progression. Unsurprisingly, mothers' baseline BMIs were consistently associated with an increase in the mothers' weight and CD4 cell count, and with a lower mean HIV-1 viral load for both EPBF and any breastfeeding groups. Associated also with the study outcomes, but in an opposite direction, was the allocation of the babies to the lopinavir/ritonavir arm, which appeared to be associated with an acceleration of the mother's HIV-1 disease progression and a higher HIV-1 viral load.

In a review of the literature on weight change in the postpartum period, there appeared to be no report of an association between breastfeeding, or generally between the mode of infant feeding, and postpartum weight loss, but a risk factor for postpartum weight loss seemed to be delivery by C-section [31], similar to our findings. However, while this review of the literature [31] found that less educated mothers (<12 years of schooling) were at risk of postpartum weight retention, we found that higher educated women (secondary school or further) were at risk of that weight retention. In a further review of literature on the effects of lactation on the mother's bodyweight, it is clear that the assumption that the postpartum weight loss is due to the high energy demand associated with lactation has been challenged by many studies [32]. Some reports conflict with our own findings, such as one in KwaZulu Natal, where HIV-1 infected mothers at between 8 and 24 weeks had a mean weight loss of 1.4 kg in contrast to a 0.4-kg weight gain in HIV-1 uninfected mothers (P=0.01) during breastfeeding [19].

Regarding the change in CD4 cell count, the South African data supports the conclusion that CD4 cell count did not differ significantly between women who breastfed and those who did not [33], which is contrary of the Kenyan Study that found that the rate of CD4 cell count decline was higher in breast-feeding mothers than in mothers who never breast-fed [17]. However, in that Kenyan study, HIV-1 RNA levels did not differ significantly between breast-feeding mothers and women feeding their babies with formula.

Regarding HIV-1 disease progression, in Durban, South Africa, one report found no deleterious effect of breastfeeding in HIV-1-infected mothers, in agreement with our own results. This study had as outcome variables the CD4 and CD8 cell count, the mothers 'illness and mortality, and their hemoglobin levels [33]. Some reports from Malawi and South Africa reached the same conclusion that breastfeeding was not associated with higher risk of maternal morbidity or mortality [33 34]. In the South Africa study, the authors specifically

assessed the change in CD4 cell count and found no deleterious effect of breastfeeding. A study in Zambia concluded much the same, in that at 12 months after delivery, there was no difference in mortality between women who breastfed for a short duration (4 months) versus those who breastfed for a duration of their own choice [21]. An individual patient data meta-analysis on mortality among HIV-1 infected mothers according to children's feeding modality confirmed that the risk of dying within 18 months postpartum was not significantly affected by the infants' feeding modality (i.e. ever versus never breastfed) [35].

However, one report from Kenya found that HIV-1 infected breastfeeding mothers were more prone to death than HIV-1 infected non-breastfeeding mothers, with a relative risk of 3·2 (95% CI 1·3–8·1, p=0·01) [18]. Along with the flaws noted in the design of the study, another explanation could be that, in the context of HIV infection, breastfeeding is more often the choice among poorer women.

Strengths and limitations

Our study has been implemented in 4 Countries in Africa, including Burkina Faso (West), South Africa and Zambia (South) and Uganda (East), which we consider representative of much of the Sub-Saharan African population. The data were also collected in the rigorous context of a clinical trial, which minimized the lost to follow-up, the missing data as well as other data collection errors, and therefore improved the quality of our data.

However, the selection associated with the environment of a clinical trial - usually quite different from a routine environment - may have slightly biased our findings. Nonetheless, our endpoints (mother's weight, CD4 cell count and HIV-1 viral load) were sufficiently robust for us to vouch for their validity. Another point of note is the stratification of the participants into two strata, i.e. South Africa versus Burkina Faso, Uganda and Zambia. This stratification reduced the sample size in South Africa. Thus some of the modelling for South Africa could be less rigorous, and the findings regarding the risk factors here may not truly reflect the reality.

CONCLUSION

Breastfeeding as far as this study can conclude was not a risk factor for the HIV-1 infected mothers weight, CD4 cell count, and HIV-1 viral load change, or HIV-1 disease progression, keeping in mind that all the participants had a baseline CD4 cell count >350 cells/ul. The mothers' baseline high weight and high hemoglobin concentration were important factors in being consistently associated with an improvement of the outcome variables at stake. A higher education level was also a factor associated with a better HIV-1 infection status. Considering the benefits of breast milk for infants, and the consensus results from different

studies elsewhere that breastfeeding does not harm HIV-1-infected mothers, this study also supports the WHO 2016 guidelines on infant feeding, which indicates that mothers living with HIV should breastfeed for at least 12 months and up to 24 months, provided that the right treatment or prophylaxis for the infection is given where formula feeding is unsafe.

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Contributorship statement:

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STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract OK
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found OK
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported OK
Objectives	3	State specific objectives, including any prespecified hypotheses OK
Methods		
Study design	4	Present key elements of study design early in the paper OK
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection OK
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up OK *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed Not applicable *Case-control study*—For matched studies, give matching criteria and the number of controls per case NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable OK
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group OK
Bias	9	Describe any efforts to address potential sources of bias OK
Study size	10	Explain how the study size was arrived at NA
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why OK
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding OK
		(*b*) Describe any methods used to examine subgroups and interactions OK
		(*c*) Explain how missing data were addressed OK
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed OK *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses OK

Continued on next page

Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed OK
		(b) Give reasons for non-participation at each stage OK
		(c) Consider use of a flow diagram used in a previous related paper
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders OK
		(b) Indicate number of participants with missing data for each variable of interest No
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount) OK
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time N/A
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included OK
		(*b*) Report category boundaries when continuous variables were categorized NA
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses OK
Discussion		
Key results	18	Summarise key results with reference to study objectives OK
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias OK
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence OK
Generalisability	21	Discuss the generalisability (external validity) of the study results OK
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based OK

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

HIV-1 disease progression in immune-competent HIV-1 infected and breastfeeding mothers participating in the ANRS12174 clinical trial in Burkina Faso, South Africa, Uganda and Zambia: a cohort design.

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HIV-1 disease progression in immune-competent HIV-1 infected and breastfeeding mothers participating in the ANRS12174 clinical trial in Burkina Faso, South Africa, Uganda and Zambia: a cohort design

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Words count: 3097

Abstract

Objective

We have assessed HIV-1 disease progression among HIV-1 positive mothers in relation to duration of any or exclusive breastfeeding in the context of ANRS12174 trial (clinical trial no NCT0064026).

Methods

The analysis was completed on 203, 212, 272 and 529 HIV-1-positive and lactating mothers with CD4 count >350 cell/µl from Burkina Faso, South Africa, Uganda and Zambia, respectively. The trial compared Lamivudine and Lopinavir/Ritonavir as a peri-exposure prophylaxis during a 50-week follow-up time. A multiple logistic regression model was run with the mothers' weight, CD4 count and HIV-1 viral load as separate dependent variables, then combined into a dependent composite end-point called HIV-1 disease progression where HIV-1 viral load were replaced by the HIV-1 clinical stage. Exclusive or predominant breastfeeding and any breastfeeding duration were the key explanatory variables.

Results

In the adjusted model, the associations between EPBF duration and weight change, CD4 cell count and the HIV-1 viral load were consistently insignificant. The CD4 cell count was associated with a significantly higher mothers' body mass index (BMI; a mean increase of 4.9 (95% CI: 2.1; 7.7) CD4 cells/µl per each extra kilogram per square meter of BMI) and hemoglobin concentration (19.4 (95% CI: 11.4; 27.4) CD4 cells/µl per each extra gram per decilitre of hemoglobin concentration).

There was no significant association between EPBF duration and HIV-1 disease progression. However, randomization to the lopinavir/ritonavir arm was related to a significant acceleration of HIV-1 disease progression (adjusted odd ratio of 1.3 (95% CI: 1.0; 1.6; p=0.04) at the multivariate model). In South Africa any breastfeeding duration was associated with an increase of the HIV-1 viral load.

Conclusion

Breastfeeding was not a risk factor for a faster progression of HIV-1 disease in mothers of this cohort with a baseline CD4 cell count >350 cells/µl.

Keywords: HIV-1 infection; breastfeeding; Sub Saharan Africa

Strengths and limitations of this study

- Our study has been implemented in 4 Countries in Africa, namely Burkina Faso (West), South Africa and Zambia (South), and Uganda (East), which made our sample representative of the wider Sub-Sahara African population.
- The data were collected in the context of a rigorous clinical trial, which minimized the lost to follow-up, the missing data as well as other data collection errors, and therefore improved the quality of our data.
- However, the selection associated with the environment of a clinical trial, usually quite different from a routine environment, may have biased our findings. Also AZT and 3TC are usually administered together. However in our data collection tool (the questionnaire), the investigators had to ask specifically and separately the question for AZT and 3TC. We suspect that they may have been some reporting errors, creating slight differences in the percentages of women who complied with the prophylaxis requirements.
- Nonetheless, the variables analysed separately as dependent variables or as part of our composite end-points (mother's weight, CD4 cell count, HIV-1 viral load or HIV-1 clinical stage) were sufficiently robust and had a high validity.

INTRODUCTION

In 2015, 36.7 [34.0-39.8] million people were infected with HIV. Among them 17.4 [16.1-20.0] million were women of childbearing age [1 2]. HIV-1 prevalence is estimated between 5.3 and 6.5% among pregnant women in Sub-Saharan Africa [3]. Because of the almost irreversible immune activation involved, HIV-1 infection creates a condition of metabolic stress that may result in wasting and immune depression [4-7]. Ten per cent weight loss and a CD4 count of <350 cells/µl in the context of HIV-1 infection have been recognized as major criteria of the diagnosis of AIDS [8]. This weight loss is also associated with a higher risk of mortality in HIV-1-infected breastfeeding mothers [9]. Furthermore, HIV-1 is a major cause of maternal mortality in affected countries in Southern Africa. About 25% of pregnancy-related deaths in Sub-Saharan Africa are attributable to HIV [10], and 88% of deaths among pregnant and postpartum women with HIV infection are attributable to the virus [11].

In women, pregnancy is, though a physiological condition, a period of increased metabolic activities and synthesis requiring a supplement of energy and nutrients. After delivery, breastfeeding prolongs the increased metabolic demands. In spite of this, WHO still recommends HIV-1-infected women to breastfeed as the best choice for the infant and the mother [12] in contexts where replacement feeding does not meet AFASS (affordable, feasible, available, safe and sustainable) criteria.

There have been conflicting results on assessment of the impact of breastfeeding in HIV-1-

infected mothers. Some studies found that breastfeeding was harmful to HIV-positive mothers by either accelerating HIV disease progression as assessed by the mother's weight loss, a decrease in CD4 cells count, or even an increased risk of maternal mortality, suggesting that metabolic, immunologic or hormonal changes associated with breastfeeding may accelerate HIV-1 disease progression in postpartum mothers [13-15]. Others found no effect on the mothers' health assessed by death, development of a low CD4 cell count, anaemia or excessive weight loss [16 17]. Some studies have found breastfeeding protective, allowing weight gain in HIV-1 infected breastfeeding mothers [15 18-22].

In the ANRS12174 trial, we assessed mothers' HIV-1 disease progression (measured by the change in weight, CD4 cells count and HIV-1 disease stage as per WHO classification) in relation to exclusive breastfeeding or duration of any breastfeeding during the infant first 6 months of life and until week 50 post-partum.

METHODS

Study design

The ANRS 12174 clinical trial in Ouagadougou (Burkina Faso), East London (South Africa), Mbale (Uganda) and Lusaka (Zambia) was conducted from 2009 to 2013. The protocol and the main outcome have been published [23 24]. Briefly, a cohort of HIV-1 infected, pregnant women, at the time not eligible for highly active antiretroviral therapy because CD4 count was >350 cells/µl, aged 18 or above, planning to breastfeed were identified from antenatal clinics between 28 and 40 weeks of amenorrhea. As part of the HIV post-test counselling session, they were informed on the different feeding options for their babies. Only women intending to breastfeed were referred to the research clinic for further assessment of the inclusion criteria during the antenatal period and again with their child within 6 days after birth, for an enrolment and randomisation at day 7 postpartum. From 28 weeks of pregnancy to day 7 after birth, programmatic mother to child transmission prophylaxis was implemented with antepartum zidovudine, intrapartum single dose nevirapine and zidovudine-lamivudine for mothers and nevirapine for infants for 7 days postpartum. Twins and triplets, infants with positive HIV-1 DNA PCR test result at day 7 (+/- 2 days) postpartum, low birth-weight or ill babies (ranked grade II or above of the ANRS classification for adverse events) were excluded [25]. The intervention provided an infant prophylaxis in the breastfeeding period plus one week from day 7 to 50 weeks of age with either lopinavir/ritonavir or lamivudine.

Data management and analysis

Data was collected on a paper case-report form or directly entered online using the Electronic Data capture system: OpenClinica™ (www.openclinica.com). Twenty-four hour and one week breastfeeding recalls were collected during the enrolment visit at day 7±2 days after birth and the 13 monthly-scheduled follow-up visits that started at week 2. During these visits, mothers were asked in particular if they gave their infants other foods/liquids as well as breastmilk. Prelacteal feeding data - defined as any food item except mothers' milk given to infants before initial breastfeeding - were also collected at the enrolment visit.

The mothers at each visit were categorized into the following groups: 1) exclusive breastfeeding, EBF (only breastmilk being given to the infant without any other food or liquid, except medically prescribed drugs or vitamins); 2) predominant breastfeeding, PBF (breastmilk with some liquid-based food, such as juice, tea, sugar-water and salt-water, including glucose without any kind of formula, or animal milk); and 3) mixed feeding, MF (breastmilk with other solid or liquid-based food, including other kinds of milk). We thereafter combined EBF and PBF into one group called "exclusive or predominant breastfeeding" (EPBF)) as PBF presented few cases and was assessed as having much the same risk as EBF, at least with regard to postnatal HIV transmission [26].

During the follow-up visits, the mothers underwent a clinical assessment, including weight measurement and HIV-1 infection staging at the first screening visit or screening one (between 28 and 40 weeks of gestation), day 7 post-partum, weeks 26 and 50; CD4 cell count analysis at screening one, weeks 26 and 50; and HIV-1 viral load at screening one, day 7, weeks 6, 14, 26, 38 and 50. The dependant variables were mothers' weight, CD4 cell count and HIV-1 viral load considered separately and measured at the same time points as per above. We generated a new variable called "weight loss", which was calculated as the mothers' weight at W26 (because of missing data mothers 'weights were not available for week 50) minus the baseline weight at day 7 postpartum, which was compared to the baseline weight to assess if the loss had reached 10%. Furthermore, we combined CD4 cell count, mothers' weight loss and HIV-1 disease stage as per WHO classification to create the composite endpoint called "HIV-1 disease progression". HIV-1 disease progression was accelerated when CD4 cell count decreased to<350 Cells/µl, or the HIV-1 infection was assessed by the trial physician at stage 3 or above, or the mothers lost >10% of their weight; otherwise, HIV-1 disease progression was deemed absent or slow. Our main independent variable was EPBF (until week 26 post-partum) or any breastfeeding (until week 50 post-partum) duration. The data were collected by trained physicians, pharmacists, biologists and counsellors. Seca-brand scales and stadiometers were used to measure the mother's height and weight. Weights were rounded to the nearest 10 grams and the height at the nearest

millimetre. Weight and height were measured twice based on the WHO guidelines (http://www.who.int/childgrowth/training/en/).

We first ran linear mixed-effect models that considered separately the mothers' weight, CD4 cell count and HIV-1 viral load changes as dependant variables, and EPBF or any breastfeeding as key independent variables. The lost to follow up were censored in a survival analysis completed to build the EPBF and any breastfeeding variables [27]. When the inter-country variability was not significant, a linear multivariate regression analysis was run. We ran a logistic regression regarding the composite endpoint. Adjustment covariates included baseline variables measured at the screening one visit (BMI, education level, marital status, hemoglobin concentration) or on day 7 postpartum (mode of delivery, breastfeeding initiation time, the baby's gender, and the trial arm). These multivariate analyses were run taking all participants together and also as 2 strata comprising South African mothers (stratum 1) and Burkina Faso, Uganda and Zambia together (stratum 2) because South Africa presented important socio-economic, cultural and demographic differences compared with the other countries. For continuous variables, the mean values with 95% confidence interval (CI) were estimated, and for categorical variables, percentages were used. Associations between variables were tested using the Chi-square test for categorical variables. STATA/SE 13.1 statistical software has been used for the analyses.

Ethics

Prior to enrolment, the mothers signed a written informed consent and assent form for themselves and their children, respectively. The trial was conducted according to the sponsor (ANRS) ethic charter, Good Clinical Practices and the principles of the Helsinki declaration. The protocol had obtained approval from the relevant ethical committees, including the Ethical Committee for Health Research in Burkina Faso (EC N° 2008-039), the Biomedical Research Ethics Committee in Zambia (EC N° 008-02-08), the Uganda National Council for Science and Technology (EC N° HS470), the Stellenbosch University ethical committees and the Medicines Control Council in South Africa (EC N° 20090938).

RESULTS

In the ANRS 12174 trial, 1,273 mother-infant pairs were randomized and 6 were excluded due to protocol violations. Of the remaining 1,267 participants, 204 were from Ouagadougou, 222 from East London, 278 from Mbale and 563 from Lusaka. In all, 42 were excluded from analysis due to lack of breastfeeding data after inclusion, 7 due to inaccurate feeding duration data and 2 women had no data on weights. The analysis included 1,216 subjects. The complete flow chart has been published elsewhere [27]. The

mean baseline weight, the percentage of educated and employed women was highest, and the mean EPBF and any breastfeeding durations shortest in South Africa where the HIV-1 viral load was also the lowest (Table 1a and 1b).

Table 1:

Table 1a: Baseline characteristics collected at screening one or on day 7 postpartum and breastfeeding duration data (continuous variables)

	Burkina Faso	**South Africa**	**Uganda**	**Zambia**	**All sites**
	N=203	N=212	N=272	N=529	N=1216
	Mean (95% CI)	mean (95% CI)	mean (95% CI)	mean (95% CI)	mean (95% CI)
Mean duration of AZT regimen post-delivery (days)	6.6 (6.5; 6.8)	7 (7.0; 7.0)	6.8 (6.7; 6.9)	7.0 (6.9; 7.0)	6.9 (6.8; 7.0)
Mean duration 3TC regimen post-delivery (days)	6.6 (6.5; 6.8)	Data not available	6.7 (6.6; 6.8)	7.0 (6.9; 7.0)	6.8 (6.8; 6.9)
Mean baseline CD4 count*10^2cel/µl	5.6 (5.4; 5.8)	5.5 (5.3;5.7)	5.6 (5.4; 5.8)	6.0 (5.8;6.2)	5.8 (5.7; 5.9)
Mean baseline viral load*10^3 copies/µl	23.0 (7.3; 38.7)	13.5 (7.5; 19.6)	34.9 (19.7; 50.0)	29.1 (21.5; 36.6)	26.4 (21.1; 31.8)
Baseline mothers' weight (kg)	62.9 (61.4; 64.5)	72.1 (70.0; 74.1)	58.1 (57.0; 59.2)	62.0 (61.0; 62.9)	63.0 (62.3; 63.7)
Mean EPBF duration (months)	6.3 (6.2; 6.4)	4.8 (4.7; 4.9)	5.6 (5.5; 5.7)	6.0 (5.9; 6.1)	5.8 (5.7; 5.9)
Mean breastfeeding duration (months)	10.5 (10.4; 10.6)	6.7 (6.6; 6.8)	8.4 (8.3; 8.5)	8.4 (8.3; 8.5)	8.4 (8.3; 8.5)

Table 1b: Baseline characteristics collected at screening one or on day 7 postpartum and breastfeeding duration data (categorical variables).

	Burkina Faso	**South Africa**	**Uganda**	**Zambia**	**All sites**
	N=203	N=212	N=272	N=529	N=1216
	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)
Mother's age group					
Below 25 years	26.2 (20.5; 32.6)	34.4 (28.3; 41.1)	39.3 (33.7; 45.3)	37.8 (33.8; 42.0)	35.6 (33.0; 38.3)
25 – 30 years	36.9 (30.6; 43.8)	31.2 (25.2; 37.7)	35.7 (30.2; 41.5)	33.1 (29.2; 37.2)	34.0 (31.3; 36.7)
30 and above	36.9 (30.6; 43.8)	34.4 (28.3; 41.1)	25.0 (20.2; 30.5)	29.1 (25.4; 33.1)	30.4 (27.9; 33.1)
HIV stage 1	93.1 (88.7; 95.9)	98.6 (95.7; 99.5)	92.3 (88.4; 94.9)	99.8 (98.7; 100.0)	96.8 (95.6; 97.6)
Education					
Uncomplete primary school	68.5 (61.7; 74.5)	8.5 (5.4; 13.1)	48.5 (42.6; 54.5)	28.2 (24.5; 32.2)	36.0 (33.4 ; 0.38.8)
Completed primary school	7.4 (4.5; 11.9)	0.5 (0.1; 3.3)	15.8 (11.9; 20.6)	18.5 (15.4; 22.1)	12.9 (11.1; 14.9)
Secondary school and more	24.1 (18.7; 30.5)	91.0 (86.4; 94.2)	35.7 (0.30.2; 41.5)	53.3 (49.0; 57.5)	51.1 (48.2; 53.9)
Marital status (married)	90.6 (85.8; 94.0)	39.1 (32.8; 45.9)	82.0 (76.9; 86.1)	88.7 (85.7; 91.1)	78.9 (76.5; 81.1)
Occupation (employed)	8.9 (5.6; 13.6)	41.5 (35.0; 48.3)	35.3 (29.8; 41.2)	17.0 (14.0; 20.5)	24.0 (21.7; 26.5)
Primipara	21.7 (16.5; 27.9)	33.5 (27.4; 40.1)	18.0 (13.9; 23.0)	20.6 (17.4; 24.3)	22.4 (20.2; 24.9)
Vaginal delivery	93.6 (89.3; 96.2)	65.1 (58.4; 71.2)	93.4 (89.7; 95.8)	96.2 (94.2; 97.5)	89.7 (87.9; 91.3)
Breastfeeding initiation time (within one hour)	6.9 (4.1; 11.3)	51.4 (44.7; 58.1)	55.9 (49.9; 61.7)	80.7 (77.1; 83.9)	57.7 (54.9; 60.5)
Lamivudine arm	49.7 (42.9; 56.6)	51.9 (45.1; 58.6)	49.6 (43.7; 55.6)	50.3 (46.0; 54.5)	50.3 (47.5; 53.1)
Female baby	41.9 (35.2; 48.8)	49.1 (42.4; 55.8)	52.9 (46.0; 58.8)	48.4 (44.1; 52.7)	48.4 (45.6; 51.2)

Overall in the adjusted model, the association between EPBF duration and weight change was negative and non-significant. Mothers who

completed secondary school had a significant mean increase of 1.1 kg compared to those who did not complete primary school (Table 2a).).

Table 2:

Table 2a: Mother's weight change according to EPBF duration adjusted to different covariates: stratification presenting South Africa versus the other sites and pooled analysis

	South Africa		Burkina Faso, Uganda and Zambia		Pooled analysis	
	Unadjusted coefficient (95% CI[a])	Adjusted coefficient (95% CI[a])	Unadjusted coefficient (95% CI[a])	Adjusted coefficient (95% CI[a])	Unadjusted coefficient (95% CI[a])	Adjusted coefficient (95% CI[a])
Dependant variable=mother's weight						
EPBF duration (months)	0.1 (-0.7; 0.9)	-0.2 (-0.6; 0.1)	-0.1 (-0.5; 0.3)	0.1 (-0.0; 0.3)	-0.2 (-0.5; 0.2)	-0.1 (-0.2; 0.1)
Baseline BMI[b]	2.5 (2.4; 2.7)	2.4 (2.3; 2.6)	2.5 (2.4; 2.6)	2.4 (2.3; 2.5)	2.5 (2.4; 2.6)	2.4 (2.3; 2.5)
Mother's age	0.8 (0.5; 1.2)	0.1 (0.0; 0.3)	0.5 (0.4; 0.6)	0.1 (0.1; 0.2)	0.5 (0.4; 0.7)	0.1 (0.1; 0.2)
HIV disease stage						
HIV stage 1						
HIV stage >1	12.4 (-4.5; 29.4)	6.7 (0.4; 13.1)	-2.8 (-6.4; 0.8)			
Education						
Not completed primary school			1	1	1	1
Completed primary school			3.9 (2.0; 5.9)	0.2 (-0.7; 1.1)	4.4 (2.2; 6.5)	0.6 (-0.3; 1.5)
Secondary school and more			2.7 (1.2; 4.1)	0.7 (0.1; 1.4)	3.1 (1.5; 4.6)	1.1 (0.4; 1.8)

10

Marital status						
Married/cohabiting mothers	1				1	
Single mothers	-3.1 (-7.2; 0.9)				-1.2 (-3.0; 0.5)	
Delivery						
Vaginal delivery	1		1		1	1
C-section delivery	3.7 (-0.4; 7.9)		4.5 (1.5; 7.6)		4.4 (2.1; 6.7)	-1.1 (-2.1; -0.1)
Parity						
Primipara	1		1		1	-
Multipara	6.1 (1.9; 10.3)		3.1 (1.4; 4.7)		3.9 (2.3; 5.4)	-
Trial arm						
Lamivudine arm	1	1	1	1	1	1
Lopinavir/ritonavir arm	2.0 (-2.0; 6.1)	0.8 (-0.7; 2.3)	-0.1 (-1.4; 1.3)	-0.3 (-0.9; 0.2)	0.3 (-1.0; 1.6)	-0.1 (-0.7; 0.4)

[a]Confidence interval

[b]Body mass index

Table 2b: Mother's CD4 count change according to EPBF duration adjusted to different covariates: stratification presenting South Africa versus the other sites and pooled analysis

	South Africa		Burkina Faso, Uganda and Zambia		Pooled analysis	
	Unadjusted coefficient (95% CI[a])	Adjusted coefficient (95% CI[a])	Unadjusted coefficient (95% CI[a])	Adjusted coefficient (95% CI[a])	Unadjusted coefficient (95% CI[a])	Adjusted coefficient (95% CI[a])
Dependent variable=CD4 count						
EPBF duration (months)	-1.0 (-8.9; 7.0)	-6.4 (-18.6; 5.8)	9.3 (2.3; 16.3)	7.9 (-4.2; 20.1)	5.4 (-0.1; 10.9)	4.5 (-6.2; 15.1)
Baseline BMI[b]			4.9 (2.4; 7.3)	5.9 (2.5; 9.2)	3.3 (1.3; 5.3)	4.9 (2.1; 7.7)
Mother's age	-3.1 (-7.5; 1.2)		-4.9 (-7.3; -2.5)	-6.2 (-8.6; -3.8)	-4.7 (-6.9; -2.6)	-6.2 (-8.4; -4.1)
Hemoglobin concentration	33.3 (12.7; 53.8)	34.8 (14.4; 55.1)	15.2 (7.8; 22.6)	12.9 (4.6; 21.2)	19.3 (12.3; 26.4)	19.4 (11.4; 27.4)
Breastfeeding initiation time						
Breastfeeding initiation within 1 h	1	1	1	1	1	-
Breastfeeding initiation after 1 h	-56.2 (-94.9; -17.4)	-39.9 (-90.3; 10.6)	-40.5 (-60.1; -20.9)		-42.5 (-61.1; -23.9)	-
Child's gender						
Male babies	1	1	1			
Female babies	-53.1 (-104.7; -	-52.9 (-103.0; -	21.8 (-3.9; 47.4)			

12

	1.6)	2.9)				
HIV disease stage						
HIV stage 1			1	1	1	1
HIV stage >1			-85.8 (-131.5; -40.2)	-86.5 (-147.1; -26.0)	-70.2 (-115.5; -25.0)	-83.7 (-144.1; -23.4)
Education						
Non completed primary school			1		1	1
Completed primary school			29.2 (-8.6; 67.1)		25.1 (-12.6; 62.9)	24.4 (-12.9; 61.6)
Secondary school and more			1.0 (-26.8; 28.9)		-7.8 (-34.9; 19.3)	-9.3 (-36.8; 18.2)
Marital status						
Married/cohabiting mothers			1	1	1	1
Single mothers			-34.5 (-73.2; 4.2)	-44.6 (-83.1; -6.03)	-24.6 (-55.9; 6.6)	-29.7 (-61.0; 1.6)
Delivery						
Vaginal delivery			1	1		
C-section delivery			71.6 (11.7; 131.4)	71.1 (11.1; 131.2)		
Trial arm						
Lamivudine arm	1	1	1	1	1	1

Lopinavir/ritonavir arm	-33.4 (-69.2; 2.3)	-65.3 (-116.4; -14.1)	-12.8 (-31.6; 6.1)	-12.9 (-38.2; 12.4)	-15.8 (-32.6; 1.0)	-19.2 (-41.9; 3.6)

[a]Confidence interval

[b]Body mass index

Table 2c: Mother's HIV-1 viral load change according to EPBF duration adjusted to different covariates: stratification presenting South Africa versus the other sites and pooled analysis

	South Africa		Burkina Faso, Uganda and Zambia		Pooled analysis	
	Unadjusted coefficient (95% CI[a])	Adjusted coefficient (95% CI[a])	Unadjusted coefficient (95% CI[a])	Adjusted coefficient (95% CI[a])	Unadjusted coefficient (95% CI[a])	Adjusted coefficient (95% CI[a])
Dependent variable=viral load (coefficient * 10^3)						
EPBF duration (months)	4.5 (-3.4; 12.4)	-3.6 (-11.5; 4.4)	5.4 (-7.1 18.0)	2.0(-11.3 15.4)	6.2 (-2.5; 14.9)	1.7 (-7.3; 10.8)
Baseline BMI[b]	-7.7 (-10.9; -4.6)	-14.5 (-17.9; -11.0)	-4.7 (-8.5; -1.0)	-5.7 (-9.8; -1.6)	-6.5 (-9.2; -3.8)	-8.0 (-11.0; -4.9)
Mother's age	-2.7 (-5.3; -0.1)	-2.7 (-5.5; 0.1)	-1.9 (-4.6; 0.8)	-4.5 (-7.7; -1.4)	-2.1 (-4.3; 0.1)	-4.5 (-7.0; -2.0)
Breastfeeding initiation time						
Breastfeeding	1	1				

initiation<1h						
Breastfeeding initiation>1h	70.5 (41.3; 99.7)	45.1 (13.5; 76.7)				
Child's gender						
Male babies	1	1	1	1	1	1
Female babies	-49.1 (-79.4-18.7)		-19.5 (-48.5; 9.4)	-36.5 (-66.0 - 7.2)	-25.3 (-49.2; -1.4)	-35.2 (-59.2; -11.1)
Education						
Non completed primary school			1	1	1	1
Completed primary school			-10.2 (-53.9;33.5)	2.9 (-41.4; 47.2)	-5.0 (-45.1; 35.2)	13.4 (-26.9; 53.8)
Secondary school and more			-76.7 (-108.1; -45.3)	-73.4 (-105.9; -41.0)	-72.7 (-98.7; -46.7)	-62.0 (-89.7; -34.3)
Marital status						
Married/cohabiting mothers	1	1				
Single mothers	55.6 (21.6; 89.5)	127.9 (92.8 163.0)				
Delivery						
Vaginal delivery	1	1	1	1	1	1
C-section delivery	118.5 (86.4; 150.5)	143.2(108.8; 177.5)	72.6 (6.8; 138.4)	84.2 (17.6;150.7)	90.8 (49.8; 131.8)	105.5 (65.2; 145.7)

Parity						
Primipara	1	1	1	1	1	1
Multipara	66.5 (34.8; 98.2)	125.9 (90.5; 161.2)	47.7 (12.1; 83.2)	56.7 (15.1; 98.2)	54.8 (26.7; 83.0)	65.1 (32.8; 97.4)
Trial arm						
Lamivudine arm	1	1	1	1	1	1
Lopinavir/ritonavir arm	-48.4 (-77.6; -19.2)	-37.6 (-67.5; -7.6)	39.9 (12.4; 67.4)	47.0 (17.9; 76.1)	22.6 (-0.0; 45.2)	31.1 (7.1; 55.0)
Birthweight	0.0 (0.0 ; 0.1)	0.1 (0.0; 0.1)	0.0 (0.0; 0.1)	0.1 (0.0; 0.1)		

[a]Confidence interval

[b]Body mass index

Table 2d: Mother's HIV-1 disease progression according to EPBF duration adjusted to different covariates: stratification presenting South Africa versus the other sites and pooled analysis

	South Africa		Burkina Faso, Uganda and Zambia		Pooled analysis	
	Unadjusted odd ratio (95% CI[a])	Adjusted odd ratio (95% CI[a])	Unadjusted odd ratio (95% CI[a])	Adjusted odd ratio (95% CI[a])	Unadjusted odd ratio (95% CI[a])	Adjusted odd ratio (95% CI[a])
EPBF duration (months)	1.0 (0.9; 1.1)		1.1 (1.0 1.2)	1.0 (0.9;1.1)	1.1 (1.0; 1.1)	1.1 (1.0; 1.2)
Mother's age			1.0 (1.0; 1.1)	1.0 (1.0; 1.1)		1.0 (1.0; 1.0)
Child's gender						

Male babies			1		1	1
Female babies			0.8 (0.6; 1.0)		0.8 (0.6; 1.0)	0.8 (0.6; 1.0)
HIV disease stage						
HIV stage 1			1	1		
HIV stage >1			4.0 (2.5; 6.2)	4.2 (2.6; 6.5)		
Marital status						
Married/cohabiting mothers			1	1	1	1
Single mothers			1.6 (1.1; 2.2)	1.8 (1.3; 2.6)	1.5 (1.2; 1.9)	1.6 (1.3; 2.1)
Trial arm						
Lamivudine arm			1	1	1	1
Lopinavir/ritonavir arm			1.3 (1.0; 1.6)	1.3 (1.0; 1.7)	1.3 (1.0; 1.6)	1.3 (1.0; 1.6)
Birthweight			0.9 (0.8; 1.0)			

[a]Confidence interval

[b]Body mass index

Table 3

Table 3a: Mother's weight change according to any breastfeeding duration adjusted to different covariates: stratification presenting South Africa versus the other sites and pooled analysis

	South Africa		Burkina Faso, Uganda and Zambia		Pooled analysis	
	Unadjusted coefficient (95% CI[a])	**Adjusted coefficient (95% CI[a])**	**Unadjusted coefficient (95% CI[a])**	**Adjusted coefficient (95% CI[a])**	**Unadjusted Odd Ratio (95% CI[a])**	**Adjusted odd ratio (95% CI[a]**
		Weight				
Any breastfeeding duration	0.3 (-0.2; 0.8)	-0.1 (-0.3; 0.0)	-0.0 (-0.3; 0.2)	0.1 (0.0; 0.3)	-0.0 (-0.3; 0.2)	-0.0 (-0.2; 0.1)
Baseline BMI[b]	2.5 (2.4; 2.7)	2.5 (2.3; 2.6)	2.5 (2.4; 2.6)	2.4 (2.3; 2.5)	2.5 (2.4; 2.6)	2.4 (2.3; 2.5)
Mothers' age	0.8 (0.5; 1.2)	0.2 (0.0; 0.3)	0.5 (0.4; 0.6)	0.1 (0.1; 0.2)	0.5 (0.4 0.7)	0.1 (0.1; 0.2)
HIV disease stage						
HIV stage 1	1	1	1			
HIV stage>1	12.4 (-4.5; 29.4)	6.4 (0.0; 12.7)	-2.8 (-6.4; 0.8)			
Education						
Non completed primary school			1	1	1	1
Completed primary school			3.9 (2.0; 5.9)	0.3 (-0.6; 1.1)	4.4 (2.2; 6.5)	0.6 (-0.3; 1.5)
Secondary school and further			2.7 (1.2; 4.1)	0.9 (0.2; 1.5)	3.1 (1.5; 4.6)	1.0 (0.4; 1.7)

Marital status						
Married/cohabiting mothers	1				1	
Single mothers	-3.1 (-7.2; 0.9)				-1.2 (-3.0; 0.5)	
Delivery						
Vaginal delivery			1		1	
C-section delivery	3.7 (-0.4; 7.9)	-1.6 (-3.2; 0.0)	4.5 (1.5; 7.6)		4.4 (2.1; 6.7)	-1.2 (-2.1; -0.2)
Parity						
Primipara	1	1	1	1	1	
Multipara	6.1 (1.9; 10.3)		3.1 (1.4; 4.7)		3.9 (2.3; 5.4)	
Trial arm						
Lamivudine arm	1	1	1	1	1	
Lopinavir/ritonavir arm	2.0 (-2.0; 6.1)	0.7 (-0.8; 2.2)	-0.1 (-1.4; 1.3)	-0.3 (-0.9; 0.3)	0.3 (-1.0; 1.6)	0.1 (-0.7; 0.4)

[a]Confidence interval

[b]Body mass index

Table 3b: Mother's CD4 cell count change according to any breastfeeding duration adjusted to different covariates: stratification presenting South Africa versus the other sites and pooled analysis

		South Africa		Burkina Faso, Uganda and Zambia		Pooled analysis
	Unadjusted coefficient (95% CI[a])	**Adjusted coefficient (95% CI[a])**	**Unadjusted coefficient (95% CI[a])**	**Adjusted coefficient (95% CI[a])**	**Unadjusted Odd Ratio (95% CI[a])**	**Adjusted odd ratio (95% CI[a]**
		CD4 cells count				
Any breastfeeding duration	0.4 (-6.8; 7.6)	-2.4 (-9.5; 4.7)	1.2 (-5.8; 8.3)	9.8 (-2.1; 21.8)	1.5 (-3.9; 7.0)	5.7 (0.4; 10.9)
Baseline BMI[b]			4.9 (2.4; 7.3)	5.7 (2.4; 9.1)	3.3 (1.3; 5.3)	4.2 (1.5; 6.9)
Mother's age	-3.1 (-7.5; 1.2)		-4.9 (-7.3; -2.5)	-6.5 (-8.9; -4.1)	-4.7 (-6.9; -2.6)	-6.2 (-8.4; -4.1)
Hemoglobin concentration	33.3 (12.7; 53.8)	33.9 (13.5; 54.3)	15.2 (7.8; 22.6)	15.7 (7.2; 24.3)	19.3 (12.3; 26.4)	16.7 (9.0; 24.4)
Breastfeeding initiation time						
Within 1 hour			1		1	
After 1 hour	-56.2 (-94.9; -17.4)		-40.5 (-60.1; -20.9)		-42.5 (-61.1; -23.9)	
Child's gender						
Male babies	1	1	1			

Female babies	-53.1 (-104.7; -1.6)	-54.1 (-104.3; -3.6)	21.7 (-3.9; 47.4)			
HIV stage						
HIV stage 1			1	1	1	1
HIV stage>1			-85.8 (-131.5; -40.2)	-91.5 (-152.9; -30.1)	-70.2 (-115.5; -25.0)	-88.8 (-148.3; -29.3)
Education						
Non completed primary school			1		1	1
Completed primary school			29.2 (-8.6; 67.1)		25.1 (-12.6; 62.9)	19.9 (-16.7; 56.5)
Secondary school and further			1.0 (-26.8; 28.9)		-7.8 (-34.9; 19.3)	-17.4 (-43.7; 9.5)
Marital status						
Married/ cohabiting mothers			1	1	1	1
Single mothers			-34.5 (-73.2; 4.2)	-43.1 (-81.5; -4.6)	-24.6 (-55.9; 6.6)	-43.3 (-72.8; -13.7)
Delivery						
Vaginal delivery			1	1		
C-section delivery			71.6 (11.7; 131.4)	71.8 (11.9; 131.7)		
Parity						
Primipara			1			

Multipara						
Trial arm						
Lamivudine arm	1	1	1	1	1	1
Lopinavir/ritonavir arm	-33.4 (-69.; 2.3)	-58.7 (-109.6; -7.8)	-12.8 (-31.6; 6.1)	-13.4 (-38.6; 11.8)	-15.8 (-32.6; 1.0)	-19.2 (-41.9; 3.6)

[a]Confidence interval

[b]Body mass index

Table 3c: Mother's HIV-1 viral load change according to any breastfeeding duration adjusted to different covariates: stratification presenting South Africa versus the other sites and pooled analysis

	South Africa		**Burkina Faso, Uganda and Zambia**		**Pooled analysis**	
	Unadjusted coefficient (95% CI[a])	**Adjusted coefficient (95% CI[a])**	**Unadjusted coefficient (95% CI[a])**	**Adjusted coefficient (95% CI[a])**	**Unadjusted Odd Ratio (95% CI[a])**	**Adjusted odd ratio (95% CI[a]**
		HIV-1 Viral load (coefficient*10^3) copies/µl				
Any breastfeeding duration	11.2 (6.8; 15.6)	7.7 (3.4; 12.1)	5.9 (-1.5; 13.2)	2.5 (-5.2; 10.2)	9.8 (4.9; 14.7)	6.1 (1.0; 11.2)
Baseline BMI[b]	-7.7 (-10.9; -	-14.0 (-17.4; -10.6)	-4.7 (-8.5; -1.0)	-5.7 (-9.8; -	-6.5 (-9.2; -3.8)	-7.6 (-10.7; -

	4.6)			1.5)		4.5)
Mother's age	-2.7 (-5.3; -0.1)	-3.4 (-6.2; -0.6)	-1.9 (-4.6; 0.8)	-4.6 (-7.8; -1.4)	-2.1 (-4.3; 0.1)	-4.8 (-7.3; -2.3)
Breastfeeding initiation time						
Within 1 hour	1	1				
After 1 hour	70.5 (41.3; 99.7)	34.2 (2.7; 65.7)				
Child's gender						
Male babies	1		1	1	1	1
Female babies	-49.1 (-79.4; -18.7)		-19.5 (-48.5; 9.4)	-37.2 (-66.6; -7.9)	-25.3 (-49.2; -1.4)	-36.2 (-60.2; -12.2)
Education						
Non completed primary school			1	1	1	1
Completed primary school			-10.2 (-53.9; 33.5)	4.7 (-39.8; 49.3)	-4.9 (-45.1; 35.2)	16.6 (-23.8; 57.0)
Secondary school and further			-76.7 (-108.1; -45.3)	-70.7 (-104.4; -37.1)	-72.7 (-98.7; -46.7)	-54.5 (-82.8; -26.1)
Marital status						
Married/ cohabiting mothers	1	1			1	
Single mothers	55.6 (21.6;	124.9 (89.9; 160.0)				

	89.5)					
Delivery						
Vaginal delivery	1	1	1	1	1	1
C-section delivery	118.5 (86.4; 150.5)	137.0 (102.7; 171.2)	72.6 (6.8; 138.4)	84.4 (18.0; 150.7)	90.8(49.8; 131.8)	104.7 (64.5; 144.9)
Parity						
Primipara	1	1	1	1	1	1
Multipara	66.5 (34.8; 98.3)	125.0 (89.8; 160.3)	47.7 (12.1; 83.2)	57.2 (15.6; 98.8)	54.8 (26.7; 83.0)	65.0 (32.7; 97.3)
Trial arm						
Lamivudine arm	1	1	1	1	1	1
Lopinavir/ritonavir arm	-48.4 (-77.6; -19.2)	-35.0 (-64.8; -5.3)	39.9 (12.4; 67.4)	47.6 (18.6; 76.7)	22.6 (-60.2; 45.2)	31.9 (8.0; 55.8)

[a]Confidence interval

[b]Body mass index

Table 3d: Mother's HIV-1 disease progression according to any breastfeeding duration adjusted to different covariates: stratification presenting South Africa versus the other sites and pooled analysis

	South Africa		Burkina Faso, Uganda and Zambia		Pooled analysis	
	Unadjusted odd ratio (95% CI[a])	Adjusted odd ratio (95% CI[a])	Unadjusted odd ratio (95% CI[a])	Adjusted odd ratio (95% CI[a])	Unadjusted odd ratio (95% CI[a])	Adjusted odd ratio (95% CI[a]
		HIV disease progress				
Any breastfeeding duration			1.1 (1.0; 1.2)	1.0 (0.9; 1.1)	1.0 (0.9; 1.0)	1.0 (0.9; 1.0)
Baseline BMI[b]			1.0 (1.0; 1.1)			
Mother's age				1.0 (1.0; 1.1)	1.0 (0.9; 1.0)	
Breastfeeding initiation time						
Within 1 h	1					
After 1 h						
Child's gender						
Male babies			1		1	
Female babies			0.8 (0.6; 1.0)		0.8 (0.6; 1.0)	
HIV stage						
HIV stage 1			1	1	1	1

HIV stage>1			4.0 (2.5; 6.2)	4.2 (2.6; 6.6)	4.4 (2.8; 6.7)	4.6 (2.9; 7.3)
Education						
Non completed primary school			1			1
Completed primary school						1.4 (0.9; 2.0)
Secondary school and further						0.7 (0.5; 0.9)
Marital status						
Married/cohabiting mothers			1	1		
Single mothers			1.6 (1.1; 2.2)	1.8 (1.2; 2.6)	1.5 (1.2; 1.9)	
Trial arm						
Lamivudine arm			1	1	1	1
Lopinavir/ritonavir arm			1.3 (1.0; 1.6)	1.3 (1.0; 1.7)	1.3 (1.0; 1.6)	1.3 (1.0; 1.6)

[a]Confidence interval

[b]Body mass index

The association between CD4 cells count and EPBF duration was non-significant (5.4 (95% CI:-0.1; 10.9) and 4.5 (95% CI:-6.2; 15.1) CD4 cells/µl increase per month of EPBF duration at univariate and multivariate analysis, respectively. The association was significantly positive between the mothers' baseline BMI, hemoglobin concentration and CD4 cell count yielding a mean increase of 4.9 (95% CI: 2.1; 7.7) CD4 cells/µl per BMI unit and 19.4 (95% CI:11.4; 27.4) CD4 cells/µl per each additional unit of hemoglobin throughout the EPBF period (Table 2b).

There was no significant association between HIV-1 viral load and EPBF duration. The heavier and older mothers, those who delivered female babies and the best educated women group had a significantly lower mean viral load in the multivariate analysis. The mothers allocated to the lopinavir/ritonavir group had a significantly higher mean viral load than the ones in the Lamivudine arm (Table 2c).

We found no significant association between EPBF duration and HIV-1 disease progression. However, randomization to the lopinavir/ritonavir arm or being single mother led to a significantly adjusted odd ratios (AOR) of 1.3 (95% CI: 1.0; 1.6; p=0.04) and 1.6 (95% CI: 1.3; 2.1), respectively (Table 2d).

There was no weight change at univariate and multivariate analysis considering any breastfeeding duration (Table 3a). Still regarding any breastfeeding, overall, there was a significant mean increase of 5.7 (95% CI: 0.4; 10.9) CD4 cells/µl per month of any breastfeeding. We found also that being a single mother was associated with a mean decrease of -43.3 (95% CI: -72.8; -13.7) CD4 cells/ µl as compared to married ones (Table 3b). Any breastfeeding duration was also associated with a significantly higher mean viral load (Table 3c). Analysis with any breastfeeding pattern and HIV-1 disease progression showed the same associations as EPBF and HIV-1 disease progression (Table 3d).

In the stratified analysis, we found that EPBF duration had no influence on mothers' weight, CD4 count, or HIV viral load, whatever the stratum. HIV disease progression was not associated either with EPBF duration (Table 2a, 2b, 2c, 2d). In stratum 2, C-section delivery was associated with an increase in CD4 cell count (Table 2b), whereas delivering a female baby and being educated beyond secondary school were associated with a decrease in HIV-1 viral load (Table 2c).

In South Africa, initiating breastfeeding one hour post-delivery and being a single mother were related to an increase in HIV-1 viral load. In both strata, C-section delivery and

multiparity were also related to an increase in HIV-1 viral load. Being randomised to the lopinavir/ritonavir arm in South Africa was associated with a decrease in HIV-1 viral load, while being randomised to the lopinavir/ritonavir arm from another country participating in the trial was associated to an increase in HIV-1 viral load (Table 2c). When it comes to HIV-1 disease progression, being randomised to the lopinavir/ritonavir arm from another country than South Africa was associated with a more rapid progression of the HIV-1 disease. However the confidence interval was border-line significant with an adjusted hazard ratio of 1.3 (95% CI: 1.0; 1.7) (Table 2d). There was no association between any breastfeeding and the mothers' weight, CD4 cells count and HIV-1 disease progression in any of the strata (Table 3a, 3b, 3d). However, any breastfeeding duration was associated with an increase of the HIV-1 viral load in South African women (Table 3c).

DISCUSSION

Considered separately, there appeared to be no variations in the mothers' weight, CD4 cell count and HIV-1 viral load related to EPBF or any breastfeeding. The same conclusion applied to these outcomes combined in a composite endpoint representing HIV-1 disease progression. Unsurprisingly, mothers' baseline BMIs were consistently associated with an increase in the mothers' weight and CD4 cell count, and with a lower mean HIV-1 viral load for both EPBF and any breastfeeding groups. Associated also with the study outcomes, but in an opposite direction, was the allocation of the babies to the lopinavir/ritonavir arm, which appeared to be associated with an acceleration of the mother's HIV-1 disease progression and a higher HIV-1 viral load. We do not have any clear explanation for this finding. We think that this may be due to chance.

In a review of the literature on weight change in the postpartum period, there appeared to be no association between breastfeeding, or generally between the mode of infant feeding, and postpartum weight loss. However C-section delivery was a risk factor for postpartum weight loss [28], similar to our findings. South Africa had markedly lower rates of vaginal deliveries versus other countries (table 1b). In the years 2000, studies were published demonstrating that elective C-section before the labour and before the rupture of membranes added protection against HIV transmission to the new born [29 30]. The lower rates of vaginal deliveries in South Africa was likely due to the country policies (influenced by the scientific evidence) which supported HIV-infected women toward delivering HIV-free babies. This support included, free formulas and probably scheduled C-section for the HIV-infected pregnant women and mothers. Why the rest of the countries did not implement the same policy is certainly a matter of affordability and availability of local resources. Another reason

is that C-section rate is «recklessly high» in South Africa where up to 90% of pregnant women deliver through this method in private hospitals (The Guardian https://www.theguardian.com/world/2014/sep/24/caesarean-section-south-africa [Accessed on 27 October 2017]. This practise may have spilled over but at a lesser extent into public health facilities. We believe this practice has not skewed our results, since these C-section deliveries were not medically indicated at first hand, at least not based on a vaginal delivery risk, then they are not done on women with poorer health status. Actually, South Africa women had the lowest mean HIV viral load and the highest mean BMI.

Yet, this review of literature [28] found that less educated mothers (<12 years of schooling) were at risk of postpartum weight retention; we found that higher educated women (secondary school or further) were at risk of that weight retention. This difference in our finding may be explained by the difference in our categorization of the education variable. In our study less educated participants included only women with primary school level, meaning around six years of schooling. Therefore, the results of the two studies are not realy comparable. In a further review of literature on the effects of lactation on the mother's bodyweight, it is clear that the assumption that the postpartum weight loss is due to the high energy demand associated with lactation has been challenged by many studies [31]. Some reports conflict with our own findings, such as the one in KwaZulu Natal, where HIV-1 infected mothers at between 8 and 24 weeks had a mean weight loss of 1.4 kg in contrast to a 0.4-kg weight gain in HIV-1 uninfected mothers (P=0.01) during breastfeeding [15].

Regarding the change in CD4 cells count, the South African data support the conclusion that CD4 cell count did not differ significantly between women who breastfed and those who did not [32]. This finding contradicts the Kenyan Study that found that the rate of CD4 cell count decline was higher in breast-feeding than in non-breast-feeding mothers [13]. However, in that Kenyan study, HIV-1 RNA levels did not differ significantly between breast-feeding and formula-feeding mothers.

Regarding HIV-1 disease progression, the same data showed no deleterious effect of breastfeeding in HIV-1-infected mothers, similar to our study-findings. The outcome variables were the CD4 and CD8 cell count, the mothers 'illness and mortality, and their hemoglobin levels [32]. Another study from Malawi reached the same conclusion that breastfeeding was not associated with higher risk of maternal morbidity or mortality [33]. A study in Zambia concluded in the same direction that at 12 months after delivery, there was no difference in mortality between women who breastfed for a short duration (4 months) versus those who breastfed for a duration of their own choice [17]. An individual patient data meta-analysis on mortality among HIV-1 infected mothers according to children's feeding modality confirmed that the risk of dying within 18 months postpartum was not significantly affected by the

infants' feeding modality (i.e. ever versus never breastfed) [34].

In healthy breastfeeding mothers, the postpartum weight loss would be around 0.5 kg per month among population with relatively high mean of BMI. The mechanism of the weight loss would be burning of 483 -538 kcal per day [35 36]. Therefore, losing weigh after birth is likely when the mother's calorie intake does not cover the calorie expense related to breast-milk production. Considering these findings, we think that energy requirement and thus the metabolic stress related to breastfeeding would be quite bearable. This may explain why in our study HIV-1-infected, immune-competent and breastfeeding mothers health status was not deteriorated by breastfeeding. This evidence inspires the idea that option A peri-exposure antiretroviral prophylaxis might still have pertinent indications since breastfeeding remained the most frequent feeding option in Sub Saharan Africa and since breast-milk might still host HIV-1 reservoirs that mother's prophylaxis could not always 100% suppress [37].

Strengths and limitations

Our study has been implemented in four countries in Africa, including Burkina Faso (West), South Africa and Zambia (South) and Uganda (East). Therefore we consider our study population representative of the Sub-Sahara African population. The data were also collected in the rigorous context of a clinical trial, which minimized the lost to follow-up, the missing data as well as other data collection errors, and therefore improved the quality of our data.

However, the selection associated with the environment of a clinical trial - usually quite different from a routine environment - may have biased our findings. Nonetheless, our endpoints (mother's weight, CD4 cell count and HIV-1 viral load) were sufficiently robust for us to vouch for their validity. Another point of note is the stratification of the participants into two strata, i.e. South Africa versus Burkina Faso, Uganda and Zambia. This stratification reduced the sample size in South Africa. Thus some of the modelling for South Africa could be less rigorous, and the findings regarding the risk factors there may not truly reflect the reality.

CONCLUSION

Breastfeeding as far as this study can conclude was not a risk factor for the HIV-1 infected mothers weight, CD4 cell count, and HIV-1 viral load change, or HIV-1 disease progression, keeping in mind that all the participants had a baseline CD4 cell count >350 cells/ul. The mothers' baseline high weight and high hemoglobin concentration were important factors in being consistently associated with an improvement of the outcome variables at stake. A higher education level was also a factor associated with a slower HIV-1 disease progression.

Considering the benefits of breast milk for infants, and the consensus results from different studies elsewhere that breastfeeding does not harm HIV-1-infected mothers, this study also supports the WHO 2016 guidelines on infant feeding, which indicates that mothers living with HIV should breastfeed for at least 12 months and up to 24 months, provided that the right treatment or prophylaxis for the infection is given where formula feeding is unsafe [12].

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Data Availability Statement: The study sponsor (the French agency for research on HIV and viral hepatitis: ANRS) offers data sharing upon request. ANRS will be the contact organisation (direction@anrs.fr). The shared data will be those presented in the article.

Contributorship statement:

Conceptualization: ENS, IMSE, NN, NM, PVP, TT.

Data curation: ENS, RV.

Formal analysis: ENS, IMSE.

Investigation: ENS, MS, NM, JKT, CK, JGH.

Methodology: ENS, IMSE, TT.

Project administration: NN, PVP, TT, NM.

Resources: TT, IMSE.

Supervision: TT, IMSE, NM, NN.

Validation: TT, IMSE, NM, NN.

Writing the original draft: ENS, IMSE.

Writing and review and editing: ENS, IMSE, NN, NM, TT, RV, CK, JKT, JGH, MS, KH

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STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract: **YES ; page 1 row 3**
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found: **YES; page2 row 8-28**
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported: **YES; page 3 row 23-37 ; page 4 row 1-8**
Objectives	3	State specific objectives, including any prespecified hypotheses: **YES; page 4 row 10-13**
Methods		
Study design	4	Present key elements of study design early in the paper: **YES page 4 row 10-34**
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection: **YES page 4 row 17-18**
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up: **YES page 4 row 20-22 and row 30-32**
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable: **YES page 5 row 19-35**
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group: **YES page 5 row 10-18 and 35-37 ; page 6 row 1-2**
Bias	9	Describe any efforts to address potential sources of bias: **YES page 6 row 2-11**
Study size	10	Explain how the study size was arrived at: **YES page 6 row 30-35**
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why: **YES page 6 row 15-17**
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding: **YES page 6 row 3-17**
		(*b*) Describe any methods used to examine subgroups and interactions: **YES page 6 row 6-8 and 11-15**
		(*c*) Explain how missing data were addressed**: YES page page 6 row 5-6 and 32-34**
		(d) If applicable, explain how loss to follow-up was addressed **YES page 6 row 5-6**
		(*e*) Describe any sensitivity analyses: **page page 6 row 11-15**
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed: **YES; page 6 row 30-35**
		(b) Give reasons for non-participation at each stage: **YES; page 6 row 30-35**
		(c) Consider use of a flow diagram: **YES; page 6 row 35**
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders: **YES tables 1a and 1b**
		(b) Indicate number of participants with missing data for each variable of interest: **Yes page 6 row 31-35**
		(c) Summarise follow-up time (eg, average and total amount) **YES (mean EPBF**

		and any breastfeeding) table 1a
Outcome data	15*	Report numbers of outcome events or summary measures over time **NO**
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included: **YES tables 2 and 3**
		(*b*) Report category boundaries when continuous variables were categorized: **YES table 1b (mothers'age)**
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period: **not relevant**
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses: **YES page 6 row 11-17**
Discussion		
Key results	18	Summarise key results with reference to study objectives: **YES page 28 row 14-23**
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias: **YES page 30 row 21-28**
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence: **YES page 30 row 25-28 and 32-34**
Generalisability	21	Discuss the generalisability (external validity) of the study results: **YES page 30 row 16-20**
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based: **YES page 31 row 20-27**

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

BMJ Open

HIV-1 disease progression in immune-competent HIV-1 infected and breastfeeding mothers participating in the ANRS12174 clinical trial in Burkina Faso, South Africa, Uganda and Zambia: a cohort study

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Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Nutrition and metabolism, Epidemiology
Keywords:	HIV & AIDS < INFECTIOUS DISEASES, Tropical medicine < INFECTIOUS DISEASES, NUTRITION & DIETETICS

HIV-1 disease progression in immune-competent HIV-1 infected and breastfeeding mothers participating in the ANRS12174 clinical trial in Burkina Faso, South Africa, Uganda and Zambia: a cohort study

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Words count: 3097

Abstract

Objective

We have assessed HIV-1 disease progression among HIV-1 positive mothers in relation to duration of any or exclusive breastfeeding in the context of ANRS12174 trial (clinical trial no NCT0064026).

Methods

The analysis was completed on 203, 212, 272 and 529 HIV-1-positive and lactating mothers with CD4 count >350 cell/µl from Burkina Faso, South Africa, Uganda and Zambia, respectively. The trial compared Lamivudine and Lopinavir/Ritonavir as a peri-exposure prophylaxis during a 50-week follow-up time. A multiple logistic regression model was run with the mothers' weight, CD4 count and HIV-1 viral load as separate dependent variables, then combined into a dependent composite end-point called HIV-1 disease progression where HIV-1 viral load was replaced by the HIV-1 clinical stage. Exclusive or predominant breastfeeding and any breastfeeding duration were the key explanatory variables.

Results

In the adjusted model, the associations between EPBF duration and weight change, CD4 cell count and the HIV-1 viral load were consistently insignificant. The CD4 cell count was associated with a significantly higher mothers' body mass index (BMI; a mean increase of 4.9 (95% CI: 2.1; 7.7) CD4 cells/µl per each additional kilogram per square meter of BMI) and hemoglobin concentration (19.4 (95% CI: 11.4; 27.4) CD4 cells/µl per each additional gram per decilitre of hemoglobin concentration).

There was no significant association between EPBF duration and HIV-1 disease progression. A higher education level was a factor associated with a slower HIV-1 disease progression

Conclusion

Breastfeeding was not a risk factor for a faster progression of HIV-1 disease in mothers of this cohort with a baseline CD4 cell count >350 cells/µl.

Keywords: HIV-1 infection; breastfeeding; Sub Saharan Africa

Strengths and limitations of this study

- Our study has been implemented in 4 Countries in Africa, namely Burkina Faso (West), South Africa and Zambia (South), and Uganda (East), which made our

sample representative of the wider Sub-Sahara African population.

- The data were collected in the context of a rigorous clinical trial, which minimized the lost to follow-up, the missing data as well as other data collection errors, and therefore improved the quality of our data.
- However, the selection associated with the environment of a clinical trial, usually quite different from a routine environment, may have biased our findings.
- Nonetheless, the variables analysed separately as dependent variables or as part of our composite end-points (mother's weight, CD4 cell count, HIV-1 viral load or HIV-1 clinical stage) were sufficiently robust and had a high validity.

INTRODUCTION

In 2015, 36.7 [34.0-39.8] million people were infected with HIV. Among them 17.4 [16.1-20.0] million were women of childbearing age [1 2]. HIV-1 prevalence is estimated between 5.3 and 6.5% among pregnant women in Sub-Saharan Africa [3]. Because of the almost irreversible immune activation involved, HIV-1 infection creates a condition of metabolic stress that may result in wasting and immune depression [4-7]. Ten per cent weight loss and a CD4 count of <350 cells/µl in the context of HIV-1 infection have been recognized as major criteria of the diagnosis of AIDS [8]. This weight loss is also associated with a higher risk of mortality in HIV-1-infected breastfeeding mothers [9]. Furthermore, HIV-1 is a major cause of maternal mortality in affected countries in Southern Africa. About 25% of pregnancy-related deaths in Sub-Saharan Africa are attributable to HIV [10], and 88% of deaths among pregnant and postpartum women with HIV infection are attributable to the virus [11].

In women, pregnancy is, though a physiological condition, a period of increased metabolic activities and synthesis requiring a supplement of energy and nutrients. After delivery, breastfeeding prolongs the increased metabolic demands. In spite of this, WHO still recommends HIV-1-infected women to breastfeed as the best choice for the infant and the mother [12] in contexts where replacement feeding does not meet AFASS (affordable, feasible, available, safe and sustainable) criteria.

There have been conflicting results on assessment of the impact of breastfeeding in HIV-1-infected mothers. Some studies found that breastfeeding was harmful to HIV-positive mothers by either accelerating HIV disease progression as assessed by the mother's weight loss, a decrease in CD4 cells count, or even an increased risk of maternal mortality, suggesting that metabolic, immunologic or hormonal changes associated with breastfeeding may accelerate HIV-1 disease progression in postpartum mothers [13-15]. Others found no effect on the mothers' health assessed by death, development of a low CD4 cell count,

anaemia or excessive weight loss [16 17]. Some studies have found breastfeeding protective, allowing weight gain in HIV-1 infected breastfeeding mothers [15 18-22].

In the ANRS12174 trial, we assessed mothers' HIV-1 disease progression (measured by the change in weight, CD4 cells count and HIV-1 disease stage as per WHO classification) in relation to exclusive breastfeeding or duration of any breastfeeding during the infant first 6 months of life and until week 50 post-partum.

METHODS

Study design

The ANRS 12174 clinical trial in Ouagadougou (Burkina Faso), East London (South Africa), Mbale (Uganda) and Lusaka (Zambia) was conducted from 2009 to 2013. The protocol and the main outcome have been published [23 24]. Briefly, a cohort of HIV-1 infected, pregnant women, at the time not eligible for highly active antiretroviral therapy because CD4 count was >350 cells/µl, aged 18 or above, planning to breastfeed were identified from antenatal clinics between 28 and 40 weeks of amenorrhea. As part of the HIV post-test counselling session, they were informed on the different feeding options for their babies. Only women intending to breastfeed were referred to the research clinic for further assessment of the inclusion criteria during the antenatal period and again with their child within 6 days after birth, for an enrolment and randomisation at day 7 postpartum. From 28 weeks of pregnancy to day 7 after birth, programmatic mother to child transmission prophylaxis was implemented with antepartum zidovudine, intrapartum single dose nevirapine and zidovudine-lamivudine for mothers and nevirapine for infants for 7 days postpartum. Twins and triplets, infants with positive HIV-1 DNA PCR test result at day 7 (+/- 2 days) postpartum, low birth-weight or ill babies (ranked grade II or above of the ANRS classification for adverse events) were excluded [25]. The intervention provided an infant prophylaxis in the breastfeeding period plus one week from day 7 to 50 weeks of age with either lopinavir/ritonavir or lamivudine.

Data management and analysis

Data was collected on a paper case-report form or directly entered online using the Electronic Data capture system: OpenClinica™ (www.openclinica.com). Twenty-four hour and one week breastfeeding recalls were collected during the enrolment visit at day 7±2 days after birth and the 13 monthly-scheduled follow-up visits that started at week 2. During these visits, mothers were asked in particular if they gave their infants other foods/liquids as well as breastmilk. Prelacteal feeding data - defined as any food item

except mothers' milk given to infants before initial breastfeeding - were also collected at the enrolment visit.

The mothers at each visit were categorized into the following groups: 1) exclusive breastfeeding, EBF (only breastmilk being given to the infant without any other food or liquid, except medically prescribed drugs or vitamins); 2) predominant breastfeeding, PBF (breastmilk with some liquid-based food, such as juice, tea, sugar-water and salt-water, including glucose without any kind of formula, or animal milk); and 3) mixed feeding, MF (breastmilk with other solid or liquid-based food, including other kinds of milk). We thereafter combined EBF and PBF into one group called "exclusive or predominant breastfeeding" (EPBF)) as PBF presented few cases and was assessed as having much the same risk as EBF, at least with regard to postnatal HIV transmission [26].

During the follow-up visits, the mothers underwent a clinical assessment, including weight measurement and HIV-1 infection staging at the first screening visit or screening one (between 28 and 40 weeks of gestation), day 7 post-partum, weeks 26 and 50; CD4 cell count analysis at screening one, weeks 26 and 50; and HIV-1 viral load at screening one, day 7, weeks 6, 14, 26, 38 and 50. The dependant variables were mothers' weight, CD4 cell count and HIV-1 viral load considered separately and measured at the same time points as per above. We generated a new variable called "weight loss", which was calculated as the mothers' weight at W26 (because of missing data, mothers 'weights were not available for week 50) minus the baseline weight at day 7 postpartum, which was compared to the baseline weight to assess if the loss had reached 10%. Furthermore, we combined CD4 cell count, mothers' weight loss and HIV-1 disease stage as per WHO classification to create the composite endpoint called "HIV-1 disease progression". HIV-1 disease progression was accelerated when CD4 cell count decreased to<350 Cells/µl, or the HIV-1 infection was assessed by the trial physician at stage 3 or above, or the mothers lost >10% of their weight; otherwise, HIV-1 disease progression was deemed absent or slow. Our main independent variable was EPBF (until week 26 post-partum) or any breastfeeding (until week 50 post-partum) duration. The data were collected by trained physicians, pharmacists, biologists and counsellors. Seca-brand scales and stadiometers were used to measure the mother's height and weight. Weights were rounded to the nearest 10 grams and the height to the nearest millimetre. Weight and height were measured twice based on the WHO guidelines (http://www.who.int/childgrowth/training/en/).

We first ran linear mixed-effect models that considered separately the mothers' weight, CD4 cell count and HIV-1 viral load changes as dependant variables, and EPBF or any breastfeeding as key independent variables. The lost to follow up were censored in a survival analysis completed to build the EPBF and any breastfeeding variables [27]. When the inter-country variability was not significant, a linear multivariate regression analysis was run. We

ran a logistic regression regarding the composite endpoint. Adjustment covariates included baseline variables measured at the screening one visit (BMI, education level, marital status, hemoglobin concentration) or on day 7 postpartum (mode of delivery, breastfeeding initiation time, the baby's gender, and the trial arm). These multivariate analyses were run taking all participants together and also as 2 strata comprising South African mothers (stratum 1) and Burkina Faso, Uganda and Zambia together (stratum 2) because South Africa presented important socio-economic, cultural and demographic differences compared with the other countries. For continuous variables, the mean values with 95% confidence interval (CI) were estimated, and for categorical variables, percentages were used. Associations between variables were tested using the Chi-square test for categorical variables. STATA/SE 13.1 statistical software has been used for the analyses.

Ethics

Prior to enrolment, the mothers signed a written informed consent and assent forms for themselves and their children, respectively. The trial was conducted according to the sponsor (ANRS) ethic charter, Good Clinical Practices and the principles of the Helsinki declaration. The protocol had obtained approval from the relevant ethical committees, including the Ethical Committee for Health Research in Burkina Faso (EC N° 2008-039), the Biomedical Research Ethics Committee in Zambia (EC N° 008-02-08), the Uganda National Council for Science and Technology (EC N° HS470), the Stellenbosch University ethical committees and the Medicines Control Council in South Africa (EC N° 20090938).

RESULTS

In the ANRS 12174 trial, 1,273 mother-infant pairs were randomized and 6 were excluded due to protocol violations. Of the remaining 1,267 participants, 204 were from Ouagadougou, 222 from East London, 278 from Mbale and 563 from Lusaka. In all, 42 were excluded from analysis due to lack of breastfeeding data after inclusion, 7 due to inaccurate feeding duration data and 2 women had no data on weights. The analysis included 1,216 subjects. The complete flow chart has been published elsewhere [27]. The mean baseline weight, the percentage of educated and employed women was highest, and the mean EPBF and any breastfeeding durations shortest in South Africa where the HIV-1 viral load was also the lowest (Table 1a and 1b).

Table 1:

Table 1a: Baseline characteristics collected at screening one or on day 7 postpartum and breastfeeding duration data (continuous variables)

	Burkina Faso	**South Africa**	**Uganda**	**Zambia**	**All sites**
	N=203	N=212	N=272	N=529	N=1216
	Mean (95% CI)	mean (95% CI)	mean (95% CI)	mean (95% CI)	mean (95% CI)
Mean duration of AZT regimen post-delivery (days)[a]	6.6 (6.5; 6.8)	7 (7.0; 7.0)	6.8 (6.7; 6.9)	7.0 (6.9; 7.0)	6.9 (6.8; 7.0)
Mean duration 3TC regimen post-delivery (days) [a]	6.6 (6.5; 6.8)	Data not available	6.7 (6.6; 6.8)	7.0 (6.9; 7.0)	6.8 (6.8; 6.9)
Mean baseline CD4 count*10^2cel/µl	5.6 (5.4; 5.8)	5.5 (5.3;5.7)	5.6 (5.4; 5.8)	6.0 (5.8;6.2)	5.8 (5.7; 5.9)
Mean baseline viral load*10^3 copies/µl	23.0 (7.3; 38.7)	13.5 (7.5; 19.6)	34.9 (19.7; 50.0)	29.1 (21.5; 36.6)	26.4 (21.1; 31.8)
Baseline mothers' weight (kg)	62.9 (61.4; 64.5)	72.1 (70.0; 74.1)	58.1 (57.0; 59.2)	62.0 (61.0; 62.9)	63.0 (62.3; 63.7)
Mean EPBF duration (months)	6.3 (6.2; 6.4)	4.8 (4.7; 4.9)	5.6 (5.5; 5.7)	6.0 (5.9; 6.1)	5.8 (5.7; 5.9)
Mean breastfeeding duration (months)	10.5 (10.4; 10.6)	6.7 (6.6; 6.8)	8.4 (8.3; 8.5)	8.4 (8.3; 8.5)	8.4 (8.3; 8.5)

[a] AZT and 3TC are usually administered together. However in our data collection tool (the questionnaire), the investigators had to ask specifically and separately the question for AZT and 3TC. We suspect that they may have been some reporting errors, creating slight differences in the percentages of women who complied with the prophylaxis requirements.

Table 1b: Baseline characteristics collected at screening one or on day 7 postpartum and breastfeeding duration data (categorical variables).

	Burkina Faso	South Africa	Uganda	Zambia	All sites
	N=203	N=212	N=272	N=529	N=1216
	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)
Mother's age group (years)					
Below 25	26.2 (20.5; 32.6)	34.4 (28.3; 41.1)	39.3 (33.7; 45.3)	37.8 (33.8; 42.0)	35.6 (33.0; 38.3)
25 – 30	36.9 (30.6; 43.8)	31.2 (25.2; 37.7)	35.7 (30.2; 41.5)	33.1 (29.2; 37.2)	34.0 (31.3; 36.7)
30 and above	36.9 (30.6; 43.8)	34.4 (28.3; 41.1)	25.0 (20.2; 30.5)	29.1 (25.4; 33.1)	30.4 (27.9; 33.1)
HIV stage 1	93.1 (88.7; 95.9)	98.6 (95.7; 99.5)	92.3 (88.4; 94.9)	99.8 (98.7; 100.0)	96.8 (95.6; 97.6)
Education					
Uncomplete primary school	68.5 (61.7; 74.5)	8.5 (5.4; 13.1)	48.5 (42.6; 54.5)	28.2 (24.5; 32.2)	36.0 (33.4 ; 0.38.8)
Completed primary school	7.4 (4.5; 11.9)	0.5 (0.1; 3.3)	15.8 (11.9; 20.6)	18.5 (15.4; 22.1)	12.9 (11.1; 14.9)
Secondary school and more	24.1 (18.7; 30.5)	91.0 (86.4; 94.2)	35.7 (0.30.2; 41.5)	53.3 (49.0; 57.5)	51.1 (48.2; 53.9)
Marital status (married)	90.6 (85.8; 94.0)	39.1 (32.8; 45.9)	82.0 (76.9; 86.1)	88.7 (85.7; 91.1)	78.9 (76.5; 81.1)
Occupation (employed)	8.9 (5.6; 13.6)	41.5 (35.0; 48.3)	35.3 (29.8; 41.2)	17.0 (14.0; 20.5)	24.0 (21.7; 26.5)
Primipara	21.7 (16.5; 27.9)	33.5 (27.4; 40.1)	18.0 (13.9; 23.0)	20.6 (17.4; 24.3)	22.4 (20.2; 24.9)
Vaginal delivery	93.6 (89.3; 96.2)	65.1 (58.4; 71.2)	93.4 (89.7; 95.8)	96.2 (94.2; 97.5)	89.7 (87.9; 91.3)
Breastfeeding initiation time (within one hour)	6.9 (4.1; 11.3)	51.4 (44.7; 58.1)	55.9 (49.9; 61.7)	80.7 (77.1; 83.9)	57.7 (54.9; 60.5)
Lamivudine arm	49.7 (42.9; 56.6)	51.9 (45.1; 58.6)	49.6 (43.7; 55.6)	50.3 (46.0; 54.5)	50.3 (47.5; 53.1)
Female baby	41.9 (35.2; 48.8)	49.1 (42.4; 55.8)	52.9 (46.0; 58.8)	48.4 (44.1; 52.7)	48.4 (45.6; 51.2)

Overall in the adjusted model, the association between EPBF duration and weight change was negative and non-significant. Mothers who completed secondary school had a significant mean increase of 1.1 kg compared to those who did not complete primary school (Table 2a).).

Table 2:

Table 2a: Mother's weight change according to EPBF duration adjusted to different covariates: stratification presenting South Africa versus the other sites and pooled analysis

	South Africa		Burkina Faso, Uganda and Zambia		Pooled analysis	
	Unadjusted coefficient (95% CI[a])	Adjusted coefficient (95% CI[a])	Unadjusted coefficient (95% CI[a])	Adjusted coefficient (95% CI[a])	Unadjusted coefficient (95% CI[a])	Adjusted coefficient (95% CI[a])
Dependant variable=mother's weight						
EPBF duration (months)	0.1 (-0.7; 0.9)	-0.2 (-0.6; 0.1)	-0.1 (-0.5; 0.3)	0.1 (-0.0; 0.3)	-0.2 (-0.5; 0.2)	-0.1 (-0.2; 0.1)
Baseline BMI[b] (kg/m^2)	2.5 (2.4; 2.7)	2.4 (2.3; 2.6)	2.5 (2.4; 2.6)	2.4 (2.3; 2.5)	2.5 (2.4; 2.6)	2.4 (2.3; 2.5)
Mother's age (years)	0.8 (0.5; 1.2)	0.1 (0.0; 0.3)	0.5 (0.4; 0.6)	0.1 (0.1; 0.2)	0.5 (0.4; 0.7)	0.1 (0.1; 0.2)
HIV disease stage						
HIV stage 1						
HIV stage >1	12.4 (-4.5; 29.4)	6.7 (0.4; 13.1)	-2.8 (-6.4; 0.8)			
Education						
Not completed primary school			1	1	1	1
Completed primary school			3.9 (2.0; 5.9)	0.2 (-0.7; 1.1)	4.4 (2.2; 6.5)	0.6 (-0.3; 1.5)
Secondary school and			2.7 (1.2; 4.1)	0.7 (0.1; 1.4)	3.1 (1.5; 4.6)	1.1 (0.4; 1.8)

more						
Marital status						
Married/cohabiting mothers	1				1	
Single mothers	-3.1 (-7.2; 0.9)				-1.2 (-3.0; 0.5)	
Delivery						
Vaginal delivery	1		1		1	1
C-section delivery	3.7 (-0.4; 7.9)		4.5 (1.5; 7.6)		4.4 (2.1; 6.7)	-1.1 (-2.1; -0.1)
Parity						
Primipara	1		1		1	-
Multipara	6.1 (1.9; 10.3)		3.1 (1.4; 4.7)		3.9 (2.3; 5.4)	-
Trial arm						
Lamivudine arm	1	1	1	1	1	1
Lopinavir/ritonavir arm	2.0 (-2.0; 6.1)	0.8 (-0.7; 2.3)	-0.1 (-1.4; 1.3)	-0.3 (-0.9; 0.2)	0.3 (-1.0; 1.6)	-0.1 (-0.7; 0.4)

[a]Confidence interval

[b]Body mass index

Table 2b: Mother's CD4 count change according to EPBF duration adjusted to different covariates: stratification presenting South Africa versus the other sites and pooled analysis

	South Africa		Burkina Faso, Uganda and Zambia		Pooled analysis	
	Unadjusted coefficient (95% CI[a])	Adjusted coefficient (95% CI[a])	Unadjusted coefficient (95% CI[a])	Adjusted coefficient (95% CI[a])	Unadjusted coefficient (95% CI[a])	Adjusted coefficient (95% CI[a])
Dependent variable=CD4 count						
EPBF duration (months)	-1.0 (-8.9; 7.0)	-6.4 (-18.6; 5.8)	9.3 (2.3; 16.3)	7.9 (-4.2; 20.1)	5.4 (-0.1; 10.9)	4.5 (-6.2; 15.1)
Baseline BMI[b] (kg/m^2)			4.9 (2.4; 7.3)	5.9 (2.5; 9.2)	3.3 (1.3; 5.3)	4.9 (2.1; 7.7)
Mother's age (years)	-3.1 (-7.5; 1.2)		-4.9 (-7.3; -2.5)	-6.2 (-8.6; -3.8)	-4.7 (-6.9; -2.6)	-6.2 (-8.4; -4.1)
Hemoglobin concentration (g/dl)	33.3 (12.7; 53.8)	34.8 (14.4; 55.1)	15.2 (7.8; 22.6)	12.9 (4.6; 21.2)	19.3 (12.3; 26.4)	19.4 (11.4; 27.4)
Breastfeeding initiation time						
Breastfeeding initiation within 1 h	1	1	1	1	1	-
Breastfeeding initiation after 1 h	-56.2 (-94.9; -17.4)	-39.9 (-90.3; 10.6)	-40.5 (-60.1; -20.9)		-42.5 (-61.1; -23.9)	-
Child's gender						
Male babies	1	1	1			

11

Female babies	-53.1 (-104.7; -1.6)	-52.9 (-103.0; -2.9)	21.8 (-3.9; 47.4)			
HIV disease stage						
HIV stage 1			1	1	1	1
HIV stage >1			-85.8 (-131.5; -40.2)	-86.5 (-147.1; -26.0)	-70.2 (-115.5; -25.0)	-83.7 (-144.1; -23.4)
Education						
Non completed primary school			1		1	1
Completed primary school			29.2 (-8.6; 67.1)		25.1 (-12.6; 62.9)	24.4 (-12.9; 61.6)
Secondary school and more			1.0 (-26.8; 28.9)		-7.8 (-34.9; 19.3)	-9.3 (-36.8; 18.2)
Marital status						
Married/cohabiting mothers			1	1	1	1
Single mothers			-34.5 (-73.2; 4.2)	-44.6 (-83.1; -6.03)	-24.6 (-55.9; 6.6)	-29.7 (-61.0; 1.6)
Delivery						
Vaginal delivery			1	1		
C-section delivery			71.6 (11.7; 131.4)	71.1 (11.1; 131.2)		
Trial arm						

Lamivudine arm	1	1	1	1	1	1
Lopinavir/ritonavir arm	-33.4 (-69.2; 2.3)	-65.3 (-116.4; -14.1)	-12.8 (-31.6; 6.1)	-12.9 (-38.2; 12.4)	-15.8 (-32.6; 1.0)	-19.2 (-41.9; 3.6)

[a]Confidence interval

[b]Body mass index

Table 2c: Mother's HIV-1 viral load change according to EPBF duration adjusted to different covariates: stratification presenting South Africa versus the other sites and pooled analysis

	South Africa		Burkina Faso, Uganda and Zambia		Pooled analysis	
	Unadjusted coefficient (95% CI[a])	Adjusted coefficient (95% CI[a])	Unadjusted coefficient (95% CI[a])	Adjusted coefficient (95% CI[a])	Unadjusted coefficient (95% CI[a])	Adjusted coefficient (95% CI[a])
Dependent variable=viral load (coefficient * 10^3)						
EPBF duration (months)	4.5 (-3.4; 12.4)	-3.6 (-11.5; 4.4)	5.4 (-7.1 18.0)	2.0(-11.3 15.4)	6.2 (-2.5; 14.9)	1.7 (-7.3; 10.8)
Baseline BMI[b] (kg/m^2)	-7.7 (-10.9; -4.6)	-14.5 (-17.9; -11.0)	-4.7 (-8.5; -1.0)	-5.7 (-9.8; -1.6)	-6.5 (-9.2; -3.8)	-8.0 (-11.0; -4.9)
Mother's age (years)	-2.7 (-5.3; -0.1)	-2.7 (-5.5; 0.1)	-1.9 (-4.6; 0.8)	-4.5 (-7.7; -1.4)	-2.1 (-4.3; 0.1)	-4.5 (-7.0; -2.0)
Breastfeeding initiation time						

Breastfeeding initiation<1h	1	1				
Breastfeeding initiation>1h	70.5 (41.3; 99.7)	45.1 (13.5; 76.7)				
Child's gender						
Male babies	1	1	1	1	1	1
Female babies	-49.1 (-79.4-18.7)		-19.5 (-48.5; 9.4)	-36.5 (-66.0 - 7.2)	-25.3 (-49.2; -1.4)	-35.2 (-59.2; -11.1)
Education						
Non completed primary school			1	1	1	1
Completed primary school			-10.2 (-53.9;33.5)	2.9 (-41.4; 47.2)	-5.0 (-45.1; 35.2)	13.4 (-26.9; 53.8)
Secondary school and more			-76.7 (-108.1; -45.3)	-73.4 (-105.9; -41.0)	-72.7 (-98.7; -46.7)	-62.0 (-89.7; -34.3)
Marital status						
Married/cohabiting mothers	1	1				
Single mothers	55.6 (21.6; 89.5)	127.9 (92.8 163.0)				
Delivery						
Vaginal delivery	1	1	1	1	1	1
C-section delivery	118.5 (86.4;	143.2(108.8;	72.6 (6.8; 138.4)	84.2	90.8 (49.8;	105.5 (65.2; 145.7)

	150.5)	177.5)		(17.6;150.7)	131.8)	
Parity						
Primipara	1	1	1	1	1	1
Multipara	66.5 (34.8; 98.2)	125.9 (90.5; 161.2)	47.7 (12.1; 83.2)	56.7 (15.1; 98.2)	54.8 (26.7; 83.0)	65.1 (32.8; 97.4)
Trial arm						
Lamivudine arm	1	1	1	1	1	1
Lopinavir/ritonavir arm	-48.4 (-77.6; -19.2)	-37.6 (-67.5; -7.6)	39.9 (12.4; 67.4)	47.0 (17.9; 76.1)	22.6 (-0.0; 45.2)	31.1 (7.1; 55.0)
Birthweight (g)	0.0 (0.0 ; 0.1)	0.1 (0.0; 0.1)	0.0 (0.0; 0.1)	0.1 (0.0; 0.1)		

[a]Confidence interval

[b]Body mass index

Table 2d: Mother's HIV-1 disease progression according to EPBF duration adjusted to different covariates: stratification presenting South Africa versus the other sites and pooled analysis

	South Africa		Burkina Faso, Uganda and Zambia		Pooled analysis	
	Unadjusted odd ratio (95% CI[a])	Adjusted odd ratio (95% CI[a])	Unadjusted odd ratio (95% CI[a])	Adjusted odd ratio (95% CI[a])	Unadjusted odd ratio (95% CI[a])	Adjusted odd ratio (95% CI[a])
EPBF duration (months)	1.0 (0.9; 1.1)		1.1 (1.0 1.2)	1.0 (0.9;1.1)	1.1 (1.0; 1.1)	1.1 (1.0; 1.2)
Mother's age (years)			1.0 (1.0; 1.1)	1.0 (1.0; 1.1)		1.0 (1.0; 1.0)

15

Child's gender						
Male babies			1		1	1
Female babies			0.8 (0.6; 1.0)		0.8 (0.6; 1.0)	0.8 (0.6; 1.0)
HIV disease stage						
HIV stage 1			1	1		
HIV stage >1			4.0 (2.5; 6.2)	4.2 (2.6; 6.5)		
Marital status						
Married/cohabiting mothers			1	1	1	1
Single mothers			1.6 (1.1; 2.2)	1.8 (1.3; 2.6)	1.5 (1.2; 1.9)	1.6 (1.3; 2.1)
Trial arm						
Lamivudine arm			1	1	1	1
Lopinavir/ritonavir arm			1.3 (1.0; 1.6)	1.3 (1.0; 1.7)	1.3 (1.0; 1.6)	1.3 (1.0; 1.6)
Birthweight (g)			0.9 (0.8; 1.0)			

[a]Confidence interval

[b]Body mass index

Table 3

Table 3a: Mother's weight change according to any breastfeeding duration adjusted to different covariates: stratification presenting South Africa versus the other sites and pooled analysis

	South Africa		Burkina Faso, Uganda and Zambia		Pooled analysis	
	Unadjusted coefficient (95% CI[a])	**Adjusted coefficient (95% CI[a])**	**Unadjusted coefficient (95% CI[a])**	**Adjusted coefficient (95% CI[a])**	**Unadjusted Odd Ratio (95% CI[a])**	**Adjusted odd ratio (95% CI[a]**
		Weight				
Any breastfeeding duration (months)	0.3 (-0.2; 0.8)	-0.1 (-0.3; 0.0)	-0.0 (-0.3; 0.2)	0.1 (0.0; 0.3)	-0.0 (-0.3; 0.2)	-0.0 (-0.2; 0.1)
Baseline BMI[b] (kg/m^2)	2.5 (2.4; 2.7)	2.5 (2.3; 2.6)	2.5 (2.4; 2.6)	2.4 (2.3; 2.5)	2.5 (2.4; 2.6)	2.4 (2.3; 2.5)
Mothers' age (years)	0.8 (0.5; 1.2)	0.2 (0.0; 0.3)	0.5 (0.4; 0.6)	0.1 (0.1; 0.2)	0.5 (0.4 0.7)	0.1 (0.1; 0.2)
HIV disease stage						
HIV stage 1	1	1	1			
HIV stage>1	12.4 (-4.5; 29.4)	6.4 (0.0; 12.7)	-2.8 (-6.4; 0.8)			
Education						
Non completed primary school			1	1	1	1
Completed primary school			3.9 (2.0; 5.9)	0.3 (-0.6; 1.1)	4.4 (2.2; 6.5)	0.6 (-0.3; 1.5)
Secondary school and further			2.7 (1.2; 4.1)	0.9 (0.2; 1.5)	3.1 (1.5; 4.6)	1.0 (0.4; 1.7)

Marital status						
Married/cohabiting mothers	1				1	
Single mothers	-3.1 (-7.2; 0.9)				-1.2 (-3.0; 0.5)	
Delivery						
Vaginal delivery			1		1	
C-section delivery	3.7 (-0.4; 7.9)	-1.6 (-3.2; 0.0)	4.5 (1.5; 7.6)		4.4 (2.1; 6.7)	-1.2 (-2.1; -0.2)
Parity						
Primipara	1	1	1	1	1	
Multipara	6.1 (1.9; 10.3)		3.1 (1.4; 4.7)		3.9 (2.3; 5.4)	
Trial arm						
Lamivudine arm	1	1	1	1	1	
Lopinavir/ritonavir arm	2.0 (-2.0; 6.1)	0.7 (-0.8; 2.2)	-0.1 (-1.4; 1.3)	-0.3 (-0.9; 0.3)	0.3 (-1.0; 1.6)	0.1 (-0.7; 0.4)

[a]Confidence interval

[b]Body mass index

Table 3b: Mother's CD4 cell count change according to any breastfeeding duration adjusted to different covariates: stratification presenting South Africa versus the other sites and pooled analysis

		South Africa		Burkina Faso, Uganda and Zambia		Pooled analysis
	Unadjusted coefficient (95% CI[a])	**Adjusted coefficient (95% CI[a])**	**Unadjusted coefficient (95% CI[a])**	**Adjusted coefficient (95% CI[a])**	**Unadjusted Odd Ratio (95% CI[a])**	**Adjusted odd ratio (95% CI[a]**
		CD4 cells count				
Any breastfeeding duration (months)	0.4 (-6.8; 7.6)	-2.4 (-9.5; 4.7)	1.2 (-5.8; 8.3)	9.8 (-2.1; 21.8)	1.5 (-3.9; 7.0)	5.7 (0.4; 10.9)
Baseline BMI[b] (kg/m^2)			4.9 (2.4; 7.3)	5.7 (2.4; 9.1)	3.3 (1.3; 5.3)	4.2 (1.5; 6.9)
Mother's age (years)	-3.1 (-7.5; 1.2)		-4.9 (-7.3; -2.5)	-6.5 (-8.9; -4.1)	-4.7 (-6.9; -2.6)	-6.2 (-8.4; -4.1)
Hemoglobin concentration (g/dl)	33.3 (12.7; 53.8)	33.9(13.5; 54.3)	15.2 (7.8; 22.6)	15.7 (7.2; 24.3)	19.3 (12.3; 26.4)	16.7 (9.0; 24.4)
Breastfeeding initiation time						
Within 1 hour			1		1	
After 1 hour	-56.2 (-94.9; -17.4)		-40.5 (-60.1; -20.9)		-42.5 (-61.1; -23.9)	
Child's gender						
Male babies	1	1	1			

Female babies	-53.1 (-104.7; -1.6)	-54.1 (-104.3; -3.6)	21.7 (-3.9; 47.4)			
HIV stage						
HIV stage 1			1	1	1	1
HIV stage>1			-85.8 (-131.5; -40.2)	-91.5 (-152.9; -30.1)	-70.2 (-115.5; -25.0)	-88.8 (-148.3; -29.3)
Education						
Non completed primary school			1		1	1
Completed primary school			29.2 (-8.6; 67.1)		25.1 (-12.6; 62.9)	19.9 (-16.7; 56.5)
Secondary school and further			1.0 (-26.8; 28.9)		-7.8 (-34.9; 19.3)	-17.4 (-43.7; 9.5)
Marital status						
Married/ cohabiting mothers			1	1	1	1
Single mothers			-34.5 (-73.2; 4.2)	-43.1 (-81.5; -4.6)	-24.6 (-55.9; 6.6)	-43.3 (-72.8; -13.7)
Delivery						
Vaginal delivery			1	1		
C-section delivery			71.6 (11.7; 131.4)	71.8 (11.9; 131.7)		
Parity						

Primipara			1			
Multipara						
Trial arm						
Lamivudine arm	1	1	1	1	1	1
Lopinavir/ritonavir arm	-33.4 (-69.; 2.3)	-58.7 (-109.6; -7.8)	-12.8 (-31.6; 6.1)	-13.4 (-38.6; 11.8)	-15.8 (-32.6; 1.0)	-19.2 (-41.9; 3.6)

[a]Confidence interval

[b]Body mass index

Table 3c: Mother's HIV-1 viral load change according to any breastfeeding duration adjusted to different covariates: stratification presenting South Africa versus the other sites and pooled analysis

	South Africa		**Burkina Faso, Uganda and Zambia**		**Pooled analysis**	
	Unadjusted coefficient (95% CI[a])	**Adjusted coefficient (95% CI[a])**	**Unadjusted coefficient (95% CI[a])**	**Adjusted coefficient (95% CI[a])**	**Unadjusted Odd Ratio (95% CI[a])**	**Adjusted odd ratio (95% CI[a]**
		HIV-1 Viral load (coefficient*10^3) copies/µl				
Any breastfeeding duration (months)	11.2 (6.8; 15.6)	7.7 (3.4; 12.1)	5.9 (-1.5; 13.2)	2.5 (-5.2; 10.2)	9.8 (4.9; 14.7)	6.1 (1.0; 11.2)

Baseline BMI[b] (kg/m^2)	-7.7 (-10.9; -4.6)	-14.0 (-17.4; -10.6)	-4.7 (-8.5; -1.0)	-5.7 (-9.8; -1.5)	-6.5 (-9.2; -3.8)	-7.6 (-10.7; -4.5)
Mother's age (years)	-2.7 (-5.3; -0.1)	-3.4 (-6.2; -0.6)	-1.9 (-4.6; 0.8)	-4.6 (-7.8; -1.4)	-2.1 (-4.3; 0.1)	-4.8 (-7.3; -2.3)
Breastfeeding initiation time						
Within 1 hour	1	1				
After 1 hour	70.5 (41.3; 99.7)	34.2 (2.7; 65.7)				
Child's gender						
Male babies	1		1	1	1	1
Female babies	-49.1 (-79.4; -18.7)		-19.5 (-48.5; 9.4)	-37.2 (-66.6; -7.9)	-25.3 (-49.2; -1.4)	-36.2 (-60.2; -12.2)
Education						
Non completed primary school			1	1	1	1
Completed primary school			-10.2 (-53.9; 33.5)	4.7 (-39.8; 49.3)	-4.9 (-45.1; 35.2)	16.6 (-23.8; 57.0)
Secondary school and further			-76.7 (-108.1; -45.3)	-70.7 (-104.4; -37.1)	-72.7 (-98.7; -46.7)	-54.5 (-82.8; -26.1)
Marital status						
Married/ cohabiting mothers	1	1			1	

Single mothers	55.6 (21.6; 89.5)	124.9 (89.9; 160.0)				
Delivery						
Vaginal delivery	1	1	1	1	1	1
C-section delivery	118.5 (86.4; 150.5)	137.0 (102.7; 171.2)	72.6 (6.8; 138.4)	84.4 (18.0; 150.7)	90.8(49.8; 131.8)	104.7 (64.5; 144.9)
Parity						
Primipara	1	1	1	1	1	1
Multipara	66.5 (34.8; 98.3)	125.0 (89.8; 160.3)	47.7 (12.1; 83.2)	57.2 (15.6; 98.8)	54.8 (26.7; 83.0)	65.0 (32.7; 97.3)
Trial arm						
Lamivudine arm	1	1	1	1	1	1
Lopinavir/ritonavir arm	-48.4 (-77.6; -19.2)	-35.0 (-64.8; -5.3)	39.9 (12.4; 67.4)	47.6 (18.6; 76.7)	22.6 (-60.2; 45.2)	31.9 (8.0; 55.8)

[a]Confidence interval

[b]Body mass index

Table 3d: Mother's HIV-1 disease progression according to any breastfeeding duration adjusted to different covariates: stratification presenting South Africa versus the other sites and pooled analysis

	South Africa		Burkina Faso, Uganda and Zambia		Pooled analysis	
	Unadjusted odd ratio (95% CI[a])	Adjusted odd ratio (95% CI[a])	Unadjusted odd ratio (95% CI[a])	Adjusted odd ratio (95% CI[a])	Unadjusted odd ratio (95% CI[a])	Adjusted odd ratio (95% CI[a]
		HIV disease progress				
Any breastfeeding duration (months)			1.1 (1.0; 1.2)	1.0 (0.9; 1.1)	1.0 (0.9; 1.0)	1.0 (0.9; 1.0)
Baseline BMI[b] (kg/m^2)			1.0 (1.0; 1.1)			
Mother's age (years)				1.0 (1.0; 1.1)	1.0 (0.9; 1.0)	
Breastfeeding initiation time						
Within 1 h	1					
After 1 h						
Child's gender						
Male babies			1		1	
Female babies			0.8 (0.6; 1.0)		0.8 (0.6; 1.0)	
HIV stage						
HIV stage 1			1	1	1	1

24

HIV stage>1			4.0 (2.5; 6.2)	4.2 (2.6; 6.6)	4.4 (2.8; 6.7)	4.6 (2.9; 7.3)
Education						
Non completed primary school			1			1
Completed primary school						1.4 (0.9; 2.0)
Secondary school and further						0.7 (0.5; 0.9)
Marital status						
Married/cohabiting mothers			1	1		
Single mothers			1.6 (1.1; 2.2)	1.8 (1.2; 2.6)	1.5 (1.2; 1.9)	
Trial arm						
Lamivudine arm			1	1	1	1
Lopinavir/ritonavir arm			1.3 (1.0; 1.6)	1.3 (1.0; 1.7)	1.3 (1.0; 1.6)	1.3 (1.0; 1.6)

[a]Confidence interval

[b]Body mass index

The association between CD4 cells count and EPBF duration was non-significant (5.4 (95% CI:-0.1; 10.9) and 4.5 (95% CI:-6.2; 15.1) CD4 cells/µl increase per month of EPBF duration at univariate and multivariate analysis, respectively). The association was significantly positive between the mothers' baseline BMI, hemoglobin concentration and CD4 cell count yielding a mean increase of 4.9 (95% CI: 2.1; 7.7) CD4 cells/µl per additional BMI unit and 19.4 (95% CI:11.4; 27.4) CD4 cells/µl per additional unit of hemoglobin throughout the EPBF period (Table 2b).

There was no significant association between HIV-1 viral load and EPBF duration. The heavier and older mothers, those who delivered female babies and the best educated women group had a significantly lower mean viral load in the multivariate analysis. The mothers allocated to the lopinavir/ritonavir group had a significantly higher mean viral load than the ones in the Lamivudine arm (Table 2c).

We found no significant association between EPBF duration and HIV-1 disease progression. However, randomization to the lopinavir/ritonavir arm or being single mother led to a significantly adjusted odd ratios (AOR) of 1.3 (95% CI: 1.0; 1.6; p=0.04) and 1.6 (95% CI: 1.3; 2.1), respectively (Table 2d).

Considering any breastfeeding duration, there was no weight change at univariate and multivariate analysis overall(Table 3a). Still regarding any breastfeeding, overall, there was a significant mean increase of 5.7 (95% CI: 0.4; 10.9) CD4 cells/µl per month of any breastfeeding. We found also that being a single mother was associated with a mean decrease of -43.3 (95% CI: -72.8; -13.7) CD4 cells/ µl as compared to married ones (Table 3b). Any breastfeeding duration was also associated with a significantly higher mean viral load (Table 3c). Analysis with any breastfeeding pattern and HIV-1 disease progression showed the same associations as EPBF and HIV-1 disease progression (Table 3d).

In the stratified analysis, we found that EPBF duration had no influence on mothers' weight, CD4 count, or HIV-1 viral load, whatever the stratum. HIV-1 disease progression was not associated either with EPBF duration (Table 2a, 2b, 2c, 2d). In stratum 2, C-section delivery was associated with an increase in CD4 cell count (Table 2b), whereas delivering a female baby and being educated beyond secondary school were associated with a decrease in HIV-1 viral load (Table 2c).

In South Africa, initiating breastfeeding one hour post-delivery and being a single mother were related to an increase in HIV-1 viral load. In both strata, C-section delivery and

multiparity were also related to an increase in HIV-1 viral load. There was no association between any breastfeeding and the mothers' weight, CD4 cells count and HIV-1 disease progression in any of the strata (Table 3a, 3b, 3d). However, any breastfeeding duration was associated with an increase of the HIV-1 viral load in South African women (Table 3c).

DISCUSSION

Considered separately, there appeared to be no variations in the mothers' weight, CD4 cell count and HIV-1 viral load related to EPBF or any breastfeeding. The same conclusion applied to these outcomes combined in a composite endpoint representing HIV-1 disease progression. Unsurprisingly, mothers' baseline BMIs were consistently associated with an increase in the mothers' weight and CD4 cell count, and with a lower mean HIV-1 viral load for both EPBF and any breastfeeding groups.

In a review of the literature on weight change in the postpartum period, there appeared to be no association between breastfeeding, or generally between the mode of infant feeding, and postpartum weight loss. However C-section delivery was a risk factor for postpartum weight loss [28], similar to our findings. South Africa had markedly lower rates of vaginal deliveries versus other countries (table 1b). In the years 2000, studies were published demonstrating that elective C-section before the labour and before the rupture of membranes added protection against HIV transmission to the new born [29 30]. The lower rates of vaginal deliveries in South Africa were likely due to the country policies (influenced by the scientific evidence) which supported HIV-infected women toward delivering HIV-free babies. This support included, free formulas and probably scheduled C-section for the HIV-infected pregnant women and mothers. Why the rest of the countries did not implement the same policy is certainly a matter of affordability and availability of local resources. Another reason is that C-section rate is «recklessly high» in South Africa where up to 90% of pregnant women deliver through this method in private hospitals (The Guardian https://www.theguardian.com/world/2014/sep/24/caesarean-section-south-africa [Accessed on 27 October 2017]. This practise may have spilled over but at a lesser extent into public health facilities. We believe this practice has not skewed our results, since these C-section deliveries were not medically indicated at first hand, at least not based on a vaginal delivery risk; therefore they are not done on women with poorer health status. Actually, South African women had the lowest mean HIV-1 viral load and the highest mean BMI.

Yet, this review of literature [28] found that less educated mothers (<12 years of schooling) were at risk of postpartum weight retention; we found that higher educated women (secondary school or further) were at risk of that weight retention. This difference in our

finding may be explained by the difference in our categorization of the education variable. In our study less educated participants included only women with primary school level, meaning around six years of schooling. Therefore, the results of the two studies are not really comparable. A higher education level was also a factor associated with a slower HIV-1 disease progression. This finding is consistent with our result that higher educated women retained more weight.

In a further review of literature on the effects of lactation on the mother's bodyweight, it is clear that the assumption that the postpartum weight loss is due to the high energy demand associated with lactation has been challenged by many studies [31]. Some reports conflict with our own findings, such as the one in KwaZulu Natal, where HIV-1 infected mothers at between 8 and 24 weeks had a mean weight loss of 1.4 kg in contrast to a 0.4-kg weight gain in HIV-1 uninfected mothers (P=0.01) during breastfeeding [15].

Regarding the change in CD4 cells count, the South African data support the conclusion that CD4 cell count did not differ significantly between women who breastfed and those who did not [32]. This finding contradicts the Kenyan Study that found that the rate of CD4 cell count decline was higher in breast-feeding than in non-breast-feeding mothers [13]. However, in that Kenyan study, HIV-1 RNA levels did not differ significantly between breast-feeding and formula-feeding mothers.

Regarding HIV-1 disease progression, the same data showed no deleterious effect of breastfeeding in HIV-1-infected mothers, similar to our study-findings. The outcome variables were the CD4 and CD8 cell count, the mothers 'illness and mortality, and their hemoglobin levels [32]. Another study from Malawi reached the same conclusion that breastfeeding was not associated with higher risk of maternal morbidity or mortality [33]. A study in Zambia concluded in the same direction that at 12 months after delivery, there was no difference in mortality between women who breastfed for a short duration (4 months) versus those who breastfed for a duration of their own choice [17]. An individual patient data meta-analysis on mortality among HIV-1 infected mothers according to children's feeding modality confirmed that the risk of dying within 18 months postpartum was not significantly affected by the infants' feeding modality (i.e. ever versus never breastfed) [34].

In healthy breastfeeding mothers, the postpartum weight loss would be around 0.5 kg per month among population with relatively high mean of BMI. The mechanism of the weight loss would be burning of 483 -538 kcal per day [35 36]. Therefore, losing weight after birth is likely when the mother's calorie intake does not cover the calorie expense related to breast-milk production. Considering these findings, we think that energy requirement and thus the metabolic stress related to breastfeeding would be quite bearable. This may explain why in our study HIV-1-infected, immune-competent and breastfeeding mothers' health status was

not deteriorated by breastfeeding. This evidence inspires the idea that option A peri-exposure antiretroviral prophylaxis might still have pertinent indications since breastfeeding remained the most frequent feeding option in Sub Saharan Africa and since breast-milk might still host HIV-1 reservoirs that mother's prophylaxis could not always 100% suppress [37].

Strengths and limitations

Our study has been implemented in four countries in Africa, including Burkina Faso (West), South Africa and Zambia (South) and Uganda (East). Therefore we consider our study population representative of the Sub-Sahara African population. The data were also collected in the rigorous context of a clinical trial, which minimized the lost to follow-up, the missing data as well as other data collection errors, and therefore improved the quality of our data.

However, the selection associated with the environment of a clinical trial - usually quite different from a routine environment - may have biased our findings. Nonetheless, our endpoints (mother's weight, CD4 cell count and HIV-1 viral load) were sufficiently robust for us to vouch for their validity. Another point of note is the stratification of the participants into two strata, i.e. South Africa versus Burkina Faso, Uganda and Zambia. This stratification reduced the sample size in South Africa. Thus some of the modelling for South Africa could be less rigorous, and the findings regarding the risk factors there may not truly reflect the reality.

CONCLUSION

Breastfeeding whatever the type (exclusive or any) as far as this study can conclude was not a risk factor for the HIV-1 infected mothers weight, CD4 cell count, and HIV-1 viral load change, or HIV-1 disease progression, keeping in mind that all the participants had a baseline CD4 cell count >350 cells/ul. The mothers' baseline high weight and high hemoglobin concentration were important factors in being consistently associated with an improvement of the outcome variables at stake. A higher education level was also a factor associated with a slower HIV-1 disease progression. Considering the benefits of breast milk for infants, and the consensus results from different studies elsewhere that breastfeeding does not harm HIV-1-infected mothers, this study also supports the WHO 2016 guidelines on infant feeding, which indicates that mothers living with HIV should breastfeed for at least 12 months and up to 24 months, provided that the right treatment or prophylaxis for the infection is given where formula feeding is unsafe [12].

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Contributorship statement:

Conceptualization: ENS, IMSE, NN, NM, PVP, TT.
Data curation: ENS, RV.
Formal analysis: ENS, IMSE.
Investigation: ENS, MS, NM, JKT, CK, JGH.
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Supervision: TT, IMSE, NM, NN.
Validation: TT, IMSE, NM, NN.
Writing the original draft: ENS, IMSE.
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35. IOM. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. Micronutrients IoMPo, editor. Institute of Medicine, Food and Nutrition Board: National Academies Press 2005:1-1357
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37. Van de Perre P, Rubbo P, Viljoen J, et al. HIV-1 reservoirs in breast milk and challenges to elimination of breast-feeding transmission of HIV-1. Sci Transl Med. 2012;**4**(143):14

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract: **YES ; page 1 row 3**
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found: **YES; page2 row 8-28**
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported: **YES; page 3 row 23-37 ; page 4 row 1-8**
Objectives	3	State specific objectives, including any prespecified hypotheses: **YES; page 4 row 10-13**
Methods		
Study design	4	Present key elements of study design early in the paper: **YES page 4 row 10-34**
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection: **YES page 4 row 17-18**
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up: **YES page 4 row 20-22 and row 30-32**
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable: **YES page 5 row 19-35**
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group: **YES page 5 row 10-18 and 35-37 ; page 6 row 1-2**
Bias	9	Describe any efforts to address potential sources of bias: **YES page 6 row 2-11**
Study size	10	Explain how the study size was arrived at: **YES page 6 row 30-35**
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why: **YES page 6 row 15-17**
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding: **YES page 6 row 3-17**
		(*b*) Describe any methods used to examine subgroups and interactions: **YES page 6 row 6-8 and 11-15**
		(*c*) Explain how missing data were addressed**: YES page page 6 row 5-6 and 32-34**
		(d) If applicable, explain how loss to follow-up was addressed **YES page 6 row 5-6**
		(*e*) Describe any sensitivity analyses: **page page 6 row 11-15**
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed: **YES; page 6 row 30-35**
		(b) Give reasons for non-participation at each stage: **YES; page 6 row 30-35**
		(c) Consider use of a flow diagram: **YES; page 6 row 35**
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders: **YES tables 1a and 1b**
		(b) Indicate number of participants with missing data for each variable of interest: **Yes page 6 row 31-35**
		(c) Summarise follow-up time (eg, average and total amount) **YES (mean EPBF**

		and any breastfeeding) table 1a
Outcome data	15*	Report numbers of outcome events or summary measures over time **NO**
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included: **YES tables 2 and 3**
		(*b*) Report category boundaries when continuous variables were categorized: **YES table 1b (mothers'age)**
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period: **not relevant**
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses: **YES page 6 row 11-17**
Discussion		
Key results	18	Summarise key results with reference to study objectives: **YES page 28 row 14-23**
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias: **YES page 30 row 21-28**
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence: **YES page 30 row 25-28 and 32-34**
Generalisability	21	Discuss the generalisability (external validity) of the study results: **YES page 30 row 16-20**
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based: **YES page 31 row 20-27**

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.